Abstracts of Papers Presented at the Fifth International Congress of Radiation Research, Seattle, Washington, USA July 14–20, 1974

A-1-1 *Energy Needs, Nuclear Power, and the Environment.* A. M. WEINBERG, Office of Energy Research and Development, Federal Energy Office, Washington, D.C. 20500, USA (*see page 316*).

A-2-1 *Fundamental Aspects of LET in Radiobiology.* J. F. FOWLER, Gray Laboratory, Mount Vernon Hospital, Northwood, London, Great Britain.

Low LET radiation is less efficient per rad than high LET radiation and also permits more repair of molecular damage to take place during and after irradiation. Are these two statements different ways of saying the same thing? If low doses (or dose rates) of low-LET radiation act by "single-hit" kinetics, i.e., if a proportion of the injury they produce is irreparable, the RBE would then rise to a finite limiting value as the dose per session (or dose rate) is reduced. In these circumstances the full proportion of "repair" that can occur after low-LET radiation *is* occurring. Further, modification by oxygen and similar radiosensitisers would be reduced.

Although the "LET" does not rise above 30 KeV/micron at the end of electron tracks, the *specific* energy imparted to submicroscopic target volumes can be enormous even for low-LET radiation. Can this effect account for the proportion of irreparable injury observed in biological experiments? Evidence for this and other alternatives will be reviewed.

Applications include neutron and heavy particle radiobiology as well as low doses per session of X-rays.

A-2-2 *Energy Deposition in Small Volumes in Relation to LET.* D. SRDOČ, Institute "Rudjer Bošković," 41001 Zagreb, POB 1016, Yugoslavia.

Energy deposition patterns produced by ionizing radiation in matter are briefly discussed as well as processes leading to physical and chemical changes of irradiated matter. The density of charged particles and excited atoms and molecules along the path of a charged particle crossing the volume of interest is of utmost importance for understanding the relative biological effectiveness (RBE) of the radiation in question. Therefore, the experimental technique for measurement of energy deposition spectra in very small volumes of tissue-equivalent material is presented and results for various types of radiation are shown. Also, the dependence of these spectra on the size of the volume of interest is shown. Current trends as well as experimental and theoretical results in this field are reviewed and some inherent limitations to the present methods are briefly discussed.

A-2-3 *The Dependence on LET of Various Types of Damage in Phage DNA in Relation to the Inactivation Efficiency.* RALPH C. CHRISTENSEN, Department of Microbiology and Radiobiology, College of Medicine, The Pennsylvania State University, Hershey, Pa. 17033, USA.

Ionizing radiation is known to produce various kinds of primary and secondary lesions in DNA. Based on ability to discriminate the various types of these physical lesions, radiation-induced DNA damage historically has been separated into the categories of single-strand breaks, double-strand breaks, cross-links, and other nucleotide damage. Ambiguity, however, arises from the fact that some such measurements were made under alkaline conditions, whereas others were made at quasi-physiological pH, and ionizing radiation is now known to produce a significant number of lesions expressed as breaks only at very alkaline pH. The paper will review the current state of knowledge regarding correlation of these various lesions with loss of DNA function, especially the "overall" function of survival. The LET dependence of the production of these lesions (where known) will be correlated with survival of phage, and will be used for crude estimates of energy requirements for specific DNA lesions.

A-2-4 *The Dependence of RBE and OER on Neutron Energy for Damage to Mammalian Cells and Plant Systems.* ERIC J. HALL, Radiological Research Laboratory, College of Physicians and Surgeons of Columbia University, New York, N.Y. 10032, USA.

Data are reported for the OER and RBE as a function of neutron energy. Ten energies ranging from 15 MeV down to 60 keV were available at the Radiological Research Accelerator Facility at Brookhaven National Laboratory. In addition, two higher energy neutron beams

were studied, at the Naval Research Laboratory (NRL) and the Texas A and M Variable Energy Cyclotron (TAMVEC). Two biological systems were used, namely growth inhibition of *Vicia* seedlings, and Chinese hamster cells cultured *in vitro*.

OER values reached a minimum of 1.3 for neutron energies of 220 to 440 keV. An increase of energy was accompanied by an increase of OER to a maximum of 1.8 for 15 MeV. Both higher energy cyclotron-produced neutrons showed an OER of 1.6.

The plot of RBE as a function of neutron energy has a similar shape for both biological test systems used; the maximum RBE occurs at a neutron energy of 350 keV. The data are consistent with theoretical predictions based on microdosimetric measurements.

A-2-5 *RBE Values of Fast Neutrons for Damage to Organized Tissues in Experimental Animals.* J. J. BROERSE, Radiobiological Institute TNO, 151 Lange Kleiweg, Rijswijk (ZH), The Netherlands.

The characteristic parameter of a type of ionizing radiation which determines its relative biological effectiveness for a specified effect, is the spatial distribution of the energy deposition in tissue, which can to a first approximation, be described by the linear energy transfer (LET) of the ionizing particles. Heavy ions with LET values in excess of 20 keV/μm are suitable for studies with single cells or thin layers of cells, but have insufficient penetration to be used for studies of RBE-LET relationships for relatively large multicellular systems including laboratory animals. This difficulty can be overcome by employing beams of fast neutrons or negative pions, which have sufficient penetrating power and deposit a considerable part of their energy through secondaries of high LET.

For a rapidly proliferating cell renewal system, such as the immunohemopoietic system, intestinal tissues, and skin, RBE values are available for neutron beams of various energies, with different LET spectra. Only recently have quantitative studies been initiated on the responses of tissues with much longer turnover times of functional cells, such as the vascular endothelium, the lung and the spinal cord. The studies on the response of these different types of normal tissues will be summarized and the RBE for fast neutron beams of various energies will be compared.

A-3-1 *Repair Studies at the Molecular, Chromosomal, and Cellular Levels: A Review of Current Work in Japan.* S. OKADA, University of Tokyo, Tokyo, Japan.

Most of cellular radiobiological effects showed dependency on dose fractionation and dose rate, suggesting that they might have 'recovery.' Among them, Elkind type recovery and restitution of chromosome aberrations were studied in detail. Sato *et al.* (Aichi Cancer Center) found absence of Elkind type recovery in a Burkitt lymphoma. One expression of Elkind recovery is seen in electrokinetic behavior of the irradiated cells. Sasaki (Tokyo Med. Den. Univ.) found a rapid restitution of X-ray-induced chromosome aberrations in lymphocytes of Down's syndrome. Lymphocytes which showed a high sensitivity to an agent(s) and which might suggest some abnormal repair(s) are: Fanconi's anemia to bialkylated Mitomycin C, and Xeroderma pigmentosum to 4 NQO. Hashimoto *et al.* (Tokyo Univ.) found a rapid rejoining of DNA scissions in Down's lymphocytes. Fujiwara (Kobe Univ.) found efficient post-replication repairs in several cell lines with different excision repair capabilities to UV.

Some of the approaches developed are the use of isolated nuclei (Matsudaira *et al.* NIRS, Chiba), demonstrating requirements of ATP and NAD. Ono *et al.* (Tokyo Univ.) compared the rejoining of DNA scissions in several tissues of animals. Comparative studies of repairs of chromatins by centrifugation method and a radioautographic method are now in progress (Terashima and Watanabe, NIRS).

A-3-2 *DNA Damage and its Repair in Hyperthermic Mammalian Cells: Relation to Enhanced Cell Killing.* E. BEN-HUR, Department of Cellular Biochemistry, The Hebrew University—Hadassah Medical School, Jerusalem, Israel, AND M. M. ELKIND, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439, USA.

A pronounced enhacement of cell killing is observed in hyperthermic mammalian cells after x-irradiation, methyl methane sulfonate (MMS) treatment, ultraviolet light-irradiation, and

incorporation of radioisotopes into DNA. Temperatures up to 41°C enhance cell killing mainly by reducing the capacity for and/or the repair of sublethal damage. Higher temperatures enhance potentially lethal damage. Since the target molecule of all of these cytotoxic treatments is thought to be DNA, sedimentation studies of cellular DNA were undertaken using alkaline sucrose gradients. These reveal damage to DNA expressed as single-strand breaks and damage to a DNA complex. Following x-irradiation, hyperthermia up to 42°C enhances the initial rate of repair of single-strand breaks, but slows the repair of the DNA complex. After MMS treatment hyperthermia slows also the repair of MMS-induced single-strand breaks. Temperatures above 41°C, applied after either x-irradiation or MMS treatment, cause endonucleolytic-like degradation of the DNA after a few hours. It is suggested that inhibition of the repair of sublethal damage reflects the interference by hyperthermia with the repair of the DNA complex. Enhancement of potentially lethal damage could be related to hyperthermia-induced DNA degradation.

A-3-3 *Use of a Purified Lesion-Recognizing Enzyme to Assay DNA Repair in Cultured Animal Cells.* M. C. Paterson, Biology and Health Physics Division, Atomic Energy of Canada Limited, Chalk River, Ontario K0J 1J0, Canada.

Enzyme-mediated mechanisms associated with the repair of damage induced in DNA by environmental agents have been identified in primary cells cultivated from numerous animals ranging from echinoderms to mammals. The elucidation of the various error-correction mechanisms in these cells has been hampered by the limitations of existing assays. Recently, however, an enzymatic assay to evaluate DNA repair in human cells has been developed which, due to its sensitivity and substrate specificity, is superior to the well-established assays. The enzymatic assay exploits the highly selective ability of an endonuclease (purified from *Micrococcus luteus*) to introduce single-strand scissions at damaged sites in DNA extracted from UV-irradiated cells. These endonuclease-induced scissions are subsequently measured by sedimentation analysis. By incubating the irradiated cells for various times before enzymatic anslysis it is possible to obtain kinetics of the disappearance of the damaged sites and thereby to monitor in retrospect the amount of DNA repair occurring *in vivo*. New data indicate that the enzymatic assay not only complements other conventional assays for DNA repair, but also lends itself to a number of studies hitherto technically infeasible. In fact, it seems likely that the enzymatic assay may be used to investigate metabolic processes acting on a variety of physicochemical lesions in DNA including those induced by UV and ionizing radiations, as well as various chemicals displaying carcinogenic, mutagenic, or teratogenic activity.

A-3-4 *The DNA Repair Capacity of Human Cells Following Application of Chemical, Physical and Viral Carcinogens.* Hans F. Stich, Cancer Research Centre, University of British Columbia, Vancouver, Canada.

In this report we will focus on several practical and theoretical implications of DNA repair including: (1) The possible use of DNA repair synthesis in large scale screening programmes for chemical and physical carcinogens; (2) The introduction of a combined in vivo/in vitro bioassay to detect DNA repair in various organs and tissues following application of organotropic carcinogens. Since DNA repair synthesis seems only to occur in those tissues from which neoplasms arise the target organs can be readily identified; (3) The use of UV-irradiated human viruses (AD12) to estimate the DNA repair capacity of cultured cells of different human patients. This method could be employed in the identification of high risk groups with an elevated sensitivity to chemical and physical carcinogens; (4) The possible role of a perfect, defective or inclomplete DNA repair in neoplastic transformation of mammalian cells following exposure to chemical, physical and viral carcinogens.

A-3-5 *Chemical Changes Induced in DNA by Ionizing Radiation and the Relationship of their Repair to Survival of Mammalian Cells.* Robert B. Painter, Laboratory of Radiobiology, University of California, San Francisco, California 94143, USA.

Base damage accompanies single-strand-break (ssb) formation during irradiation of mammalian cells; these two kinds of DNA damage are repaired with very similar kinetics. Double-

strand-breaks, which account for 1/10 or less of total strand breaks, are repairable. The oxygen enhancement ratio for strand breaks is 3.8–4.0; there is no compelling evidence for any kind of "ultrafast" enzymatic repair of ssb at 0°C. The chemical nature of ssb end groups formed by aerobic irradiation of mammalian cells is different from that of end groups formed by irradiation of DNA in solution.

Single-strand-breaks induced by ^{125}I incorporated into mammalian DNA are not repaired as completely as are those caused by ^{3}H within DNA or by exogenous radiation ^{125}I within DNA is much more efficient in killing cells than are the other two kinds of radiation. When Chinese hamster cells are x-irradiated and retained in extreme hypoxia (<25 ppm) no repair of sublethal damage occurs. Under the same condition about 10% fewer ssb are repaired than under conditions of 200 ppm oxygen, where sublethal damage is repaired normally, even though the original yield of ssb is the same in both conditions of oxygenation. The results with both the ^{125}I experiment and the experiment with extreme hypoxia show that failure to repair ssb is accompanied by increased lethality and suggest, but do not prove, that unrepaired strand breaks are an important cause of cell killing. (Work performed under the auspices of the U.S. Atomic Energy Commission.)

A-4-1 *Mechanism of Radiation Carcinogenesis.* D. W. VAN BEKKUM, Radiobiological Institute TNO, 151 Lange Kleiweg, Rijswijk (ZH), The Netherlands.

In radiation carcinogenesis the immune reactivity of the host may theoretically be involved in two different ways. Firstly, the inducing radiation exposures may damage a substantial part of the lymphoid system as for instance when whole body irradiation is employed thus causing a decreased immune reactivity at the time when transformed cells are likely to be around. If these transformed cells were to possess specific tumor antigens, their inactivation and subsequent removal by host immune defense mechanisms could be impaired at a critical stage of tumor initiation. There is substantial evidence that such immune suppression is not a strict requirement for tumor induction by radiation. Several instances have been reported in which an irradiated tumor was transplanted immediately after either in vivo or in vitro irradiation to an isologous host where the carcinogenic process continued unabated. In many systems employed for tumor induction, e.g., with radioactive isotopes, information on the way the inducer affects the immune system is simply not available.

The second mechanism which may implicate the immune responsiveness in the process leading to tumor formation is the antigenicity of radiation induced tumors. So far only a limited number of studies has been reported where this property was examined. The results suggest that most radiation induced tumors possess neo-antigens, although they tend to be weaker than those observed in chemically induced tumors. In several instances, evidence of the involvement of a tumor virus has been presented, notably in leukemia and osteosarcomas, but the significance of this factor for interference in the process of carcinogenesis by the immune system is not well understood. Other mechanisms of radiation carcinogenesis will be discussed in relation to the immune surveillance theory.

A-4-2 *The Interplay of Viruses and Radiation in Carcinogenesis.* ARTHUR C. UPTON, State University of New York at Stony Brook, Stony Brook, New York 11790, USA.

Viruses have been implicated in the pathogenesis of a growing diversity of neoplasms in animals of various species. In most instances, however, the association between virus and neoplasia has been demonstrated in nonirradiated animals, a notable exception being certain forms of leukemia. In no case thus far has the interaction between radiation and virus been defined in detail, nor have the respective roles of the two agents in the carcinogenic process been fully delineated. Nevertheless, research in relevant model systems suggests a number of possible mechanisms of interaction, ranging from subcellular effects, such as might be analogous to induction of phage in lysogenic bacteria, to more general effects on cell population kinetics, immunological resistance, and hormonal balance in the host animal. Current advances in methodology point to promising approaches for investigation of alternative possibilities within the foreseeable future.

A-4-3 *General Aspects of Tumor Immunology.* T. JUHANI LINNA, Temple University School of Medicine, Philadelphia, Pennsylvania 19140, USA.

The discovery of tumor-associated transplantation antigens has provided the foundation for much of the work in tumor immunology. Obviously, if tumors have unique antigens, they can initiate immune responses. Much data support the concept that an important function of the immunological system may be surveillance, i.e., detection and destruction of malignant cells. While the early work emphasized cell-mediated immunity in these functions, it is increasingly evident that the antibody-forming system as well has a host-protective function, surveillance if you will. It has been found that "blocking" serum factors, first thought to be antibodies and later as antigen-antibody complexes, could enhance tumor growth. While enhancement certainly can be produced by passive administration of appropriate antisera or by particular immunization schemes, the phenomenon of "blocking" has come to be seen in a new light recently. It seems that circulating soluble tumor antigens, or antigen-antibody complexes in antigen excess, can interfere with the access of the cytotoxic cells to tumor cells, and in this way permit enhanced tumor growth. In tumor therapy, it seems rational to boost immunological host defenses against tumors in a specific and/or in a non-specific way. However, there is a call for great care in defining such immunotherapy schedules for clinical use, so that the immunostimulation really results in a boost of host defense mechanisms.

A-4-4 *Macrophage Functions in Immune Response to Tumors.* H. COTTIER, Universitat Bern, Bern, Switzerland (*see page 316*).

A-5-1 *Radioecology Studies Applied to Electronuclear Siting.* A. GRAUBY, CEA Radioecology Laboratory, Saint-Paul-Lez-Durance, France.

For many years, radioecology research has been dominated by laboratory studies and experiments on operational nuclear facilities, while relatively few studies have been made of suitable sites for future nuclear installations.

The ecological diversity of the natural environment is nevertheless responsible for the wide variability in the critical channels of radioelement transfer—temporal as well as spatial variability, largely as a result of human activities.

For this reason, reports of nuclear siting studies (Environmental Statements) must encompass more than mere extrapolations from radioecology laboratory findings: they must take into account on-site or simulated experimental results.

In view of the predictable development of nuclear power stations, the environmental study of suitable future nuclear sites represents a whole new field for radioecology research.

In this context, the author defines both the role and scope of radioecology as applied to nuclear site studies, and describes the methodology developed for this purpose. Finally, using two radioecological models applied to two types of river basins, the author demonstrates the importance of this research application in safeguarding man and his environment.

A-5-2 *Effects of Limnological Variables on Bioaccumulation Factors.* H. A. VANDERPLOEG AND J. R. KERCHER, Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee,[1] 37830, USA.

Bioaccumulation factors, ratios of radionuclide concentration in organisms to that in water, are used to predict radiological hazards to biota and man from chronic releases of radionuclides into aquatic systems. Bioaccumulation factors reported in the literature show wide variation. To account for this variation, an extensive analysis of laboratory and field studies of stable element and radionuclide distributions of Cs, Sr, Co, and Mn in freshwater ecosystems attempted to relate bioaccumulation factors to various limnological variables. Laboratory-derived bioaccumulation factors usually differ from bioaccumulation factors derived from field studies, a difference which results from the unnatural conditions imposed in the laboratory.

One important variable which clearly deserves more attention is the proportion of the isotope appearing in different physico-chemical forms; this proportion, in turn, is a function of limnological variables including eutrophy and amount of suspended particulates. We were limited in developing quantitative bioaccumulation factor relations because the appropriate limnological

data were not taken in most studies. If meaningful bioaccumulation factor relationships are to be derived, stable element and radionuclide distributions must be studied in nature, and special attention must be paid to the collection of appropriate limnological data.

[1] Operated by Union Carbide Corporation under contract with the U.S. Atomic Energy Commission.

A-5-3 *Effect of Some Important Physio-Ecological Factors on the Accumulation of Radionuclides by Freshwater Fish.* A. BERG, Biology Division, EURATOM Joint Research Centre, Ispra, Italy.

The increased utilization of nuclear energy in the near future will need better predictions of accumulation levels of radionuclides along the food chain. The effect of some physio-ecological factors must be particularly considered in this respect, in order to enable a more valid extrapolation of experimental results to the aquatic environmental conditions. By using simple mathematical formulae, it has been attempted to illustrate the role played by the following factors: capacity of metabolic regulation (in case of Zn and of Sr/Ca for example, in opposition to Cr) relative to the concentration of the stable element in water and to the interference of eventual chelating agents; contribution of the two ways of absorption (from water and from food); rate of exchange of the stable element which determines the uptake rate of the radionuclide; growth processes which bring the growing tissues directly in equilibrium with the environment.

A-5-4 *Behavior of Plutonium in Terrestrial Ecosystems.* F. WARD WHICKER, Colorado State University, Fort Collins, CO 80521, USA.

This paper will review some current concepts and problems regarding the behavior of plutonium in arid and semi-arid environments. Data from a grassland zone around the Rocky Flats Plant near Denver, Colorado, from desert ecosystems at the Nevada Test Site, and from canyons at Los Alamos, New Mexico provide the principal support for ideas presented in the paper. Emphasis will be given to dispersal mechanisms, ecosystem distribution, and inter-compartmental transport processes. Physical, chemical, and biological factors affecting environmental transport and distribution will be considered. Since comparatively little is known about plutonium transport processes in natural ecosystems, some discussion will be devoted to problems being encountered in current investigations. Such problems include sampling and sample heterogeniety, analytical methods, and experimental limitations. Comparative differences in transport mechanisms between prevailing physico-chemical forms of plutonium and the more soluble radionuclides which readily pass through food chains will be mentioned. The paper will conclude with an assessment of our state of knowledge in this field and important questions which deserve attention.

A-6-1 *Ground State Structure of Trapped Electrons in Glassy Matrices.* LARRY KEVAN. Department of Chemistry, Wayne State University, Detroit, Michigan 48202, USA.

The molecular dipole arrangement around excess trapped electrons in aqueous and organic glasses has recently been deduced from a combination of types of electron magnetic resonance measurements. Trapped electrons in glassy matrices typically show only a single unresolved EPR line. However, spin flip satellite lines have now been observed at 35 GHz for electrons in 10 M NaOH glass. This allows one to determine the distance to a particular number, n, of nearest neighbor protons, but n is not uniquely determined. Comparison of these data with electron spin echo results, and with calculations based on a semicontinuum potential both indicate that $n = 8$ and that four oriented water dipoles surround the electron with their protons 1.75 Å distant. Electron spin echo signals of trapped electrons in deuterated matrices show modulation phenomena from electron-nuclear dipolar interactions which are related to the spatial arrangement of surrounding deuterons. In 10 M NaOH glass these results have been analyzed to show that the second shell water dipoles are partially oriented toward the electron. In deuterated and partially deuterated methyltetrahydrofuran (MTHF) glasses, electron spin echo results show that the trapped electron interacts with most of the protons on the MTHF molecule and that these are most probably four MTHF molecules surrounding the electron. These results are further reinforced by their agreement with semicontinuum model calculations.

The picture of the electron trapping potential being formed by orientation of surrounding molecular dipoles in response to the field of the excess electron is also supported by these structural results.

A-6-2 *Electron Transport in Alkane Glasses.* K. FUNABASHI, University of Notre Dame, Notre Dame, Indiana, USA.

The excess electron mobility in 3-methylpentane (3MP) is in the range of 0.02–0.1 $cm^2/v.s.$ for 4.2–85°K. The mobility is nearly independent of temperature below 35°K, while the activation energy is about 0.01 eV for 35°K–85°K. The magnitude of mobility and its temperature dependence are consistent with the hopping and tunneling motion of electron between trapped (or localized) states. The decay kinetics of the absorption spectrum of trapped electrons in 3MP also suggest the presence of many trapping sites, and a small mean free path of retrapping for a quasi-free electron. It is conjectured that the electron-transport in 3MP glass is the phonon-assisted hopping or tunneling and the mean free path (or the mobility) at the quasi-free state is not as large as 100 Å (or 150 $cm^2/v.s.$). The mean free path of scattering for an excess electron at the quasi-free level in various alkane glasses can be found approximately from measurement of attenuation constants for electron beams (Chang and Berry). The relationship of these attenuation constants with V_0 (quasi-free state) will be discussed.

The effect of electron-phonon coupling on the effective mass of excess electrons will also be discussed in terms of a simple model. The effective mass is a sensitive function of the ratio of the relaxation energy to the phonon energy.

A-6-3 *Deferred Luminescence in Organic Matrices at Very Low Temperatures.* F. KIEFFER, C. LAPERSONNE-MEYER, AND J. RIGAUT, Laboratoire de Physico-Chimie des Rayonnements, 91405 Orsay, France.

The recombination of trapped electrons and cations formed by γ-irradiation or photoionization of organic glasses containing appropriate solutes gives rise to a deferred luminescence which can be observed at the temperature of irradiation for up to several hours. In all glasses studied, at sufficiently low temperatures, this isothermal luminescence (ITL) is found to decay in accordance with a linear relationship (1) $I_0/I(t) = 1 + \alpha(t - t_0)$ which seems to hold whenever recombination occurs predominantly by a tunneling mechanism. (I_0 = luminescence intensity at time t_0 of the beginning of observation, $I(t)$ = intensity at time t, α = a constant depending only on irradiation time and time elapsed between irradiation and observation).

By irradiating at a relatively high temperature, low enough though for the ITL decay to occur according to (1) (e.g., 77°K for methylcyclohexane), and subsequently cooling to 4°K, a decrease in luminescence intensity with temperature is observed, from which an activation energy can be deduced. This is of the order of 0.04 eV between 77° and 60°K, and practically zero from 60 down to 4°K. The effect of solute concentration is discussed.

A-6-4 *Trapped Electrons and Anions in Rigid Organic at 4°K.* T. HIGASHIMURA, Research Reactor Institute, Kyoto University, Japan.

States of the trapped electrons before solvation in organic glasses are reviewed with emphasizing advantages in the gamma radiolysis method at 4°K.

Secondary electrons are slowed down, being thermalized and then stabilized at potential wells which exist in the glasses. These wells are formed by surrounding electric dipoles which are oriented randomly. Trapped electrons transform very rapidly to solvated electrons at usual low temperature. Trapped electrons can be observed successfully by the gamma radiolysis at 4°K. The distribution of the trap depth, sizes of trap cavities and the yield of the trapped electrons are discussed.

The electrons are captured by scavenger molecules, forming anions. Some anions are unstable even at 77°K but they become stable at 4°K. Examples are presented and their absorption spectra are discussed.

A-6-5 *Reactions of Electrons in 3-Methylhexane Glass.* HUGH A. GILLIS, NORMAN V. KLASSEN AND GEORGE G. TEATHER, Division of Physics, National Research Council of Canada, Ottawa, Canada.

The growth of anion has been followed from 20 nsec to several minutes following pulse irradiation of solutions of either biphenyl or pyrene in 3-methylhexane at 76°K. Scavenging by unsolvated electrons seems to be particularly important in the pyrene solutions, whereas scavenging of solvated electrons by a tunneling mechanism seems more important for the biphenyl solutions.

The kinetics of electron decay in irradiated pure 3-methylhexane at various temperatures above 76°K have been studied. Absorption has been followed at several wavelengths so that electron decay could be distinguished from the blue shift in the absorption spectrum. Our results are compared with some published treatments of the kinetics of geminate combination.

A-6-6 *Formation and Decay of Trapped Electrons in Frozen Media.* JOHAN MOAN,* Biophysics Department, Norsk Hydro's Institute for Cancer Research, The Norwegian Radium Hospital, Montebello, Oslo 3, Norway.

The characteristic of electron trapping and decay in various media are described. The model of electron trapping in shallow traps seems to account for most of the experimental data such as: 1) The change in optical and ESR-spectra from 4° to 77°K, 2) the time dependence of the absorption spectra, 3) the increase in e_t^--yield and thermoluminescence yield with irradiation temperature, 4) the decrease in optical bleaching efficiency with temperature, 5) the decrease in spontaneous recombination luminescence with temperature, and 6) the recombination of electrons with cations to form triplet states, a process which needs spin relaxation and probably cannot occur within the time scale of recombination of free ions.

Several mechanisms of electron decay may be important. Electron tunneling certainly takes place, notably at 4°K. Tunnelling is probably also the most important mechanism of electron scavenging below the glass transition point of the solvent. However, a decay rate which is dependent on the solvent viscosity is often observed, especially in the recombination of e_t^- with positive ions as revealed by thermoluminescence. The trapped electrons seem to be capable of moving as an entity in the field from the positive ions. Thus thermoluminescence glow curves often show a maximum near the glass transition temperature of the solvent. Thermal release of the electrons out of the traps seems to be of small importance.

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* Fellow of the Norwegian Research Council for Science and the Humanities.

A-6-7 *Tunneling Phenomena in Irradiated Rigid Media.* J. KROH AND CZ. STRADOWSKI, Institute of Radiation Chemistry, Technical University, Łódź, Wróblewskiego 15, Poland.

It has been proved that electrons trapped in frozen matrices at 77°K may decay either spontaneously or in the presence of certain electron scavengers. The transfer of electrons from their traps to the scavenger molecules can proceed via quantum-mechanical tunneling. The most important experimental facts supporting such suggestion are: time and temperature dependence of electron decay, dependence on the scavenger concentration and radius, wavelength effect ("red electrons" decay faster than the "blue" ones) and electron transfer in frozen systems as measured by pulse radiolysis. The experimental data are compared with the predictions of theoretical model for electron tunneling. The applicability of this model is discussed in terms of significance of such parameters as the trap depth (characteristic for the nature of the matrix) and the density of vibrational states in scavenger molecule (characteristic for the scavenger).

A-7-1 *Effects of Plasmid Genes on Repair and Mutagenesis in Strains of* Salmonella typhimurium. DONALD G. MACPHEE, Genetics Department, La Trobe University, Bundoora, Victoria 3083, Australia.

The drug-resistance transfer factor R-Utrecht reduces the susceptibility of wild-type cells of *Salmonella typhimurium* to the lethal effects of UV and ionizing radiation while increasing their susceptibility to the mutagenic effects. The protective and mutation enhancing effects of R-Utrecht are still found in strains of *S. typhimurium* which are deficient in excision repair (*uvrB* mutants) or in the enzyme DNA polymerase I (*polA* mutants), but are not found in recombination-deficient (*recA*) mutants. R-Utrecht restores the host-cell reactivation (HCR) ability of otherwise HCR-deficient *polA* mutants to wild-type levels, which suggests that the plasmid carries a gene whose product is functionally equivalent to DNA polymerase I. This has been

confirmed by enzyme assays. The radiation-protecting effects of R-Utrecht can readily be explained in terms of the plasmid-coded DNA polymerase, but it is not yet clear whether the mutation-enhancing effects result from the same gene product or from another one.

(Supported by the Australian Institute of Nuclear Science and Engineering.)

A-7-2 *A Temperature-Dependent Ultraviolet-Sensitive* uvrA *Mutant of* Escherichia coli *K12: URT-43.* YOSHIE SHIMAZU, MITUSOKI MORIMYO AND KENSHI SUSUKI, National Institute of Radiological Sciences Chiba-shi, Japan.

URT-43 isolated from KMBL49 is a mutant which gives a larger number of UV survivors on plates at 30°C than it does on plates at 41°C. Complementation analysis revealed that the mutation is located in the same cistron as *uvrA6*. Sedimentation profiles in sucrose density gradients of irradiated URT-43 showed that nicks are formed in the DNA of irradiated bacteria when they were incubated at 30°C but not at 41°C. It was also found that UV survival of the mutant is greatly enhanced by the presence of 0.35M NaCl in the medium for post-irradiation incubation. In parallel to the survival data, pyrimidine dimers were released from DNA when irradiated growing bacteria were incubated at 30°C in the presence of NaCl, but there was no release when the bacteria were incubated at 41°C in the absence of NaCl. Interesting was the fact that there was no release of pyrimidine dimers from the DNA of amino acid starved bacteria incubated under either of these conditions. The host-cell reactivation of bacteriophage λ was negative at low and high temperatures. Chloramphenicol inhibited the recovery of irradiated bacteria. It was inferred from this evidence that the mutation is involved in the incision step of excision repair mechanism, but that the mechanism seems to be far more complicated than has been believed to be.

A-7-3 *Repair of DNA Daughter Chains after Depression of Dimer Excision.* M. SEDLIAKOVÁ, F. MAŠEK, J. BROZMANOVÁ, V. SLEZÁRIKOVÁ, AND L. MAŠKOVÁ, Cancer Research Institute, Bratislava, Czechoslovakia.

Preirradiation inhibition of DNA and protein syntheses applied simultaneously may depress dimer excision in various *E. coli* strains uvr^+rec^+ if proper conditions of starvation for thymine and amino acid are met. These treatments did not influence viability of cells, producing but a negligible effect on survival after UV.

When the isogenic *E. coli* strains uvr^-rec^+ were irradiated with a dose which produced amounts of dimers similar to those persisting in uvr^+ cells, the latter displayed much higher surviving rate.

Sedimentation analysis of DNA daughter chains revealed that uvr^+ cells could restore normal-sized DNA while uvr^- cells could not.

The data indicate that controlling the excision mechanism is not the only function of *uvr* markers. Besides, they do control a repair step that is involved in restoration of DNA daughter chains and functions independently of excision. Owing to this function of *uvr* markers, depression of dimer excision does not influence fractions of survivors. At the same time it makes it possible for excision-proficient strains to tolerate an essentially higher amount of dimers than may be tolerated by excision-deficient cells.

A-7-4 *Postreplication Repair in* Bacillus subtilis. HIROSHI TANOOKA, National Cancer Center Research Institute, Tsukiji, Tokyo, Japan.

Vegetative cells of various *B. subtilis* strains differing in their repair capacity, i.e., excision-deficient mutant (*hcr42*, Munakata), excision- and recombination-deficient mutant (*hcr42 recA1*), and parental thymine-requiring strain (Farmer and Rothman), were irradiated with u.v. light (20–50 erg/mm²), pulse-labeled with 100 μCi ^{3}H-thymidine per ml, and reincubated. The newly synthesized DNA was analyzed by the alkaline sucrose density gradient method (centrifugation: 35,000 rpm, 2 h, 20°). Small molecular weight DNA pieces were observed depending on u.v. dose. The single-strand gaps between the DNA pieces were repaired by further incubation. In excision-deficient mutant *hcr42*, the repair was rapid and saturated in 40 min incubation. By further incubation, newly synthesized DNA underwent degradation unlike in *E. coli*. In *hcr42 recA1*, the rapid repair was still observed to occur, although limited to a certain extent. Whether this is due to the leaky character of the *rec* mutant of *B. subtilis* or the *rec*-independent postreplication repair operates in *B. subtilis* is under investigation.

A-7-5 *Influence of Cell Volume and Cell Weight on UV-Sensitivity in* Saccharomyces. B. SCHAARSCHMIDT AND C. UMLAUF, Zentralinstitut für Biochemie und Biophysik, Freie Universität Berlin, West Germany.

Populations of yeast cells grown up under atmospheres of air, N_2, O_2, or CO_2 up to 10 kp/cm² pressure differ in their mean cell volume by a factor up to 4. With the same strain there is a proportionality between cell volume and cell dry weight, except at high O_2-pressure owing to its toxicity. With increasing volume higher D_0-values of inactivation after UV-irradiation are found. As a similar dependence appears with the survival curve after liquid-holding procedure a close relationship between cell size and repair ability is supposed. Microcalorimetrically-obtained data on the metabolism give evidence that those cells, which grew up to great size, are able to store a higher proportion of intracellular reserve carbohydrates than smaller cells. The dependence of the repair mechanisms after UV-irradiation on the energy metabolism of the cell is discussed.

A-7-6 *Evidence for Excision Repair of Base Damage Produced by Ionizing Radiation.* DAVID A. YOUNGS AND KENDRIC C. SMITH, Department of Radiology, Stanford University, Stanford, California 94305, USA.

Cells of *Escherichia coli* K-12 were grown to log phase in a minimal medium containing ^{3}H-thymine. The cells were resuspended in phosphate buffer and incubated at the desired temperature for 30 min before, during, and after x-irradiation. The number of single-strand breaks in the DNA was determined by alkaline sucrose gradient techniques. In wild-type cells the number of strand breaks present immediately after x-irradiation rapidly decreased. In *polA* and *polA polC* (the *polC* mutation results in production of a temperature-sensitive DNA polymerase III) cells, repair of the strand breaks occurred at 30°C, but at 42° or 46°C the number of strand breaks in DNA increased 2 to 3-fold during the first 10 min of post irradiation incubation. Longer incubations at 42°C resulted in some repair of the breaks, but the *polA polC* strain always showed less repair than the *polA* strain. At 46°C, very little repair occurred in the *polA polC* cells. A similar increase in the number of strand breaks during incubation at 46°C was observed in *lig* t_{s-7} and *polA uvrA* cells after either aerobic or anoxic x-irradiation.

These results agree with the hypothesis that an excision repair system acts on base damage produced by ionizing radiation (M. C. Paterson and R. B. Setlow, PNAS 69: 2927, 1972; P. V. Hariharan and P. A. Cerutti, JMB 66: 65, 1972). Our results suggest that the "incision breaks" may normally be efficiently repaired by DNA polymerase I or III in conjunction with polynucleotide ligase. When these enzymes are inactivated, the strand breaks rapidly accumulate in the DNA.

A-7-7 *An Enzyme Possibly Involved in the Early Step of Repair of γ-Ray Damaged DNA in* Bacillus subtilies. T. NOGUTI AND T. KADA, National Institute of Genetics, Mishima, Shizuoka-ken 411, Japan.

A significant recovery of biological activity of cellular DNA occurred in γ-ray irradiated and toluenized *B. subtilis* cells when they were incubated in presence of substrates and cofactors of DNA-polymerase and DNA-ligase (J. Mol. Biol., 67, 507, 1972). Experiments with a mutant strain deficient in DNA-polymerase I revealed that this enzyme was really involved in the above repair. Gamma-ray irradiation is known to produce inactive as well as active DNA lessions for DNA polymerase I. The inactive lessions have been supposed to be modified by certain cellular enzymes before they are subjected to repair involving the DNA polymerase. We found an enzymatic activity in *B. subtilis* cells which enhances preferentially priming activity of irradiated DNA for the purified DNA polymerase I. This cellular factor was partially purified with DEAE- and phospho-cellulose chromatography. Two active peaks appeared in phosphocellulose fractions. Sucrose density gradient analysis suggested that one of them introduced additional cuts in γ-ray irradiated DNA but not in non-irradiated DNA.

A-7-8 *Chemical Nature of the Ends of DNA Single-Strand Breaks Induced by γ-Radiation in Vivo and the Ways of their Reparation.* A. I. GAZIEV, S. A. SERGEEVA, D. T. ZAKRZHEVSKAYA, A. M. KUZIN. Institute of Biological Physics, USSR Ac. Sci., Pushchino, 142292, USSR.

Single-strand breaks with 5'OH, 5'PO_4, 3'OH ends determined by alkaline phosphatase, poly-

nucleotide kinase, DNA ligase and DNA polymerase reactions are registered in DNA from the cells *E. coli* of wild type and DNA polymerase I deficient one, isolated immediately after γ-irradiation. The total quantity of single-strand breaks with 5′ ends corresponds to those registered by the method of sedimentation in the gradient of alkaline sucrose. Unlike the yield of general single-strand breaks in DNA in vivo, that of breaks with different ends is different depending on the dose. The number of $5'PO_4$ groups is larger than that of 3′OH ones. Greater part (40–70% with doses 10–30 krad) of single-strand breaks with $5'PO_4$ and 3′OH ends is bound by ligase. The DNA polymerase I deficient end groups of ligase specific breaks of DNA cells, transform into unbindable forms during a short period of post-irradiation incubation. It occurs due to exonuclease attacks of $5'PO_4$ and 3′OH ends of DNA single-strand breaks. But ligase transformation into ligase adenylate complex at the moment of irradiation contributes to quick reparation of a part of single-strand breaks in DNA *E. coli* independent of DNA polymerase. The other part of breaks is restored only after DNA polymerase reassembling of a polynucleotide chain. It is shown in the process that ATP dependent DNA polymerase participates in reparative synthesis of DNA, along with DNA polymerase I.

A-8-1 *Evidence for Two Modes of Host-Cell Reactivation of UV-Irradiated Phage T1.* WALTER HARM, University of Texas at Dallas, Dallas, Texas 75230, USA.

UV-irradiated Tl phages infecting excision repair-proficient *E. coli* cells (B/r) undergo host-cell reactivation (HCR) i.e., their survival is much higher than when they infect repair-deficient cells (B_{s-1}). The result is usually a hetero-component survival curve, with the shallower component extrapolating to zero UV dose at an ordinate value $p < 1$. Moderate inhibition of HCR at caffeine concentrations from 0.08 to 0.5 mg/ml accentuates the biphasic character of the curves by decreasing p with little change in slope of the shallower component. At 2 mg/ml caffeine only the steep curve component is observed ($p < 10^{-3}$), which resembles the Tl survival obtained in B_{s-1} cells. Similarly Tl in B_{s-1}, with either maximum photoreactivation (PR) or PR partially inhibited by caffeine, fails to show hetero-component survival, which therefore seems to be typical of HCR.

Holding complexes of UV′*d*Tl in B/r cells in buffer, prior to plating on nutrient agar containing 2 mg/ml caffeine, results in increased phage survival ("liquid-holding recovery" or LHR). The survival curves, whose slopes decrease with increasing periods of holding, are monophasic, suggesting there are two modes of HCR. The hetero-component curves, obtained by direct plating on nutrient agar (without or with moderate HCR inhibition) reflect non-random distribution of repair events in the population, whereby within a given individual either all (most) or none (few) lesions are repaired. The monophasic curves obtained under LHR conditions indicate that excision repair of lesions (or at least its initiation) occurs randomly in the population, suggesting that within each individual the repair events are independent of one another, as in the case of PR.

A-8-2 *Repair of DNA Damaged by Ultraviolet Light and Formaldehyde.* HAJIME NISHIOKA, Doshisha University, Kyoto, Japan.

When four mutant strains of *E. coli* K12 with differing DNA repair systems (*uvrA*$^+$*recA*$^+$, *uvrA*$^-$*recA*$^+$, *uvrA*$^+$*recA*$^-$, *uvrA*$^-$*recA*$^-$) were exposed to formaldehyde (FA) at different concentrations at 37°C for 10 min, the cells were inactivated in correlation to their UV-sensitivities. The inactivation was enhanced at higher temperature during the treatment. FA sensitized the UV-killing in certain strains. FA resistant mutants isolated from those strains showed higher UV resistance than their original ones. FA and UV mutagenesis (to kanamycin resistance) in those strains corresponded with each other. T_3 phage exposed to FA was reactivated with those K12 strains similarly in the case of the phage inactivated by UV.

These results suggest that FA-lesion on DNA may be subject to common enzymatic-repair-systems with pyrimidine dimer formed by UV. One type of FA-lesion may be adenine dimer in which two adjacent adenine in a DNA strand are linked together through methylene bridge at amino groups. Since the configuration of adenine dimer seems to be topologically similar to that of pyrimidine dimer, it could be assumed that both kinds of dimers are removed from DNA in similar ways.

A-8-3 *Dependence of Ultraviolet Radiation-Sensitivity on Cell Age in Bacterial Strains of Known Repair Capability.* MERIJEAN S. KELLEY AND CLAUD S. RUPERT, University of Texas at Dallas, Dallas, Texas 75230, USA.

Variations of radiation sensitivity with stage in the cell division cycle have been noted for eucaryotic cells. Cells of *Escherichia coli*, growing in minimal medium on poor carbon sources with a single genome per cell, resemble eucaryotes in having a DNA synthesis period shorter than the generation time. Since they can be obtained with known repair deficiencies, and can be synchronized, they provide a convenient model. DNA synthesis occurs *late* in the interdivisional period of both K12 and *B/r* strains—contrary to common belief, based on the (incorrect) assumption that the Helmstetter membrane procedure delivers new-born cells immediately after their division. Wild type, repair-capable cells show maximum ultraviolet sensitivity early in the *S* period, like the Chinese hamster cells reported by Sinclair and co-workers, but excisionless (UVR^-) strains show minimum sensitivity in this period, and maximum sensitivity during the gap. Recombinationless (REC^-) strains (which carry out only excision repair) and REC^- UVR^- strains show no detectable variation around the cell cycle. (Supported by USPHS research grant GM 16547 and by training grant 5-T01-CA05136-12 to the U. T. Health Sciences Center at Dallas.)

A-8-4 *Radioresistance Induced by Bacteriophage Lambda in* Escherichia coli. ZELJKO TRGOVCEVIC AND W. DEAN RUPP, Yale University, New Haven, Connecticut 06510, USA.

Transient induction of λ*cI857*, which does not kill the host cells, can alter the response of some *E. coli* strains to x-irradiation by causing an increase in radioresistance. The products of λ *red* genes—λ exonuclease and β protein—are needed to produce this effect in *recB*, *recB recC xonA*, and *recB recC sbcA* strains. All these strains lack exonuclease V. It seems likely therefore that λ *red* products can effectively substitute for the missing exonuclease V in the repair of x-irradiated DNA. On the other hand, the product of λ *gam* gene ("γ protein") is necessary for the increased radioresistance of wild type and *polA* cells. The mechanism of this γ-promoted resistance appears to be indirect: γ protein inhibits DNA degradation at early stages of repair, thereby allowing more efficient repair of DNA single-strand breaks.

(This work was supported by United States Public Health Service Grant, CA 06519.)

A-8-5 *Radiation-Sensitive Mutants of* Micrococcus radiodurans. SHIGERU KITAYAMA AND AKIRA MATSUYAMA, Institute of Physical and Chemical Research, Wako-shi, Saitama-ken, 351, Japan.

Radiation sensitive mutants were isolated from adenine auxotroph of *M. radiodurans* R_1 after nitrosoguanidine treatment. Among them, two mutants which denoted as H_{84} and F_{221} will be compared with wild type. H_{84} gives almost the same D_{10} value as *E. coli* at the exponential part of survival curve for γ-rays. It is unlikely that H_{84} has multiple mutation on genes which control radiosensitivity, because it reverts at high frequency.

The shoulder of sigmoidal survival curve which is characteristic for *M. radiodurans* disappeared in survival curve of F_{221}. They were transformed at the almost same frequency as the wild type. DNA polymerase activities in each deoxyribonuclease deficient mutant of wild type and F_{221} were almost the same, while incorporated ^{3}H-TMP in the extract of H_{84} had been rapidly lost. But the latter nuclease activity of H_{84} might not account for its high radiosensitivity, because DNA synthesis in the extract of a radiation resistant revertant was not recovered. DNA degradation *in vivo* during postirradiation incubation supports this possibility.

A-8-6 *Recovery from Enhancement by Caffeine of Radiation-Induced Inactivation.* NORMAN E. GENTNER, Biology Branch, Atomic Energy of Canada Limited, Chalk River, Ontario KOJ 1J0, Canada.

The dark repair inhibitor caffeine, if present in the post-irradiation plating medium at concentrations that do not affect the viability of unirradiated cells, causes increased inactivation of γ-irradiated *Micrococcus radiodurans* or UV- or γ-irradiated *Schizosaccharomyces pombe*. Irradiated cells, if incubated in liquid medium without caffeine before plating, become increasingly less sensitive to lethal enhancement by caffeine; study of this recovery process appears to be a convenient method of determining the time course and requirements of the system for

repair of radiation damage. Data from *M. radiodurans*, which was used as a test system, show that parameters for recovery from caffeine inhibition mirror those reported for DNA repair processes in this very radiation-resistant bacterium. This method was then used to obtain information on the recovery process in the haploid fission yeast *S. pombe*; these results have been corroborated by dose fractionation experiments. When logarithmic and stationary phase cells are compared, furthermore, the rate of recovery from caffeine inhibition reflects their relative radiation resistance.

A-8-7 *Response of* Micrococcus radiophilus *to Combinations of UV and Gamma Radiation.* NORMAN F. LEWIS AND UMESH S. KUMTA, Biochemistry & Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay 400085, India.

Micrococcus radiophilus, an exceptionally radio-resistant bacterium isolated in this laboratory, is found to possess a more efficient capacity than *Micrococcus radiodurans* for repair of damage induced by UV or gamma radiation. Since the damage and repair of lesions produced by these radiations in cellular DNA are quite distinct, the combined effect of UV and gamma radiation on *M. radiophilus* was examined.

After subjecting cells to various UV doses followed by step-wise enhancement in the dose of gamma radiation, or reversing the sequence of radiation treatment, it was found that both the UV and gamma radiation survival curves which are normally sigmoidal, showed a progressive loss of shoulder yielding straight line exponential survival curves. These findings suggest (i) that cellular ability for repair of scissions in DNA strands that contain pyrimidine dimers (caused by pre-UV exposure) is much less than in cells subjected to gamma radiation alone, and (ii) that dimer excision (and subsequent repair) from DNA fragments (caused by pre-gamma treatment) is much less efficient than in cells subjected only to UV radiation. Possible mechanisms will be presented.

A-9-1 *Induction of Skin Tumors by a Single Dose of UV-Radiation or by Freezing.* P. D. FORBES, The Skin and Cancer Hospital, Temple University Health Sciences Center, 3322 N. Broad Street, Philadelphia, Pa. 19140, USA.

Radioactive microspheres can induce profound changes in localized areas of skin, including neoplasia. Since physical trauma has sometimes been associated with skin tumorigenesis, it is important to assess the component of damage and wound healing in the process of radiation-induced neoplasia. The skin of mutant hairless (*hrhr*) mice has proven to be susceptible to tumor induction by certain types of physical damage. Twelve mice were exposed to an FS20T12 fluorescent "sun" lamp (12×10^4 j/m^2) *once*, resulting in necrosis, sloughing, contraction and scarification. Over the subsequent 40 weeks, 20 tumors developed at the edges of healed burns on 6 mice. On 24 other hairless mice, the skin surrounding a sutured incision was frozen with CO_2 dry ice; 10 tumors developed on 7 mice. No tumors developed on an equal number of mice receiving either the incision or freezing treatment alone. The tumors in both experiments were predominantly papillomas, in contrast to the carcinomas and sarcomas which follow low-level chronic radiation exposures. Thus it may be possible to distinguish between components of the neoplastic process in hairless mouse skin on the basis of the type of tumors produced.

(Supported by AEC contract No. AT(11-1)-2366.)

A-9-2 *Effect of Radiation Penetration and Growth Stage of Hair Follicles on Skin Tumor Induction.* R. E. ALBERT, F. J. BURNS, AND M. VANDERLAAN, NYU Medical Center, 550 First Ave., New York, NY 10016, USA.

An earlier experiment suggested that in growing phase skin, tumor induction did not require irradiation to the full depth of the hair follicles. The purpose of the present experiment was to obtain an estimate of the minimum penetration required for tumor induction and to determine how the stage of follicle development at the time of irradiation affects the tumor yield. Dorsal rat skin received electron irradiation at surface doses ranging from 500 rads to 8000 rads and penetrations from 0.5 mm to 2.0 mm. Some rats were irradiated at 1, 3, and 5 days after hair plucking during various phases of follicle elongation. The dependence of tumor incidence on time was similar to the pattern noted in previous resting phase experiments. The stage of

follicle elongation had no effect on the final tumor yield, and the tumor incidence for the shallowest penetration suggested that a minimum radiation penetration of about 0.3 mm was necessary to produce tumors, while a minimum penetration of about 1.0 mm was necessary to produce permanent follicle atrophy. The results suggest that the proliferative stage of the hair follicle cells at the time of irradiation is of little or no consequence to the induction of tumors, and tumorigenically responsive tissues are located within the outermost 0.3 mm of skin.

A-9-3 *Proliferation of Early Tumor Foci in Irradiated Rat Skin.* F. J. BURNS, M. VANDERLAAN, AND R. E. ALBERT, NYU Medical Center, 550 First Ave., New York, NY 10016, USA.

In order to assess the possible importance of tumor growth and proliferation rate on appearance time, rat skin tumors in the subvisible size range (0.1 mm to 1.0 mm diameter) were located histologically and their cell proliferation rates were determined stathmokinetically. Tumors were induced by irradiation with 1000 R, 2000 R or 3000 R of 20 KVP x-rays. They were scored by visual observation and by clearing the skin and searching for small cellular foci. The incidence curve for the small lesions showed a consistent displacement about 10 weeks earlier than the comparable curve for visible tumors. Of the lesions small enough to be assigned a location, 62% were in the outermost 0.15 mm of skin (epidermal location) while 14% were found deeper than 0.5 mm. Keratinizing and keratosebaceous tumors exhibited average mitotic rates of $1.10 \pm 0.4\%$/hr independent of size, while undifferentiated tumors proliferated significantly more slowly at $0.24 \pm 0.1\%$/hr. No correlation was found between growth rate and tumor appearance time. Vascular patterns detected by India ink infusions indicated that all the tumors, even the smallest, had dense vascular networks. The results suggest that new tumors constantly appear throughout the rat's life and that slow tumor growth is a result of high cell loss rate.

A-9-4 *Ovarian Tissue Radiosensitivity as Determined by Cell Types in Radiation-Induced Ovarian Tumors.* NEAL K. CLAPP, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

Non-treated female RF mice developed 3.8% ovarian tumors, while 50–60% incidences were seen after single whole-body doses of 50–400 rads with a negative dose response from 100–400 rads. Tumor cell types (primarily adenoma, luteoma, and granulosa-cell) were identified histologically in 2400 mice, and tumors were characterized as single- or mixed-cell type. From 0–100 rads, percentages of mixed-cell tumors increased and single-cell tumors decreased; they were about equal numerically from 100–400 rads. The greatest percentage increase after radiation occurred in luteal tumor elements (from 5% in controls to 50%), followed by adenomas (from 30% to 65%) and granulosa-cell tumors (15%). Maximum incidences for adenoma tumor cells were at 50 rads and for luteal cells at 100 rads with negative dose responses at higher doses, while the percentage of granulosa-cell tumors generally increased from 50–400 rads. Using the difference between control values and the incidence of various cell types seen at the lowest dose (50 rads), adenoma cells were most radiosensitive, luteal intermediate, and granulosa cells were least sensitive.

(Research sponsored by the U. S. Atomic Energy Commission under contract with the Union Carbide Corporation.)

A-9-5 *Changes in the Thymic Cell Populations During the Development of Radiation-Induced Lymphomas.* J. BONIVER, L. J. SIMAR, AND E. H. BETZ, Institute of Pathology, University of Liège, Liège, Belgium.

After a fractionated irradiation inducing thymic lymphomas in C57 Bl mice, blast cells accumulate in the subcapsular zone of the thymus. A quantitative analysis with the stereological methods indicates that this accumulation is not due to a real increase of the absolute number of blast cells but to a lymphocytic depletion.

Ultrastructural investigations demonstrate the heterogeneity of the blast cell population and permit to distinguish 3 major cell types: lymphoblasts, "X-cells," and so called "ring shaped nucleolus cells." These two last cell types are not present in the normal thymus of mice over 2 months of age but have been observed during the perinatal period. Furthermore "X-cells" have been also previously described in the bone marrow after a single or fractionated irradiation.

During the primary regeneration following each of the successive doses of irradiation, "X-cells" appear in the thymus beside lymphoblasts. On the contrary after the last dose during the pre-leukemic atrophy, the proportion of "X-cells" is low whereas "ring shaped nucleolus cells" become frequent. This last cell type corresponds perhaps to a resting stage of "X-cells" and lymphoblasts, these 3 cell-types belonging to the same cell line. The role of these elements (target cells for the leukemogenic virus) will be discussed.

A-9-6 *Systemic Tumors in Mice after X-Ray Irradiation of the Head.* J. F. ESTABLE-PUIG, AND R. F. DE ESTABLE-PUIG, Département de Pathologie, Faculté de Médecine, Université Laval, Québec G1K 7P4, P.Q., Canada.

109 swiss mice received a single dose of 64 r of x rays in the head, with body shielded. The source of irradiation was a G. E. Maxitron. The physical factors were: 300 KVP, 1.74 mm copper filter and a rate of 130/minute.

Irradiated animals and 42 controls were kept under identical housing and feeding conditions. 1½ years after irradiation, surviving animals from both series were sacrificed and autopsies were performed. Systemic tumors were found outside the irradiated zone. Controls of the same strain were negative. Tissue was routinely fixed for histopathological study and particular areas processed for electron microscopy after Dalton's fixation. Epitheliomas and sarcomas were observed. Structural and ultrastructural features of some tumors will be presented. The mechanisms probably involved in the production of tumors in this pilot experiment will be discussed.

A-9-7 *Experimental Rectal Carcinoma Induced by X-Rays in Mice.* FUMIO HIROSE, KAICHI FUKAZAWA, AND NOBUO TAKEICHI, Department of Cancer Research, Research Institute for Nuclear Medicine and Biology, Hiroshima University, Hiroshima, Japan.

The pelvic regions of 5 to 8 weeks old ICR strain mice were irradiated with 2 or 3 doses of 1,000 rads, 1, 2, or 3 doses of 2,000 rads or one dose of 3,000 rads. The same regions of 13 weeks old CF_1 strain male mice were exposed to 2 doses of 2,000 rads. The animals were maintained until their natural death.

The development of rectal adenocarcinoma was seen in only one of 13 ICR mice irradiated with 2 doses of 2,000 rads. No case of carcinoma was seen in mice irradiated with 2 doses of 2,000 rads, in spite of the development of atypical hyperplasia in 94% of these animals. On the contrary, rectal adenocarcinoma was found in 6 of 10 CF_1 mice irradiated with 2 doses of 2,000 rads and 2 of these were accompanied by additional foci of squamous cell carcinoma.

A-9-8 *Carcinogenic Effect of Radioactive Phosphorus.* B. CHUDECKI, Royal Devon and Exeter Hospital, Exeter, Great Britain.

32p has been used routinely in treatment of polycythaemia vera long enough for radiation-induced tumours to come to light now.

Several series indicate that cancer can be produced by ionizing rations in most organs if not in all. There is no real evidence that a threshold dose exists below which carcinogenesis does not occur.

There is strong evidence that the incidence of acute leukaemia in 32p-treated polycythaemia patients is higher than in non-radiation group.

Two cases of radiation cancer are reported.

Primary carcinoma of the liver. This occurred in a 68-year-old white woman eight years after the first dose of 32p. The total dose absorbed by the liver was estimated at 720 rads.

Primary carcinoma of the kidney. This occurred in a 72-year-old man seventeen years after the first dose of 32p. The total dose absorbed by the kidney was estimated at 820 rads.

The author suggests that at least some if not all renal carcinomas reported in 32p-treated polycythaemia vera patients are radiation-induced tumours and there is no causal relationship between the two conditions.

A-10-1 *Stimulation by Adrenalectomy of Radiation-Induced Mammary Carcinogenesis in Mammotropic Pituitary Tumor-Bearing Rats.* KELLY H. CLIFTON AND EVAN B. DOUPLE, University of Wisconsin Medical School, Madison, Wisconsin 53706, USA.

The hormonal product(s) of grafted mammotropic pituitary tumors (MtT) increase the incidence and reduce the latency of radiation-induced rat mammary carcinomas (Yokoro et al, *Cancer Research* 21, 178, 1961), and cause full ducto-alveolar development of the mammary glands of adreno-gonadectomized male rats without steroid hormone replacement (Clifton and Furth, *Endocrinology* 66, 893, 1960). Cortisol treatment, but not deoxycorticosterone trimethylacetate treatment, causes secretion in mammary glands of adreno-gonadectomized MtT hosts. In the current experiment, 330–380 day old F/344 multiparous female rats were divided into six experimental groups. Groups I and IV were unirradiated; Groups II and V received 200 rads fission-spectrum neutrons to the whole body; Groups III and VI received 600 rads ^{137}Cs gamma rays to the whole body. Three days later, all animals received intradermal grafts of MtT-F4. Three days after grafting, all rats in Groups IV, V and VI were adrenalectomized and given 2.5 mg deoxycorticosterone acetate per week until termination of the experiment 98-100 days after irradiation. Twice during the course of the experiment when the MtT grafts were large, they were surgically resected, and the rats were regrafted. Rats with histologically confirmed mammary carcinomas/rats per group were as follows: I, 0/8; II, 0/9; III, 0/3; IV, 1/12; V, 6/7; VI, 6/6. It is suggested that milk secretion is a terminal differentiative step which precludes further proliferation. When this step is blocked by steroid hormone deficiency, more of the MtT-stimulated radiation-damaged cells may retain proliferative capacity and the ability to express malignant damage. (Supported by NIH Research Grant CA-13881 and Center Grant CA-06295. E.B.D. was trainee, NIH Grant TO1-CA-05104.)

A-10-2 *Radiobiological Manipulation of the Preleukemic State.* FREDERIC C. LUDWIG, MARY M. MORAT, AND WOLFGANG SCHUG. College of Medicine, University of California, Irvine, USA.

Radiation which induces leukemogenesis in the unirradiated mouse, inhibits leukemogenesis if given again to the previously irradiated, but not yet leukemic mouse. The objective of this experiment was to identify non-malignant late effects of radiation, the modification of which may account for this postponement or prevention of the above type of leukemia. To this end, 40 male RFM/U mice received leukemogenic whole-body exposure when they were 100 days old; half of them were re-irradiated under identical conditions 70 days later; 20 additional mice served as controls. Sixty days after the second irradiation (or 130 days after the first), the absolute number of cells of the femoral marrow was determined. The controls showed 11.6×10^6 myeloid cells/femur, 8×10^6 or 69.7% of which were mature, oxidase positive forms. In the leukemogenically irradiated mice, these values were 10.7×10^6 and 5.3×10^6 or 31.9%, respectively. In the re-irradiated mice, in which leukemia is postponed or prevented, these values were 9.8×10^6 and 5.4×10^6 or 56%. Leukemia incidence is thus positively correlated with the number of the immature myeloid cells, and negatively correlated with the percentage of the mature forms in the entire myeloid series. Re-irradiation restores a situation approximating that of the controls. The implications of this for the understanding of preleukemic change are discussed.

A-10-3 *Illumination and X-Ray Effects on Tumorigenesis in Rodents.* KIKI B. HELLMAN AND C. DAVID LYTLE, Bureau of Radiological Health, Rockville, Maryland 20852, USA.

An *in vitro-in vivo* tumor model system was developed from an x-radiation induced rat mammary adenocarcinoma. Inoculating female weanling Osborne-Mendel rats with an average of $5–10 \times 10^5$ cultured cells (RMT) resulted in 80–100% tumor incidence 7 days post-inoculation. This response was used to investigate the effects of light illumination or x-irradiation on tumor initiation and progression. Rats were exposed to a 12 hour cycle of overhead illumination from fluorescent lights filtered to provide broad wavelength bands of either red or blue light (100 μ W/cm^2; 30 Ft. Cdl.). Blue light illumination resulted in decreased tumor incidence and more rapid tumor regression not seen for control or red light illumination. Rats were also exposed to x-irradiation prior to being inoculated with 1×10^5 RMT cells. A linear dose response in tumor incidence at 7 days was noted, ranging from 30–40% at 0 rads to 100% by 250 rads. The rate of tumor regression decreased with increased X-ray exposure. This system represents a good model for studying the effects of different radiations on tumor initiation and progression.

A-10-4 *Effect of ^{144}Ce-^{144}Pr and Partial Hepatectomy on Production of Liver Tumors in Chinese Hamsters.** STEPHEN A. BENJAMIN, ANTONE L. BROOKS, ROGER O. MCCLELLAN, AND ROBERT K. JONES, Inhalation Toxicology Research Institute, Lovelace Foundation, 5200 Gibson S.E., Albuquerque, N. Mex. 87108 USA.

The combined effect of partial hepatectomy and injected ^{144}Ce-^{144}Pr citrate on production of liver tumors in Chinese hamsters is being studied. Six hundred and fifty animals were injected intraperitoneally with activity levels from 0.0 to 0.25 μCi ^{144}Ce/gm body weight. Animals from each activity level, including controls, were partially hepatectomized at 20 days before or 20 or 200 days after injection or given no further treatment and were sacrificed at 700 days post-injection (d.p.i.). To date, 440 animals have died or been sacrificed; 320 at activity levels of 0.036, 0.04, 0.05, and 0.25 μCi/gm and 120 controls. The remaining 210 animals were injected with 0.0, 0.1, or 0.12 μCi/gm at a later time to better define the dose response relationship between liver tumor incidence and injected ^{144}Ce. The radiation dose to liver at sacrifice ranged from 1000 to 14,000 rads and produced from 0.4 to 3.2 chromosome aberrations per cell. Animals injected with ^{144}Ce had a hepatic neoplasm incidence approaching 20 percent with no significant difference in incidence between groups injected with ^{144}Ce and partially-hepatectomized or injected with ^{144}Ce alone. No liver tumors were observed in controls.

* Research performed under AEC Contract AT(29-2)-1013.

A-10-5 *Dose-Incidence Data for Mouse Reticulum Cell Sarcoma.* P. METALLI, V. COVELLI, M. DI PAOLA, AND G. SILINI, Laboratory of Animal Radiation Biology, C.N.E.N., C.S.N. Casaccia, Casella Postale 2400, Roma, Italy.

Analyses were made of the frequency and age-specific rate of mortality for Reticulum Cell Sarcoma (RCS) in (C57BL/Cne $\times$ C3H/Cne)Fl male mice, untreated or irradiated with doses of X-rays ranging from 50 to 900 rad. To insure long-term survival after the highest dose level, bone-marrow cells were injected from either normal or irradiated isogenic donors. Average frequency of the disease was about 60% in controls; it was not affected by sublethal X-ray doses below 500 rad, and was decreased by the mid-lethal dose of 700 rad. 900 rad + isologous marrow reduced the total incidence to about 5%. At the lower doses there was some indication of shortening of the latency time. The effects were not correlated with the life-span shortening. Although a true induction process in terms of new or more tumors appearing after irradiation was not clearly identified, the inhibition of RCS at doses higher than 500 rad indicates that the critical dose level for reduced incidence is not far from the "turnover dose" shown by other types of murine leukaemias. In addition, results from lethally irradiated animals restored with isologous or autologous bone marrow clearly showed that this tissue does not contain cells capable of malignant transformation to reticulum cell sarcoma.

A-10-6 *Cell Proliferation and Induction of Thyroid Tumors by X-Rays.* KONSTANTIN CHRISTOV, Center of Oncology, Sofia-56 Bulgaria.

A number of observations indicate that irradiation of the neck region in childhood leads to development of thyroid tumors. It was suggested that the oncogenic effect of irradiation in the growing thyroid might result from a higher number of dividing cells in the gland. In an experimental study relating to this problem we have found that in the first two weeks after birth the number of proliferating thyroid cells in rats is 60 times higher than those of 60 day-old animals. Twelve months after irradiation in 16 percent of the animals irradiated at the age of 10 days and in 4 percent of the animals irradiated at the age of 60 days with 300 rads X-rays, thyroid tumors appear. Subsequent treatment of the animals with MTU increases the number of the thyroid tumors to 82 percent in the 10-day-old group and to 39% in the 60 day-old-group.

A-11-1 *The Role of Na^+ K^+ ATPase in Radiation-Induced Inhibition of Active Transport of Potassium in Thymocytes.* IAN V. CHAPMAN AND MICHAEL G. STURROCK, Department of Medical Biophysics, The University, Dundee DD1 4HN, Scotland, Great Britain.

It has been demonstrated that the radiation induced drop in adenosine triphosphate concentration in thymocytes in vitro cannot be the primary cause of the observed decrease in active transport of potassium ions.

Isolation of Na^+ K^+ Adenosine triphosphatase and Mg^{2+} ATPase from thymocytes post irradiation reveals that the enzyme primarily concerned with transport of potassium, Na^+ K^+ ATPase, is considerably more sensitive to ionising radiation. The results show that inhibition of enzyme activity approaches a maximum at 8–10 Krads in the dose range 1–20 Krads. This finding is in close agreement with results achieved for active transport inhibition.

Na^+ K^+ ATPase contains several active sulphydryl groups. The pattern of the radiation effect over the low dose range 1–8 Krads suggests that the radiation effect on the enzyme may be brought about by oxidation of sulphydryl groups resulting in conformation changes at the active centre.

Experiments have been designed to relate these radiation induced structural changes of membrane bound Na^+ K^+ ATPase to inhibition of the active transport mechanism.

A-11-2 *Selective and Differential Photodynamic Effects on* E. coli *Membrane Processes and Their Inhibition.* HENRY SCHNEIDER, J. Y. D'AOUST, L. R. BARRAN, AND W. G. MARTIN, National Research Council of Canada, Ottawa, K1A OR6 Canada.

Effects of wavelengths above 400 nm on membrane processes in *E. coli* were investigated with emphasis laid on active transport and energy coupling mechanisms. Relatively specific and differential inactivations were observed. Inactivation rates were essentially identical for glycine, threonine, leucine and methionine uptake and were the most rapid observed. The rate of phenylalanine was the slowest and that for methyl thio-β-D-galactoside intermediate. Transport of each of these materials utilizes a different carrier. Despite the large effects on transport several membrane or cytoplasmic enzymes were only minimally affected. Also, viability was not altered, although longer illuminations had pronounced effects. The data suggest relatively few lesions are produced, but these inactivate several processes because of damage to a common component. For the amino acids whose uptake is inactivated at similar rates the lesion may involve a molecular feature common to the carrier systems. An alternative is that the damage site is part of the respiratory chain since rates of photoinhibition of respiration were similar and electron flow can energize active transport. Flavoenzymes, although inactivated, are probably not the site of damage for amino acids behaving like glycine as indicated by kinetic studies. Protection as well as sensitization effects were observed in the presence of adenine, anthranilic acid and cysteine.

A-11-3 *Effect of Ionizing Radiation on Sodium Ion Permeability in Various Mammalian Erythrocytes.* TAKAKO KANKURA, WATARU NAKAMURA, HIDEO ETO, AND MAKOTO NAKAO, National Institute of Radiological Sciences, Chiba, Japan and Tokyo Medical and Dental University School of Medicine, Tokyo, Japan.

^{22}Na uptake by erythrocytes from several mammalian species, i.e., man, monkey, rabbit, rat, mouse, dog, sheep and ox was measured after 2,000 R γ-irradiation *in vitro*. A considerable increase was generally observed in the uptake. These animals could be classified into two groups based on values of the ratio of ^{22}Na uptake by irradiated erythrocytes to that by non-irradiated cells. In the case of man, monkey, rabbit, rat and mouse, the ratio was between 2 and 3, while it was between 1 and 2 in the case of dog, sheep and ox. These differences were discussed in relation to the ratio of K/Na in red cells, types and abundance of phospholipid and fatty acid composition in erythrocyte membranes of these species of animals.

A-11-4 *Free-Radical Damage to Mammalian Erythrocytes.** D. R. KALKWARF AND W. D. FELIX, Battelle, Pacific Northwest Laboratory, Richland, Washington 99352, USA.

Experiments were performed to determine the role which specific free radicals play in radiation-induced damage to erythrocyte membranes. Radicals were prepared by exposure of water-soluble crystals of pure biochemicals to gamma radiation, and their concentration and general structure were determined by electron spin resonance measurements. Their abilities to act as chemical oxidizing or reducing agents were tested by dissolving them in solutions of oxidation-reduction indicators. Weighed samples of the irradiated crystals were dissolved in saline suspensions of porcine erythrocytes, and cell damage was evaluated by measuring hemolysis as a function of time after crystal disolution. These processes occurred rapidly after introductions of radicals capable of acting as chemical reducing agents, such as those formed in irradiated galactose or lactose. Cell suspensions showed a significantly lower hemolysis rate if exposed only to non-irradiated crystals

or to solutions of the irradiated crystals in which only the stable radiolysis products remained. No significant hemolysis was observed when porcine erythrocytes were exposed to similar concentrations of oxidizing radicals such as those formed in irradiated glycine and α-alanine.

* This paper is based on work performed under United States Atomic Energy Commission Contract AT(45-1)-1830.

A-11-5 *Radiation Effect on Phosphatidyl Glycerol Turn-over, and Its Comparison with* DNA *Synthesis.* Osamu Yamamoto, Hiroshima University, Hiroshima, Japan.

It is known that membrane synthesis couples with phosphatidyl glycerol (PG) turn-over. PG turn-over was compared with TdR incorporation using ^{60}Co-irradiated *E. coli* cells. When random cells of the radiosensitive mutant R 15 and the radioresistant mutant H/r 30 were irradiated, a decrease in the incorporation rates of PG-^{14}C and TdR-^{14}C in the R 15 cells and an increase of these rates in the H/r 30 cells were observed, post incubation. When synchronized cells of the thermosensitive mutant N 167 were irradiated at various time in cell cycle, the incorporation rate of PG-^{14}C decreased and that of TdR-^{14}C increased, post incubation, in the radiosensitive stages. When random N 167 cells were irradiated after the incorporation of PG-^{14}C, the release of ^{14}C-radioactivity decreased, post incubation.

A-11-6 *Membrane-Associated Radiation Damage In Human Lymphocytes.* Chuck Aoki, Judith Berliner, David Kwan, Renato Mello, Walter Nikesch, and Amos Norman, Department of Radiological Sciences, UCLA School of Medicine, Los Angeles, California, 90024, USA.

Ionizing radiations induce lymphocytes to incorporate radioactive thymidine into nuclear DNA. An autoradiographic study of nuclear sections shows that the majority of the radioactivity is localized in the DNA adjacent to the nuclear membrane. Extraction and fractionation of the DNA on density gradients also indicates that the majority of the radioactivity is in the membrane bound DNA fraction. Radiation changes the properties of the cell membrane. Using a flow microfluorimeter we have detected changes in the rates of transport of fluorescein diacetate into the cells, and of the hydrolysate product, fluorescein, out of the cells within a few hours after irradiation. Studies of the intensity and polarization of the fluorescence of ANS and perylene bound to the membrane suggest that the interior of the membrane is more affected by radiation than the exterior. Establishing a relationship between membrane associated damage detected early after irradiation and cell membrane failure, which is the proximal cause of interphase death, is complicated by the heterogeneity of the lymphocyte populations.

A-11-7 *Loss of Negative Charge on Cell Membrane and Nuclear Membrane after Irradiation, and its Modification by SH-Blocking Agents or PHA.* Chikako Sato and Kiyohide Kojima, Aichi Cancer Center Inst., Nagoya 464, Japan.

Alterations in the negative charge of whole cells or isolated nuclei after x-irradiation were investigated by cell electrophoresis in three different radiosensitive cell lines. The electrophoretic mobility decreased with time after irradiation and reached a minimum 4 hours after exposure. The mobility reduction was dose-dependent and same in three cell-lines. The decrease in mobility recovered in melanoma cells B16-C2W (n = 20, D_0 = 160R) but not in Burkitt lymphoma P3H-R1 (n = 1, D_0 = 67R). Good statistical correlation was obtained between the surviving fractions (colony-forming ability) and the fractions of electrophoretically intact (or recovered) cells. The mechanism of mobility decrease after x-irradiation is tentatively envisaged as conformation change of the membrane based upon the following results: 1) The amount of acidic sugars, responsible for the negative charge of cell surface, did not decrease after irradiation. 2) Phytohaemagglutinin and Concanavalin A, which induce the structural change of the membrane, blocked the mobility change after irradiation. 3) SH-blocking agents (PCMB, NEM, iodoacetamide) blocked the mobility change at the concentrations of 1×10^{-8} 10^{-6} M.

A-11-8 *Further Investigations on the Surface Coat of Cell Membrane.* Bogdan M. Woźniewicz, Department of Pathomorphology, Institute of Pediatrics, Medical School, Marszałkowska 24, Warsaw 00-628, Poland.

Special method was introduced for visualisation of the surface coat of the thymocytes (glycocalyx) using Ruthenium Red procedure (Groniowki et al.). Thymus cells of the mouse were examined in the so called latent period after a total body of X-ray irradiation and preparation of thymocyte suspension for electron microscopy. Previously, morphological changes in the mitochondria and nucleus occurring but 20 min. to 1 hour after irradiation were observed. The results from the experiments presented above showed "disappearance" of the surface coat of the thymus cells. It is worth to note that glycocalyx of the thymocytes showed changes already in the first few minutes after irradiation i.e., prior to the other cell structures. Studies on cell membrane damage after irradiation using cryo ultramicrotomy, scanning microscope and cytophotometry are presented.

A-11-9 *Disturbance in Membrane-Interactions Between Subcellular Organelles of Irradiated Rat Liver.* I. K. KOLOMIJZEVA, YU. S. KAZNACHEEV, A. M. KUZIN. Institute of Biological Physics Acad. Sci. USSR, Moscow Region, Pushchino, USSR.

The exchange of P^{32} and C^{14} labelled phospholipids and C^{14} labelled cholesterol between microsomes and mitochondria or microsomes and nuclei *in vitro* served as a criterion of intermembrane interactions. The rate of the transfer of C^{14} and P^{32} labelled phospholipids between microsomes and unlabelled mitochondria or nuclei in the presence of a labelled 105000 g supernatant was shown to be 40% lower as compared to the control. Subcellular organelles were isolated from rat liver one hour after irradiation with a dose of 1200R and injection of labelled precursors (1-C^{14}-acetate and P^{32}-phosphate). In the same systems the rate of the transfer of C^{14} cholesterol between the organelles of irradiated rat liver was 30% higher compared to the control.

A-12-1 *An Effect of X-Irradiation on Homing Patterns of Mouse Mesenteric Lymph Node Cells.* DAVID A. CROUSE, TITUS C. EVANS, AND THOMAS L. FELDBUSH, University of Iowa, Iowa City, Iowa 52242, USA.

Mesenteric lymph node (MLN) cell suspensions were prepared from BDF_1 female mice and labeled with ^{51}Cr *in vitro* prior to irradiation and i.v. transfer to syngeneic hosts. Pooled MLN cells were divided into aliquots for control and irradiated groups. Irradiated groups received 25–1000 R of 250 kVp X-rays just prior to injection. Various tissues were removed, weighed, and assayed for activity at 24 hours post-injection; mice of selected cell exposure groups were assayed between 1 and 96 hours after injection.

Irradiation altered the homing pattern and it appeared to be related to the amount of exposure. With exposures of $\geq$50 R, cell localization in the spleen was significantly increased while that in the MLN and Peyer's patches was decreased. In contrast, heat-killed cells appeared to be concentrated in the liver. With an exposure of 100 R, significant alterations in distribution of cells were observed at 6 hours post-injection and remained relatively stable through 48 hours; by 96 hours, control and irradiated groups appeared to be nearly the same.

(Supported by NDEA IV Fellowship and American Cancer Society Grant ET-37M.)

A-12-2 *Very Rapid Changes in the Binding of H^3-Ouabain to Membrane Na-K-ATPase After PHA and Leucoagglutinin (LA) Administration to Lymphocytes.* M. R. QUASTEL, Human Development Division, Environmental Health Centre, Tunney's Pasture, Ottawa, Canada.

Lymphocyte activation by mitogens such as phytohemagglutinin is fundamental to the cytogenetic preparation of chromosomes used in the assessment of radiation exposure, and represents an immunological phenomenon which particularly involves the T cell population. The mechanism of the activation remains unknown, but early membrane changes are now considered as primary to the subsequent increases of nucleic acid synthesis and cell division. Past results showing enhanced potassium^{42} uptake into lymphocytes following PHA induced stimulation led us to examine the binding sites on the cell surface using H^3-ouabin as a surface label for the potassium site of the Na-K-ATPase. It is shown that marked increases in the degree of binding occur within minutes after administration of PHA or LA. However, no such rapid effect occured after Concavalin A or pokeweed mitogen, suggesting either that these agents have different mechanisms of action, or that different subpopulations of lymphocytes might be involved in stimulation by these mitogens. If the latter is the case the use of different mitogens of this sort in cytogenetic assessments of

radiation exposure by measurement of the type of frequency of chromosome aberrations, may lead to different results according to the mitogen used. The mechanism of the change in binding is not understood but is characterized by a change in the rate of uptake and an increase in the number of binding sites, rather than in the rate of release. Conformational changes in the protein and phospholipid of the cell membrane are postulated in a model.

A-12-3 *Effects of Radiation upon Lymphocyte Replicating Ability.* P. L. T. Ilbery, Radiobiology, School of Public Health and Tropical Medicine, The University of Sydney, Sydney, N.S.W. 2006, Australia.

Recent improvements in the quantitation of lymphocyte response to mitogens have allowed the effect of cancer therapy on the immune response to be more accurately described and consequently varying degrees of depression have been noted within small groups of cancer patients. Depression of the lymphocytes' ability to incorporate labelled DNA precursor in stimulated cultures has been related to *in vitro* radiation dosage in the kilorad range. The fall in lymphocyte replicating ability (LRA) is shown for a number of radiotherapy/chemotherapy schedules as the patient's response in comparison with that of a particular normal donor, expressed as the relative response. The autoradiographic results suggest the depression of LRA is due to changes in the size of the S-phase population rather than changes in the rate of synthesis.

The accumulated radiation damage seen cytogenetically, expressed as dicentrics/cell, has been interpreted from a calibration curve derived from *in vitro* irradiation of lymphocytes. The value for the *in vivo* damage seen cytogenetically in lymphocytes is in all cases less than the *in vivo* damage to lymphocytes obtained in terms of LRA values from the kinetic *in vitro* calibration curve. Since a 'serum factor' could not be identified the results support a T cell depression hypothesis resulting from a change in the fraction of lymphocytes in the circulating pool normally responsive to mitogenic stimulation.

A-12-4 *Effect of Serum from X-Irradiated Tumor-Bearing Mice on Migration of Mouse Spleen Cells.* Harold Moroson and B. Rucker, Department of Radiology, New York Medical College, New York, NY. 10029, USA.

Sensitive lymphocytes stimulated by antigen release a number of biologically active substances, one of which is migration inhibitory factor (MIF), a correlate of delayed hypersensitivity. The assay developed by David, Al-Askari, Lawrence and Thomas (1964) and by Bloom and Bennett (1966) for inhibition of migration of lymphocytes from capillary tubes, has been employed to detect tumor specific antigen of a mouse fibrosarcoma in serum of tumor-bearing mice before and after local irradiation. By this means antigen released by local tumor X-irradiation may be detected and quantified. Antigenic determinants are shed by tumor and leak into the host serum, possibly acting as inhibitors of cell mediated anti-tumor reaction.

Migration of washed spleen cells from tumor-bearing C57/BL mice was inhibited by adding 2.0–20 mg/ml serum from tumor-bearing mice to the medium, and unaffected by 2.0–20 mg/ml serum from control mice. Local irradiation of non-tumor mice has no effect on migration of their spleen cells, nor does the addition of serum from tumorous mice, while whole body irradiation (400 r) markedly inhibits spleen cell migration.

Serum from X-irradiated tumor-bearing mice (1000 r $\times$ 3) had no greater effect than serum from unirradiated tumor mice. Hence, irradiation of this tumor does not enhance release of tumor specific antigen into serum. These experiments may provide models for human cancer radiotherapy.

(Supported by Research Grant CA 14374 from the National Cancer Institute, NIH.)

A-12-5 *Radiation Effect on Immune Cell-Mediated Cytolysis in Vitro.* Taehwan Kim, Chang W. Song, and Seymour H. Levitt. University of Minnesota Medical School, Minneapolis, Minnesota 55455, USA.

P-815-X2 mastocytoma cells of DBA/2J mice were labeled with ^{51}Cr-chromate and ^{125}I-IUdR and the cytolysis of these cells by X-irradiation, immune lymphocytes, or by both, was quantitated by the amount of isotopes released *in vitro* up to 48 hrs. The immune lymphocytes were the splenic lymphocytes of BL57 mice immunized against the mastocytoma cells. When the tumor cells were X-irradiated and then incubated with the lymphocytes, the cytolysis of the tumor cells was

significantly higher than that by either X-irradiation or lymphocytes alone. The X-irradiation of lymphocytes with 2000 rads in a single exposure reduced the cytotoxicity of lymphocytes slightly. To simulate a clinical situation in which tumor cells and lymphocytes are irradiated simultaneously at the tumor site, we irradiated a mixure of tumor cells and lymphocyte, and found a higher cytolysis of tumor cells than that by irradiation of tumor cells alone. The regression of tumors in clinical radiotherapy may not be due to the radiation damage on tumor cells alone, but also to the cell-mediated immune reaction against the tumor cells. (Supported by a research grant from the Minnesota Division of American Cancer Society.)

A-12-6 *Development of the MOD-MEM Test. A Possible Diagnostic Test for Cancer.* J. A. V. PRITCHARD, J. L. MOORE, W. H. SUTHERLAND, AND C. A. F. JOSLIN. Tenovus Laboratory, Velindre Hospital, Whitchurch, Cardiff, Great Britain.

Field & Caspary (Lancet II, 1337, 1970) reported that circulating lymphocytes from patients could be used to detect presence of disease. Lymphocytes were sensitized to a basic protein derived from brain and produced a substance called Macrophage Slowing Factor (MSF). MSF is capable of altering the charge density on the surface of Guinea pig macrophages and the reduced mobility seen in a Zeiss Electrophoretic apparatus can be used to prove the presence of a malignant condition. A test called the Macrophage Electrophoretic Mobility (MEM) test has been developed (Pritchard, Moore, Sutherland & Joslin. Br. J. Cancer. **27**, 1, 1973).

A modified test (MOD-MEM) has been introduced and the percentage positivity for cancer patients has been increased from the original range of 15–20% to 23–40% without any corresponding increase in the normal range 0–4%.

Certain biochemical and morphological studies with lymphocytes and macrophages have been carried out. These results and attempts to characterise the MSF will be presented.

A study of over 100 patients from a general hospital who have other diseases has been carried out and their clinical significance will be discussed. The MEM test has possibilities of measuring lymphocyte interaction as a rapid matching technique for organ transplantation.

A-13-1 *Results and Applications about γ-Irradiated Sodium Heparinate.* CHARLES BAQUEY AND CHARLES DARNEZ, Institut National de la Santé et de la Recherche Médicale, Unité 53, Domaine de Carreire, Rue Camille Saint-Saëns, Bordeaux, 33000, France.

In a previous paper, we have dealt with the paramagnetic species present in γ irradiated dry and deoxygenated sodium heparinate. Here we report some results about the nature and the reactivity of these species in various conditions. Gases evolved from the irradiated product have been analysed and we give the corresponding radiochemical yield. Besides, we deal with biological applications of this study: sodium heparinate keeps its anticoagulant activity after irradiation and can either be radiosterilized or modified by radiochemical methods.

A-13-2 *γ-Radiolysis and UV-Photolysis of S-*(cis-1-*Propenyl*)-L-*Cysteine.* HIROYUKI NISHIMURA AND JUNYA MIZUTANI, Department of Agricultural Chemistry, Hokkaido University, Sapporo, Japan.

In connection with the sprout-inhibition of onion by γ-irradiation and with food-flavor deterioration accompanied by irradiation, γ-radiolysis and UV-photolysis of S-(*cis*-1-propenyl)-L-cysteine-(PeCS), which is found in *Allium* plants (onion, garlic, etc.), were investigated in aqueous systems.

From the comparisons of GLC, MS, and IR spectra with reference compounds, the major products from γ-irradiated PeCS were identified as 1-propenethiol, *n*-propyl 1-propenyl sulfides-(*cis* and *trans*), di-1-propenyl sulfides(*cis-cis* and *cis-trans*), and alanine. By the experiments with N_2O, KBr, or NaCN addition during irradiation, principal roles of the chemical species(·OH, e_{aq}^- and ·H) from γ-radiolysis of water in the degradation processes were elucidated, and further a new *cis-trans* isomerization mechanism of the 1-propenylthiyl radicals from irradiated PeCS was suggested.

On the other hand, UV-photolysis of PeCS was remarkably different from its γ-radiolysis, and despite the formation of 1-propenylthiyl radicals from both γ-radiolysis and UV-photolysis of PeCS, such thiophene derivatives as 2,4- and 3,4-dimethylthiophenes and H_2S were mainly produced by UV-photolysis. The unique mechanism of the formations of these thiophenes will be discussed.

A-13-3 *Study of Radiation Chemistry of Thymine and Thymidine Through Their Photolysis in the Presence of Hydrogen Peroxide.* Bo-Sup Hahn and Shih Yi Wang, Department of Biochemistry, The Johns Hopkins University, Baltimore, Maryland 21205, USA.

Photolysis (313 and 360 nm) of thymine and thymidine in the presence of H_2O_2 has been performed for a comparative study of photo- and radiation chemistry of nucleic acids. While many photoproducts are formed under this reaction condition, most of these are identical to the radiation products of thymine and thymidine such as glycols, hydroxy-hydroperoxides and others. Both qualitative and quantitative aspects of the findings will be presented in order to correlate the effects of γ-radiation in aerated aqueous solution with the effects of HO· and/or HOO· radicals for these compounds. (A similar study on Uracil and Cytosine will be presented at the Second Annual Meeting of American Society for Photobiology.)

(This research is supported by U. S. Atomic Energy Commission Contract AT(11-1)-3286 and is identified as No. COO-3286-7.)

A-13-4 *Measurement of Oxygen Consumption in Aqueous Solutions of Organic Molecules Irradiated with Sparsely Ionizing Radiation.* W. Pohlit, Gesellschaft für Strahlen- und Umweltforschung, D 6 Frankfurt/Main, Paul-Ehrlich-Strasse 20, West Germany.

Oxygen consumption of organic molecules in cells leads to anoxia in the cell and consequently reduces radiation sensitivity. Some of the well known protective agents can be assumed to react in this way if applied in aerated cell suspensions or in cell cultures. The oxygen consumption of different organic molecules in aqueous solutions ranging from 10^{-6} up to about 10 mol/l have been measured by an O_2-electrode technique. The analysis of these experiments give data on the distance of energy depositions responsible for these indirect radiation reactions, the mean oxygen consumption per absorbed dose, the energy necessary for the destruction of the organic molecule and on the concentration where direct effects with these molecules occur. It can be determined also, if a one step or a two step reaction is involved. In this way solutions of methanol, ethanol, propanol, glycerol, cysteine, cystine and serine have been analyzed. The oxygen consumption in cell suspensions of yeast cells show that additional to the measured oxygen consumption by irradiation, the oxygen consumption due to respiration and the oxygen production from metabolism of irradiation products has to be included into a quantitative model for oxygen ballance in irradiated cells.

A-13-5 *Radiation Chemistry of Uric Acid in Aqueous Solutions.* Gabriel A. Infante, Shirley Santiago, Juanita Campos and Odette Portuondo, Catholic University of Puerto Rico, Ponce, Puerto Rico 00731, USA.

Alloxan, allantoin, uric acid glycols, parabanic acid, oxaluric acid, dialuric acid, 4-amino, 5-formamido-2,6-dihydroxy pyrimidine, triurea and urea have been determined quantitatively as major products of gamma irradiated aqueous C-14 labelled uric acid using radiochromatographic and spectroscopic techniques. Using appropriate scavengers, yields of these products have been determined for the reactions of e_{aq}^-, ·OH and H· in water. Good material balances between G(-uric acid) and G(total products) have been obtained in all cases. Mechanisms for these radiation induced reactions will be proposed, explained and compared with other important biological systems.

A-13-6 *Radiolysis of Aromatic Amino Acids in Aqueous Solutions: Change in Site of Attack with Change in pH.* Jai P. Mittal, Chemistry Division, Bhabha Atomic Research Centre, Trombay, Bombay 400085, India.

Site of attack of primary reactive species produced in the γ-radiolysis of water with various aromatic amino acids in aqueous solutions have been studied. A change in the site of attack was observed with change in pH. In neutral or weakly acidic air saturated and degassed solutions, the principle site of attack of ·OH radical is at the phenyl ring, whereas at alkaline pH (pH > 13), the results indicated that O^- which is in rapid equilibrium with ·OH ($OH + OH^- \overset{pK=11.9}{\rightleftharpoons} O^- + H_2O$) attacks the aliphatic side chain resulting into α-hydrogen atom abstraction as the dominant reaction. The products were detected and characterized with the help of emission and absorption spectrophotometry and chromatographic analysis. Earlier results[1] of the pulse radiolysis

study of site of attack of e_{aq}^- with phenylalanine and phenylglycine have been corraborated. Significant and interesting differences are found in the reactivity and the nature of products formed from the reaction of e_{aq}^-, the above compounds. Whereas phenylglycine undergoes almost exclusive demination by e_{aq}^-, part of the e_{aq}^- formed appears to add to the aromatic ring of phenyl alanine. Similar studies have been carried out with aromatic amines, oligopeptides and acetylated aromatic amino acids.

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[1] J. P. Mittal and E. Hayon, J. Phys. Chem. (submitted) 1974.

A-13-7 *Alterations of Light Scattering Caused by Gamma-Irradiation of Dilute Aqueous Lysozyme-Polyinosinic Acid Complexes.* YOGESH DAVE AND CONRAD N. TRUMBORE, University of Delaware, Newark, Delaware 19711, USA.

An investigation has been carried out on gamma radiation-induced changes in light scattering of dilute lysozyme (L)-polyinosinic acid (PI) complexes in aqueous solutions at different concentration ratios of L/PI (J. W. Preiss and D. A. Stevenson, *Biophysical J.* 12, 80 (1972)). The results suggest that both light-scattering intensity and dissymmetry of scattering are functions of the concentration ratio, L/PI, and radiation dose. At most concentration ratios of L/PI, the irradiated complex assumes a greater dimension than the unirradiated complex at the same concentrations. However, at certain critical concentrations, the change in light scattering induced by irradiation is much larger than at other concentrations. There is evidence that these changes are similar to those induced by heating the L-PI complex. However, if the complex is heated before irradiation, the light scattering from the resulting complex is relatively insensitive to irradiation,

A-13-8 *Concentration Effects in Amino Acid Radiolysis.* N. A. DUJENKOVA AND A. V. SAVITSCH. Institute of Biophysics, Ministry of Public Health, Moscow, USSR.

The radiolysis of aliphatic and cyclic amino acids was studied in aqueous solutions in a wide concentration range. Two kinds of concentration dependence of G-values were established: 1) the power-low rise $G = bC_0$; such dependence was found for amino acid degradation, deamination of aliphatic amino acids and for destruction of ring structures in cyclic amino acids. 2) The "constant" dependence, when G-value rises with the rise of concentration at concentration lower than about 10^{-3} M and remains constant at higher concentrations; such dependence was found for deamination of cyclic amino acids.

These data show, that the radiosensitivity of amino acids can be characterized only if the concentration dependence of G-value is known. As a criterion of the radiosensitivity of amino acids the power index "α" can be used. The following values of "α" were found: 0.25 for glycine, alanine, serine and phenylalanine; 0.5 for glutamine and tryptophane. The greatest value of "α" was found for histidine and proline: 0.5 at low concentrations (up to 10^{-2}) and 1.2 for concentrations, higher than 10^{-2} M.

A-14-1 *Redox Potentials of Free Radicals of Biochemical Interest.* P. S. RAO* AND E. HAYON. Pioneering Research Laboratory, U.S. Army Natick Laboratories, Natick, MA 01760, USA.

Using the technique of pulse radiolysis, the redox potentials (E^{01}, V, and pH 7.0 and 25°C) of a number of free radicals of biochemical interest have been determined in aqueous solution. Among the systems examined are $\cdot O_2^-$, NAD^+, nicotinamide, riboflavin, disulfides, pyrimidines, N-ethyl maleimide, and various N-heterocyclic compounds. The free radicals are produced by reaction of the substrates with e_{aq}^- or OH radicals. The E^{01} values for the acid and base forms of the free radicals were determined. In all cases, the basic form have more negative potentials, i.e., are stronger reducing agents. These and other redox properties of free radicals will be discussed.

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A-14-2 *A Possibility of Singlet-to-Ground Transitions in Thermoluminescence and Radiation-Induced Phosphorescence of Nucleic Acid Bases.* V. G. TATAKE AND T. S. DESAI, Biology & Agriculture Division, Bhabha Atomic Research Centre, Trombay, Bombay 400 085, India.

Quantitative and qualitative studies on the thermoluminescence (Tl) and radiation induced

phosphorescence (RIP) of some purines, pyrimidines and their analogues have shown that the two phenomena have the same origin. The integrated Tl and RIP values have been found to add up to a constant for a given compound exposed to a fixed gamma dose. The Tl emission spectrum is also seen to be identical with that of RIP. The studies on the emission spectra of Tl of these compounds have shown that the quality of the spectral emission can be correlated to their chemical structures. The spectral range of emission of some of the bases can be explained on the basis of singlet-to-ground transitions.

A-14-3 *Kinetic Aspects of the Radiation-Induced Inactivation of Papain in Aqueous Solution.* J. R. CLEMENT, D. A. ARMSTRONG, Chemistry Department, The University of Calgary, 2920–24 Avenue N.W., Calgary, Alberta, T2N 1N4, Canada, AND N. V. KLASSEN AND H. A. GILLIS, Physics Division, National Research Council, Ottawa, Ontario KIA OSI, Canada.

The proteolytic enzyme papain has a well characterized structure with an SH group at the active site, which is essential for activity. For these reasons it is an interesting compound in which to study the enhanced radio-sensitivity of sulfydryl enzymes.

The reactions of the transient species produced by attack of OH and e_{aq}^- on papain have been investigated by pulse radiolysis at times up to 0.5 seconds. No indication of reaction modes other than first order was found for the decay of these species. Changes in the absorption spectrum induced by Co^{60} γ-radiation were also investigated as was the inactivation of the enzyme. The implications of these results and other findings will be discussed and important conclusions relating to the mechanism of radiolytic inactivation will be presented.

A-14-4 *Steady-State Radiation Chemistry in Aqueous Micellar Systems.* JANOS H. FENDLER, Texas A&M University, College Station, Texas 77843 USA.

Cationic hexadecyltrimethylammonium bromide (CTAB), anionic sodium dodecyl sulfate (NaLS) and uncharged Igepal CO-730 surfactants dynamically associate in water to form large aggregates or micelles. In aqueous micellar CTAB, NaLS and Igepal CO-730 solutions the radiation chemistry of many systems differ substantially from that in pure water. Differences are manifested not only in the rate constants for the reactions of e_{aq}^-, $\cdot OH$, $\cdot H$ and $Cl_2^-\cdot$ with substrates but also in *G*-values and product distributions. Thus *G*-values for the products formed on the interaction of e_{aq}^-, $\cdot OH$, $\cdot H$, and $Cl_2^-\cdot$ with uracil and thymine are markedly affected in the micellar environment. Similarly, the extent of radiation protection by sulfhydryl compounds is altered when they constitute part of a tailor-made micelle. Significance of these results and their relevance to radiation biology are discussed.

A-14-5 *Micelles as Models for Electron Transfer Reactions in Bioaggregates.* MICHAEL GRAETZEL AND JOHN K. THOMAS, Radiation Laboratory and Chemistry Department, University of Notre Dame, Notre Dame, Indiana 46556, USA.

Pyrene solubilized in the interior of sodium lauryl sulfate micelles is photoionized by a 347.1 nm laser pulse. Photoejected electrons react with suitable scavengers (Cu^{2+}, Cd^{2+}, benzoic acid) residing in the periphery region of the micelles. The subsequent back transfer of the electron from the reduced form of the scavenger to the parent cation occurs with a half-life time of 10–20 μs. Reactions between electron donors (e_{aq}^-, CO_2^-) formed in the aqueous phase by pulse radiolysis and acceptors (pyrene, biphenyl) present inside the micelles are controlled by the micellar surface potential. Negative potentials inhibit these reactions while positive potentials greatly promote the reaction rate and even enable electron transfer processes which do not occur in homogeneous solution. Details of the transfer mechanism are obtained by changing the ionic strength of the solution which determines the thickness of the micellar double layer and the surface potential and by selecting acceptors located at different sites of the micelle.

A-14-6 *Singlet-Triplet Quantum Efficiencies and Isomerization Yields of Visual Polyenes.* R. BENSASSON,[1] M. COOPER,[1] E. A. DAWE,[2] AND E. J. LAND.[3]

Nanosecond pulse radiolysis and laser flash photolysis are being used to study the triplet excited states of isomers of retinal, the chromophore of the visual pigment rhodopsin. Absorptions and lifetimes of the triplet states of 9-, 11-, 13-*cis* and all-*trans* retinal have been characterized. Their

triplet extinction coefficients were measured against biphenyl triplet as standard by a development of the energy transfer method which takes into account the short lifetimes of these polyene triplets. These extinctions were employed to determine the corresponding singlet-triplet crossover efficiencies, ϕ, using naphthalene as standard for 265 nm excitation, and anthracene or duroquinone for 353 nm excitation. ϕ was found to vary widely from one isomer to another. Photoisomerization was observed by comparing initial and final absorptions around 365 nm, the wavelength maximum of singlet ground state retinals, after laser irradiation. Photoisomerization yields were measured by comparing the final absorption changes at 365 nm with the initial triplet yields as measured at their maxima around 450 nm. In the case of 11-*cis* retinal, the isomer normally present in rhodopsin, comparison of absorption changes at 365 and 445 nm during the first 10 μsec after 265 nm excitation suggests that the major route for photoisomerization to all-*trans* retinal is via the triplet state. Similar studies of the *n*-butylamine and lysine Schiff bases of these retinals, closer models of rhodopsin, are in progress.

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A-14-7 *Radiation-Chemical Transformations in the Ion-Stabilized Colloidal Systems.* Irene G. Bakhtadze, Larisa V. Lyashenko, Helen M. Nanobashvili, The A. S. Pushkin Georgian State Pedagogical Institute, Tbilisi 380079, USSR.

Colloidal sulphides of different metals change their oxidation-reduction state and therefore directional changing their stability is of scientific interest. A study of the action of γ- and uv irradiation on the sulphide sols of argentum, thallium, arsenic and other metals has shown that the irradiation causes a decrease of the electrokinetic potential and changes the intermicellar solution and dispersion phase. Sulphide-ions stabilizing a sol go, under the irradiation, into sulphite-, thiosulphite- and sulphate ions. The change of chemical composition of the intermicellar solution results in the denudation of a particle as a result of which a colloidal particle itself is subjected to the oxidation-reduction processes and As^{45} ions become available in the solution. The irradiation of Tl_2S sols results in a complete destruction of the dispersion phase with the transition of colloidal solution into a true solution. These changes may be prevented by adding the certain quantity of gelatine a stabilizing action of which is greater the larger the quantity that has been introduced for protection. Regularities obtained for the given systems can be extrapolated to the other colloidal systems as well.

A-15-1 *Electron Impact-Induced Reactions of Condensed Ethane and Cyclopropane.* H. Hiraoka, IBM Research Laboratory, San Jose, California 95193, USA.

Electron impact effects on condensed ethane and cyclopropane show energy dependence as well as significant differences from those of gas phase gamma ray radiolysis and vacuum uv photolysis. In 3 keV electron impact, condensed ethane-1,1,1-d_3 mainly yielded H_2 and D_2 far more than HD, and some acetylene and methane with ratio 2:1. Only a trace amount of ethylene was found, indicating direct formation of acetylene from ethylidene as the intermediate. At 3 keV, there was no significant *n*-butane and propane formation. Below 100 eV, however, *n*-butane and propane became major products. The presence of $C_2H_2D_4$ from $C_2H_3D_3$ indicates abstraction of hydrogen from neighboring ethane by methylene and by ethylidene, followed by either recombination or disproportionation of the two radicals formed, thus yielding propane, methane, and ethylene from methylene, and *n*-butane, ethane, and ethylene from ethylidene. Electron impact of 100 eV on condensed cyclopropane resulted in four primary reactions: formation of trimethylene biradical, methylene + ethylene, cyclopropylidene + hydrogen, and cyclopropane + hydrogen, in the order of importance. With cyclopropane-1,1,2,2-d_4, the ratio of $(H_2 + D_2)/HD$ formed was 1.2. The result on condensed ethane and cyclopropane was not affected by presence of N_2O_3 in the matrix. The study are being extended to cyclobutane and cyclopentane.

A-15-2 *Gamma-Radiation-Induced Decomposition of Methylsulfonylchloride Solutions in Cyclohexane.* Avraham Horowitz, Soreq Nuclear Research Center, Yavne, Israel.

The gamma-radiation-induced free radical reactions in solutions of methylsulfonylchloride in cyclohexane-(RH) were studied over the temperature range of 25–200°C. It was found that $MeSO_2Cl$ is decomposed by a free radical chain reaction the steps of which are;

Initiation (schematic)	$2RH \rightarrow 2R + H_2$	(I)
Propagation	$R + MeSO_2Cl \rightarrow RCl + MeSO_2$	(1)
	$MeSO_2 + RH \rightarrow MeSO_2H + R$	(2)
Termination	$2MeSO_2 \rightarrow (MeSO_2)_2$	(3)

The rate constant ratios k_2/k_3 and the respective relative Arrhenius parameters were determined by kinetic analysis. Competition between reaction (1) and the addition of cyclohexyl radicals to tetrachloroethylene was used for the determination of k_1.

A-15-3 *γ-Ray-Induced Oxidation of Benzene and Toluene in the Carbon Dioxide Solution at 0°C.* KANAME ISHIZAKI AND SHIN SATO, Tokyo Institute of Technology, Ookayama, Meguro-ku, Tokyo 152, Japan.

Products observed with the solution of benzene are carbon monoxide and phenol, the *G*-values of which from the 1:1 solution are 1.2 and 0.7, respectively. From the solution of toluene, carbon monoxide ($G = 0.4$), *o*-cresol ($G = 0.2$), *m*-cresol ($G = 0.09$), *p*-cresol ($G = 0.06$), and bibenzyl containing unspecified products ($G = 0.1$) are found. With an increase in the fraction of carbon dioxide, all of the products are found to increase. The effect of additives is also studied. A possible reaction mechanism will be discussed.

A-15-4 *Estimation of Free Radical Contribution in the Radiolysis of Solid Alkanes and Cycloalkanes.* B. TILQUIN, J. ALLAERT, D. MAUER, AND P. CLAES, Department of Chemistry, University of Louvain, B-1348 Louvain-la-Neuve, Belgium.

Scavenging techniques were largely used in order to measure the radical contribution in the radiolysis of liquids. These results must be used with discretion because of the lack of specificity of the additives. Extension of these studies to solid materials has been tried and is justified only in the case of glasses where the mobility of the additives allows a quantitative scavenging of radicals.

This report describes results obtained by means of post-irradiation scavenging technique, which is applied to the study of polycrystalline samples. The solid sample irradiated at 77 K is dissolved in a solution of a scavenger at a temperature at which radical combination does not occur in the matrix. Comparison of the G yields measured for such samples with those obtained in the case of molten samples gives information about the yields of trapped radicals and about the reactions of the radicals during the warming up of the sample (Combination-disproportionation ratios).

Experimental results are reported and discussed for *n*-pentane, *n*-hexane, cyclopentane and cyclohexane. A comparison with ESR results is proposed.

(The authors are much indebted to the Fonds de la Recherche Fondamentale et Collective for financial assistance.)

A-15-5 *Transient Species in Pulse-Irradiated Acetonitrile.* R. S. DIXON, A. SINGH, C. R. ROY, AND S. P. VAISH, Atomic Energy of Canada Limited, Whiteshell Nuclear Research Establishment, Pinawa, Manitoba, ROE 1LO, Canada.

Acetonitrile reacts with electrons in water or alcohols with rate constants from $\sim 2 \times 10^7$ to $5 \times 10^8\ M^{-1}\ S^{-1}$ at 298K. Addition of acetonitrile to water or alcohol at room temperature reduces the intensity of the solvated electron spectrum but does not change the position of the absorption maximum. Eventually, at very high acetonitrile concentrations, the spectrum of the solvated electron disappears completely. However in pure acetonitrile small absorptions are present, one of which has its maximum in the 600 nm region. This absorption disappears in the presence of various additives, including water and alcohol. Reaction of acetonitrile with electrons and the possible nature of the transient species in pure acetonitrile are discussed.

A-15-6 *Nanosecond Pulse Radiolysis of 2,2-Paracyclophane.* T. E. GANGWER AND J. K. THOMAS, Radiation Laboratory, University of Notre Dame, Notre Dame, Indiana 46556, USA.

The compound 2,2-paracyclophane (PCP) consists of two benzene rings held in a sandwich configuration by para-substituted ethylene bridging groups. This molecule is unique since it provides a model compound whose investigation reveals properties of the short-lived excimers in aromatic systems. Upon pulse irradiating *p*-xylene or cyclohexane solutions of PCP three broad absorptions occur in the $\lambda \sim 320$ nm, $\lambda \sim 390$ nm and $\lambda \sim 600$ nm regions and a fluorescence band is observed at $\lambda \sim 350$ nm. The species giving rise to the fluorescence and 600 nm absorption has a half life of 18 nanoseconds and is the first excited singlet state of PCP. This result confirms the assignment of similar emission and absorption bands in neat benzene and the alkyl benzenes to excimers whose half lives lie in the 10–30 nanosecond region. From correlation with the theory on PCP a ring separation of ~3.3 Å is estimated for these excimer systems. The species giving rise to the 390 nm absorption has a half life of 2.4 microseconds and is due to a free radical of PCP. The 320 nm region is a complex absorption due to PCP triplet and radical transitions. From energy transfer studies the PCP triplet half life was found to be 800 nanoseconds. Further information which supports and expands the interpretation of the radiation-produced processes in benzene and related systems has been obtained from the study of PCP.

A-15-7 *Investigation of Some Irradiated Inclusion Compounds of Thiourea by the EPR Method.* ANZOR D. BICHIASHVILI, LALI V. IVANITSKAYA, HELEN M. NANOBASHVILI, AND NANULI N. TSOMAIA, Institute of Inorganic Chemistry and Electrochemistry, Tbilisi 380093, USSR.

The nature and properties of the paramagnetic centers formed during γ-radiolysis of inclusion compounds of thiourea with some organic compounds have been investigated by the EPR method at 77°K. During radiolysis of inclusion compounds of thiourea with decalin and tetralin a photosensitive free radical is formed having the doublet EPR spectrum which is not observable either during irradiation of pure addenda (decalin and tetralin) or during irradiation of pure thiourea. The EPR spectra of irradiated inclusion compounds of thiourea with isobutylbromide and trimethylacetic acid are the superposition of the above doublet and of the spectrum of pure addenda. The doublet spectrum is assumed to belong to the radical formed from thiourea. The EPR spectra of irradiated addenda are interpreted. In the case of radiolysis of isobutylbromide the isobutyl radical $CH_3CH(CH_3)CH_2$ is formed by the mechanism of dissociative capture of an electron giving five lines of the EPR. The ten-line spectrum with the binomial ratio of intensities to be due to the radical $(CH_3)_3C$ observed during radiolysis of decalin is assigned to the radical of bicyclo-(4,4,0)-decyl-1 formed from trans-decalin by the detachment of a hydrogen atom from the tertiary carbon atom. Geometrical parameters of this radical are characterized.

A-15-8 *Radiolysis of Di- and Trithiols.* GEORGE G. CHIRAKADZE, HELEN M. NANOBASHVILI, GURAM A. MOSASHVILI, Institute of Inorganic Chemistry and Electrochemistry, Tbilisi 380093, USSR.

Investigation of radiation-chemical transformations of di- and trithiols is of considerable interest for obtaining on their basis the compounds having a practical application as well as for modelling of some important physiological processes. The isooctane and alcohol solutions of dithiols (1,2-C_2, 1,4-C_4, 1,5-C_5, 1,10-C_{10}) and 1,2,3-trithiopropane have been investigated. γ-Irradiation was performed under different conditions (the Co-60 unit, dose rate $= 0.8 - 1.6 \times 10^{10}$ eV/ml-sec. The dependence of a degree of the radiation oxidation of sulphydryl groups on the amount of absorbed energy and on the length of the alkyl radical has been established. $G[-(CH_2)_n-(SH)_2]$ for $n = 2, 4, 5, 10$ decreases from 25 to 5 molecs./100 eV, respectively. The dependence of the nature of the formed products of radiolysis on the length of the alkyl radicals and on the concentration of dithiols has been ascertained by the methods of chromatographic, electrometric, spectrophotometric and other analyses. Optimal conditions of the formation of some products of radiolysis: cyclic disulphides (1,2-dithiacyclohexane, 1,2-dithiacycloheptane) and sulphides (1,2-thiacyclohexane), high-molecular sulphur-containing compounds, hydrogen sulphide and others have been found. A possible mechanism of the described processes has been considered.

A-16-1 *Ionic Processes Initiated by Radical Reactions in Polar Solvents.* ROLF E. BÜCHLER AND HCH. BÜCHLER, Laboratory for Physical Chemistry, Swiss Federal Institute of Technology, 8006 Zürich, Switzerland.

By pulse radiolysis of pure alkyl iodide solvents and of aqueous solutions of alkyl iodides it was possible to detect equilibria between radical charge-transfer complexes [(I·RI) or (OH·RI)] and corresponding ionic inner complexes [$(I_2^- \cdot R^+)_{solv}$ or $(HOI^- \cdot R^+)_{aq}$]. They are built-up from radicals (I or OH) reacting with alkyl iodide and their decay through the inner complexes is ionic. Details of these complexes, their equilibria and the reaction mechanisms involved will be discussed. It represents a novel path from radical reactions to ionic processes in polar solvents.

A-16-2 *Heavy-Ion Radiolysis of Liquid Aliphatic Ketones.* MASAO MATSUI AND MASASHI IMAMURA, The Institute of Physical and Chemical Research, Wako, Saitama, 351, Japan.

Acetone, methylethyl ketone, and diethyl ketone were subjected to radiolysis with He-, C-, and N-ions accelerated with the IPCR cyclotron at various energies. It was found that both $G(H_2)$ and G(CO) for these ketones increase with increasing LET and reach maxima in the LET region of 50–70 eV/Å. These significant results are explained by assuming that the decomposition of keton molecules takes place inside the reaction zone of a fixed radius. An enlarging of the reaction zone appears to result by adding a small amount of water into acetone, where the peaks of $G(H_2)$ and G(CO) are not observed but both yields increase steadily with increasing LET. The thermal-spike effect is also assumed from the fact that the ratios of $G(H_2)$ to G(CO) increase with increasing LET, which is indicative to be due to decomposition of free radicals in the heavy-ion tracks.

A-16-3 *Chemical Effects of Low Energy Electrons on Hydrocarbons.* JEANNE DANON AND RENÉE DERAI, Laboratoire de Physico-chimie des rayonnements, Associé au CNRS, Université de Paris-Sud, Centre d'Orsay, Orsay, France 91405.

Stable products formed in propene and cyclopropane by electron impact below the ionization potential (4 to 10 eV) are studied. Information about precursors and mechanisms are obtained from the products apparition curves versus electron energy. These curves are compared with electronic excitation spectra obtained by trapped electron method and optical absorption. This comparison shows that products are formed through decomposition of excited states reached by, not only optically allowed transitions, but also optically forbidden ones. An interesting point is the determination of decomposition process of propene and cyclopropane in the first triplet state. Comparison with Hg-photosensitized experiments is made in the case of propene: first triplet state is also reached in such experiments but by energy transfer and not directly as with electron impact.

A-16-4 *Radiation-Initiated Hydrocarboxylation of Olefins in the Presence of Dicobaltoctacarbonyl as a Catalyst.* A SAUS AND S. MASOJI, Institut fur Technische Chemie und Petrol Chemie, RWTH Aachen, West Germany.

The cobalt carbonyl catalyzed hydrocarboxylation of *n*-octene-1 with carbon monoxide and methanol in presence of Co-60 gamma radiation gives high yields of alkanoic methyl esters using pressures of 75–250 atm and temperatures of up to 100°C. With *n*-octene-1 a conversion of 92% of the olefin was achieved at 75 atm and 100°C (61% without irradiation). The reaction product was an isomeric mixture containing 99% of octanoic acid methyl ester (95% without irradiation), equivalent to an ester yield of 91% (58% without irradiation). The isomeric mixture of octanoic acid methyl ester consisted of 70% octanoic acid methyl ester—(1), 20%—(2), 6%—(3), and 4%(4) independent of the reaction conditions used. Increasing the radiation dose results in higher yields without any influence on the composition of the reaction product. In all cases the unreacted olefin was recovered in its original form without any signs of isomerization.

A-16-5 *The Radiolysis of Olefins in Liquid Saturated Hydrocarbon Solutions.* F. BUSI AND A. RODA, Laboratorio di Fotochimica e Radiazioni d'Alta Energia, C.N.R., Bologna, Italy.

The concentration dependence of product yields from solutions of olefins (*n*-hexene, cyclohexene, 1–3 cyclohexadiene, 1–4 cyclohexadiene) in cyclohexane, in the presence of nitrous oxide, has been investigated.

The reactivity of the different unsaturated compounds for the transient species produced by irradiation of the solvent is highly dependent on the nature of the solute. Reaction mechanisms for the different olefins are discussed on the basis of gamma and pulse radiolysis experiments.

A-16-6 *Luminescence of Irradiated Organics; Enhancement by Electrical Fields.* A. CHARLESBY, Royal Military College of Science, Shrivenham, Swindon, Wiltshire, Great Britain.

Irradiated organic materials can show luminescence when warmed after irradiation. This is ascribed to thermal untrapping of electrons. Even at constant low temperature, luminescence is observed (preglow) which may be due to tunnelling. This luminescence is temporarily enhanced by an external field, and computer calculations relate this enhancement to the trap depth. Reversal of external field direction causes renewed enhancement, indicating that geminate electrons are involved. Trap depth can therefore be determined by two methods, degree of instantaneous enhancement, and subsequent decay rate.

A-16-7 *A Kinetic and Tracer Study of Aromatic Hydroxylation Induced by Radiation.* N. A. VYSOTSKAYA, V. S. ZHIKHAREV, L. G. SHEVCHUK, AND L. V. PISARZHEVSKY, Institute of Physical Chemistry, Ukr. SSR Academy of Sciences Kiev, USSR.

The reactivities of benzene, furan, pyridine and their derivatives with the hydroxyl radicals in the gamma-radiolysis of aqueous solutions depending upon pH values have been studied by competitive reaction method.

We have shown that gaseous oxygen O^{18} is involved in the benzene hydroxylation and elucidated the mechanism of this process. Disolved O_2^{18} enters into final product, phenol, via successive formation of hydrogen peroxide from O_2^{18} and hydroxyl radicals. The latter attack the aromatic molecule to give hydroxylation products. The amount of oxygen from gas phase into phenol is decreased with increasing medium acidity. It can be due to two factors. First, the pH value affects the properties of radiolytic primary products. Second, the intermediate oxycyclohexadienyl radicals formed in the benzene oxidation undergo acidic fragmentation which results an exchange of their oxygen with water.

A-17-1 *Heats of Reaction of Trapped Intermediates in γ-Irradiated Organic Glasses.* S. L. HAGER AND J. E. WILLARD, Department of Chemistry, University of Wisconsin, Madison, Wis. 53706, USA.

Differential thermal analysis of 3-methylpentane (3MP) and other organic glasses following γ-irradiation at 72°K has been used to determine: 1) the heats of reaction of trapped electrons when they are detrapped by light at 77°K; 2) the heats evolved by reactions of the trapped anions, cations and radicals when the matrices are warmed to temperatures where rapid diffusion occurs; 3) enthalpy changes accompanying annealing of the glasses at 77°K. The heat of reaction of the electrons (*ca.* 140 kcal mole^{-1} after a γ-dose of 2×10^{19} eV g^{-1}) decreases with dose, presumably reflecting increasing carbanion formation relative to neutralization. An exothermic DTA peak at 85°K in 3MP on warm-up grows linearly with dose. If attributed to a species with a G value of 3 the heat of reaction is *ca.* 70 kcal mole^{-1}. An endothermic peak at 82° in unirradiated 3MP grows with time of annealing at 77°K in parallel with the decrease in decay rate of the electrons observed in γ-irradiated samples annealed for various times. The apparatus used can detect 0.1 mcal g^{-1} sec^{-1} in a 0.3 ml sample of organic glass.

A-17-2 *Benzyl Radical Formed by Dissociative Electron Attachment in Rigid Matrices.* HIROSHI YOSHIDA, MASAHIRO IRIE, AND MASAAKI SHIMIZU, Hokkaido University, Kita-ku, Sapporo, 060, Japan.

Benzyl radical was formed by electron attachment to benzyl derivatives such as benzyl chloride or their direct photolysis in several organic rigid matrices. It was found by means of a recording fluorescence spectrometer that the electronic transitions of benzyl are different depending on the way of formation, radiolysis or photolysis, in non-polar hydrocarbon matrices. This is attributed to the interaction between benzyl and the fragment anion such as Cl^- formed in the case of radiolysis as a counterpart. The nearby anion causes the red-shift of all the visible, near-uv and far-uv excitation spectra and the visible emission spectrum of benzyl, the broadening of the spectral lines, and the appearance of a new band at around 360 nm. These features are not observed in polar matrix. In soft hydrocarbon matrix, the effect of anion becomes less and less even at 77°K until the spectra are identical to those of benzyl formed by photolysis. These results give an indication of the intermediate stage of the dissociative electron attachment reaction to form benzyl radical by the radiolysis in rigid matrices.

A-17-3 *Polychromatic Kinetics of Low Temperature Reactions in Irradiated Molecular Solids.* A. I. MIKHAILOV, YA. S. LEBEDEV, I. M. BARKALOV, V. I. GOLDANSKII, Institute of Chemical Physics, Academy of Sciences of the USSR, Moscow, USSR.

A model of "polychromatic" kinetics has been examined for description of bimolecular reactions in irradiated molecular solids. This model takes into consideration that identical chemical processes can occur with different rate constants. One of the reasons seems to be the different diffusion mobility of reactants near extended defects and in ideal crystals. It has been found that the concentrations of reactants in polychromatic processes decrease as $n(t) = a + b \ln t$ resulting in kinetic arrest of the reaction. An algorithm for determination of principal kinetic parameters, when polychromaticity is caused by distribution either of the activation energy or by that of the preexponential factors (for example, in tunneling processes with different barriers penetrabilities (see the report by A. I. Mikhailov and V. I. Goldanskii) has been developed. This algorithm was found to be valid for kinetics of free radical decay /1/, of low-temperature (90–100°K) oxcidation of macroradicals /2/, of solid state polymerization 3, etc. A. I. Mikhailov *et al.* /1/ Soviet Physics-solid state **14**, 1000 (N4, 1972); /2/ Dokl. Akad. Nauk SSSR **204**, 383 (1972).

A-17-4 *Relative Concentration of Isomeric Alkyl Radicals in n-Alkane Single Crystals.* TOMAS GILLBRO,[1] ANDERS LUND,[1] AND PER-OLOF KINELL.[2]

A difference in saturation behaviour has been observed between the radicals CH_3CHCH_2R (I) and $R'CH_2CHCH_2R''$ (II) present in γ-irradiated *n*-alkane single crystals. The radical type (I) saturates at an incident microwave power of 1 mW, the type (II) at 0.01 mW. The microwave frequency used was 9.5 GHz. A point of interest of this finding is that the concentration of radical type (II) can easily be underestimated. Values of the concentrations of the radicals type (I) and (II) are: alkane/C_I%/C_{II}%: C_6H_{14}/65.6/34.4; $C_{10}H_{22}$/42.5/57.5; $C_{16}H_{34}$/33.1/66.9; $C_{20}H_{42}$/29.3/70.7. The mechanism of radical formation is discussed.

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A-17-5 *Electron Transfer by Quantum Mechanical Tunneling in Rigid Matrices.* J. R. MILLER, Chemistry Division, Argonne National Laboratory, Argonne, Illinois 60439, USA.

Pulse radiolysis of aqueous and alcoholic matrices at 77°K shows that solvated electrons trapped in these matrices react with added electron acceptors. Unusual kinetic features are: 1) The reactions proceed approximately logarithmnically with time, over our experimental time range of 10^{-8} to 10^2 sec. 2) The reaction rates increase exponentially with electron acceptor concentration. 3) The rates do not depend on temperature over a range of at least 77 to 143°K, in 6 *M* NaOH glass. The rates depend much more strongly on the electron affinity of the electron acceptor than reactions rates with hydrated electrons in liquid water. For the best electron acceptors, model tunneling calculations of electrons penetrating potential barriers match the experimental data almost quantatively. Similar reactions are observed in which the electron donor is a molecular anion rather than a solvated electron. There is also evidence that "dry electrons" are captured in glasses, but less efficiently than previously supposed.

(Work performed under the auspices of the U.S. Atomic Energy Commission.)

A-17-6 *Study of Electron Tunneling in Alkaline Glasses after γ-Irradiation at 4 and 77 K by Luminescence Method.* B. G. ERSHOV* AND F. KIEFFER.**

The isothermal (ITL) and thermoluminescence of pure and phenol-containing alkaline (6 *M* NaOH) glasses after γ-irradiation at 4° and 77°K have been studied. ITL is due to the recombination of the "electron-positive center" pairs as a result of long distance electron tunneling. The overall process is determined by the disappearance of pairs with different distances of separation. ITL decay kinetics has been described by hyperbolical law. It is independent of dose, but is determined by the duration of irradiation. The intensity of both ITL and TL is considerably increased by the addition of phenol to the glass. Thus ITL is increased about 10 times for a phenol concentration of 3×10^{-4} mol dm^{-3}, and a linear increase with concentration is observed up to 3×10^{-2} mol dm^{-3}. The kinetics of ITL decay for identical irradiation times are independent of phenol concentration and of temperature.

The glow curve of glasses irradiated at 77°K consists of two peaks: first peak at about 92°K and second at about 182°K.

The dependence of luminescence intensity on the temperature within the range from 4 to 90°K has been studied. It has been found that the activation energy gradually decreases from 7 kkal mole^{-1} and below 65°K reaches to the zero value. This effect is connected with electron tunneling.

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A-17-7 *Tunneling of Electrons in Low Temperature Radiolysis.* A. I. Mikhailov and V. I. Goldanskii, Institute of Chemical Physics, Academy of Sciences of the USSR, Moscow, USSR.

Various phenomena induced by the tunnel effect in low temperature radiolysis have been examined. The electrons were found to tunnel through the barrier at about 1 to 5 ev (typical value for structural traps and other scavengers) to considerable distances (30 to 50 Å). The kinetics of low-temperature decay of trapped electrons (e_{tr}^-) and their accumulation in the presence of acceptors were investigated in frozen matrices by the ESR technique. The kinetics of accumulation of e_{tr}^- in presence of the acceptors is described in terms of the "excluded volume" model (V^*). It has been found that $V^* \sim 10^3 \div 10^4$ Å. The rate of e_{tr}^- decay is almost temperature independent over the range of 4.2 to 120°K. This shows that electron transfer occurs by the tunneling mechanism. Estimation has shown that electrons are tunneling through barriers of very low penetrability $P \sim 10^{-20}$. The kinetics of tunneling reactions are described in terms of polychromatic kinetics (see the report by A. I. Mikhailov, Ya. S. Lebedev *et al.*). Tunneling transfer of electrons to distances exceeding the size of a molecule (or of the monomer link) in solids and viscous matrices can induce reactions between spatially distant particles, alter their mechanisms and even the spectrum of final products.

A-17-8 *The Phenomenon of Quantum Low-Temperature Limit of Chemical Reaction Rates.* V. I. Goldanskii, M. D. Frank-Kamenetskii, and I. M. Barkalov, Institute of Chemical Physics of the Academy of Sciences of the USSR, Moscow, V-334, Vorobjevskoje Shausse, 2b, 117334, USSR.

Use of sensitive adiabatic and diathermic microcalorimeters allowed us to measure directly at 140–4°K the rates of growing of polymer chains in radiation γ-induced solid-state polymerization of formaldehyde. The radiation yield of polymerization: $G \sim 10^7$ (140°K), 10^5 (80°K), 10^3 (4°K). The average time of an addition of a new link to the polymer chain $\tau_0 \simeq \tau/G$ (τ is the time of growing of the whole chain) increases at 140–80°K according to Arrhenius law: $E \simeq 0.1$ eV and reaches $\tau_0 \sim 10^{-5}$ sec. At lower temperatures the increase of weakens and at 10–4°K τ_0 reaches a plateau value: $\tau_0 \sim 10^{-2}$ sec. The existence of low-temperature limit of chemical reaction rates is semi-quantitatively explained, as a quantum effect of non-adiabatic tunneling from the zero-vibrational level of primary state, i.e., as an electron relaxation combined with the change of the whole geometry of molecules, lengths and angles of valence bonds.

The observed phenomenon can be significant in chemical and biological evolution, because at very low temperatures the entropy doesn't effect the equilibrium, and the low-temperature formation of even most complicated products in radiation-induced exothermic chain reactions is possible (cold pre-history of life?).

A-18-1 *Infrared Spectroscopy of Pulse Irradiated Gases.* Harold A. Schwarz, Brookhaven National Laboratory, Upton, New York 11973, USA.

Equipment has been developed for measuring infrared spectra of intermediates with lifetimes of a few microseconds or longer produced by a Van de Graaff pulse radiolysis system. The gas sample is an atmosphere of argon containing a small amount of the substrate of interest. The intermediates are produced by ionization or excitation transfer from the argon to the subtrate. The equipment consists of a globar light source, a multiple reflection White cell with optical path lengths to 24 meters, a 500 mm grating monochromator, an In-Sb detector (2–5 micron range), a signal amplifier, a 20 point Waveform Digitizer, and a signal averager. From 1000 to 10,000 waveforms are averaged to reduce the noise level to 1 part per million of the light

signal at a resolution of 0.05 microns. Absorptions ranging from 10 to 100 parts per million have been observed with water and alcohols as substrates. The principle peak, between 2.8 and 3.3 microns, is attributed to ionic species, based on decay kinetics and the effect of added SF_6 on the lifetime. This work was supported by the AEC.

A-18-2 *The Hydrogen Sulphide Radiolysis in the Gas Phase. The Effect of Butadiene-1,3 and Carbon Tetrachloride.* MIECZYSŁAW FORYŚ, IWONA SZAMREJ AND ANTONI JÓWKO, University Teachers College, 08-110 Siedlce, Poland.

The results has been obtained on $G(H_2)$ and G(S) from the hydrogen sulphide in the presence of butadiene-1,3, and CCl_4. Butadiene lowers $G(H_2)$ as an H atom scavenger. Corresponding constant ratios and G_H has been calculated. The lowering of G(S) from 7.1 to a 0.8 ± 0.3 value has also been obtained.

Carbon tetrachloride enlarges sulphur yield to a value G(S) = 9 and does not affect $G(H_2)$ up to 4 mol.%.

When both CCl_4 and butadiene are present in the mixture irradiated, $G/H_2/$ is exactly equal to that in the presence of butadiene alone and G/S/ falls down to zero.

A mechanism is proposed including S_2^+ ions formed as the precursors of molecular sulphur not scavenged by butadiene. The effect of CCl_4 is ascribed to the change in the nature of the negative species taking part in the ion-recombination process.

A-18-3 *The Gamma Radiolysis of Mixtures of Ethane and Hexafluoroethane in the Gas Phase.* MICHAEL D. SCANLON AND ROBERT J. HANRAHAN, Department of Chemistry, University of Florida, Gainesville, Florida 32611, USA.

An investigation has been made of the gamma radiolysis of gas phase mixtures of ethane and hexafluoroethane with compositions ranging from pure fluorocarbon to pure hydrocarbon. This system obeys the generalization (Fallgatter and Hanrahan, J. Phys. Chem. 1970) that the radiolysis products resemble those of the pure hydrocarbon, rather than the pure fluorocarbon. However, the process seen in mixtures of higher molecular weight fluorocarbons and hydrocarbons, in which the fluorocarbon protects the hydrocarbon against decomposition to give molecular hydrogen in a sacrificial process involving C–F bond rupture, is apparently absent in the present system. Instead, there is marked sensitization of the decomposition of ethane to give hydrogen, butane, and several other products. The major mixed fluorocarbon-hydrocarbon products are trifluoromethane and 1,1-difluoroethylene; maximum yields are 0.45 and 0.28 molec./100 e.v. respectively in systems where 90% of the energy is deposited in the fluorocarbon. Postulated mechanisms involve hydride transfer to the CF_3^+ ion and combination of methyl and trifluoromethyl radicals to give an excited ethane which eliminates HF. In 50 mole % mixtures of ethane and hexafluoroethane, major products and their yields are as follows: hydrogen, 2.4; methane, 0.21; ethylene, 0.12; acetylene, 0.10; propane, 0.82; propylene, 0.24; *n*-butane, 2.43; trifluoromethane, 0.28; and 1,1-difluoroethylene, 0.17. (This work was supported by the U.S. A.E.C. under Contract No. AT-(40-1)-3106.)

A-18-4 *A One Nanosecond Time Resolution Study of Excited I_2 Produced by the Irradiation of Gaseous Argon-I_2 Systems with 50 Picosecond Pulses.* FRANZ GRIESER AND RONALD COOPER, University of Melbourne, Parkville, Vic., Australia, AND MYRAN C. SAUER, JR. AND WILLIAM A. MULAC, Chemistry Division, Argonne National Laboratory, Argonne, Illinois 60439, USA.

The 342 nm emission from the D state of I_2 to the ground state is observed to be the most intense feature of the emission spectrum, and the formation and decay of this emission have been examined using time sampling techniques. The D state is formed after the pulse, and reaches a maximum level in about 5 nsec in the case of a sample consisting of 700 torr argon and 0.3 torr I_2; the subsequent decay proceeds with a rate comparable to that of the formation. Decreasing the argon pressure lengthens the formation period, but changing the iodine pressure has little effect. Data collection and analysis are completely computerized. The formation of the D state is too fast to be due to collisions between excited argon species and I_2, or to ion-recombination processes. Possible explanations of the experimental observations will be discussed.

(Work performed under the auspices of the U.S. Atomic Energy Commission.)

A-18-5 *OH($X^2\pi$) Reactions Studied by Pulse Radiolysis of Water Vapor and by Kinetic Spectroscopy.* SHEFFIELD GORDON, W. A. MULAC, AND E. HAMILTON, Chemistry Division, Argonne National Laboratory, Argonne, Illinois 60439, USA.

OH($X^2\pi$) radicals were produced by pulse radiolysis of pure water vapor, water vapor containing OH scavengers and mixtures of water vapor and argon with and without OH scavenger. Rate constants were determined for reactions of the OH radical with NH_3, N_2O, NO, NO_2, SO_2, O_2, and allyl alcohol. The OH radicals were monitored by following the decay of the OH($A^2\Sigma^+ \leftarrow X^2\pi$) absorption at 3087 Å. The experiments were carried out in a pressure range of 10 torr to 1000 torr water vapor and in a temperature range of 25°C to 162°C. The reaction with NH_3 and SO_2 will be discussed in more detail. The reaction with NH_3 produces NH_2, whose rate of formation we have followed by monitoring its absorption at 597.6 nm. This method enables us to relate optical density of the OH radical under our experimental conditions to its concentration making it possible for us to evaluate the bimolecular rate constant of the recombination of two OH radicals. Rate constants for OH with SO_2 at different temperatures will be presented and the effect of third bodies on this reaction will be discussed.

(Work performed under the U.S. Atomic Energy Commission.)

A-18-6 *Long-Lived Organic Negative Ions.** J. G. CARTER, L. G. CHRISTOPHOROU,† AND D. L. MCCORKLE,‡ Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

A number of biologically important molecules were found to capture slow electrons and form long-lived (lifetimes $>10^{-6}$ sec) parent-negative ions. The lifetimes, τ, were found to depend on the ion's internal energy and distinct structural characteristics. Studies of such ions for NO_2-containing disubstituted benzene derivatives indicated that τ depends on the electron donor-acceptor properties of the substituents and the interaction between them. Mass-spectrometric studies and τ-measurements showed that -NO_2-containing benzene derivatives, CN-substituted organic molecules, higher aromatic hydrocarbons, strained structures, and organic molecules containing the functional groups -COCO-, -COCH(OH)-, -COOH, and =CHCHO- capture thermal and near-thermal electrons very efficiently (often with cross sections >100 Å^2) and form long-lived parent negative ions.

* Research sponsored by the U.S. Atomic Energy Commission under contract with Union Carbide Corporation.

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A-18-7 *Average Energies, W, Required to Form an Ion Pair in Liquefied Rare Gases and Liquefied Rare Gas Mixtures.* T. TAKAHASHI, S. KONNO AND T. HAMADA, Institute of Physical and Chemical Research, Wako, Saitama, Japan, AND M. MIYAJIMA, National Laboratory for High Energy Physics, Tsukuba, Japan, S. KUBOTA, Department of Physics, St. Paul's University, Ikebukuro, Tokyo, AND A. NAKAMOTO, Institute for Atomic Energy, St. Paul's University, Yokosuka-shi, Japan, AND H. SHIBAMURA, A. HITACHI, AND T. DOKE, Science and Engineering Research Laboratory, Waseda University, Shinjuku-ku, Tokyo, Japan.

The W-value in liquid argon for internal-conversion electrons emitted from ^{207}Bi was determined to be 23.6 ± 0.3 eV by the pulse method. The W-value in liquid argon, krypton and xenon were also determined by measuring steady conduction currents produced by ^{63}Ni β-rays and were found to be 23.7 ± 0.7, 20.5 ± 1.5 and 16.4 ± 1.4 eV, respectively, which were clearly lower than the corresponding W-values in the gaseous state. (Takahashi *et al.*, Phys. Lett. **44A**, 123 (1973), Miyajima *et al.*, Phys. Rev. **A9**, in press, Takahashi *et al.*, J. Phys. **C7**, in press.)

According to our preliminary experiment, the W-value in liquefied Ar + 0.1% Xe mixture was slightly lower than that in pure liquid argon. Precise measurement is now in progress.

A-19-1 *Biological Effects of Cadmium and Radiation.* WILLIAM L. LAPPENBUSCH AND JAY D. GILE, U.S.E.P.A., Region X, Seattle, Washington 98101, USA.

Adult male rats were injected (I.P.) twice weekly for 30 days with 0, 12.5, 62.5, 125 or 250 μg $CdCl_2$ solution, then (1) maintained another 30 days (Day 60) without further treatment if

used for the $CdCl_2$ mortality study or (2) administered x rays if used for either the radiosensitivity study or the investigations concerned with the effect of the co-insults on the blood picture (number of circulating RBC and WBC, including differentials).

Concentrations of 125 μg $CdCl_2$ solution or less caused mortality while the higher concentration killed 20%. Complete tissue accumulation and distribution analysis by atomic absorption showed that 125 ug $CdCl_2$ solutions resulted in an accumulation of 2200 and 3850 ppm ash in the kidney by days 30 and 60, respectively.

The co-insult of $CdCl_2$ and radiation resulted in a linear decrease in $LD_{50/30}$ values as $CdCl_2$ concentrations were increased. For the animal groups listed above the $LD_{50/30}$ values were 695(637–772), 694(641–750), 648(570–735), 601(538–673) and 491(430–561) rads, respectively. Co-insults drastically altered the number of circulating RBC and WBC and significantly lowered the lymphocyte/neutrophil ratio for extended periods of time. Hematological disorders found in the experiment partially explain the increased radiosensitivity observed. Cadmium chloride is definitely a radiosensitizing agent and its biological effect is synergistic.

A-19-2 *Serum Proteins in Mice Inflicted with Irradiation Combined with Open Skin Wounds (Electrophoretic Studies).* Otfried Messerschmidt and Roland Henneberg, Laboratorium für experimentelle Radiologie, 8042 Neuherberg/Munich, West Germany.

A whole body irradiation (600 R) changes the protein fractions in the serum of mice. There is a decrease of prealbumins, albumins and α_1-globulins one week after irradiation and an increase of α_2- and β-globulins at the same time. The γ-globulins show a high increase two weeks after irradiation.

Open skin wounds produced some days *before* irradiation or simultaneously with the irradiation intensify the irradiation effects on the protein composition of the serum. By producing the skin wound *after* irradiation the protein fractions are extremely changed. There is a great loss of prealbumins and albumins, beginning a short time after wounding. This decrease will not stop until the death of the animals. The relations between the loss of proteins and the high lethality of this kind of combined injuries will be discussed.

A-19-3 *Quantitation of Radiation-Induced Changes in Man.* R. C. Ricks and C. C. Lushbaugh, Oak Ridge Associated Universities, Oak Ridge, Tennessee 37830, USA.

Efforts to evaluate human prodromal responses and increased fatiguability after total-body irradiation have generally been qualitative or quantal rather than quantitative. We have been attempting to quantitate the severity of gastro-intestinal responses to irradiation by remote physiologic monitoring using power spectral analysis of pulmonary impedance. This system measures changes in respiratory pattern secondary to physical exertion or to feedback from vagal nervous activity induced during such GI distress as nausea and vomiting. It is used clinically to maintain remote surveillance of patients undergoing total-body irradiation therapy for various chronic hematologic malignancies. In such patients, prodromal responses produce well-defined changes in the power spectrum of pulmonary impedance. The variance of this power spectrum is proportional to the severity of the incident, being significantly less for nausea than for vomiting. In some cases, chlorpromazine blocks these responses and the typical changes in pulmonary impedance do not occur. Recently these correlations have also been studied in a normal man accidentally irradiated. Currently we are employing ergometric exercise to increase respiratory work and thereby produce changes in pulmonary impedance, CO_2 output and O_2 consumption as a means of measuring increased fatiguability in therapeutically irradiated patients. (Supported by USAEC and NASA.)

A-19-4 *Effects of Whole-Body X-Irradiation and Clofibrate on the Peroxisome Population of Mouse Liver.* F. M. Hill and S. Kleinbergs-Krisans, Dept. of Biology, San Diego State University, San Diego, California 92115, USA.

It has been shown that the administration of the hypolipemic drug ethyl α-*p*-chlorophenoxyisobutyrate (CPIB, Clofibrate) greatly increases the number of peroxisomes in the livers of male rats (Svoboda, Grady and Azarnoff, J. Cell Bio. **35**, 127–151, 1967). Our electron micrograph studies of male mice confirm this observation. Dietary administration of CPIB resulted in a large increase in the peroxisome population.

It has also been noted that after whole-body irradiation, an increase in peroxisome proliferation occurs in hepatic tissue. An ultra-structural analysis of the peroxisome populations was performed on the control, control plus irradiated, treated, and treated plus irradiated groups. Results indicated that irradiation at low exposures had very little effect on peroxisome proliferation. CPIB-treated animals showed similar peroxisome numbers as CPIB-treated, irradiated animals. The pattern was the same for mice maintained on a regular diet.

An $LD_{50/30}$ was determined for both groups. Preliminary data suggests that mice fed the Clofibrate diet have longer survival times, particularly at the higher levels of exposure (1,000–1,600 R). (Supported in part by a grant from the NIH through the San Diego State University Foundation.)

A-19-5 *Hypophysial-Adrenal Control on the Survival Time of Irradiated Fish.* HISAMI ETOH Division of Biology, National Institute of Radiological Sciences, Chiba 280, Japan.

Survival time of goldfish kept at 25°C after x-irradiation with 4 to 32 kR was about 10 day, irrespective of dose size. However, survival time of hypophysectomized fish was shorter than that of intact fish under the same irradiation condition.

Life span of epithelial cells of intestine and gills was estimated from turnover time of 50% of labeled DNA of epithelial cells. Turnover time of epithelial cells in hypophysectomized fish was 20 days for intestine and 22 days for gills, whereas that in intact fish was 25 days for intestine and 37 days for gills.

Intact and hypophysectomized fish were irradiated with 2 kR of x-rays. Irradiated fish were injected with ^{3}H-TdR at 1 to 7 days after irradiation. Changes in incorporation of ^{3}H-TdR into the intestine and gills were examined in operated fish and compared to those in intact fish. No difference between the intact and hypophysectomized fish was found in recovery pattern of incorporation of ^{3}H-TdR. Role of hormone from hypophysis (ACTH) on the life span of epithelial cells of intestine and gills, and on the survival time after irradiation is suggested.

A-19-6 *Prediction of Radiosensitivity of a Mouse with Its Physiological Characteristics before X-Irradiation.* WATARU NAKAMURA, FUMIAKI SATO, YOSHIO NISHIMOTO, AND NAOYUKI KAWASHIMA, National Institute of Radiological Sciences, Chiba, Japan.

Animals used were 143 female mice. They were housed individually in metabolism cages. The observation of their physiological characteristics was continued from the 8th week of age for 10 days. In this period of observation the mice were deprived of laboratory chow for 2 days. At 11th day after the period of the observation they were subjected to a total body x-irradiation with 700 R which resulted in $LD_{45(30)}$. Nineteen parameters adopted as indicators of physiological characteristics of individual mice were induced from the daily changes in body weight, water consumption, urine volume and amount of 5-HIAA excreted in urine. When tested with their mean values none of these parameters showed statistically significant difference between the survivors and the decedents. However, by the method of quantification of Hayashi, a kind of multidimensional analyses of these parameters, it was possible to predict death of individual mice with a high rate of success.

A-19-7 *Main Radiobiological Regularities in the Light of Radiosensitivity of Species.* N. G. DARENSKAYA, G. M. AVETISOV, AND L. E. KOZNOVA. Institute of Biophysics, Ministry of Public Health, Moscow, USSR.

The difference in radiosensitivity among species becomes evident when one studies relationships between the dose and the biological effect and the distribution of dose in space and time.

Dose-dependent death-rates and average duration of life have been established for six species of animals given irradiation under the same conditions and in a wide dose range. The values of $LD_{50/30}$ were varied from 250 rads for dogs to 588 rads for rats. Dose ranges that cause development of various forms of radiation damage in the animals of different species have been found.

The mode of changes in these relationships and peculiarities of the clinical display of radiation damage have been established for animals of different species under the conditions of a non-uniform total-body radiation exposure. These peculiarities have been studied in relation to the geometry of irradiation when the ratio of non-uniformity was changed from 1 to 11.

The influence of dose-rates on the values of dose levels causing the development of various forms of radiation disease and the existence of critical values of dose rates in their development have been established for animals of various species and for man as well.

The differences that have been found are discussed in connection with the radiosensitivity of various organs and systems of animals of different species and in relation to the values of threshold doses and critical dose rates causing various forms of damage.

A-20-1 *The Effect of Irradiation on Normal and Hyperemic Blood Flow in Mouse Limbs.* NEAL L. HORN,[1] M. THOMPSON, A. E. HOWES, J. MARTIN BROWN, R. F. KALLMAN, AND JOHN C. PROBERT, Department of Radiology, Stanford University School of Medicine, Stanford, California 94305, USA.

The right hind limbs of male C3H mice were treated with fractionated doses of x-radiation ranging from a total of 4,000 to 7,000 rads. Measurements of mean limb blood flow were obtained at intervals up to 30 weeks after irradiation by studying the clearance of inhaled radioactive xenon-133. Clearance values were obtained simultaneously from the irradiated and non-irradiated hindlimb of each mouse under resting conditions and also following two minutes of limb ischemia produced by the application of a tourniquet. With unirradiated limbs, a marked increased rate of clearance is seen in the limbs following ischemia indicating a normal hyperemic response. Under resting conditions the irradiated limbs showed a significantly increased clearance rate, particularly with the higher dose levels. Following temporary ischemia a significant and progressively developing inability of the irradiated limb to mount a hyperemic response was found. A preliminary comparison of blood flow changes and the appearance of late radiation induced deformities suggests that the impairment of the hyperemic response is an important factor in the development of chronic radiation changes and may have prognostic significance in clinical radiation therapy. (Research supported by grants CA-10372 and CA-05008.)

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A-20-2 *The Effects of Fast Neutron and X-Ray Radiation on the Microvasculature of the Rabbit Ear Chamber.* GEORGE S. DIMITRIEVICH, MELVIN L. GRIEM, AND FRANCA T. KUCHNIR, Department of Radiology, University of Chicago, and the Franklin McLean Memorial Research Institute (operated by The University of Chicago for the United States Atomic Energy Commission), Chicago, Illinois, USA.

One of the principal lesions which concerned Stone in the early fast neutron clinical trials was the injury of the vasculo-connective tissue. The RBE of various normal tissue end points is known to vary with dose and with differential amount and rate of repair of sublethal damage for each tissue.

Our assays were carried out in a mature modified Sandison-Clark Rabbit Ear Chamber in order to evolve a method of scoring radiation injury to the microvasculature, so as to provide an end point of measure of vascular injury over two orders of magnitude.

Scoring consisted of measuring of morphological changes in the vascular wall with quantitation and illustration of related physical evidence including endothelial swelling, irregularities of the vascular wall, microvesiculations and WBC infiltration indicating the transition period between the early and late radiation effects.

This scoring has been compared with analysis of flow patterns, size, and plasma loss through damaged endothelium and surrounding structures, in capillaries, venules and arterioles and with the degree of instracellular and perivascular edema.

Our evaluation will be compared with recent studies of Reinhold and co-workers.

A-20-3 *Post-Irradiation Recovery in Capillary Endothelium.* H. S. REINHOLD AND G. H. BUISMAN, Radiobiological Institute TNO, Rijswijk (Z. H.), The Netherlands.

The radiosensitivity of capillary endothelium as determined previously in the rat subcutis showed a D_0 of 170 rad, an extrapolation number of 7, a DQ of 340 rad, and, with split dose experiments, a $D_2 - D_1$ of 290 rad. The latter value indicated that capillary endothelium is capable of repairing sublethal damage. In recent experiments, the time-recovery response of the repair of sublethal damage was investigated. The results indicate that the (non-proliferating) endothelium shows a similar time-recovery pattern as those published for proliferating systems. In another series of experiments, the repair capacity of endothelial cells over a course of two months after a single dose of irradiation of 1000 rad was investigated. It seems that there is extensive repair in the first week, amounting to a factor of four. Additional repair, again of a factor of about four, takes place between ten and sixty days post-irradiation. Some of this repair may be due to repair of potentially lethal damage.

Preliminary results with respect to the shape of the "survival" curve, obtained by the induction of proliferation in the endothelium one month after irradiation seem to indicate that there is an increase in extrapolation number as well as a possible change in the slope of the "survival" curve.

A-20-4 *Cardiovascular Syndrome and Radiation-Induced Shock with Its "Point of No Return."* CHARLES C. BERDJIS, M.D., Armed Forces Institute of Pathology, Washington, D.C. 20306, USA.

High doses of irradiation are known to produce a CV syndrome almost always accompanied by a shock situation. Ultrastructural studies of the capillaries and small blood vessels in heavily irradiated animals demonstrated endothelial vacuolization often associated with subendothelial electron-dense deposits. Although the significance of these lesions and their relationship to irradiation is obscure, there is a distinct relationship between the degree of vascular injury, and the evolution of shock. Blisterings and vacuolization are reversible and characterize the early phase of shock (JAMA **204**, 191, 1968). Subendothelial deposits, however, indicate the irreversible phase of shock or the "point of no return" (fatal shock).

A-20-5 *Reactions of Isolated Vascular Smooth Muscle Preparations of Some Vertebrates to X-Irradiation.* URSULA E. WELSCHER AND MICHAEL CHR. MICHAILOV, Institut für Biologie der Gesellschaft für Strahlen- und Umweltforschung Neuherberg-München, West Germany.

The mechanical activity of helical strips of arteries and veins continuously superfused with Krebs-Henseleit solution at 37°C was recorded isotonically. Filtered x-rays (50 kV, 0.3 mm Al) induced in *arteries* three types of mechanical reactions:

1. Immediate (latent time some seconds), reproducible tonic contractions after 0.25–1 kR (dose-rate 6 kR/min)—(a) reversible after some minutes (rat and frog aorta) or (b) semi-reversible (rabbit aorta).
2. Immediate (latent time some seconds), reproducible, reversible relaxations after doses of 1–3 kR (6 kR/min), that were potentiated by adrenaline (rat and rabbit renal artery).
3. Late (latent time some minutes) and very slow (maximum after more than one hour) tonic contractions with long duration (more than 3 hours) after doses of about 3 kR (6 kR/min) (aorta and pulmonary artery of guinea-pig, pigeon and chicken).

Veins reacted to x-irradiation with reproducible, reversible tonic contractions and changes in the phasic activity (v. cava caud., portal and other veins of rabbit, rat, guinea-pig and pigeon).

The influence of several physical and chemical factors (tension, $t°$, pH, ionic milieu, etc.) will be discussed.

A-20-6 *Immediate Reaction of Isolated Rat Aorta to X-Irradiation.* MICHAEL CH. MICHAILOV, HELMUT AMELUNG, AND URSULA E. WELSCHER, Institut für Biologie der Gesellschaft f. Strahlen und Umweltforschung, Neuherberg-München, West Germany.

The mechanical activity of helical strips of rat aorta continuously superfused with Krebs-Henseleit solution at 37°C was recorded isotonically and isometrically with Harvard, resp. Statham transducers.

The aortic strips reacted to x-irradiation (50 kV, 0, 3 mm Al; dose-rate 6/kR/min, threshold dose 250 R) with a reversible tonic contraction which is dose and dose-rate dependent according to statistical analysis. After repeated irradiation pulses a tachyphylactic reaction appeared, yet the preparations were still radiosensitive after a total dose of more than 100 kR.

During temperature changes to 20°C or 40°C the contraction disappeared, but was restored at 37°C. The antiadrenergic drugs dibenamine (10^{-6} *M*), regitine (10^{-6} *M*) and bretylium (10^{-4} *M*) blocked the effect of adrenaline but not that of x-irradiation. Tetrodotoxin (up to 10^{-6} g/ml) was also without effect. The action mechanisms of the x-irradiation on the aorta and other smooth muscle preparations will be discussed in relation with the electro- and pharmacomechanical coupling and the cyclic-AMP-system.

A-20-7 *Effects of Gamma-Irradiation on Myocardial Contractility and Coronary Flow.* ANAS M. EL-NAGGAR, MAMDOUH A. ZAKI, AND FATHY S. GALAL, Atomic Energy Establishment, Cairo, Egypt.

Isolated perfused heart preparations of the rabbit were exposed to gamma irradiation from a Radium 226 source to study the effects on myocardial contractility, and coronary flow after acute exposures of 300 to 1700 roentgens. The effects on frog's heart was studied for purpose of comparison.

Results indicated that contractility of isolated rabbit's heart was inhibited starting at 900 roentgens, and reached 47.9 ± 3.8% at 1700 roentgens. Myocardial coronary flow was decreased starting at 300 roentgens and reached 61.0 ± 3.0% at 1700 roentgens. No change was evident in the contractility of the frog's myocardium up to 1800 roentgens.

The results of these experimental procedures were interpreted and discussed in the light of the data obtained, and the reports of previous relevant investigations. Final postulations were drawn reparding the nature of cardiac radiosensitivity in particular, and well differentiated tissue in general.

Statistical procedures were carried out to evaluate the results. Data were supported by original tracings of the heights of amplitudes of contractility, and coronary flow.

A-20-8 *Effect of Metal Ions on the Radiosensitivity of Cardiac Muscle.* JOSEPH TIGYI AND ANTHONY NIEDETZKY, Biophysical Institute of Medical University, Pécs, Hungary.

Excitability of frog's cardiac muscle were examined after irradiation with external gamma [60 Co] and internal beta and mixed beta-gamma radiation [32 P, 45 Ca–24 Ha, 42 K] respectively. The isolated frog hearts were previously treated by different sorts of metal salts in a concentration of 10^{-3}–10^{-5} M. It was found that three metal ions: Cr, Mn, and Ba increased the radiosensitivity of the hearts in very significant measure. The experimental results together with other data of the laboratory seem to support our earlier hypothesis about semiconductor mechanism both of excitation and radiation effect.

A-21-1 *Exposure-Rate Response in the Prenatally Irradiated Rat: Effects of 50 R on Day 18 of Gestation to the Developing Testis.* JOHN R. STRANGE, Georgia Institute of Technology, Atlanta, Georgia 30332, USA.

Pregnant rats were irradiated on the 18th day of gestation with a total exposure of 50 R at rates of 1, 3, 10, 25 and 47 R/min. Testes of the progeny were evaluated at 28, 49, 70, 91 and 210 days *post partum*. At 28, 49, 70 and 91 days an exposure-rate response was present as determined by evaluating the most advanced cell type in 500 seminiferous tubule cross-sections in 16 animals from each group. A rapid increase in damage was associated with increasing exposure rate up to 10 R/min. The effects of 25 and 47 R/min appeared to be less damaging than those of the 10 R/min rate. By 210 days of age all groups showed no differences from the controls. The total recovery time appeared to be a function of initial damage rather than recovery rate since all irradiated groups recovered in a parallel fashion.

A-21-2 *Radiation Response of Spermatogonial Stem Cells in the Mouse.* E. F. OAKBERG, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn. 37830, AND CLAIRE HUCKINS, Dept. of Cell Biology, Baylor College of Medicine, Houston, Texas 77025, USA.

Adult 101 X C3H F_1 hybrid males were given either multiple or single injections of ^{3}H-thymidine, and 159 hours after the last injection, exposed to 150 R x rays. Mice were killed 48 hours after irradiation, which was 207 hours or one cycle of the seminiferous epithelium after ^{3}H-thymidine injection. Autoradiographs of 3 μ testis sections were scored for labeling of A_s spermatogonia. For the group receiving multiple injections, percentage of labelled cells was 42% for controls and 32% for irradiated mice, and after a single ^{3}H-thymidine injection, the values were 12% for controls and 11% for irradiated animals. The only spermatogonia which survive a 150 R dose are of the single, isolated (A_s, A_{is}) type. Therefore, the presence of a significant number of labeled cells at 207 hours demonstrates that the spermatogonial stem cells are in continuous cycle; there is no evidence for a non-dividing, "reserve stem cell" population. (Research sponsored by U.S. Atomic Energy Commission under contract with Union Carbide Corp.)

A-21-3 *Age Changes in Testicular Radiation-Sensitivity Associated with Changes in Androgen Synthesis and Lipid Peroxidation.* LeGrande C. Ellis, Utah State University, Logan, Utah 84322, USA.

Recent investigations have shown that there are marked increases in the radiosensitivity of rat testes 16–18 days prenatally and 35 days postnatally. Although these changes in radiosensitivity occur with an onset of androgen synthesis at these two periods associated with development of the gonad, there is no ready explanation for this phenomenon. On this basis, a detailed study was undertaken to evaluate changes in androgen synthesis and lipid peroxidation during the period of 1–90 days of postnatal development. A marked increase in lipid peroxidation was observed on day-28 just prior to a decrease in testosterone synthesis on day-34. Lipid peroxidation was reduced on day-36, but was rapidly elevated and remained high during the remaining observation period. Both lipid peroxidation and androgen synthesis were shown to be gonadotrophin dependent, but responded differently to various metabolic inhibitors. The change in radiosensitivity of rat testes at 35 days of age correlates well with rather abrupt changes in androgen synthesis and lipid peroxidation. (Supported by U.S. Atomic Energy Commission Grant No. AT(11-1)-1602.)

A-21-4 *Effect of Dose and Dose Rate on the Stem Spermatogonia of the Prepuberal Rat.* Bert H. Erickson, Michael J. Blend, and Polly G. Martin, UT-AEC Comparative Animal Research Laboratory, Oak Ridge, Tennessee 37830, USA.

While much is known of the radioresponse of spermatogonia, the spermatogonia of the prepuberal testis have received little attention. Rats in age groups varying from 6–60 days received 300 rads of γ-radiation (40 rads/min). Spermatogonial effects were assayed in tubular cross sections and tubular whole-mounts at 5 and 80 days postirradiation.

At 5 days postirradiation spermatogonial survival was greatest in testes irradiated when 6 days of age (41% of control) and least in 9-day testes (13% of control). Differences between testes aged 20 or more days at irradiation (17–19% of control) were nonsignificant. At 80 days postirradiation, spermatogonial values remained least in 9-day testes (50% of control). Six-day testes were at 78% of control and values derived from testes irradiated at later ages were only slightly higher (80–85% of control).

The effect of 400 rads on stem spermatogonia was enhanced by a factor of 2.0 when delivered at 40 rather than 1 rad/min to testes aged 6–20 days at irradiation. The rate effect declined to 1.7 in 30-day testes and reached a low of 1.3 in 60-day testes. (Supported by U.S. AEC under contract AT-40-1-GEN-242 with University of Tennessee.)

A-21-5 *Effects of X-Irradiation on Mammalian Oocytes at Specific Stages of Meiosis.* B. K. Batra and V. A. Kalra, Cancer Research Institute, Tata Memorial Centre, Bombay 4000 12, India.

Mammalian oogenesis which has chronologically programmed sequences lends itself to critical analysis of radiosensitivity at well defined stages of cell cycle. This aspect was taken advantages of in this study.

C_{57}Bl/Icrc mice were given a single dose of 150 r whole body x-irradiation on different days during pregnancy when oocytes in embryos were at pre-leptotene, leptotene, pachytene and

zygotene stages of the meiotic prophase. Radiation effects were evaluated in chromosomes at second meiotic metaphase. Oocytes of untreated animals served as controls.

Leptotene stage irradiation resulted in the maximum incidence of breaks. A reciprocal correlation between the incidence of breaks and that of deletions and additions was evident at all stages of irradiation. The occurrence of advanced stages of oogenesis, aneuploidy and stickiness were some of the other chromosomal abnormalities.

Temporal correlation between different stages of meiotic prophase and radiosensitivity is indicated by the results. The possible differential correction of x-ray insult and its significance in the expression of the original damage are discussed.

A-21-6 *Causes of Sterility in Male Mice Derived from Germ Cells X-Irradiated in Spermatogonial or Postspermatogonial Stages.* N. L. A. CACHEIRO AND M. S. SWARTOUT, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn. 37830, USA.

It has long been known that complete sterility is rare in F_1 males derived from irradiation of spermatogonia, but much more frequent in males derived from irradiation of postspermatogonial stages. Males from these two derivations are now being analyzed histologically and cytologically in an attempt to determine and compare causes of sterility.—We were able to detect chromosomal aberrations in 9 of 13 sterile sons (69%) derived from postspermatogonial stages of x-irradiated (101 X C3H) F_1 males (Genetics, in press), and a further group is under study. In 18 sterile sons derived from x-irradiated (101 X C3H)F_1 spermatogonia (experiments of W. Generoso), there were, however, only 5 (28%) with chromosomal anomalies. The difference between the groups is on the borderline of significance ($P = 0.03$). While all 9 chromosomally abnormal steriles in the postspermatogonial group probably carried translocations, the 5 in the gonial group were comprised of two XYY males and three with reciprocal translocations, one of the latter forming a metacentric chromosome.—Another way in which the two groups were found to differ is with respect to frequency and stage of spermatogenic arrest. Among the 13 steriles derived from postspermatogonial irradiation, there were 7 in which spermatogenesis was blocked in pachytene or diakinesis. On the other hand, only one of the sterile males derived from spermatogonial irradiation was blocked in pachytene, 17 others having mature spermatids in the testis. The difference is significant ($P < 0.01$). (Research sponsored by U.S. Atomic Energy Commission under contract with Union Carbide Corp.)

A-21-7 *Spermatogonia Survival in Mice after Exposure to Low Doses of ^{60}Co γ-Rays and Neutrons of 14 and 400 MeV.* M. BIANCHI AND J. BAARLI, European Organization for Nuclear Research (CERN), 1211 Geneva 23, Switzerland.

The effect of small doses on the survival of different kinds of spermatogonia cells in mice have been investigated after exposure to ^{60}Co γ rays and neutrons of 14 and 400 MeV at low doserates. The animals were given whole-body irradiations and were killed 46 hours after the start of the exposures. The RPS (resting primary spermatocytes) in seminiferous tubules in stage VII and VIII and type B spermatogonia together with RPS in stage VI were counted. The survival curves for the different stages are reported and discussed together with the measured RBE values.

A-21-8 *Effect of AET and 5-Hydroxytryptamine (5 HT) on Dominant Lethal Mutations after X-Irradiation of Mouse Spermatogonia.* KRISHNA SUNDARAM AND JOHN PHILIP, Bio-medical Group, Bhabha Atomic Research Centre, Bombay 400085, India.

Male Strain A mice were given whole body irradiation of 200 R of x-rays when 10 to 12 weeks old. One group was given an intraperitoneal injection of AET at a dose of 300 mg/kg body weight and another group 5-HT at a dose of 75 mg/kg body weight 30 minutes prior to x-ray exposure. During the succeeding weeks virgin strain A females were mated for up to 9 weeks following irradiation. The females were dissected 17 days after the appearance of the vaginal plug. The numbers of corpora lutea, implants, dead and living embryos were determined. The induced dominant lethal mutation rate per roentgen for spermatozoa, spermatids, spermatocytes, Type B and Type A spermatogonia have been computed for different experimental groups. The Dose Reduction Factor (DRF) for AET and 5 HT for various stages of spermatogenic

cycle have been calculated. With 5 HT, a DRF of 9 is obtained for spermatozoa in Vas Deferens, and 7 for spermatocytes. It is also observed that while 90 per cent of animals are sterile at 7 weeks post-irradiation in both 200 R and 200 R plus AET groups, all the animals in the 200 R plus 5 HT group were fertile. The reduction in the rate of induced Dominant lethal mutations is reflected in an increase in the number of living pups/litter in both AET and 5 HT treated groups.

A-21-9 *Spermatogonial Cell Renewal under Low Level Irradiation. XIII. Response and Cell Population Kinetics of the Spermatogonial Stem Cell under Continuous Irradiation.* THOMAS H. S. HSU AND JACOB I. FABRIKANT, Department of Radiology, University of Connecticut School of Medicine, Farmington, Connecticut 06032, USA.

Cellular response and cell population kinetics in the premeiotic stages of spermatogenesis in the C57BL/6J mouse under continuous gamma irradiation have been studied using the techniques of quantitative histology and high resolution autoradiography. At 45 rads/day, although there was depopulation of the type A, intermediate and type B cell compartments, the type A_s spermatogonial stem cell population could be maintained at near-normal levels. In a radiation-free environment following 45 rads/day continuous irradiation for 2 weeks, cellular restitution occurred, the recovery starting sequentially from the surviving A_s stem cells. A re-examination of the cell population kinetic data obtained at 1.8 rads/day and 4.5 rads/day has also shown that the A_s cell population does not appear to be perturbed at these levels. The experimental results are discussed in terms of factors affecting tissue homeostasis in the mammalian testis under low level irradiation, primary cellular radiosensitivity and compensatory cell proliferation. (Research supported by USAEC Contract CH AT(11-1)-3013).

A-21-10 *Action of X-Radiation on DNP of Sexual Cells.* V. G. KONDRATENKO, V. A. STAKANOV AND A. M. KUZIN, Institute of Biological Physics, USSR Academy of Sciences, Pushchino, USSR.

Chromatin of spermatogenic cells has been microfluorometrically studied within 24 hours after the irradiation in situ. The degree of DNP dissociation and DNA denaturation has been found to change as early as one hour following the irradiation (25–400 r). Changes in DNP dissociation degree depend on the original chromatin state and dose of irradiation; they are maximum in 16 hours. More pronounced changes are observed in DNA. Within the first 60 minutes after the irradiation the degree of DNA denaturation of irradiated cells decreases as compared with normal ones; later (beginning with the 4th till 8th hour) it gradually increases, depending on the dose, to reach maximum also in 16h (the more condensed the chromatin in cells, the faster is the increase). Model experiments, involving partial body shielding and with physiologically active substances (or lipid and quinoid nature) as well as sexual hormones being used, led to the conclusion that early response of chromatin of sexual cells was dependent on the direct effect of radiation; later (in 4 h) a relationship was observed between radiation effect and disorganization of hormonal regulation and radiation anabolites formed.

A-22-1 *Radiation Responses of Neuroblastoma Cells in Vitro.* T. C. H. YANG, J. RISIUS, AND C. A. TOBIAS, Lawrence Berkeley Laboratory and Donner Laboratory, University of California, Berkeley, California 94720, USA.

Mouse neuroblastoma cells (Cl300), cultured *in vitro* for many generations, have a doubling time of 24 hours at 37°C and are highly tumorigenic. The colony-forming ability of those tumor cells has been examined and used as a criterion for studying its radiosensitivity. Exponentially growing cells in 10% fetal calf serum DMEM were exposed to 220 kVp x-rays with a 0.25 mm Cu and 1.0 mm Al filter and a dose rate of 200 R/min, and the number of colonies formed two weeks later was checked. A dose-response curve with D_0 around 200 R and an extrapolation number about 3 was found. Cells irradiated with two doses of 150 R at various intervals showed a biphasic pattern of recovery: reached a maximum of survival at 6 hr, decreased to a minimum around 8 to 10 hr, and increased again with longer intervals. The growth of cells was decreased to 50% of control by a dose of 150 R as the total number of cells was counted at 5 days post-irradiation. In addition to the effect on cell proliferation, radiation appears to induce some

morphological changes in neuroblastoma cells. A significant increase of the percentage of "differentiated" cells in the irradiated cell population was observed. Three days after cells exposed to 600 R, for example, about 30% cells formed long neurites, as compared to only 15% in control. Movies of individual cells under microscope taking during and immediately after exposure to radiation indicate that some swelling of the soma occurs after irradiation and that actively growing neurites often exhibit sudden retraction of the growth cone. A definite toxic effect on the cell proliferation has been found when cells were exposed to 0.2 m*M* to 1.0 m*M* hydroxyurea for 1.5 hr, and an exposure of cells to hydroxyurea post-irradiation produced a further inhibition of cell growth.

A-22-2 *Differentiation Inhibitors and Their Effects on Stem Cell Sensitivity to Irradiation in Regenerating Planarians.* VERNON E. STEELE AND CHRISTOPHER S. LANGE, The University of Rochester School of Medicine and Dentistry, Rochester, New York 14642, USA.

The planarian owes its extensive powers of regeneration to the possession of a totipotential stem cell system. The survival of the animal after irradiation depends mainly upon this system. In this respect the planarian is analogous to mammalian organ systems such as bone marrow or gut epithelium. The differentiated cells control the course of stem cell mediated tissue renewal by the secretion of differentiator and/or inhibitor substances. One such inhibitor substance (one that inhibits brain formation; specific to the organ but not species) is evaluated in terms of its biochemical nature and its effect on the post-irradiation fate of the stem cells; and ultimately on the fate of the whole animal. The differentiative integrity of the stem cells is measured at various intervals during the post-irradiation period of recovery and/or mortality by using the inhibitor substance and measuring its effect on the regenerating planarian *Dugesia etrusca.* The data presented show that the probability exists to alter the post-irradiation recovery pattern by shifting the differentiative demands placed on the stem cells. (This work was performed under contract with the U.S. Atomic Energy Commission in the Dept. of Experimental Radiology [Contract AT(30-1)-4284] and at the University of Rochester Atomic Energy Project and has been assigned Report No. UR-3490-458. V. E. S. would like to acknowledge the support of the Rochester A.E.C. Laboratory Graduate Participant Program. C. S. L. would like to acknowledge the support of an N. I. H. Career Development Award.)

A-22-3 *Differentiation of Neuroblastoma Cells in Culture: X-Irradiation vs. cAMP.* KEDAR N. PRASAD, Dept. of Radiology, University of Colorado Medical Center, Denver, CO 80220, USA.

Cyclic AMP induces many differentiated functions in mouse neuroblastoma (NB) cells in culture, some of which can also be induced by x-irradiation. In addition to cell death, x-irradiation induces neurites which are electrically excitable and thus exhibit the physiological characteristic of mature neurons. The neurite formation is blocked by vinblastine sulfate, cytochalasin B and cycloheximide, but not by actinomycin D. The radiosensitivity of NB cells in producing neurites markedly vary from one clone to another; however, all clones show a marked increase in the size of soma and nucleus. The DNA, RNA and protein contents markedly increase. In an adrenergic clone (contains tyrosine hydroxylase, but no choline acetyltransferase) x-irradiation increases acetylcholinesterase (AChE) activity without changing the TH activity. In a cholinergic clone (contains choline acetyltransferase but no tyrosine hydroxylase) x-irradiation increases both choline acetyltransferase and AChE activities. Cyclic AMP increases all three enzymes. X-irradiation does not change the intracellular level of cAMP. From these data the following conclusions are made: (a) x-irradiation promotes the organization of microtubules and microfilaments for the expression of differentiated phenotype similar to that observed in cAMP-treated cell culture; (b) neurites are formed in the absence of any increase in TH activity. (Supported by USPHS-NS-09230 and CA-12247.)

A-22-4 *Differential Radiosensitivy of Reproductivity and Differentiative Trait in Chick Cartilage Cells Cultured in Vitro.* H. UTSUMI AND T. SUGAHARA, Kyoto University, Kyoto, Japan.

As the recent development of cell culture techniques made it possible for several differentiated cell types from chick embryos to maintain their differentiated phenotype in clonal cell culture,

quantitative comparison of radiosensitivity for reproductivity and differentiative traits were studied by a new technique.

Single chondrogenic cell suspensions from the chick embryonic sterna were inoculated into medium Ham's F12 according to the method by Coon 1966. After 16 day incubation period, the cartilage cell suspensions yield two colonies; cartilage-making colony (CMC) recognized by the presence of a metachromatic matrix and fibroblastlike (non CMC). We measured survivals of reproductivity (CMC and non CMC) and differentiative traits (CMC or non CMC) at various x-ray doses. The cell of CMC and that of non CMC showed the same radiosensitivity, but the ratio of non-CMC to CMC was increased by irradiation, especially in low dose range demonstrating higher radiosensitivity of differentiative traits than of reproductivity. The result may have an implication on the interpretation for the effect of radiation on cell renewal systems.

A-22-5 *A Major Cause of Radiation Damage: Interference with Endocellular Control of Mitotic Activity and Differentiation.* K.-H. v. WANGENHEIM, Nuclear Research Establishment, 517 Jülich, West Germany.

In radiotherapy, and radiobiology in general, it is assumed that irreversible genetic defects are a prerequisite to radiation damage. Some facts, however, do not agree with this assumption, although it is well established that the nucleus is the most sensitive organ of the cell. The reason has been elucidated in plants. Parallel observations suggest that zoological systems do not differ in this respect. According to a control mechanism detected in the endosperm, a rise of the ratio of cytoplasmic amount to ploidy at a certain level—in contrast to former proposals (nucleocytoplasmic tension, hertwig)—triggers differentiation. During the period of mitotic delay following x-irradiation cytoplasmic growth, including propagation of mitochondria und proplastids, continues. The cells differentiate and cease mitotic activity either before the end of mitotic delay or after a reduced number of mitotic cycles, depending on the surplus amount of cytoplasm gained. Thus, irreversible genetic defects are not a prerequisite to inhibition of colony forming ability. The contribution of this "early differentiation" to non-survival varies according to the conditions of irradiation and may outweigh genetic defects.

A-23-1 *Radiosensitivity of Human Hematopoietic Stem Cells as Measured Using the Diffusion Chamber Culture Technique.* A. L. CARSTEN, A. BOYUM,* AND E. P. CRONKITE, Brookhaven National Laboratory, Upton, New York 11973, USA.

Determination of the radiosensitivity of the human hematopoietic stem cell has significance in Health Physics, Nuclear Medicine and Mammalian Radiobiology. The degree of injury to this system is a prime determinant of survival of the radiation-exposed individual. The D_0 for x-ray exposure of normal human bone marrow has been measured using the diffusion chamber culture technique. Bone marrow was obtained from healthy volunteers. The red cells were removed and the remaining nucleated cells placed in millipore diffusion chambers which were implanted into the peritoneum of mice. The mice received split x-ray exposures so that the total dose to the mouse was 600 rads and the dose to the implanted chambers varied from 25–400 rads. The cell inocula were varied from 3×10^5 to 5×10^6 nucleated cells to ensure an approximately equal cell harvest after 12 days. Differential counts on chambers obtained in 4 replications indicated a D_0 ranging from 72–80 rads for proliferative granulocytes, when calculated over the dose range of 25–400 rads. These findings will be discussed in relationship to values obtained for *in vitro* exposures of human bone marrow. (Supported by the U.S. Atomic Energy Commission.)

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* Norwegian Defence Research Establishment Division for Toxicology, Norway.

A-23-2 *Effect of X-Irradiation upon the Hematopoiesis-Supporting Stromal Tissues of the Mouse Spleen.* PREM RADHESHWAR AND NORMAN WOLF, University of Washington, Seattle, Washington 98195, USA.

The loss of ability by the splenic stroma to support hematopoiesis after a single x-irradiation of the spleen alone was measured by the spleen colony technique and by ^{59}Fe uptake, using a following dose of whole-body irradiation plus intravenously injected bone marrow cells. The dose

of primary irradiation and the time between irradiations varied. Primary dose sham controls were used. In mice tested 90 days later, a primary splenic irradiation of as little as 100 R produced a significant drop in visible spleen colonies formed and in ^{59}Fe uptake, while 2000 R produced maximal reduction within a 4000 R upper dose limit. Using a 2000 R splenic irradiation, the ability to support splenic hematopoiesis began to decline by 24 hours, reaching a nadir at 72 hours which was maintained through 90 days. Measurements utilizing intravenously injected ^{59}Fe-marked donor red cells indicated that blood flow to spleens irradiated with 2000 R was reduced, manifesting a time course similar to that for the reduction of hematopoiesis support. Light microscope studies provided little evidence of prolonged tissue damage. Partially supported by NIH grants 5 RO1 Am 15280-03 and TO1 FD 01011.

A-23-3 *Effects of Single or Fractionated Doses of Neutron or Gamma-Radiation on Hematopoietic Stem Cells.* E. JOHN AINSWORTH, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439, USA.

The effects of dose fractionation on stem cell depression and repopulation are not well defined for gamma radiation, and less is known for neutron radiation. Changes in the femur content of stem cells (CFU-S) have been measured over 180 days after single or fractionated doses of fission neutrons from the JANUS reactor or of ^{60}Co gamma radiation. When total doses of 288 neutron or 740 gamma rad were given in 9 equal fractions over 3 weeks rather than as a single dose, a significant sparing effect was observed for both radiations, but only during the first 23 days. Thereafter, the counts did not differ significantly, so fractionation does not produce a sustained increase in the rate or degree of repopulation. Fractionation of the gamma dose produced a greater sparing effect than did fractionation of the neutron dose, so the RBE is greater than 2.6 during at least the early phase of hematopoietic recovery. (Work supported by the U.S. Atomic Energy Commission.)

A-23-4 *The Distribution of Colonies in Spleens of Lethally Irradiated Mice Given Isologous Bone Marrow.* KENNETH L. MOSSMAN AND ARTHUR L. KRETCHMAR, University of Tennessee Memorial Research Center and Hospital, Knoxville, Tennessee 37920, USA.

In order to investigate the underlying mechanisms involved in the spleen colony assay for hemopoietic stem cells, we investigated the statistical distribution of colonies in the assay mice. It was found that the distribution of colony counts in C3H mice does not fit the Poisson as expected for the assay procedure. Instead, our data are consistent with a binomial distribution. Data from other laboratories, which we have analyzed, confirm this finding in at least two other strains of mice. However, in C3H mice, if irradiated three days prior to bone marrow injection; in C57BL/6 mice; and in C3H assay mice given irradiated cells, the variance was greater than expected for a binomial. In addition, the three day pre-irradiated mice yielded lower values for the assay and lower values for "f" numbers than mice injected on the day of irradiation.

A consistent explanation of our data can be constructed on the assumption that the principle determining factor in the assay is the number of niches (microenvironments) in the spleen competent to support the growth of a gross nodule.

A-23-5 *Effect of Endotoxin on CFU Kinetics in Control and Irradiated Mice.* OTTO VOS AND ROB E. PLOEMACHER, Medical Biological Laboratory TNO, Rijswijk, and Erasmus University, Rotterdam, The Netherlands.

Seven days after i.v. injection of 0.5 mg endotoxin (*S. typhosa*) the numbers of CFU in blood and spleen have increased 10 and 100 fold respectively, in the liver the presence of hemopoietic foci and 5,000–10,000 CFU can be demonstrated, in bone marrow subnormal numbers of CFU are found. Growth curves of CFU derived from bone marrow and spleen of normal mice and from spleen and liver of endotoxin pretreated mice were followed after transplantation into lethally irradiated recipients. CFU from liver appeared to have a longer doubling time than CFU from normal femur. The growth curve of CFU from spleen of endotoxin pretreated mice did not differ from the growth curve of CFU from a normal spleen. The capacity of CFU from the different sources to save lethally irradiated mice from mortality was also investigated. High doses of endotoxin administered to irradiated and bone marrow treated mice did not

affect the doubling time but led to a higher number of CFU eventually reached in the spleen. It is postulated that the effect of endotoxin on radioresistance of mice and growth kinetics of CFU is mainly exerted by an effect on microenvironment.

A-23-6 *A Study on Competitive Proliferation between Normal and Irradiated Bone Marrow or between Normal Bone Marrow and Spleen in Syngeneic Radiation-Chimeras.* S. MURAMATSU* AND J. F. DUPLAN, Division of Radiation Hazards, National Institute of Radiological Sciences, Chiba, Japan* and Unite INSERM-117, Fondation Bergonie, Bordeaux, France.

Two syngeneic strains of mice (AKR and AKR/TlAld) bearing a chromosome marker have been used to study the competitive proliferation of hemopoietic cells of various origins in radiation-chimeras. In the first series, competitive proliferation between normal and irradiated bone marrow was studied. The bone marrow CFUs surviving a dose of 200 R have an apparent proliferative advantage over the normal CFUs, but this high rate of proliferation resumes 10 days after transplantation. When normal bone marrow and spleen cells were injected simultaneously, cells of splenic origin have a definitely lower rate of proliferation than bone marrow-derived cells. This proliferative disadvantage lasts as long as 50 days. During the first 3 weeks after transplantation, the spleen stem cells do not participate in the repopulation of the thymus and it takes more than 30 days before they produce a significant amount of thymic precursors.

A-23-7 *Effects of Neutron or Gamma Dose Fractionation on the Hematological Response of Mice.* MARIETTA MILLER, EUGENIA M. COOKE AND E. JOHN AINSWORTH, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439, USA.

Hematological responses were measured in male $B6CF_1$ mice for 3–6 months after single or fractionated exposures to fission neutrons from the JANUS reactor or to 60-Cobalt gamma radiation. To evaluate the effects of fractionation, the same total radiation doses of 288 neutron or 740 gamma rad were administered either as a single dose or in 9 doses over a period of 3 weeks. A marked sparing effect of dose fractionation, based on depression of lymphocyte or platelet counts, was only observed during the first 15 days after either neutron or gamma irradiation; a sparing effect of fractionation was not apparent at most later sample times. Dose fractionation with either neutron or gamma radiation did not produce an overall acceleration of cell repopulation in the peripheral blood. Thus, during the first 2–3 weeks after irradiation, the neutron RBE is greater than 2.6, due to a comparatively greater sparing effect of the fractionated gamma radiation, but during the later phase of hematopoietic recovery, no marked departure from an RBE of 2.6 was consistently observed. (Work supported by the U.S. Atomic Energy Commission.)

A-24-1 *Relationship of Stem Cell Pool Size to Onset of Differentiation.* SALLIE S. BOGGS AND DANE R. BOGGS, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261, USA.

In previous studies of growth and differentiation of endogenous hematopoietic spleen colonies (E-CFU), the time of resumption of erythropoiesis following irradiation was directly proportional to irradiation dose. This suggested that differentiation might not occur until a critical stem cell pool size was attained. To further study this question mice were injected with endotoxin or foreign plasma before irradiation, substances known to increase the E-CFU compartment. Groups were killed on days 3 through 10 following 600, 700, 800 or 900 rads and spleen colonies, ^{59}Fe uptake and weight determined. Iron uptake resumed more rapidly in treated than in control groups; for example after 600 rads plasma treated groups resumed iron uptake 3 days before controls. Time of onset was delayed an average of 1.5 days for each increment of 100 rads for both treated and control groups. These data are in accord with the concept that onset of differentiation is geared to stem cell pool size.

A-24-2 *The Simultaneous Determination of in Vivo Radiation Survival Characteristics for Two Clonogenic Populations of Bone Marrow Cells Using Methylcellulose Culture Systems.* FLOYD D. WILSON, MARVIN GOLDMAN, SUSAN L. MUNN, CAROLINE J. MCNEILL, AND LOIS O'GRADY, Radiobiology Laboratory, School of Veterinary Medicine, and the Department of Internal Medicine, School of Medicine, University of California, Davis, California 95616, USA.

Using methylcellulose supported bone marrow culture systems, we have observed the forma-

tion of surface-related (adherent) fibroblastic plaque formation in addition to classical hematopoietic colonies.

Preliminary studies indicated that the methylcellulose culture system allows for the simultaneous quantitation of at least two populations of bone marrow-derived stem cells—the classical radiosensitive hematopoietic stem cell (colony forming units in culture, CFU-C) and a relatively radioresistant stem cell with different *in vitro* morphology and growth characteristics, perhaps related to precursors of marrow stromal elements (plaque forming units in culture, PFU-C).

Preliminary data also indicate that plaques serve as a source of colony stimulating activity (CSA). These findings suggest a possible relationship between *in vitro* PFU-C-derived plaques and *in vivo*, radioresistant, CSA-producing "stromal" cell populations described by Chan and Metcalf.

In this report, we present methodology and results for the simultaneous determination of radiation survival characteristics for these two populations in mice in situations of acute, whole-body x-irradiation.

A-24-3 *Extracorporeal Irradiation of the Blood: A Model for Studies on Cellular Kinetics of Leukemia.* A. HAGENBEEK, A. C. M. MARTENS, AND D. W. VAN BEKKUM, Radiobiological Institute TNO, Rijswijk (Z. H.), The Netherlands.

Although extracorporeal irradiation of the blood (ECIB) has been applied to patients with leukemia, relatively few data on the cellular kinetics of leukemia in conjunction with ECIB have become available. In particular, the changes in the intercompartimental exchange of leukemic cells need further investigation.

We have developed an animal leukemia model where changes in leukemia cell kinetics can be investigated more extensively. Because of the technical feasibility of an extracorporeal blood circuit and the availability of reproducibly growing transplantable leukemias, rats were chosen as experimental animals. A procedure for ECIB in the rat which permits repeated treatment sessions during a period of 7 days will be presented. Using conventional x-rays, a transit dose to the blood ranging from 400 to 900 rad was calculated.

As a leukemia, we selected a slowly growing myeloid leukemia, which is serially transplantable in BN rats. This leukemia shows an increasing leukocytosis during a period of 14 days before death, thus allowing a suitably long period for ECIB studies. In this model, repeated sessions of ECIB retard the development of the leukemia and, therefore, significantly increase the survival time. Changes in pool sizes of leukemic cells and exchange of cells between the tissue pools and the blood as calculated by the blood disappearance and tissue distribution of infused 51-chromium labeled leukemic cells, will be discussed.

An additional advantage of this rat model is that the number of hemopoietic stem cells (HSC) can be quantitatively measured *in vivo* (spleen colony assay) as well as *in vitro* (CFU-C). Preliminary results on the effects of ECIB on the distribution of HSC will be presented.

A-24-4 *A Radioresistant Thymic Stem Cell Population in the Adult Mouse.* J. G. SHARP AND D. B. THOMAS, UNMC, Omaha, Nebraska 68105, USA, and University of St. Andrews, Great Britain.

Following the administration of a lethal wholebody dose of x-irradiation to mice there is a phase of regeneration in the thymus, maximal on the 10th day, which occurs in the absence of regeneration elsewhere in the lymphomyeloid complex. This thymic regeneration is observed in either sex of several strains of mice at various ages and suggests that the adult mouse possesses a radioresistant thymic stem cell population similar to that described by Kubai & Auerbach (Proc. Soc. Exptl. Biol. Med. **142**, 554, 1973) in embryonic mouse thymus. In subsequent experiments the thymus was exposed to local x-irradiation in addition to the wholebody dose to test if the stem cell population effecting regeneration originated within the thymus. In albino mice, local thymic irradiation did not alter the subsequent pattern of regeneration which, therefore, appears to be effected by a cell population of extra-thymic origin. In CBA mice the extent of thymic regeneration decreased with increasing doses of local x-irradiation but was not completely abolished until the total dose to the thymus exceeded 1300 rads. Either the stem cell population is not exclusively of extra-thymic origin or, alternatively, the highest doses of local

x-irradiation alter the thymic environment sufficiently to impair the intra-thymic proliferative activity of a stem cell population of extrathymic origin. The proliferative activity of this thymic stem cell population is demonstrated by an eleven fold increase in the tritium content of unit weight of thymus, from the 3rd to 5th days post-irradiation. At the same time, a striking increase in the number of heavily labelled cells is observed in autoradiographs of thymic sections. Autoradiographs of smears of thymic cell suspensions prepared during this period have been examined in an attempt to define the morphological characteristics of this stem cell population. Preliminary results indicate that these cells are mononuclear cells morphologically similar to hematopoietic stem cells. (Supported by MRC.)

A-24-5 *Histopathologic Appearance of Irradiated Bone Marrow Following Single and Fractional Dose Exposures.* NABILA ELBADAWI, PHILIP RUBIN, AND R. A. E. THOMSON, University of Rochester, Medical Center Rochester, New York, 14642, USA.

Using ^{99m}Tc-S colloid and ^{59}Fe uptake as a measure of the functional activity of regenerating bone marrow, a parallelism in the activity of erythron and R-E compartments after segmental bone marrow irradiation occurring as early as 1–12 months was found.

This study aims at histopathologic examination of bone marrow from the same above experiment. Thirty female rabbits were subjected to single dose exposures of 1000–5000 rads. Fractional dose exposures: 120 animals were given the following schedule, 1000 rads weekly with total exposure range of 2000–5000 rads. The findings confirm that the bone marrow tolerance is higher for fractional irradiation than for single dose exposures to the same levels. Destruction of microcirculation and fibrosis was seen in single dose exposures and was lacking or minimal at comparable levels of fractional irradiation. (Regeneration starts at a period ranging from 3–9 months, is dose dependent and involves equally the myeloid and erythroid compartments. The regeneration pattern is diffuse, extends along the endosteum and epiphyseal plates, the central marrow cavity regenerating last. Regenerating bone marrow extends through Haversian canals in the dense compact cortex.) Based on these findings, it is suggested that the regeneration occurs locally in the Haversian canals inside the compact bone cortex, possibly by heterotopic transformation or perhaps maturation of undifferentiated cells located in the Haversian canals to myeloid and erythroid elements and subsequent migration into the bone marrow cavity. This may explain the discrepency in the observation of radioisotopic detection of regeneration at an earlier onset, due to regenerating bone marrow nests located in the cortex.

A-24-6 *Changes in the Number of Circulating Lymphocytes with Chromosome Aberrations Following a Single Exposure of the Pelvis to γ-irradiation in Cancer Patients.* H. TAMURA, Y. SUGIYAMA, AND T. SUGAHARA,* Kyoto National Hospital, and Kyoto University,* Kyoto, Japan.

It has been reported that the recirculation of lymphocytes between peripheral blood and extravascular spaces is suggested by following cells with chromosome aberrations after extracorporeal irradiation. In the present study the number of lymphocytes with chromosome aberrations in the peripheral blood was followed up to 48 hours after single exposure of the pelvis to ^{60}Co γ-irradiations to find out the similarity between extracorporeal irradiation and partial body irradiation. Contrary to expectation, the percentage of cells with aberrations increased to reach a peak six hours after irradiations, then decreased during next 42 hours. These findings may have important implications in lymphocyte kinetics and in biological dosimetry.

A-24-7 *Radiation Sensitivity of Lymphocytes in Health and Chronic Lymphocytic Leukemia (CLL).* S. VAUGHAN SMITH AND A. E. R. THOMSON, Department of Cytochemistry Imperial Cancer Research Fund Laboratories, London, Great Britain.

Considerable knowledge has recently accumulated on the existence in health of functionally different sub-populations of non-dividing small lymphocytes. In CLL, there exist additional abnormal sub-populations of these cells, one of which is ultrasensitive *in vitro* to cytocidal action by colchicine.[1] Colchicine ultrasensitive lymphocytes commonly constitute 80% of the total circulating population in CLL[2] and are potentially separable from the remaining (colchicine-resistant) cells.

Measurements of death of peripheral blood lymphocytes in cultures, lasting up to 6 days

after irradiation, have revealed difference in radiosensitivity between the colchicine ultrasensitive abnormal sub-populations in CLL and lymphocytes from healthy people (NHL) and also between the abnormal sub-populations from different patients. Heterogeneity of radiosensitivity has been detected within the sub-populations from different patients and within NHL. The heterogeneity of radiation response for NHL has been partly resolved in subsequent preliminary experiments on partially purified sub-populations. All radiosensitivity data could be reproduced with cryopreserved lymphocytes.

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[1] A. E. R. Thomson, T. W. E. O'Connor, and G. Wetherley-Mein, Scand. J. Haemat 9, 231 (1972).

[2] A. E. R. Thomson, T. W. E. O'Connor, and G. Wetherley-Mein in "8th Leucocyte Culture Conference," Academic Press (in press).

A-25-1 *Use of the Semiconductor Devices in Dosimetry of High-Energy Electrons.* F. SCARLAT AND N. BĂLȚĂȚEANU, Institute for Atomic Physics, Bucharest, P.O. Box 35, Romania.

A study is made of the possibilities of using semiconductor devices for the determination of the absorbed dose in biological media irradiated with high-energy electron beams. The study was carried out on a large number of semiconductor electronic devices (diodes and transistors) irradiated with electrons beams generated by the 30 MeV IAP betatron and the 3 MeV IAP linear electron accelerator. Several types of semiconductor devices were found to have a linear variation of a basic parameter versus dose in a given range of absorbed doses and energies. The results obtained so far show that it is advantageous to measure the absorbed dose with semiconductor devices and the application of the method leads to an accuracy of absorbed dose measurements of the order of 5%.

A-25-2 *Automated Determination of Depth-Dose Distribution from Diagnostic X-Ray Beams.* S. JULIAN GIBBS AND ROBERT J. KING, Vanderbilt University, Nashville, Tennessee 37232, USA.

This progress report describes the modification of an automated system for determining depth-dose distribution in radiotherapy treatment planning for use with diagnostic-energy beams. The basic system consists of a computer-operated scanner for determining dose distribution in a homogeneous fluid phantom, and applying it to any cross-section of human anatomy (input through a graphical analog data input device), to generate, display, and plot isodose curves corrected for oblique and curved-surface entry, with the ability to sum multiple beams and to utilize moving beams. It has been modified to include two-channel dosimetry (moving and stationary reference tissue-equivalent ionization chambers to correct for fluctuations in beam intensity), use with diagnostic x-ray machines in multiple short exposures, and corrections for internal anatomical inhomogeneities (hard tissues, air-filled cavities, etc., also input through the graphical device), using modified Monte Carlo calculations. Calculated distributions with geometrically regular inhomogeneities are compared with those measured in the fluid phantom containing the same inhomogeneities, providing for rational step-wise development of the analytical correction system. Initial applications are to dental radiology, in which widely divergent techniques of exposure geometry, beam energy, etc., are in current use. Data generated from the system should provide a rational basis for selection of optimum techniques in terms of patient hazard. The system is capable of handling virtually any medical x-ray beam or procedure, without the requirement for laborious internal dosimetry in a human cadaver or tissue-equivalent phantom for each x-ray beam or technique studied. It will further provide the same corrections for more accurate isodose contours for radiotherapy treatment planning.

A-25-3 *A Small Ionization Chamber for Measurement of Neutron and Photon Dose.* JOHN J. SPOKAS, Illinois Benedictine College, Lisle, Illinois 60532, USA, AND FRANK S. WILLIAMSON AND GORDON L. HOLMBLAD, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439, USA.

A small thimble-type three-terminal chamber of ionizing volume equal to 0.5 cm^3 has been developed for accurately determining depth doses in human muscle. The chamber is homogeneous in that the entire sensitive portion of the chamber is constructed of Shonka tissue-equivalent

(muscle) conducting plastic and has provision for circulating a matching tissue-equivalent gas continuously through it. Precise definition of the sensitive volume is achieved by means of a buffer region. Leakage currents are of the order of 10^{-16} Amperes and voltage soakage phenomena are virtually nonexistent. The angular dependence as well as the energy dependence will be discussed experimentally and theoretically. The measured calibration factor is 5 rad/nanoCoulomb for Cesium-137. (Work supported by the U.S. Atomic Energy Commission.)

A-25-4 *Discrimination Against Neutron-Induced Events in a Gas-Filled Gamma-Ray Dosimeter.* THOMAS J. YULE AND EDGAR F.BENNETT, Argonne National Laboratory, Argonne, Illinois 60439, USA.

One is often required to measure gamma-ray doses in mixed fast-neutron and gamma-ray fields. While various gamma-ray dosimeters with low neutron sensitivity exist, their sensitivity is still such as to introduce sizeable uncertainties. An alternative approach is to use a device in which neutron-induced events are discriminated against. We have investigated the operation of a gas-filled chamber operated in the proportional mode in which pulse rise time is used for the discrimination. Pulse-shape rejection is based upon the large difference in the number of ion pairs per unit track length between tracks from heavy-ion recoils and from fast electrons. A prototype counter was constructed with steel walls and filled with an A-CO_2 mixture. The relation between energy and ionization was determined by observing the mono-energetic protons from the $^{14}N(n,p)^{14}C$ reaction with thermal neutrons and electrons from the decay of ^{37}A, when small quantities of these gases were added to the mixture. Measurements were made in a ^{60}Co gamma-ray field and in a mixed environment. Electron energy-loss distributions were obtained, which can be used to determine dose rates or be converted to LET distributions. (Work performed under the auspices of the United States Atomic Energy Commission.)

A-25-5 *Estimation of Effective Gamma Energy by Differential Responses of TLD-100 and TLD-200 Dosimeters.* MICHAEL H. MOMENI, MARVIN GOLDMAN, TOM COUNTIS, AND LOU WORDEN, Radiobiology Laboratory, University of California, Davis, California 95616 USA.

The differential energy dependence of TLD-100 and TLD-200 to gamma rays was utilized to measure the effective beam energy distribution and radiation buildup in an indoor/outdoor ^{60}Co field. Thermoluminescent dosimeters TLD-100 (LiF) and TLD-200 (CaF_2, Dy) showed, respectively, responses of about 1.4 and 16 times greater to 32 keV than to 1.25 MeV photon exposures. Beam intensity profiles corrected for energy dependence of the thermoluminescent dosimeters showed good agreement with the intensity profile measured by Victoreen chambers. The dose rate buildup factor for oblique incidence to the ground was unity or less, indicating that beam transmission exceeded scattering at the air-ground interface.

A-25-6 *Verification of the Cavity Principle in High Energy Electron Dosimetry by Means of a Double Extrapolation Graphite Chamber.* B. MARKUS, Abt. Strahlenphysik u. Strahlenbiologie der Radiol. Klinik und Betatron-Abt. der Hautklinik d. Universität, 34 Göttingen, Germany.

The application of the Bragg-Gray principle to high energy electron dosimetry and the concept of the used ionization chamber are described. The cylindrical cavity is variable in 3 dimensions and may be extrapolated to zero with high precision. Thus the chamber serves as a parallel plate chamber down to 0.01 mm plate distance, and with removable insertions allowing for side wall effects down to 3 mm diameter. Polaritiy effects are suppressed by construction. The degree of particle flux perturbation by geometry is measured. True particle equilibrium is given up to a depth still before dose maximum, followed by increasing deviation with depth, however a transient equilibrium is well defined along depth dose curve at least for 5 to 15 MeV.

A-25-7 *Investigations on the Behavior of the Fe^{++}-Cu^{++} Dosimeter.* ERLING BJERGBAKKE AND KNUD SEHESTED, Danish AEC Research Establishment, Risø, DK-4000 Roskilde, Denmark.

The Fe^{++}-Cu^{++} dosimeter is an aqueous chemical dosimeter usable in the dose range 50–1000 krad. The composition of the dosimeter is suggested by previous workers to be 10^{-3} *M* Fe^{++}, 10^{-2} *M* Cu^{++}, pH 2.1 H_2SO_4, air-saturated.

This work shows that the response [$G(Fe^{+++})$] is highly influenced by the solute concentrations especially O_2. A reaction sceme that can explain all known experimental results has been developed. The rate constants for the reactions $Cu^+ + O_2$, $Cu^+ + H_2O_2$ and $Cu^+ + Fe^{+++}$ have been established. ^{60}Co-gamma rays and 10 MeV electrons were used for irradiations.

The reactions during irradiation can be described by a set of approx. 20 differential equations. A computer program has been developed for solving these equations. (This work was partly done under the IAEA research contract no. 1173/R1/RB.)

A-25-8 *Saturation Characteristics of Ionization Chambers in the Pressure Range of a Few Microns to a Few mm of Mercury.* M. N. VARMA AND J. W. BAUM, Health Physics & Safety Div. Brookhaven National Laboratory, Upton, New York 11973, USA.

Ionization current vs voltage curves were studied at various pressures in a large ionization chamber and in a small, mesh wall ionization chamber located within the large chamber. Results indicate that saturation in the internal probe depends on (a) competition between collection in the probe and in the large ionization chamber, (b) recombination at very low voltages, (c) gas amplification at higher voltages and (d) probe position. Saturation in the large chamber depends on recombination at low voltages and on gas amplification at higher voltages. For both the probe and the large ionization chamber, collection of positive ions proved best; however, saturation voltages had to be chosen carefully at each pressure in order to achieve optimum balance of the above mentioned effects.

A-26-1 *Bone Tumor Risk in Mice after Single and Repeated Injections of ^{224}Ra.* WOLFGANG GÖSSNER, ARNE LUZ, WALTER A. MÜLLER, AND OTTO HUG, Institut für Biologie, Gesellschaft für Strahlen- und Umweltforschung, D-8042 Neuherberg, West Germany.

In previous experiments we have shown that single injections of ^{224}Ra between 2.5 and 50 μCi/kg result in an osteosarcoma incidence of about 10 to 20%.

We now report the results of experiments with female NMRI mice 4 weeks of age which received i.p. injections of ^{224}Ra twice weekly with different total activities and protraction times.

12 μCi/kg total activity over a period of 1 month showed an osteosarcoma incidence of 39%; 36 μCi/kg total activity over a period of one month showed no effect on tumor incidence, but earlier occurrence of the tumors. After protraction of 36 μCi/kg over 3 months the tumor incidence is about threefold higher (62%) as compared to a single injection of the same total activity. Further protraction over 9 months results in the highest increase of tumor risk.

The tumor incidence also increases with extension of the 36 μCi/kg experiment from 3 months to 6 months and a total of 72 μCi/kg. Ossifying fibromas and osteosarcomas of the head behave differently, i.e., they do not show this increased risk after dose protraction. (Work performed in association with EURATOM.)

A-26-2 *Dosimetric Aspects after Repeated Injections of Short-Lived Alpha-Emitters in Mice with Regard to the Risk of Late Effects.* WALTER A. MÜLLER, WOLFGANG GÖSSNER, OTTO HUG, UTZ LINZNER AND ARNE LUZ, Gesellschaft für Strahlen-und Umweltforschung, Institut für Biologie, D-8042 Neuherberg, West Germany.

Several protracted injection series were performed with young female NMRI mice. Injections of ^{224}Ra plus daughter products were basically given in intervalls of 3.5 days. The total protraction time varied between 4 and 36 weeks. The activities applied lay between 0.5 and 5 μCi ^{224}Ra per kilogram body weight for the individual injection. The total mean skeletal doses reached 360–2200 rad. Data for skeletal doses and dose rates are given and are compared with those of one time injections of ^{224}Ra and of other longer-lived alpha-emitters (^{226}Ra, ^{227}Th).

Some theoretical and practical consequences of protracted doses for bone tumor induction are discussed using our own data as well as of those in the literature. (Worked performed in association with EURATOM.)

A-26-3 *^{239}Pu in Human Bone: An Autoradiographic Study.* ROBERT A. SCHLENKER AND BILLIE G. OLTMAN, Argonne National Laboratory, Argonne, Illinois 60439, USA.

Bone samples were taken from the remains of a person who received a known amount of ^{239}Pu

in citrate solution 18 months before death, in 1947, from Cushing's Syndrome. Autoradiographs are being prepared using the technique of neutron-induced fission autoradiography.

Measurements are being made to characterize the distribution of ^{239}Pu in this bone. These include measurements of surface concentrations, surface dose rates and percentage of surface overlying volume deposits of ^{239}Pu. Estimates of the ratio of surface to uniform dose rates and the rate of volumization of ^{239}Pu are being made for use in Marshall's model of ^{239}Pu/^{226}Ra RBE.

Preliminary results indicate that a substantial redistribution of the ^{239}Pu from bone surface to bone volume has taken place at the endosteal surfaces of the rib and in rib trabeculae.

Quantitative results of measurements on sections from rib, vertebrae, and femur will be presented. (Work supported by U.S. Atomic Energy Commission.)

A-26-4 *Kinetic Model for Skeletal Retention of ^{239}Pu.* DAVID R. ATHERTON, BETSY J. STOVER, AND WALTER STEVENS, Department of Anatomy, Division of Radiobiology, Salt Lake City, Utah 84132, USA.

The initial deposition of ^{239}Pu and the rate of decrease in retention are both higher in bones which have a relatively greater amount of trabecular bone. For this reason the ulna, which has relatively less trabecular bone, was chosen for an extensive comparison with the humerus and third lumbar vertebra. These three bones were obtained from 40 beagles injected as young adults, at dose levels from 0.00064 to 0.095 μCi ^{239}Pu/kg (P0.1 to P2) and at times from 35 to 4549 days after injection. Analyses showed that the initial concentration of ^{239}Pu in the ulna was about 25% of that in the other two bones. Retention of ^{239}Pu in the ulna was approximately constant over the 12.5 year period. In contrast, retention in both the humerus and third lumbar vertebra decreased during the first several years and then approached approximately constant values. A kinetic model for the observed retention has been formulated. [Supported by USAEC Contract AT(11-1)-119.]

A-26-5 *Quantitative Measurement of Changes in ^{226}Ra- and ^{90}Sr-^{90}Y-Labeled Humeri: A Comparative Study.* MICHAEL H. MOMENI, JEAN R. WILLIAMS, L. S. ROSENBLATT, AND M. GOLDMAN, Radiobiology Laboratory, University of California, Davis, California 95616, USA.

Relative bone changes of ^{226}Ra- or ^{90}Sr-^{90}Y-labeled Beagle humeri were radiographically measured to aid in correlation of dose-effect relationships. Bone damage was recorded by an index of injury from 0 (normal) to 6 (bone tumor). Mean index of injury increased with dose and age for both ^{226}Ra- and ^{90}Sr-labeled bones. For equal average radiation doses Ra-226 was shown to have about 3 times the mean index of injury as that for Sr-90. The dose distribution in humeri of Beagles given 8 semimonthly injections of ^{226}Ra were measured in 6 cross-sectioned regions of each bone by α-track counting. The nonuniformity in ^{226}Ra concentration in humeri ranged up to 78% of the mean measured concentration; whereas, the concentration of ^{90}Sr-^{90}Y was relatively uniform in the humeri of Beagles which continuously ingested the radionuclide during their first 1½ years of life.

Tumor induction at the head and the condyle of the humerus, showing the highest regional index of injury, may be due to high uptake of ^{226}Ra in regions of high bone vascularity. Comparison of radium distribution in humeri of Beagles administered radium beginning at 120 or 435 days of age showed a higher uniformity of label and structural damage in the animals injected at the earlier age. In Beagles injected at the older age, the radium was distributed as hot spots which were found primarily on the periosteal and endosteal bone surfaces.

A-26-6 *Binding of Actinide Elements by Bone Protein.* F. W. BRUENGER, B. GRUBE, E. BRUENGER, AND W. STEVENS, Department of Anatomy, Division of Radiobiology, University of Utah, Salt Lake City, Utah 84132, USA.

After administration of actinide elements, an appreciable fraction of the nuclide is incorporated into the skeleton. Knowledge of the molecular associations formed by actinide elements with bone proteins is important for future decorporation therapy. Ground bones from beagles injected with ^{241}Am or ^{243}Cm were extracted with EDTA, Gu(guanidinium)-Cl or KCl solutions. Protein fractions were subjected to differential dialysis and precipitation procedures. Proteins extracted with KCl or Gu-Cl contained ^{241}Am and ^{243}Cm. Binding of ^{241}Am was confirmed by *in vitro* tagging and chromatography. The proteins were characterized by the presence of about 30% amino sugars and other carbohydrates and an abundance of glutamic and aspartic acid. *In vitro* tagged polyaspartic

and polyglutamic acid showed only relatively low binding constants for ^{241}Am. Proteins which bound ^{241}Am or ^{243}Cm had a minimum M.W. of $>5 \times 10^5$. No activity was found with proteins of M.W. < 30,000. These proteins may be identical with americium binding proteins which appear in canine fetuses at the onset of ossification. (Research supported by the U.S. Atomic Energy Commission).

A-27-1 *Determination of the Redox Potential of Free Radicals Using the Technique of Pulse Radiolysis.* P. S. RAO* AND E. HAYON, Pioneering Research Laboratory, U.S. Army Natick Laboratories, Natick, MA 01760, USA.

The technique of pulse radiolysis and kinetic absorption spectrophotometry was used to generate free radicals in aqueous solution and determine their redox potentials. The one-electron oxidation and reduction of free radicals (RH·), is dependent upon the redox properties and potentials of the acceptor (A) or donor (DH) present in solution. The efficiency of the reactions $RH\cdot + A \rightleftharpoons A\cdot^- + R + H^+$ and $RH\cdot + DH \rightleftharpoons RH_2 + D\cdot^-$ is dependent upon the redox potential E^{01} (V, at pH 7.0, 25°C) of A and DH. From the "titration" curves obtained as a function of the E^{01} of A and DH, and the Nernst equation, the E^{01} values for the oxidation and/or reduction of free radicals are derived. The redox potentials of organic, biochemical and inorganic free radicals can be determined by this new method.

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A-27-2 *A Study of Platinum(I) and (III) Complexes by Pulse Radiolysis and Flash Photolysis.* W. L. WALTZ, D. K. STORER, AND R. L. EAGER, Department of Chemistry and Chemical Engineering, and the Saskatchewan Accelerator Laboratory, University of Saskatchewan, Saskatoon, Saskatchewan S7N OWO, Canada.

The reactions of e_{aq}^-, H atom, OH, and Cl_2^- in aqueous media with a series of Pt(II) complexes containing amine, amino acid, and chloride ligands have been investigated by pulse radiolysis in order to elucidate the mechanisms of these oxidation-reduction reactions leading to unstable Pt(I) and Pt(III) products. The associated second-order rate constants, which range from 4×10^8 to 2×10^{10} M^{-1} sec^{-1}, approach those of diffusion-controlled limits, and consequently show only small variations in regard to the nature of the coordinated ligand and the overall electrostatic charge on the complex.

In contrast, the spectra and kinetic behavior of the Pt(III) species arising from the reactions of the strong oxidizing agents, OH and Cl_2^-, show marked differences. The products of the reaction of OH with the Pt(II) complexes all exhibit several intense absorption bands in the region of 300–550 nm whereas those resulting from Cl_2^- oxidation exhibit a single intense band at 270–290 nm. Generation and characterization of these species has also been carried out by flash photolysis of the corresponding Pt(IV) complexes and through the reactions of e_{aq}^- and isopropanol radical with the Pt(IV) complexes respectively. The nature of the Pt(III) transients and their decay mechanisms will be discussed.

A-27-3 *Radiation-Chemically Induced Oxidation and Reduction of $Ru(NH_3)_5CO^{2+}$ in Aqueous Solution.* Q. G. MULAZZANI, M. D. WARD, AND A. BRECCIA.* Laboratorio di Fotochimica e Radiazioni d'Alta Energia, C. N. R., Bologna, Italy.

In the course of a previous work[1] it was shown that $Ru(NH_3)_5N_2^{2+}$ reacts with the OH radical to give ultimately $Ru(NH_3)_5OH^{2+}$ and N_2 and it was suggested that the first stage of the reaction is an electron transfer between the metal and the radical.

We have studied the isoelectronic compound $Ru(NH_3)_5CO^{2+}$ and the results obtained, using gamma and pulse radiolysis techniques, can be summarized as follows:

a) the compound reacts either with e_{aq}^- and the OH radical and the rate constants for these reactions have been determined;
b) in the presence of N_2O or O_2 as electron scavengers, CO and $Ru(NH_3)_5OH^{2+}$ have not been detected after gamma irradiation: the main final products of the reaction between the complex and the OH radical are CO_2 and some unidentified Ru compound.

c) Transient spectra and rate constants for the decay of the intermediates have also been obtained. (This work was supported in part by N.A.T.O. research grant No. 636.)

* Facoltà di Farmacia, Università di Bologna, Italy.

[1] J. H. Baxendale and Q. G. Mulazzani, J. Inorg. Nucl. Chem. **33**, 823 (1971).

A-27-4 *Pulse Radiolysis of Lanthanide and Actinide Elements.* Sheffield Gordon, Klaus Schmidt, W. A. Mulac, and W. T. Carnall, Chemistry Division, Argonne National Laboratory, Argonne, Illinois 60439, USA.

Fundamental solution chemistry studies of the lanthanide and actinide elements have been of great importance in elucidating the role of 4f and 5f electrons in chemical reactions and in providing the basis for developing models for the interaction of these electrons with their chemical environments. We will present data involving the reduction and oxidation of these elements by the hydrated electron and OH radical, respectively, produced by our pulsed electron linac. Spectra of transient species produced have been observed using our recently developed single pulse streak camera technique which enables us to study these rare and radioactive actinides with a minimum amount of material and handling. Rate constants for the reactions of these transients with various oxidizing and reducing reactions will be presented and correlations with redox potentials will be made. (Work performed under the auspices of the U.S. Atomic Energy Commission.)

A-27-5 *Electron Transfer Reactions in Some Copper(II)-Peptide Complexes.* M. Faraggi, Nuclear Research Centre-Negev, Beer Sheva, Israel.

The pulse radiolysis method has been used to study the electron pathway in some Cu(II)-peptide complexes. The hydrated electron and the formate radical ion were used as electron donors. It has been found that the electron transfer reaction in these complexes is a multistep electron migration process. Using these results the reduction mechanism of copper and other proteins will be discussed.

A-27-6 *Determination of Hydrogen Ion Yield at Pulse Radiolysis of Aqueous Solutions of Potassium Chromate.* A. K. Pikaev, S. A. Kabakchi, and A. A. Zansokhova, Institute of Physical Chemistry of USSR. Academy of Sciences, Moscow, USSR.

The initial yield of H_3O^+ ions in irradiated water has been determined by pulse radiolysis method in the case of aqueous solutions of potassium chromate. It has been found that a decrease of optical density within the absorption band of CrO_4^{2-} and the appearance of a new band (λ_{max} = 350 nm) belonging to $Cr_2O_7^{2-}$ take place. After the pulse the new band disappears and the initial optical density of the solution is reestablished completely. This effect has been explained by the occurence of reactions: $CrO_4^- + H_3O^+ \rightleftarrows HCrO_4^- + H_2O$ (1) and $2HCrO_4^- \rightleftarrows Cr_2O_7^{2-} + H_2O$ (2). In the solutions saturated with N_2O $G(-CrO_4^-)$ is only due to the reactions (1) and (2). It permitted determination of $G(H_3O^+)$ to be equal to 4.15 $\pm$ 0.15 ions/100 eV. On the basis of the results obtained the distance between the center of a "spur" and the place of e_{aq}^- localization has been evaluated (ca. 55 Å). The influence of Cl^- ions, which are possible scavengers of H_2O^+, on $G(H_3O^+)$ has been also studied.

A-28-1 *Optical Properties of Solutes Formed by Radiolysis of Liquid Ammonia Solutions.* Jacqueline D. Belloni and Marie-Odile Delcourt, Laboratorie de physico-chimie des Rayonnements, 91405 Orsay, France, and Eichi Saito, CEN Saclay, B.P.n°2, Gif.s. Yvette, France.

To identify optical absorption spectra obtained from pulse radiolysis of liquid ammonia solutions, we investigated the absorption properties of solutes likely to be formed by irradiation. The evolution of the spectra of ammonia solutions at 20°C of $Cu(ClO_4)_2$, N_2H_4, or NaN_3 was followed with increasing γ dose. The optical cells, used for irradiation and spectroscopic measurements, were entirely made of silica with thick suprasil parallel windows. They were capable of withstanding internal pressures up to 20 bars and allowed direct observation of species present in liquid ammonia, thus avoiding possible secondary reactions occuring upon the usual transfer into aqueous phase for analytical purposes.

A-28-2 *Reactions of the Hydroperoxy Radical $HO_2\cdot$ and Its Anion $O_2^{-}\cdot$.* HARRY C. SUTTON, Institute of Nuclear Sciences, Department of Scientific and Industrial Research, Lower Hutt, New Zealand.

The electron beam irradiation of oxygen saturated aqueous solutions of formic acid or sodium formate in a flow system provides a convenient source of $HO_2\cdot$ or $O_2^{-}\cdot$ radicals, free of $OH\cdot$, which may be mixed a few milliseconds after their formation with selected reagents. Such experiments have shown that electron transfer reactions of $O_2^{-}\cdot$ with Br_2 and Br_3^- proceed at nearly diffusion controlled rates whilst the corresponding reactions of $HO_2\cdot$ are much slower. Iodide ions react with $HO_2\cdot$ in acid ($pH < 4$) but not neutral solution.

The reaction of $HO_2\cdot$ with tetranitromethane (TNM) is complex in acid solution. Experiments will be discussed which show that nitroform is produced from this reaction in two stages: (a) a fast reaction of the $O_2^{-}\cdot$ in equilibrium with $HO_2\cdot$ which produces nitroform and NO_2, followed by (b) a slow reduction of TNM occurring over several seconds by a species, possibly pernitric acid (HNO_4), which is formed by a rapid reaction of $HO_2\cdot$ with NO_2. At low TNM concentrations the reaction of $HO_2\cdot$ with NO_2 is nearly quantitative for the NO_2 released in (a).

A-28-3 *Radiation Chemistry of Aqueous NH_4NO_2.* W. CARL GOTTSCHALL JR., University of Denver, Colorado 80210, USA.

Considerable effort has been expended investigating the radiation chemistry of aqueous nitrate systems yet little work has been published involving aqueous nitrites. In addition to the obvious importance in back reactions of nitrate systems it was felt additionally that these systems were interesting to inorganic radiation chemists *per se* and might help elucidate dissociation and energy deposition phenomena. Because of the simple stoichiometry: $NH_4NO_2 \rightarrow N_2 + 2H_2O$ a radiolytic investigation of ammonium nitrite solutions exposed to cobalt 60 gamma radiation was undertaken. Nitrogen gas produced by the irradiations was determined and the influence of pH, initial ammonium nitrite concentration and the presence of other ionic or surface-active species indicated. The nitrate, nitrite, hydrogen ion, and ammonia-ammonium concentrations were also monitored as a function of dose and the mechanistic details consistent with the analytical results will be presented. The competition between simple decomposition and oxidation-reduction processes observed will also be discussed.

A-28-4 *High Dose-Rate Radiolysis of Aqueous Chloroacetic Acid Solutions.* PAUL Y. FENG, Marquette University, Milwaukee, Wisconsin 53233, USA, and National Tsinghua University, Hsinchu, Taiwan, China.

Radiolysis of aqueous solutions of chloroacetic acid at very high dose rates, and hence conditions conducive to the recombination of solvated electrons and other radiation-produced intermediates, has been carried out using the pulsed electron beams from the linear accelerators at the U.S. Army Natick Laboratories and the Argonne National Laboratory. The following experimental parameters were studied: dose rate (2×10^9 to 1.5×10^{11} rads/second), pulse length (0.5 to 6 microseconds), solute concentration (2×10^{-3} to 10^{-2} molar), and additives (N_2O and O_2). It was found that $G(Cl^-)$ decreased with increasing dose rate and decreasing solute concentration, but was essentially unaffected by the length of the radiation pulse. Under otherwise comparable conditions, e.g., irradiations using 6 microsecond pulses at the highest dose rates, $G(Cl^-)$ was 1.3, 0.9 and 0.6 respectively for aerated, deaerated, and N_2O-saturated solutions 10^{-2} molar in chloroacetic acid.

Kinetic analysis of the experimental data, coupled with available knowledge on the radiation chemistry of chloroacetic acid solutions at low dose rates, showed that satisfactory correlations can be obtained on the basis of enhanced interactions of the various radiation-produced intermediates for both the deaerated and the N_2O saturated solutions. A more complicated mechanism is required, however, for the oxygenated solutions, e.g., the participation of the peroxy radicals.

A-28-5 *Radiolysis of H_2O and D_2O at Temperatures up to 300°C.* KAMAL N. JHA AND GORDON R. FREEMAN, University of Alberta, Edmonton, Alberta, T6G 2G2, Canada.

The effect of temperature on the yield of reducing species is being investigated in the γ-radiolysis of H_2O and D_2O in the region 0° to 300°C. The fluoride ion yield from mM solutions of sulfur hexafluoride in D_2O is approximately 0.5 G units larger than that in H_2O at all temperatures.

Addition of cadmium chloride reduces the yield of fluoride ion while potassium bromide does not have significant effect in the concentration range 1 mM to 1 M. An attempt is being made to extend the study to the critical region.

A-28-6 *Investigation of Radiolysis of Hydrosulphide Ions in Aqueous Solutions.* GULNARA R. NATROSHVILI AND HELEN M. NANOBASHVILI, Institute of Inorganic Chemistry and Electrochemistry of the Georgian SSR Academy of Sciences, Tbilisi, 380093, USSR.

A radiation-chemical transformation of hydrosulphide-ions in dilute and concentrated aqueous solutions of sodium sulphide has been investigated. It has been ascertained that the stable products of radiolysis of disulphide, sulphite, thiosulphite are the secondary products of the transformation of S^- ion-radical. The yield of the radiation-chemical transformation of hydrosulphide-ions increases considerably with the growth of the concentration of primary products. The concentrated solutions were also investigated by the EPR method at the temperature of liquid nitrogen. It has been found that different paramagnetic centers: OH, e_{aq}^-, O^-, S^-, and SO_2^- are stabilized in the systems depending on the concentration of sodium sulphide an on the phase state. The mechanism of the influence of radiolytic products of water on the dilute sulphide has been considered.

A-29-1 *The Effect of Accelerated Oxygen Beams on Human Kidney Cells.* R. ROISMAN, D. KALOFONOS, BAMBINO MARTIN, JOHN LYMAN, AND C. A. TOBIAS, Donner Laboratory/Lawrence Berkeley Laboratory, Berkeley, CA. 94720, USA.

Oxygen nuclei accelerated to about 300 MeV per nucleon were used to assess the radiobiology of human kidney cells. A special tissue-culture device, the "submarine" was used for the exposures, which allowed us to construct depth-survival curves corresponding to the changing dose and beam quality along the Bragg ionization curve. The parameters of the survival curves and of the oxygen effect depend on particle flux density, LET and particle velocity. Some survival curves were obtained for mixed beams with a ridge filter. The relationship of these results to therapy and to models of radiation action will be discussed.

A-29-2 *Radiation Response of Mammalian Cells after Irradiation with a Beam of Negative Pi-Mesons.* ANDREW J. MILL AND JOHN D. LEWIS, St. Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ, Great Britain.

HeLa cell suspensions have been irradiated at various depths of perspex in the beam of 80 MeV negative pi-mesons obtained from the "Nimrod" synchrotron at the Rutherford High Energy Laboratory. The dose-rate at the Bragg peak varied between 35 and 70 rad h^{-1} depending on conditions. The peak to plateau ratio for ionization was 1.5:1. The cells were assayed, *in vitro*, for loss of reproductive integrity.

The survival curves obtained showed there to be little difference in the relative biological effectiveness (r.b.e.) for pions in the plateau and peak regions. When compared to irradiation with ^{60}Co gamma rays at 50 rad h^{-1} the r.b.e. for both depths was $\sim$2.5.

When the radiation was delivered as two doses, with a variable time interval between exposures, it was found that cells, irradiated in the peak and plateau positions, showed a significant increase in survival compared to the equivalent single dose survival. However, cells irradiated in a post-peak position showed no such increase in survival for radiation-free periods up to 10 hours.

The project was supported by a grant from the Cancer Research Campaign. The co-operation of the staff of the Rutherford High Energy Laboratory is greatly appreciated.

A-29-3 *The Relative Biological Efficiency (RBE) of α-Particles for Single- and Double-Strand DNA Breaks in Mammalian CHO Cells.* FRANCIS J. SHONKA AND ARTHUR COLE, The Univ. of Texas System Cancer Center, M. D. Anderson Hosp. & Tumor Inst., Houston, Tex. 77025, USA.

An alpha particle cell irradiator was designed and constructed by the authors using Americium-241 as a source of high LET ionizing radiation. Chinese hamster ovary cells were irradiated with radiation doses of from 50 to 85 KR for double strand analysis and doses of 2 to 10 KR for single strand DNA breakage analysis. The analysis of double strand breaks involved constant velocity centrifugation in 5 M salt-sucrose gradients with detergent cell lysis. Single strand work used 1 M

salt, alkaline sucrose gradients with alkaline lysing conditions. Alpha particle breakage efficiency was compared to damage caused by Cesium gamma irradiations using similar techniques for cell handling. The RBE for double strand breaks appears to be about 2.0. The RBE for single strand breaks appears to be 0.25. All irradiations were performed in air.

A-29-4 *Comparison of Skin Responses of Mice after 230 kV X-Irradiation with Responses after Irradiation with Cyclotron-Accelerated Helium and Oxygen Ions.* JOHN T. LEITH, W. A. SCHILLING, J. T. LYMAN, J. HOWARD, C. A. TOBIAS, AND D. G. BAKER, Lawrence Berkeley Laboratory, Berkeley, California 94720 and Claire Zellerbach Saroni Tumor Institute, Mount Zion Hospital, San Francisco, California 94115, USA.

Mouse legs were irradiated with cyclotron-accelerated helium ions at the LBL 184-inch cyclotron in either the plateau or modified Bragg peak regions of ionization. Responses after single exposures to helium ions were compared to responses produced after 230 kV X-irradiation. The plateau portion of the helium ion Bragg curve produced skin responses not significantly different from X-rays, both in magnitude and in terms of the temporal development of reactions. Modified Bragg peak helium ions showed an RBE of about 1.3 as compared to X-rays, with the RBE decreasing with increasing helium ion dose. A recovery experiment using equally sized doses of either X-rays or helium ions in the modified Bragg peak, given in two fractions separated by 24 hours was also done, and it was found that the recovery after modified Bragg peak helium ion irradiation was similar to the recovery found between two fractions of X-rays. Evaluation of late skin reactions (95–105 days postirradiation) indicated that the late RBE of modified Bragg peak helium ions was 1–1.2 and the RBE of plateau helium ions was 1.0, for single doses. Comparison of split doses of modified Bragg peak helium ions to split doses of X-rays showed no significant difference. Irradiations of mouse legs with single doses of modified Bragg peak oxygen ions at the LBL Bevatron showed an RBE increasing from 1.6 to 2.4 over a dose interval of 750 to 2000 rads. (Research supported jointly by the United States Atomic Energy Commission by the National Aeronautics and Space Administration.)

A-29-5 *Differential Analysis of Inactivation of Yeast Cells by X-Rays, ^4He and ^{16}O Ions.* HANS BLATTMANN, Radiobiological Institute, University of Zurich, P.O. Box, CH-8029 Zürich, Switzerland.

The survival of haploid yeast (S. pombe) was investigated with x-rays, α-particles and heavy ions (1 MeV/amu). Cells were irradiated in stationary phase as monocellular layers on membrane filters. Criterion for survival was the division probability (DP) of the first three post-irradiation mitotic divisions and microcolony forming ability (MFA). For the high-LET experiments (10^3 to 10^4 MeV/g/cm^2) the track segment method was employed. Independent of radiation quality and milieu during irradiation, the first post-irradiation mitotic division was less affected than the second one, DP did raise again after the second one, but replication instability was observed for at least 8 generations. Survival-curves were exponential for DP of the first two divisions, but not so for MFA, where some of the curves had shoulders. OER was reduced from 1.7–2.15 for x-rays, depending on the criterion, to 1 for ^{16}O ions. The highest RBE values were measured for α-particles (4 for MFA in N_2) and nearly 1 for ^{16}O ions. The saturation inactivation cross section for MFA was $\frac{1}{3}$ of the geometrical cross section of the nucleus and even less for the other criteria. (Supported by SNSF, grant 2.511.71.)

A-29-6 *Studies on Bacterial Inactivation and DNA-Strand Break Formation by the Cyclotron Beam.* AKIRA MATSUYAMA, TAN TAKAHASHI, KAZUI IGARASHI, AND FUMIO YATAGAI, Institute of Physical and Chemical Research, Wako-shi, Saitama-ken 351, Japan.

Using α-particles and heavy ions of carbon, nitrogen and oxygen accelerated in the IPCR cyclotron, bacterial cells on a membrane filter were irradiated in air. LET dependence of the cellular radiosensitivity of different bacteria studied was classified into three types. To examine the correlation between cellular radiosensitivity and DNA-strand break formation at different LETs, log-phase cells of *E. coli* B_{s-1} labeled with ^3H-thymidine were bombarded by cyclotron beams. The decrease in the efficiency of single-strand break formation with increasing the track-core LET is represented with the same linear relationship even for different particles by employing

a cut-off energy value of 500 eV for the δ-ray correction. With increasing LETs, the number of particles passed through the DNA strand per D_{37} decreased approaching to unity and the number of single-strand breaks per D_{37} produced by the track-core effect decreased toward 2. These facts may suggest an important role of DNA double-strand breaks induced by the particle-traversal in cell death at high LET.

A-30-1 *Some Effects of Irradiation of Mice in Utero with Tritiated Thymidine.* B. E. Lambert and M. L. Phipps, The Medical College of St. Bartholomew's Hospital, Charterhouse Square, London, Great Britain.

An efficient but simple method of continuous infusion of mice is described. The technique has been developed for the continuous infusion of pregnant mice with tritiated thymidine in order to produce fully labelled offspring. These offspring are being used to study a number of early and late effects, in particular gonad cell effects and carcinogenesis, following this form of internal irradiation.

Autoradiographic labelling patterns and the biochemical distribution and loss of tritium with time are discussed and compared with other modes of administration.

Preliminary results of radiation effects on cell turnover and on cells of the reproductive system of the labelled offspring are described.

A-30-2 *Radiotoxicity of Tritium on the Developing Rat: the Effectiveness of Tritiated Thymidine (^{3}H-TdR) and Tritiated Water (HTO).* Wolfgang Schreml, Rainer J. Haas, and Theodor M. Fliedner, Department of Clinical Physiology, University of Ulm, 79 Ulm, West Germany.

The continuous infusion of ^{3}H-TdR and HTO into pregnant rats from day 9 of pregnancy to term offers a model for comparative study of the effectiveness of DNA-bound and homogeneously distributed tritium on the developing mammal. Data of circulating activity during infusion, distribution pattern between biochemically extracted fractions of various organs and specific activity of DNA were used to estimate the dose absorbed by the nuclei of critical organs from DNA-bound and homogeneously distributed activity. Comparing the internal relative effectiveness of ^{3}H in regard to its localization within the cell, DNA-bound tritium appeared about 3 times as effective in causing damage to the somatic development and to the haemopoietic system, and about 5 times as effective in reducing the total oocyte number in newborn rats than homogeneously distributed activity. The dose absorbed from homogeneously distributed tritium necessary to induce a specific radiotoxic effect corresponded well both to data reported for continuous γ-irradiation and to results of a fractionated x-irradiation in our model, where the reduction of oocyte number was studied. In contrast, DNA-bound activity appeared more effective. This RBE of DNA-bound tritium seemingly different from 1 is discussed as either reflecting nonradiological characteristics of the model or as indicating a real difference in the induction of radiation damage. (Supported by EURATOM Contract No. 079-69-I BIAC and Bundesministerium für Bildung und Wissenschaft.)

A-30-3 *Low-Level Exposure to Tritium and Gamma-Irradiation Compared in Mouse Oocytes.* R Lowry Dobson and T. Chinnie Kwan, Biomedical Division, Lawrence Livermore Laboratory, Livermore, California 94550, USA.

Mice were exposed continuously to ^{3}HOH at 1, 3, 6, and 9 μCi/ml in their body water from conception to 14 days after birth. At that time, primary oocytes in ovaries were counted and compared to controls. Numbers of oocytes decreased exponentially with dose; there was no threshold; the LD_{50} level was 2 μCi/ml, which delivers 0.56 rad/day. Oocytes were similarly enumerated in mice exposed from conception to ^{60}Co γ-rays. Cell numbers from control animals and those exposed at 1.25 and 2.50 rad/day lay on an exponential line with LD_{50} at 1.3 rad/day, more than twice that for tritium. At 3.75 rad/day, however, survival was significantly below this line, closer to that expected for ^{3}H. The increasing slope for γ-rays in this region accords well with the theory of dual radiation action. These and other findings indicate similar effectiveness for the two radiations at higher dose-rates, while below some 3 rad/day tritium has an RBE of approximately 2. (Work performed under auspices of U.S. Atomic Energy Commission.)

A-30-4 *Tritium-Induced Specific-Locus Mutation Rate in Germ Cells of the Male Mouse.* R. B. CUMMING, W. L. RUSSELL, AND G. A. SEGA, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn. 37830, USA.

Potential genetic hazards arise from the large amounts of tritium produced by thermo-nuclear detonations and possible fusion reactors.—Specific-locus mutations at 7 loci were scored in the offspring of male mice injected with 0.5 or 0.75 mCi of tritiated water per gram of body weight.—In 7942 offspring from germ cells exposed to tritium in postspermatogonial stages, 11 presumed specific-locus mutations were obtained. The mean radiation dose per germ cell is estimated to be approximately 430 rads. Assuming this is not greatly in error, the mutation rate is well within the statistical limits of what would have been expected from a comparable dose of externally applied X or gamma radiation.—In 20,522 offspring from germ cells irradiated predominantly as spermatogonia, 16 mutations were scored. If the present estimated dose of approximately 700 rads proves correct, the mutation frequency represents roughly twice that expected from low-dose-rate gamma irradiation. However, with the present sample size and incomplete measurement of dose received by spermatogonia, a relative biological effectiveness (RBE) of 1.0 for the tritium radiation relative to external gamma radiation is not excluded. A very high RBE does already appear to be excluded.—These results are the first ever obtained on gene mutation induction by tritium in any mammal. They indicate that the genetic hazards of tritium are not much, if any, greater than would be expected from comparable absorbed doses of X or gamma radiation. (Research sponsored by U.S. Atomic Energy Commission under contract with Union Carbide Corp.)

A-30-5 *Biological Concentration of Tritium.* A. A. MOGHISSI,* R. E. STANLEY, J. C. MCFARLANE, E. W. BRETTHAUER, R. G. PATZER, AND S. R. LLOYD, U.S. Environmental Protection Agency, National Environmental Research Center, Las Vegas, Nevada 89114, USA.

In a study to investigate the possible biological concentration of tritium, rabbits were maintained in a uniformly tritiated environment through three generations. Tritiated water, incorporated into a hydroponic solution, was used to grow alfalfa which was used as the only food consumed by the rabbits. The water consumed by the rabbits was also tritiated. Both food and water contained tritium with a specific activity of 1 nCi/ml of water, or its equivalent ratio of tritium to hydrogen in the alfalfa. The animals were housed in an environmentally controlled facility, which had elevated tritium levels in the air resulting from respiration of the animals. Hence, the animals were essentially immersed in a totally controlled environment. The foundation group consisted of eighteen female rabbits maintained in the tritiated environment for at least two weeks prior to conception. The two subsequent generations were maintained in this environment entirely. Animals were sacrificed at intervals, and selected tissues were analyzed for tritium in both the tissue water and organically bound states. Although some variations were observed in the tritium concentrations in various tissues, the specific activity of tritium in aqueous and organic fractions of tissues of all animals remained essentially the same as the concentration of tritium in the food and water consumed. Results indicate that under the steady state conditions of this experiment, no significant biological concentration of tritium occurs.

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A-30-6 *Urinary Excretion of Tritium Following Accidental Absorption of HTO.* SUE CRAIN, BILL FARMER, AND BOB ROBINSON, Monsanto Research Corporation, Mound Laboratory, Miamisburg, Ohio 45342, USA.

Data on urinary excretion of tritium from a 38-year-old pipefitter, who was accidentally exposed to tritium in the form of HTO, is presented. Interpretations of the data are given in relative units. The data for the first 30 days following the exposure indicates an effective half-life of less than 6 days. No medical treatment was given with the exception of accelerated liquid intake. Data were collected for both distilled and undistilled samples in an attempt to assess the total amount of HTO that was tissue-bound. Tissue bound HTO contributed a very small percentage of the total dose.

A-31-1 *Relation of Auxin and Ethylene to Gamma-Irradiation Effects on Leaf Abscission.* ROBERT B. DWELLE, DAVID E. BILDERBACK, AND MEYER CHESSIN, Department of Botany, University of Montana, Missoula, Montana 59801, USA.

Ionizing radiation has been shown to cause premature leaf abscission of irradiated woody species. We studied the effect of gamma-irradiation from a Cs-137 source on leafblade or petiole abscission of explants of bean, *Coleus* and *Impatiens*. Lower doses typically accelerated abscission while extremely high doses resulted in a complete inhibition of abscission. We suggest that low doses reduce endogenous auxin, thus shortening the transition to ethylene-sensitivity in addition to causing a direct increase in ethylene evolution. High dose inhibition of abscission may be mediated by a lowering of cellulase activity.

A-31-2 *Radiation-Induced Organogenesis in Tobacco Tissue Culture.* NOAH DEGANI, Nuclear Research Centre, P.O.B. 9001, Beer Sheva 84190, Israel.

Irradiation induced the formation of leafy buds in irradiated dark grown tobacco tissue culture (*Nicotiana tabacum* Var. Wisconsin no. 38). Organ formation was induced also by indirect effect of irradiation; i.e., culturing non-irradiated tissue on irradiated medium. Experiments were made to resolve which component part of the medium was effective in the induction of organ formation. Fractions of the medium were irradiated singly and combined with non-irradiated fractions to form the growth medium. The results showed that formation of leafy buds was not concomitant to the irradiation of IAA. Omission of IAA from non-irradiated medium induced differentiation as expected. Irradiated myo-inositol, under certain experimental conditions induced organogenesis more consistently than the other irradiated components.

The age of the inoculum tissue and its passage number from the original tobacco stem, were determinant in the expression of the potency to organize. In most experiments, cultures did not differentiate if extracted from a stock younger than 45–50 days. The control mechanism for differentiation deteriorated after about 13 passages. After this number of passages the cultures differentiated in the darkness even on non-irradiated medium.

A-31-3 *Accelerated and Increased Development of Generative Organs in Tomato Plants from Irradiated Seeds.* R. SIDNEY KAHAN, Soreq Nuclear Research Centre, Yavne, Israel.

Mecheast-22 cultivar seeds, irradiated pre-sowing with 250–1000 rads from ^{137}Cs, and the transplanted seedlings were greenhouse-grown in 20, individual-pot, replicates. The number of flower clusters (FC), buds (B), and open flowers (F) were scored at D25, D45, D70 and D90, and weight of green plant at D90 (D ≡ days after transplanting).

Clusters were observed at D17. By D25, there was increased FC by 250 and 1000 rads. From D45, 500 and 750 rads induced significantly more FC and B. All doses gave an extra 1.5–2FC per plant; 250 rads was most effective at D45 (+18%), while 1000 rads gave 15% more FC at all dates.

Irradiation significantly increased F at D45 (4.5–5.2F versus 2.5F for controls). At D70, 500 and 750 rads significantly increased control F = 30.5, by 6–6.5F.

No dose changed vegetative growth, FC anatomy, or physiology of flower opening.

Radiation caused earlier, and more FC, possibly by stimulating the unknown mechanism initiating change from vegetative to generative growth. (Research at Bavarian Agricultural Institute, Munchen, partly supported by Deutsches Akademische Austauchdienst.)

A-31-4 *Fractionation Dose Effects with Gamma-Rays in Maize Pollen.* TARO FUJII, National Institute of Genetics, Misima, Sizuoka-ken, Japan.

Pollen grains with *Bz*-gene of maize were exposed to gamma-rays in single or two equal fractions and crossed to recessive stocks to observe mutations to *bz* F_1 seeds. A linear dose-mutation frequency relationship was confirmed up to 3 kR in the sum of whole and partial mutations expressed as bronzy aleuron color. While the frequency of partial mutation did not show any significance by single or fractionation treatment, that of whole mutations was significantly decreased by fractionation with an interval period of 2 hours or less. No temperature effect was found by holding at 5–30°C. It is concluded that the mutagenic action of gamma-rays was reduced by dose-fractionation probably due to certain recovery events taking place under experimental conditions applied.

To obtain further information about the extent of recovery, 2 and 3 kR doses split into two

equal parts with 2 and 3 hours intervals. The mutation frequency did not vary much between the two interval periods. The results confirm the earlier findings that up to 2 hours interval period recovery increased, and no further recovery was observed beyond this interval. In the present material, the 2 hours interval period appears to bring about the maximum recovery and the rate in recovery of premutational damage induced by gamma-rays seems to be approximately 30 percent.

A-31-5 *Effects of Shoot X-Irradiation on Water Uptake by a Single Root in Intact Onion Plants.* JAMES R. LOTT AND F. H. CHANG, North Texas State University, Denton, Texas 76203, USA.

The shoots of a single onion root plant were x-irradiated at varying dosages (500 r–1500 r) using a GE x-ray unit (120 KVP-5 ma); Dose rate: 512 r/min. The water uptake by a single isolated root of the plant was then measured using the potometric technique, and recorded as microliters water/area/min. Measurements were made, following a one hour equilibration, before and immediately following x-irradiation. The plants were grown in one quarter strength Hoagland's solution for 5 days. Upon removal from the growth solution, the bulb was placed in an irradiation chamber. All but a single root was then cut from the bulb. The single root extended down into a lower, lead shielded chamber and into a glass potometer. A horizontal microscope fitted with a micrometer followed the movement of the meniscus in the glass tubing during the course of the experiments. A clear quantitative enhancement of water uptake was observed in most of the irradiated plants. The results will be discussed on the basis of possible radiation effects on respiration, photosynthesis, and osmotic changes in the plant.

A-31-6 *Changes in ^{35}S Uptake, GOT (1.4.9.2-4) and Nitrogenase of Soybean Bacteroids Induced by Gamma Radiation.* JAYA N. DUBE, SUKH L. NAMDEO, AND JOSEPH THOMAS, Jawaharlal Nehru Agricultural University, Jabalpur-4(MP)-482004, and Bhabha Atomic Research Centre, Bombay 400085, India.

Soybean bacteroids were subjected to a lethal dose of gamma rays for causing alteration in their nuclear DNA. The influence of the DNA derangement was examined upon GOT, nitrogenase, and $^{35}S_2O_4^{2-}$. The changes in the bacterium- bacteroid ratio, and in the antigenic nature were also studied to quantitate teratogenicity. Nitrogenase activity and teratogenicity are salient characteristics of the plant-bacterium symbiosis, and the associated eukaryotic- prokaryotic transformation is characterized in terms of alterations in the enzyme and in the morphology.

A-31-7 *Radiosensitivity of 84 Substitution Lines of Bread Wheat and Their Parents.* BENITO GIORGI, PAOLINO MANNINO, AND BASILIO DONINI, Laboratorio Applicazioni in Agricoltura, C.N.E.N., C.S.N. Casaccia, S. Maria di Galeria, Roma, Italy.

Seeds of 84 intervarietal chromosome substitution lines of wheat involving the cultivars, Chinese Spring, Hope, Cappelle-Desprez, Cheyenne and Timstein were irradiated in Gamma Cell (^{60}Co source) at exposure rate ranging from 14 to 35 Kr. The radiosensitivity has been evaluated in terms of plant height reduction and loss of dry weight of both shoots and roots. Treated and control seeds were cultured using the seedling "growing rack techniques" and put in growth chamber at 23 $\pm$ 1°C under continuous light. For each treatment plants were measured after 160 hrs. and both leaves and roots were desiccated at 70°C and weighed 48 hrs. later.

The results obtained show: i) all donor varieties are more resistant than the recipient variety Chinese Spring; ii) among the substitution lines those belonging to the homologous groups 1, 3, and 5 appear to be of different radiosensitivity as compared with the others, thus indicating that genetic factor(s) affecting the control of radiosensitivity seem(s) to be mainly located in such chromosomes.

A-31-8 *Effect of γ-Rays upon the Yield and Composition of Peppermint Oil.* AŁA SADOWSKA, Agricultural University, Warsaw, Poland.

Stolons of peppermint obtained from one mother plant were treated with doses of 1000, 2500, 5000, 7500 and 10000 rtg of γ-rays in late November and then planted in the greenhouse. A parallel portion of stolons from the same plant was grown without any irradiation. A part of the shoots developed from the irradiated and a part of those developed from non-irradiated stolons were

exposed again to γ-rays at the above doses in March; the other parts remained unexposed. Three weeks later one-bud cuttings were taken from each of the 16 treatments (including completely non-irradiated control), rooted in sand and planted in the field. The experiment was repeated four times in 1969–1973 at Radzików, Central Poland.

The total weight of cut tops per plant ranged from 285 to 1077 g in the first year harvests and from 550 to 2209 g in the second year. The highest yields of mint and oil were obtained from the treatment where only stolons were irradiated with 2500 rtg.

The cineol content of the mint oil of individual plants varied from 0.4 to 10.2%, menthone—from 9.5 to 37.0% and menthol—from 27.9 to 60.5%. The greatest number of plants showing a low cineol and high menthol content was found as a result of irradiation only stolons with 2500 rtg.

(The author is grateful to Mr. J. Beer, Fermentation Institute, Warsaw, for analyses performed by the gas liquid chromatography method as well as to Dr. A. Rumińska and Dr. Z. Staszewski for helpful advice.)

A-32-1 *Radioprotective Effects of Ascorbic Acid in Barley Seeds.* Bob V. Conger, UT-AEC Comparative Animal Research Laboratory, Oak Ridge, Tennessee 37830, USA.

Experiments were conducted to test the radioprotective effects of ascorbic acid, a naturally occurring reducing agent, on seeds (caryopses) of barley. Results are reported as seedling growth reduction.

When seeds were soaked for 1 hour at ambient temperature in distilled water or ascorbic acid (0.062 to 1.000 *M*) prior to irradiation and then soaked for 18 hours after irradiation in air-bubbled water at 0°C, an 8 and 20% decrease in seedling injury was observed for neutron and gamma radiations, respectively, with increasing ascorbic acid concentration. Additional studies suggested that the protective effect was related to reduced hydration of the embryos of seeds soaked in ascorbic acid.

When seeds were soaked in ascorbic acid after irradiation, no protective effect was observed in seeds of 13% water content. A protective effect was observed for seeds of 2% water soaked in oxygen-bubbled ascorbic acid (0.5 *M*) but not when soaked in nitrogen-bubbled ascorbic acid. The protective effect against oxygen-dependent damage may be due to the interaction of ascorbic acid with radiation-induced free radicals. (Supported by U.S. AEC under contract No. AT-40-1-GEN-242 with University of Tennessee.)

A-32-2 *Evidence for Repair of Radiation Damage in Barley Seeds.* C. Sander and R. A. Nilan, Washington State University, Pullman, Washington 99163, USA.

γ-irradiated (20 Kr) barley seeds were incubated in O_2 or N_2 solutions for different periods prior to treatment with caffeine (10^{-1} *M*). Chromosome aberrations, dicentric bridges, and fragments were scored in anaphase cells of shoot-tips. With zero incubation time, caffeine treatment in O_2 produced a synergistic increase of about 150% in the total number of aberrations while in N_2 the increase was less—about 50%. With longer incubation periods in O_2 prior to caffeine treatment, changes were observed in the frequency of bridges and fragments—indicating repair during the first $2\frac{1}{2}$ hours of incubation. This repairable damage is attributed to secondary O_2 effects. The frequency of single strand breaks in the DNA extracted from dry seeds irradiated at 25 Kr showed no change due to prior caffeine treatments. DNA from seeds irradiated wet at 100 Kr showed some restitution of single strand breaks after 6 hrs. of O_2. The frequency of M_2 chlorophyll-deficient mutations showed no significant change due to incubation and/or caffeine treatments. Possibilities of metabolic control of radiation damage and repair mechanisms are discussed. (Research supported by AEC Contract AT[45-1]-2221.)

A-32-3 *The Effects of γ-Rays on the Accumulation of Reducing Sugar and α-Amylase During Germination of Barley Seeds.* Itsuo Kurobane and R. A. Nilan, Washington State University, Pullman, Washington 99163, USA.

γ-rays enhanced the accumulation of reducing sugar and α-amylase in the water in which irradiated seeds were germinated. Analysis of seed extracts revealed some stimulation of reducing sugar and slight inhibition of α-amylase activity by the radiation. γ-irradiation also inhibited the shoot and root development and this inhibition was stronger than that for α-amylase synthesis.

The stimulatory effects occurred between 25 Kr and 1,000 Kr with a maximum at 500 Kr. These findings suggest that the stimulatory effect of γ-rays on the accumulation of reducing sugar is due to a balance between the effects on α-amylase synthesis and on the development of embryos. Very high doses, e.g., 8,000 Kr, produced another peak of reducing sugar accumulation and severely inhibited α-amylase synthesis. It appears that the mechanism of these effects at very high doses may be different from those at 500 Kr. (Senior author is a Rotary International Graduate Fellow.)

A-32-4 *The Effects of Caffeine on Chromosome Aberrations, Mutations, and DNA Lesions of γ-Irradiated Barley Seeds.* C. SANDER AND R. A. NILAN, Washington State University, Pullman, Washington 99163, USA.

Ungerminated barley seeds, G_1 stage, were treated with caffeine (10^{-2} *M*) for 2 hrs. at 20 C then dried back to 13% water content prior to γ-irradiation. Dose response curves (0–36 Kr) for frequencies of chromosome aberrations scored in the anaphase cells of embryonic shoot-tips showed a marked synergism over noncaffeine-treated irradiated controls. However, the two components of aberrations, namely, dicentric bridges and fragments, did not exhibit parallel frequency curves over the doses used. The heights of M_1 seedlings showed a marked reduction while the frequencies in M_2 chlorophyll-deficient mutations showed no increase in caffeine plus radiation treatments. Alkaline sucrose gradients of DNA extracted from seeds irradiated at 25 Kr in the presence or absence of caffeine showed no significant change in the frequency of single strand breaks. The implications of this data on repair mechanisms will be discussed. (Research supported by AEC Contract AT[45-1]-2221.)

A-32-5 *Synergistic Effect of Combination Treatments with Gamma-Rays and Chemical Mutagens in Barley.* A. S. KHALATKAR AND C. R. BHATIA, Biology and Agriculture Division, Bhabha Atomic Research Centre, Bombay 400 085, India.

In combination treatments of x- or gamma rays and chemical mutagens on seeds, mutagenic effects were reported to be synergistic when radiations were given first, followed by the chemical treatment. When treatments were given in the reverse order, i.e., chemical and then radiation, the mutagenic effects were not synergistic. Since radiations are known to alter the integrity and permeability of biological membranes, it was thought that the synergistic effect observed in the combination treatments could be due to increased uptake of the chemical mutagen. Uptake of chemical mutagen and alkylation of macro-molecular fraction were followed using ^{3}H-ethyl methanesulfonate in barley seeds. Gamma radiation (10, 20 and 30 kR) inhibited uptake of ^{3}H-EMS by the seeds and consequently alkylations in the macro-molecular fraction were also less. In parallel experiments using cold EMS, seedling injury, mitotic and meiotic chromosomal aberrations were less than additive in the combination treatments. However, in the M2, a synergistic effect on the frequency of induced mutations was observed in the combination treatments. These results rule out increased uptake of chemical mutagen as the cause of the observed synergism between gamma radiation and EMS, and suggest interaction of radiation and chemically induced lesions.

A-32-6 *Changes in the Magnitude and Pattern of Translocation of Photoassimilated $^{14}CO_2$ in Soybean Plants Following a Single Exposure to Gamma-Radiation.* DONALD J. URSINO AND HANS SCHEFSKI, Brock University, St. Catharines, Ontario, Canada.

Young soybean plants were exposed on a single occasion to ~4000 rads of γ-radiation. Two hours after the irradiation, the mature trifoliate leaf was permitted to photoassimilate $^{14}CO_2$ and one hour later, the plant was sacrificed and the distribution of ^{14}C compounds determined. In the non-irradiated plants 18% of the ^{14}C was outside the fed leaf blade and of this translocated ^{14}C, 28% was above the node of the fed leaf, 66% below, and 6% in the petiole. In irradiated plants, only 6% of the ^{14}C was translocated out of the fed leaf and of this, only 20% was recovered above the node. Replacement of the shoot apex with 20 ppm IAA following irradiation of the intact plant partially increased the magnitude of translocation and completely restored the pattern of distribution to that found in the non-irradiated plants. These results will be discussed in reference to radiation effects on photosynthesis, the vein-loading of photoassimilates, and on hormone levels in the shoot apex.

A-32-7 *Mutations by Gamma-Irradiation on Dormant Seeds of a Partially Asynaptic Strain in Rice.* HIKOYUKI YAMAGUCHI, University of Tokyo, Tokyo 113, Japan.

Dormant seeds of two partially asynaptic strains, K-642 and K-648, in which two to eight univalents were present at the first metaphase of meiosis, and a normal cultivar Kinmaze were irradiated with γ-rays of 0 to 30 kR. The survival rate at maturity of the X_1 plant was reduced to a marked extent in K-642 and K-648 compared with Kinmaze. The mean seed setting in K-648 was slightly less than in Kinmaze. The incidence of chlorophyll mutations in Kinmaze increased linearly with the radiation dose. In contrast, the mutation frequency in K-648 was found to increase more rapidly than a single power of the dose of γ-rays. Supposing that the loose association of homologous chromosomes in somatic cell is correspondent with the failure of chromosome pairing at meiosis, the results obtained in this experiment provide the evidence of a common basis for repair and recombination mechanisms even in rice.

A-33-1 *Factors Determining Differential Responses to Irradiation of Ehrlich Ascites Tumor Cells and Host Organism.* IANCU MUSTEA, Oncological Institute, Cluj, Romania.

Investigations will be described concerning the possibilities to enhance selectively the lethal effect of X-ray upon Ehrlich ascites tumor cells by redox state modification due to the switching of the cellular metabolism predominantly on the glycolytic pathway. This change of metabolic direction was obtained inducing the Crabtree effect in cancerous cells after exogenous addition of glucose of glucose + insulin. A characteristic change was observed in both cases measuring the redox state of ascites fluid and a good correlation between redox state and irradiation response was found. The maximum enhancement effect took place at the lowest values of rH. By irradiation with different doses within the range 250–1000 R, the enhancement effect was observed at 1000 R only. The irradiation of ascites cells in conditions of the Crabtree effect induction and in the presence of a thymine analogous (5-amino-6-hydroxythymine hydrazine) gave an enhanced effect at low doses (250 R) without any significant modifications of the host animals response. The possible mechanism of action of this procedure and the interrelationships with DNA synthesis and cellular cycle, as well as the results of clinical trials with the object of improving the tumor radiotherapy will be also discussed.

A-33-2 *Radiosensitization of Pulmonary Metastases with Intravenous Infusion of Pyrimidine Analogs.* J. MARTIN BROWN, Stanford University, Stanford, California 94305, USA.

A technique is described which enables a determination to be made of the total number of viable EMT6 tumor cells in the lungs of BALB/c mice at any time following an intravenous injection of these cells. Following such an injection the individual cells grow exponentially for approximately 6 days, but as the size of each nodule increases past 1,500 cells, growth slows. A hypoxic fraction (of 0.5%) is found when the nodules reach a size of roughly 9,000 cells. During the period of exponential growth essentially all of the viable cells are sensitized to radiation (by a DMF of 1.5) by a two-day infusion of 5 mg per day of 5-bromo-2′-deoxycytidine. Data will be presented on multiple fraction regimes both with and without the addition of fluorodeoxyuridine (to inhibit endogenous thymidine synthesis). The effect of infusion of these compounds on the radiosensitivity of normal lung tissue will also be presented. The potential use of intravenous infusion of halogenated pyrimidines in combination with radiation for treatment of lung metastases will be discussed. (Research supported by USPHS grant CA-15201.)

A-33-3 *The Combined Effect of Bleomycin with Radiation on Murine Epithelioma* in vivo. K. SAKAMOTO, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan.

Bleomycin, an antibiotic, has been used clinically as an effective anti-tumour agent to human tumours, especially squamous carcinoma of man. The present study is to obtain quantitatively the dose-survival relationships with an epithelioma (squamous carcinoma) of mice *in vivo* and to investigate a possible potentiating effect of bleomycin to radiation in this tumour system.

The survival curve of bleomycin on murine epithelioma was composed to two components, the first sharp exponentially declining component followed by the second slowly declining component at a dose level of 0.2 mg/kg.

The drug was administrated either one hour before or after X-irradiation. The analysis of the

resulting survival curves showed that bleomycin failed to potentiate the effects of X-rays and that bleomycin and X-rays killed the cells independently.

A-33-4 *The Control of Solid Tumor Growth using Combined Fractionated Radiotherapeutic and Chemotherapeutic Regimens.* LAWRENCE POULAKOS, LARRY L. SCHENKEN, AND RONALD F. HAGEMANN, Cell and Radiation Biology Labs, Allegheny General Hospital, Pittsburgh, Pennsylvania 15212, USA.

A treatment concept for the control of solid tumor growth involving weekday radiotherapy and weekend chemotherapy was tested. Male DBA/2 mice were injected subcutaneously with 10^6 P815X2 mastocytoma cells on the lower back (Day 0). Treatment groups were: tumor-only X-irradiation with 200 R/day (Day 7–11, 14–18); chemotherapy with either 20 mg/kg BCNU or 5 mg/kg Adriamycin (Days 6 and 13); and combined chemotherapy and radiotherapy. Thrice weekly tumor size measurements and animal survival times were recorded.

Untreated control tumors grew from 0.60 cm^2 at Day 5 to a tumor size at death of 4.82 cm^2. Radiotherapy alone initiated tumor control by Day 15 with an eventual tumor size of 3.20 cm^2. Combined therapy proved the most effective treatment regimen. Irradiation + Adriamycin markedly slowed tumor growth and had a tumor size of 2.02 cm^2. Irradiation + BCNU controlled tumor growth from the first day of treatment, with five tumors regressing completely. The tumor size was 0.23 cm^2. Although no treatment regimen prolonged survival, the results from weekend combination therapy indicate it may be an important concept in future cancer therapy.

A-33-5 *Organ Culture Techniques for Studying the Effects of Bleomycin and Radiation on Solid Tumors.* J. K. MORAN, G. L. DETTMAN, AND J. HARRISON. University of California, Irvine, California 92664, USA.

Our laboratory has developed organ culture techniques for maintaining explants of solid tumors under conditions in which the explants simulate *in vivo* responses. We have been interested in evaluating the responses of tumor explants to radiation and/or chemotherapy by measuring specific biochemical parameters such as DNA and protein synthesis. This information can be projected to serve as a guide to clinical management.

Recently bleomycin, a polypeptide antibiotic, has demonstrated excellent cytotoxic responses in treatment of lymphoma and squamous cell carcinoma. We have evaluated bleomycin in our mouse mammary adenocarcinoma system as a model for future studies with human oral squamous cell carcinoma. Preliminary studies have revealed a synergistic inhibitory effect of bleomycin and radiation therapy on DNA synthesis in our model system. Data will be presented on the effects of combined bleomycin and radiation on protein synthesis. We will also report on data evaluating the timing of radiation in relation to treatment with bleomycin and its effects on DNA and protein synthesis in our model solid tumor system.

A-33-6 *4-4′-Dihydroxybiphenyl, Diphenylquinone and Diphosphate of Diphenylquinone, Comparative* in vivo *Radiosensitizing Effect.* M. D. ASTUDILLO, A. GOICOECHEA, M. V. ALVAREZ, P. PARTIDA, AND F. SANZ, C.S.I.C. Instituto de Química Física, Radiobiología, Serra no. 119, Madrid (6), Spain.

The compounds diphenylquinone and diphosphate of diphenylquinone are prepared from 4-4′-dihydroxybiphenyl.

In order to follow distribution, retention and metabolism, 4-4′-dihydroxybiphenyl-^{3}H is administered to rats. Radiometric measures are taken from several organs and urine. The latter is also submitted to radiochromatography and autoradiography.

The radiosensitizing ability of these compounds is determined *in vivo* in mice. At the same time a study of these compounds is made on solid tumor (M.A.C.) in relationship to radiotherapy of said tumor.

Results obtained will be discussed comparatively in relation to radiosensitizing effect of said compounds.

A-33-7 *Radiosensitization of Bleomycin on Lethal Effect of Mouse Cancer Cells in vitro.* T. MATSUZAWA, K. TAKAZAWA, T. AWANO, H. YAMAURA, AND S. OKUYAMA, Radiology and Nuclear Medicine, The Research Institute for Tuberculosis, Leprosy and Cancer, Tohoku University, 4–12 Hirosemachi, Sendai 980, Japan.

Different from other antitumor agents Bleomycin (BLM) has exhibited a significant antitumor activity on differential squamus cell carcinomas and, to a lesser effect, undifferentiated cell carcinomas and the bone marrows. The FM3A cells (originally C3H mouse mammary carcinoma) and the B16-C2W cells (originally C57BL mouse skin melanoma) growing *in vitro* were used through the experiment. The X-ray dose survival curves of both cells pretreated with BLM have a marked reduction in extrapolation number (n), and to a lesser effect, a mean lethal dose (Do). The parameters of the FM3A cells were: $n = 2.8$, $Do = 110$R for the control, and $n = 1.4$, $Do = 100$R for the BLM treated cells. The values of the C2W cells were: $n = 14$, $Do = 125$R for the control, and $n = 6$, $Do = 110$R for the BLM treated cells. These results reveal that BLM has clear radiosensitizing effect on cell killing of two mouse cancer cell lines of different origin. The mechanism of radiosensitizing action of BLM was studied in X-ray fractionation experiments.

A-33-8 *The Effect of Quinacrine and Chloroquine on EMT6/M/CC Mouse Tumor Cells in Exponential and Plateau Phases of Growth, and their Dose Modifying Potential for Radiation and Chemodamage.* NORMAN M. BLEEHEN, PETER R. TWENTYMAN, AND ANN P. WHEELER. Middlesex Hospital Medical School, London, England.

The toxicity of quinacrine and chloroquine to EMT6/M/CC cells has been measured *in vitro*. For both agents it has been found that exponentially growing cells are more sensitive than cells in "plateau phase," where the rate of proliferation is reduced. These drugs appear to act as potentiators of radiation damage when present after irradiation but not if only present at the time of irradiation. In contrast, the cytotoxicity of the antineoplastic drug bleomycin (BLM) is potentiated by the presence of quinacrine or chloroquine during exposure of cells to BLM. Further cell killing however may be obtained if these potentiators remain present in the medium for a period after the exposure to BLM.

These *in vitro* potentiating effects are seen at less toxic doses of chloroquine than of quinacrine. Data on *in vivo* studies will also be presented.

The results will be discussed in terms of possible mechanisms of radiation and drug damage.

A-33-9 *The Radiation Cell Reaction in Dependence on their Functional State.* IRINA I. PELEVINA, GEORGIJ G. AFANASJEV, VALENTINA YA. GOTLIB, SUSANNA S. VORONINA, AND ZAMIRA A. MAKSUMOVA, Institute of Chemical Physics, Moscow 117334, USSR.

It was shown that increase of cell radiation reaction by the action of radiosensitizing chemical compounds is not connected with the DNA synthesis intensity. The same degree of increase of cell radiation lethality is observed by the addition of DNA synthesis inhibitor-hydroxyurea (HU) and is rising with the exposure time before the irradiation. The inhibition of DNA synthesis after irradiation can induce the increasing of cell survival perhaps because of the effectiveness of repair processes. The repair of radiation damages in different periods of cells growth in tissue culture was investigated. The nonproliferating cells in the stationary phase of growth (HeLa) can repair potentially lethal damages. HU can increase the effectiveness of repair and survival of nonproliferating cells. It was shown that in mice solid lymphosarcoma NKLy the individual variations in the fraction of nonproliferating cells are observed. These variations can be responsible for the differences in individual tumor reactions. The role of DNA synthesis in radiation cell lethality, reaction of nonproliferating cells in tumors and the possibility of its modification, and differences in individual tumors reaction are discussed.

A-33-10 *The Factors which Determine the Modification of Tumor Cells Radiosensitivity.* NIKOLAI M. EMANUEL, AND IRINA I. PELEVINA, Institute of Chemical Physics, Moscow 117334, USSR.

The quantitative parameters determining modification of cells and tumors radiation reaction by the action of phenolic compounds (propylic ether of gallic acid, PG), iminoxyl stable free radical (TAN) and nitrosomethylurea (NMM) were investigated. These compounds can increase the DNA damage and sensitize tissue culture cells (HeLa and LL) and tumors (mice solid NKLy tumor) to irradiation. PG, TAN and NMM can increase or decrease the radiation cell lethality in dependence on the exposure time, drug doses and the dose of radiation. Because of the cell heterogeneity in tumors it is necessary to determine the critical populations and the modes of action on the different types of cells in tumors. TAN was used to increase hypoxic cell radiosensitivity. The

radiosensitizing effect was observed only when TAN free radicals were registered in tumor cells by ESR method. NMM and PG were used for the action on the nonproliferating cells (HeLa in the exponential and stationary periods of growth). NMM increases the radiation lethality of proliferating cells to a greater degree. The effectiveness of PG was the same when it acted on the proliferating and nonproliferating cells. The ways to increase the effectiveness of tumor radiotherapy in terms of damage to critical structures in cells and critical populations in tumors are discussed.

A-34-1 *Frequency Distributions of Radiocesium on the AEC Savannah River Plant.* JOHN E. PINDER AND MICHAEL H. SMITH, Savannah River Ecology Laboratory, Aiken, South Carolina 29801, USA.

The normal, lognormal and Weibull distributions were compared to the frequency distributions of radiocesium (primarily ^{137}Cs) in various eco-system components. Data were collected from several contaminated eco-systems including lakes and the flood plain of a contaminated stream, Steel Creek. Within each ecosystem samples were taken from several trophic levels. Thus, it was possible to compare frequency distributions across both trophic levels and ecosystems.

As a general model of radiocesium distributions the lognormal was more accurate than either the normal or Weibull distributions. However, the differences in accuracy between the lognormal and Weibull were slight. Although the lognormal and Weibull were accurate models of radiocesium distribution, goodness-of-fit tests indicated that neither of them could be considered as the actual radiocesium distribution. Comparisons of distribution shapes, using the shape parameter of the Weibull distribution, indicated most components of the Steel Creek ecosystem had similarly shaped distributions. However, comparisons with other ecosystems indicated that they may have different shapes to their distributions and that the shapes may change across trophic levels.

A-34-2 *The Effect of Twelve Years of Chronic Gamma Radiation upon Litterfall and Soil Humus of an Oak-Pine Forest.* T. V. ARMENTANO AND G. M. WOODWELL, Biology Department, Brookhaven National Laboratory, Upton, New York 11973, USA.

After 12 years of chronic gamma irradiation, the gradient of change in structure of the Brookhaven oak-pine forest is approaching stability. An annual litter fall of 261 g/m^2 normally contributes to the maintenance of a standing crop of humus of 4574 g/m^2. Along the gradient a 50% reduction in litter fall occurred at a twelfth-year daily exposure of 6.9 R/day (total 35,400 R); humus at this exposure was 3209 g/m^2. The half value for leaf litter production of *Quercus alba* was 7.4 R/day; for *Q. coccinea* it was 6.9 R/day and for *Pinus regida*, 2.5 R/day. An earlier study based on nuclear volume had predicted that *Q. coccinea* would be the most resistant tree; these data suggest that *Q. alba* is the most resistant tree.

The half-value for humus standing crop in the twelfth year exceeded 126 R/day, the highest exposure rate studied. Exposures as low as 3 R/day reduced the humus standing crop to 94% of the control.

This work shows that if there is a threshold for effects of chronic gamma radiation on a forest, this threshold, when measured on the basis of litter and humus, lies below 3 R/day. (Research supported by the U.S. Atomic Energy Commission.)

A-34-3 *Effect of Gamma Radiation on Secondary Treated Sewage Effluent.* DAVID D. WOODBRIDGE, W. R. GARRETT, AND P. C. COOPER, Florida Institute of Technology, Melbourne, Florida, 32901, USA.

Under the sponsorship of the U.S. Corps of Army Engineers a detailed study has been performed on both the chemical and bacteriological effects of irradiated contaminates in waste water. The study first examined the effect of irradiation on specific chemical and bacteriological additives to sterile water solutions. Later phases have utilized secondary treated sewage effluent for investigating the effectiveness of nuclear irradiation. Effectiveness of gamma rays in the reduction of organic and inorganic substances can best be explained in terms of the relatively recent discovery of the hydrated electron. The hydrated electron is a highly reactive negative ion and acts as a more powerful reducing agent than the hydrogen atom. Results have shown

that gamma rays are effective in the reduction of chemicals such as phenols, surfactants, pesticides and chlorine both in sterile solutions and in secondary effluent. Gamma rays and chemicals such as ozone and chlorine result in stronger synergistic effects on bacteria. Addition of these chemicals greatly reduce the required radiation dosage for complete annihilation of bacteria.

A-34-4 *Predictions and Implications of Probable Limits of Acute and Chronic Gamma-Radiation-Induced LD_{50} for Some Families and Genera of Gymnosperms and Herbaceous Angiosperms.* SUSAN S. SCHWEMMER, A. H. SPARROW, AND ANNE F. NAUMAN, Biology Department, Brookhaven National Laboratory, Upton, New York 11973, USA.

The applicability of lognormal frequency distributions in establishing the range of various nuclear parameters including interphase chromosome volume (ICV) and DNA/chrom. has been demonstrated for a large number of taxa of gymnosperms and herbaceous angiosperms. Parameters of the cumulative frequency-probit plots based on the lognormal distributions and the known correlations between ICV and acute and chronic whole plant radiosensitivity permit the prediction of the probable limits of radiosensitivity for all unsampled species for a given taxonomic group when the number of lognormal distributions is known. These distributions and probable limits of acute and chronic LD_{50} will be given for the gymnosperm families Pinaceae, Cupressaceae and Podocarpaceae and six genera within these families. The probable acute LD_{50} limits for herbaceous members of the family Solanaceae will also be given. Extension of this approach to other families, and possibly, genera, is in progress. The implications of these probable limits will be discussed with regard to the estimation of radiation effects on a broad scale. (Research supported by the U.S. Atomic Energy Commission.)

A-34-5 *The Effect of Ionizing Radiation and Salinity on the Grass Shrimp,* Palaemonetes pugio. D. W. ENGEL, M. G. SHELTON, AND J. C. WHITE, JR., National Marine Fisheries Service, Atlantic Estuarine Fisheries Center, Beaufort, North Carolina 28516, USA.

Grass shrimp, *Palaemonetes pugio,* a euryhaline crustacean, are more sensitive to radiation than other crustaceans studied in our laboratory. Grass shrimp were exposed to different combinations of radiation (200–4, 800 rads) and salinity (5–35 parts per thousand, ppt) to determine interacting effects on their survival and osmoregulatory capabilities. The 40 day LC_{50}s which were determined were salinity dependent (15 ppt, 600 rads; 20 ppt, 450 rads; 25 ppt, 405 rads; and 30 ppt, 210 rads). The total free amino acid concentrations in the muscle of the grass shrimp were directly dependent upon salinity, and also dependent upon radiation dose and time after irradiation. The muscle concentrations decreased with time after irradiation at 5 ppt and increased at 35 ppt. Analysis of the individual free amino acids indicates that the concentrations of glycine (50% of total amino acid) changed with time after irradiation and salinity. The fluctuations in glycine concentration indicate radiation sensitivity in the metabolic pathways leading to the synthesis of the nonessential amino acids used in osmotic regulation. (A.E.C. Contract No. AT (49-7)-5).

B-1-1 *Status and Future Prospects for Fusion Power.* ROBERT L. HIRSCH, Division of Controlled Thermonuclear Research, U.S. Atomic Energy Commission, Washington, D.C. 20545, USA.

Of the four or five fusion reactions of interest for a fusion power reactor, the deuterium-tritium (DT) reaction provides the largest energy release at the lowest temperature. It is therefore considered the most probable for first generation fusion reactors. This selection means that these reactors will operate on a thermal conversion power cycle and thereby roughly amount to a new type of "fire-box" for power plants whose character would be similar to those now in use.

The characteristics of DT fusion reactors are as follows. 1) Their basic fuels are deuterium and lithium, from which tritium is bred, and these fuels can provide millions of years of energy at negligible fuel cost. 2) DT fusion reactors will be inherently safe against nuclear runaway and will have no emergency core cooling problem. 3) Environmental and safety characteristics will be very attractive. However, radioactive materials will be involved, and they will impose restrictions on plant design and operation.

There are four major approaches to fusion power in the U.S. program. Three of these involve

magnetic plasma confinement—tokamak, theta pinch and magnetic mirror—and one utilizes inertial confinement—laser-fusion. The status of the fusion research and development will be described as will be the program plan that aims at the goal of commercial fusion power by the year 1995.

B-1-2 *Fusion Research in Japan.* SHOICHI YOSHIKAWA, Department of Physics, University of Tokyo, Tokyo, Japan 113.

The importance of fusion power to Japan is perhaps the greatest among nations of the world. The scarcity of other energy resources such as oil, coal, and even solar energy (as the land is small) makes it almost imperative to depend on nuclear power for her primary energy need in the future. A high population density means that the radiation hazard problems are very much greater than in other nations such as the USA. Earthquakes and unstable geological features pose a rather difficult problem in storing radioactive wastes which tend to be produced more from ordinary fission reactors, including breeders, rather than from fusion reactors, if the latter are practicable. Japan has two research groups actively engaged in fusion research, one is under the direction of Japan Atomic Energy Commission, and the other is under the direction of Ministry of Education. Work covers both reactor core plasmas and reactor technology. Japan is presently making a five-year plan to do scientific feasibility experiments in the scale comparable to the so-called D-T burner (USA) or JET (Euratom).

B-2-1 *Carcinogenesis by Ionizing Radiation and Its Lessons for Other Pollutants.* R. H. MOLE, Medical Research Council, Radiobiology Unit, Harwell, Oxfordshire OX11 ORD, England.

A broad survey will be attempted of what has been established about carcinogenesis by ionizing radiation and about the differences in the kinds of facts derivable from groups of 10^1–10^2 individuals and from human populations numbering 10^3–10^7. The probability per cell for induction of cell sterilisation (or of visible chromosomal aberrations) is much larger than for that form of mutation called carcinogenesis and this has important implications for interpreting observational data in terms of a linear hypothesis. There are still surprising gaps in knowledge about elementary questions such as the influence of dose rate.

The practical needs of radiological protection have emphasised the quantitative aspects of cancer induction which is increasingly a question for other pollutants. For a variety of reasons chemical carcinogens can never provide the clear experimental situation which makes ionizing radiation a uniquely valuable tool for examining the biological processes of carcinogenesis. Moreover human populations with known radiation exposures are available for quantitative validation of hypotheses based on experimental observations. Corresponding validation for chemical carcinogens may never be possible since human populations with known exposures do not exist.

B-2-2 *Carcinogenesis at the Cellular Level.* G. KLEIN, Department of Tumor Biology, Karolinska Institute, S-104 01 Stockholm, Sweden.

Demonstrated mechanisms of oncogenesis at the cellular level will be discussed in relation to a) DNA viruses, b) RNA viruses and c) non-viral carcinogens. Subsequently, the lesion responsible for the behavior of the target cell will be considered. Particular attention will be given to the results of somatic hybridization between normal and malignant cells, and between two different kinds of malignant cells, and to the reversion of neoplastic transformants. An attempt will be made to choose between the two extreme alternatives of one common mechanism, vs. diverse cellular mechanisms involved in neoplastic transformation.

B-2-3 *Direct Evidence that Damaged DNA Results in Neoplastic Transformation—A Fish Story.* R. B. SETLOW AND R. W. HART, Carcinogenesis Program, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

There is a strong association between exposure to the UV component of sunlight and skin cancer. The fact that individuals with xeroderma pigmentosum are defective in the ability to excise pyrimidine dimers from their DNA is eivdence for the hypothesis that such DNA damage may result in malignant transformation. Fish contain photoreactivating enzyme. The enzyme

monomerizes pyrimidine dimers. If UV-induced tumor induction in fish is photoreversible such a finding would indicate that dimers in DNA lead to neoplastic transformation. The preliminary data indicate that they do so. Cell suspensions of tissue from various organs of the gynogenetic (non-sexually reproducing) fish *Poecelia formosa* were exposed to several fluences of 254 nm radiation. Portions of the suspensions were injected into the middorsal region of 100–200 members of the clone from which the tissue originated and the fraction of fish with tumors were determined 3–9 months afterwards. Tumor incidence rose proportionately to the fluence at a rate dependent on the tissue. For liver an average incident fluence of 10 Jm^{-2} yielded an average of 1.9 tumors per fish. Ten percent of the tumors were very large and usually killed the animal. If the irradiated liver-cell suspension was illuminated with PR light (320–450 nm) before injection, the yield of tumors was reduced by an amount that increased with the illumination time. The PR sector was >0.8. PR illumination before 254 nm exposure did not reduce the numbers of tumors. Thus we seem to be observing true enzymic PR. These preliminary experiments indicate that pyrimidine dimers in DNA can lead to tumors. (Research sponsored jointly by the NCI and the USAEC under contract with the Union Carbide Corporation.)

B-3-1 *Use of Chromosome Aberration Data to Estimate Man's Genetic Hazard from Ionizing Radiations.** J. G. BREWEN AND R. J. PRESTON, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

Chromosome aberrations constitute a significant portion of the genetic damage produced by ionizing radiations. The two most commonly transmitted aberration types are chromosomal interchanges and deletions, the latter either conferring lethality on the zygote or appearing as simple gene mutations. Interspecific comparative studies on the production of two classes of chromosomal interchanges and deletions at various dose rates in both somatic and germinal cells are used to make estimates of the genetic consequences to man from exposure to ionizing radiations. The organisms studied include man, marmoset, Chinese hamster, and mouse, and the studies involve somatic and germ cell stages from all species and from prenatal as well as adult mice. Both chronic and acute radiation exposures are considered in light of known and theoretical radiological responses of the chromosome. Doubling doses are calculated on the basis of cytological observations and are compared to those derived from specific-locus studies.

* Research supported by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.

B-3-2 *Quantitative Studies of Mutation and Mutagenesis with Human Cells.* ROBERT I. DEMARS, University of Wisconsin, Madison, Wisconsin 53706, USA.

B-3-3 *Mutation and the Amount of Human Ill Health.* HOWARD B. NEWCOMBE, Biology and Health Physics Division, Atomic Energy of Canada Limited, Chalk River, Ontario, Canada.

Estimates of genetic risks to man from radiation exposures are needed to provide a basis for setting safety standards. Conventional risk estimates have tended to assume that a rather large fraction of the normal load of hereditary disease is maintained in the population by repeated mutations, and would increase in direct proportion to any artificial elevation of the mutation rate.

It is proposed here, however, that this assumption leads to an over-estimation of the risks by more than 10-fold. The numerous and important irregularly inherited diseases, like diabetes and cleft palate, are probably not mutation-maintained. Only the simple dominant diseases would be expected to increase in direct proportion with the mutation rate, but these appear from new data to be rarer than has been supposed, affecting only about 0.1 individual per 100 born alive instead of 1 per hundred as previously believed. A revised estimate of risk suggests that a population exposure of a million man-rem probably results in no more than 20 additional cases of hereditary defects.

Where money is spent to reduce population exposures (e.g., by additional containment of wastes from nuclear power reactors) risk estimates of this kind can indicate the amount of safety purchased per unit cost.

B-3-4 *Use of the Mouse to Fill Gaps in Our Risk Assessments.* ANTHONY G. SEARLE, Medical Research Council, Radiobiology Unit, Harwell, Didcot, Oxfordshire OX11 ORD, England.

After 25 years of large-scale effort in mammalian radiation genetics some numerical (but very tentative) estimates of probable risks to man are beginning to appear in print. The remaining areas of uncertainty can be roughly classified as due to (i) technical difficulties (ii) doubts about relevance to man and (iii) the need to know more about human population genetics and cytogenetics. The first cause is being steadily eroded, although the design of feasible low-dose experiments remains a major problem. A good *in vivo* somatic mutation system is one possibility. A 3-pronged attack (cytological, genetic and *via* foetal lethality) is being mounted to study radiation effects on the frequency of non-disjunction and therefore of trisomy in the mouse. The genesis of tertiary trisomy from translocations is also being investigated. The question of relevance to man is concerned both with extrapolation and with choice of appropriate mutational end-points. Perhaps early-acting dominant and recessive lethals have been over-emphasised at the expense of detrimentals, more important in terms of human suffering. Inversions may help to detect detrimentals and special methods are needed for work on "genetic ill-health" in general.

B-4-1 *Biophysical Implications of Radiation Quality.* HARALD H. ROSSI, Radiological Research Laboratory, Columbia University, New York, NY 10032, USA.

The pattern of energy deposition by ionizing radiation markedly influences its biological effectiveness. This is particularly evident at low absorbed doses where effects are produced by the agency of single particles and where the RBE (of high relative to low LET radiations) can reach values in excess of 100. It can be readily shown that if effects were simply proportional to energy deposition different radiations would be equally effective. It must therefore be concluded that effects depend on a higher power of energy density. The application of the concepts of microdosimetry to a great variety of effects on higher organisms indicates that their probability of occurrence is related to the square of the energy concentration (specific energy) in regions that have dimensions of the order of a few micrometers. This finding implies that one is dealing with a second order reaction in which pairs of radiation produced entities interact to cause observed effects.

These findings make it very unlikely that certain concepts of the biological action of radiation are correct but they support other theories. They also furnish qualitative and sometimes quantitative explanations for a variety of radiobiological observations.

B-4-2 *The Analysis of Radiation-Induced Chromosome Aberrations.* J. BRENOT AND N. PARMENTIER, France.

Recent developments, such as the chromosome banding techniques, promise a more detailed understanding of the radiation induction of chromosome aberrations and particularly of such notions as the site model or the distorsion hypothesis. In view of this possibility it is desirable to reconsider the biophysical basis of our understanding of chromosome damage produced by different types of ionizing radiation, and to examine the statistical procedures applied to the analysis of dose-effect relations.

The classical models, for example the treatment developed by Lea, are linked to LET and can therefore give only a simplified account of radiation quality. These models are compared to the treatment based on the more recently developed microdosimetric concepts. The experimentally observed linear-quadratic dose dependences are interpreted in terms of microdosimetry, and the derivation of site diameters or interaction distances is discussed. RBE-dose relations for aberrations in mammalian cells and the linear components which have been found at low doses of sparsely ionizing radiations are considered.

A rigorous treatment of radiation quality must be accompanied by an accurate statistical analysis of the observed dose-effect relations. The least-squares method is therefore applied to the linear-quadratic model; the least-squares criterion, the fit and the determination of the joint confidence region of the estimated parameters are discussed. The statistical procedures, although they are here related to the study of radiation induced chromosome aberrations, are of general relevance to quantitative radiation biology.

B-4-3 *Cell Cycle Kinetics and Radiation Therapy.* MORTIMER L. MENDELSOHN, Biomedical Division, Lawrence Livermore Laboratory, University of California, Livermore, California 94550, USA.

Radiation therapy as currently practiced balances the all-too-common recurrence of tumor due to undertreatment against the very real threat of complication due to overtreatment. These two risks (and hence the therapeutic ratio and the quality of therapy) can be modified by dose and dose distribution, fractionation, radiation quality, and combined therapy. One major source of leverage to generate differential sensitivity and an improved therapeutic ratio is the cell cycle of both the tumor and the relevant dose-limiting host tissues. Experimentally, the exploitation of this leverage poses the problems of identifying the relevant cells, of measuring their kinetic parameters, and of understanding how the kinetics relate to radiation sensitivity.

Theoretical design of optimal therapy then involves the interactions of several complex multiparametric dynamic systems: 1) the various cell cycles with their velocities, dispersions, and sensitivities; 2) the effects of the therapeutic agents directly on the cell cycle of surviving cells; 3) the effects of cellular depletion on the kinetics and the recruitment of cells from within and without the treatment field; and 4) the endpoints that define ablation of tumor versus complications arising from aplasia, scarring, infection, vascular damage and structural defects. (This work was performed under the auspices of the U.S. Atomic Energy Commission with the support of the U.S. Public Health Service (Grant No. 5 RO1 14533).)

B-4-4 *Theoretical Aspects and Implications of the Oxygen Effect.* JÜRGEN KIEFER, Strahlenzentrum der Justus Liebig-Universität, Giessen, West Germany.

On the basis of recent experimental results a simple unified kinetic model of the oxygen effect has been developed; the mathematical formalism leads to the well known "Alper-formula" for the dependence of OER on oxygen concentration showing that the assumption of type O and type N damage affecting different parts of the cell is not a necessary prerequisite for the experimentally found behaviour. The model allows an estimation of the life-time of radiation induced intermediates reacting with oxygen being compatible with recent experimental results. The influence of LET, as well as of repair and recovery processes will be discussed in terms of the model proposed.

B-5-1 *Recombination and UV-Mutagenesis in recB⁻ recC⁻ sbcB⁻ recF⁻ Strains of E. coli K-12.* TAKESI KATO,* ROBERT H. ROTHMAN,** AND ALVIN J. CLARK.**

Mutations may occur during the repair of gaps which are produced when UV-irradiated DNA is replicated without prior excision or photodestruction of pyrimidine dimers. UV-mutagenesis and gap-repair are blocked by *recA* mutations which also block the RecBC and the RecF pathways of genetic recombination. We have examined recombination, UV-mutagenesis and gap-repair in *recB⁻recC⁻sbcB⁻recF⁻* strains. The *recB* and *recC* mutations block the RecBC pathway and the *recF* mutation blocks the RecF pathway. The *sbcB* mutation raises the recombinational efficiency of the RecF pathway in a *recB⁻recC⁻* strain and may be irrelevant to our conclusions. In the quadruple mutant we have found recombination to be almost completely blocked, gap-repair to be partially blocked and UV-mutagenesis to be not blocked. Mutagenesis by UV was detected by appearance of histidine independent revertants of an UAA *his⁻* mutant and by appearance and linear increase with increasing UV dose of clear mutations in wild type lambda phage. Perhaps there is a minor recombinational pathway of recombination and repair which is error-prone. Other hypotheses are possible.

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B-5-2 *Nature of the Molecular Damage in Gamma-Irradiated Cells and Mechanisms Involved in Induced Mutagenesis in Haemophilus influenzae.* N. K. NOTANI AND V. R. JOSHI, Biology and Agriculture Division, Bhabha Atomic Research Centre, Bombay 400085, India.

Competent ^{3}H-labeled, *Haemophilus influenzae* cells, when exposed to gamma-rays to yield approximately 63% inactivation of colony formation, released upon incubation at 37°C, a small

amount of ^3H acid-soluble radioactivity in the medium. The amount of release of radioactivity increased somewhat as a function of incubation time. Intracellularly, some amount of DNA free from chromosome (and therefore presumably fragmented) was observed at early times which decreased upon incubation. Streptomycin resistant (30 μg/ml level) mutations could be induced maximally (138 per 10^6 surviving cells) in exponential-phase wild-type cells at a dose of 27 kR. In both recombination-defective strains DB117 and rec_2^-, maximum mutation frequency was respectively 3 and 21 per 10^6 surviving cells. DNA chain breakage, degradation (including base damage) and possible membrane damage are implicated in gamma-ray inactivation of colony formation. Mutation induction conceivably requires fragmentation followed by (error-prone) recombination, since we also find that unirradiated transforming DNA by itself is mutagenic in our system.

B-5-3 *The Effect of Caffeine, Acriflavine and Streptomycin Resistance on Ultraviolet-Induced Reversion in Strains of Escherichia coli.* THOMAS R. BARFKNECHT AND DELBERT M. SHANKEL, University of Kansas, Lawrence, Kansas 66045, USA.

The effect of caffeine and acriflavine on ultraviolet (UV) induced reversion in *Escherichia coli* B/r WP2 hcr^+, its excision minus derivative, hcr^- and streptomycin resistant mutants of each parent was studied. These strains are isogenic tryptophan (try) requiring auxotrophs. Try^+ revertants were picked and tested for their ability to support the growth of T4 amber and ochre mutants. Nutrient broth supplementation (5% v/v) of M9 minimal agar enhanced try^+ reversion in both hcr^+ and hcr^- strains. Induced reversion frequency was reduced in the Str^R strains. Caffeine (500 μg/ml) further enhanced reversion in the hcr^+ strains as did acriflavine (2 μg/ml); acriflavine, however, had its greatest effect on reversion when added to minimal agar supplemented only with 2 μg/ml try. Caffeine reduced the UV induced reversion frequency in the hcr^- strains and acriflavine may also reduce reversion frequency in the hcr^- strains. Therefore, Str^R acts as an antimutagen by inhibiting the expression of ochre suppressors and caffeine and acriflavine act as chemical antimutagens in hcr^- strains by inhibiting an error prone step in post replication repair.

B-5-4 *Pyrimidine Dimer Excision and Mutation Frequency Decline.* RICHARD A. HAAK AND RICHARD C. BOCKRATH, Medical Biophysics Program, Indiana University Medical School, Indianapolis, Indiana 46202, USA.

Defective strains of *E. coli* lacking excision repair are sensitive to ultraviolet light and have been shown incapable of mutation frequency decline (MFD). Therefore excision repair has been implicated in MFD. However certain physiological conditions are essential for MFD and their effect on excision repair has only been indicated qualitatively. Quantitative data on the kinetics of excision repair obtained by conventional paper chromatography with ^3H-TdR labeled *E. coli* in several different post-irradiation incubation environments has been obtained.

E. coli (WWU) grown in defined media lacking a required amino acid excise dimers to a similar extent as in fully supplemented media. In an MFD configuration containing chloramphenicol less excision is observed when compared to controls. MFD thus seems either independent of or inversely related to excision repair, rather than dependent on excision repair.

MFD is essentially complete in a half-hour, with an approximate "half-life" of 10 minutes. This is contrasted to excision repair with a "half-life" of more than 50 minutes. Thus the rate of MFD is markedly different than the rate of excision repair. If excision is involved in MFD, either directly or indirectly, this implies that excision rates at a specific site must be greater than the general rate of excision.

B-5-5 *Protein-Nucleic Acid Complexes Produced by UV Light and Chemical Carcinogens.* H. KUBINSKI, University of Wisconsin, Madison, Wisconsin 53706, USA.

Escherichia coli DNA and RNA were analyzed by velocity centrifugation in sucrose, gel electrophoresis, equilibrium density gradient centrifugation and chromatography on methyl-esterified albumin-kieselguhr (MAK). Complexes with bovine serum albumin, lysozyme and calf thymus histone were produced by UV irradiation and by a monoalkylating carcinogen, β-propiolactone (BPL). Such artificially produced nucleoproteins were detected by their altered

mobilities in centrifugal and electric fields, their decreased density in cesium salts and by the change in elution patterns from MAK columns as compared with untreated controls or with nucleic acids treated with UV or BPL alone. During treatment with BPL the complexes were initially soluble in ionic detergents, but subsequently a transition to an insoluble structure was observed. Similar complexes were induced *in vitro* by several other ultimate carcinogens tested under the same experimental conditions. There is no evidence that efficient mechanisms exist to excise and to repair the bulky and physically diverse nucleoproteins produced by radiation or a chemical carcinogen. It is suggested, therefore, that the formation of intermolecular associations should be considered as a possibly important and perhaps even necessary step toward the induction of mutations and malignant transformation. (Supported by US Public Health Service Grant CA 08959.)

B-5-6 *Gamma-Ray and Proton-Induced Mutagenesis: Test of the Misrepair Hypothesis in an Organism Lacking Excision-Repair.* D. S. NACHTWEY,[1] L. E. ROCHA[1], AND L. A. BRABY.[2]

Mutations may arise by the misrepair of potentially lethal lesions by one or more of the various types of enzymatic repair mechanisms. *Chlamydomonas reinhardi* lacks the excision-repair system that removes UV-induced pyrimidine dimers (Swinton and Hanawalt) and it does not exhibit split-dose recovery or low-dose rate recovery from UV-induced lethal damage. *Chlamydomnas* does, however, exhibit recovery from the lethal effects of γ-ray and 1.55 MeV proton irradiations, presumably by means of some other repair mechanism(s). To test whether this unknown repair mechanisms(s) might be involved in mutagenesis by misrepair, the mutation frequencies to a slow growth phenotype were compared for cells irradiated with (Co-60) gamma rays at dose rates of about 2 krad min^{-1} (high) and 0.08 krad m^{-1} (low). The ratio, D_{low}/D_{high} for a given level of mortality, increased almost linearly from 1.3 at 10% mortality to 5.6 at 90% mortality. However, the ratio of mutation frequencies, MF_{low}/MF_{high} at a given level of mortality remained constant at about 1.3.

Thus, cells irradiated at a low dose rate sustained 1.3–5.6 times more damage before reaching a given mortality than did cells irradiated at a high dose rate and presumably they repaired 1.3–5.6 times more radiation damage. However, they did not exhibit a proportionately increasing mutation frequency. Comparable studies with 1.55 MeV protons support these findings. These results suggest that misrepair of potentially lethal lesions is not a major factor in radiation mutagenesis in *Chlamydomonas*.

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B-5-7 *On Specific Character of Molecular Mechanisms of Mutagenesis Affected by High Energy Hadrons.* I. G. AKOEV AND S. S. YUROV. Institute of Biological Physics, Acad. Sci. USSR, Pushchino, USSR.

Mutagenic effect of secondary radiation from 70 GeV protons, caused basically by hadrons, i.e., by nuclear particles capable of strong interactions, was studied. The spectrum of r-mutations and the quantity (in per cent) of reversions ($rII \rightarrow rII^+$) caused by chemical mutagens in bacteriophage T4B were different. When studying cytogenetic changes in *Vicia faba* these results together with those obtained earlier allow us to relate the observed somatic and genetic effects of high energy hadrons to their characteristic peculiarities of strong interaction: a) possibility of nuclear interaction and fission of any of the chemical elements of macromolecules; b) plurality of generation of secondary particles, including antiprotons and multicharge ions; c) their distribution in the form of a narrow cluster. The result of the effect of high energy hadrons on the DNA molecule can be: a) at the atomic level—interaction with any atomic nucleus of the elements constituting the DNA molecule of accompanied by fission or nuclear transformation; b) at the molecular level—its break in any atom of the molecule (including sites with the strongest chemical bonds) and the plurality of molecule damage; c) on the chromosome level—a more plural damage with a greater probability of formation of some fragments of chromosomes, i.e., microdeletion.

B-6-1 *Some of the Binding Sites of the Radiosensitizer N-Ethylmaleimide in Chinese Hamster Cells.* JOY F. ARCHER AND WARREN K. SINCLAIR, Argonne National Laboratory, Agonne, Illinois 60439, USA.

Studies with N-ethylmaleimide in synchronous cultures of Chinese Hamster Cells showed that it is more effective in reducing cell survival at the end of S-phase than at any other stage of the cell cycle. These studies also suggested that N-ethylmaleimide in low concentrations inhibits the repair of radiation damage in oxygenated cells. This sensitizing ability appears to arise from the formation of stable complexes of N-ethylmaleimide with certain molecules inside the cell. N-ethylmaleimide was shown to bind to both cellular protein and non-protein sulfhydryl groups. The level of binding fluctuates with the stage of the cell cycle, being greatest in S-phase when the radiosensitizing effect is at a maximum. It was also found to bind to cellular DNA, and to affect some of the physical properties of isolated DNA.

These binding effects of N-ethylmaleimide and their possible role in the radiosensitizing ability of this compound in oxygenated cultures of Chinese Hamster Cells will be discussed.

B-6-2 *The Sensitization of X-Irradiated HeLa Cells by N-Ethylmaleimide.* ANTUN HAN, WARREN K. SINCLAIR, AND BRUCE F. KIMLER, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439, USA.

Previous studies with cysteamine and N-ethylmaleimide (NEM) in Chinese hamster cells showed that besides DNA synthesis a portion of the intracellular sulfhydryl appears to control the fluctuations in sensitivity of cells to x-rays during the cell cycle. In order to determine the relationship of fluctuations in survival in G_1 to intracellular sulfhydryl in mammalian cells the effect of NEM upon survival in HeLa cells was examined. The toxicity of NEM was found to be almost identical to that reported for Chinese hamster cells in the concentration range from 0.5–2.0 μM. When asynchronous HeLa cells were x-irradiated in presence of 0.5 μM NEM a sensitization factor of 1.3 was observed, the effect being primarily on the slope of the survival curve rather than on its shoulder. In synchronous cells, NEM sensitizes both early G_1 and late S cells when present during exposure. The cells are equally well sensitized, however, if NEM is added immediately after exposure, a finding previously reported by one of us (W. K. S.) for V-79 cells. Treatment of G_1 of S cells with NEM as a function of time after exposure shows that its effect decreases in magnitude with time for several hours after irradiation for S cells and for longer times for cells irradiated in G_1. Further experiments along these lines are in progress.

B-6-3 *Modification of Radiation Response of Mammalian Cells. Implications for Initial Radiochemical Events.* JAMES A. BELLI AND GRETA D. NAGLE, Harvard Medical School, Boston, Massachusetts, 02115, USA.

Chinese hamster cells (V79) in culture were x-irradiated in the presence of oxygen, nitrogen or nitrous oxide with or without tertiary butanol (70 mM) and survival curve characteristics compared. Tertiary butanol was protective when the radiation response was determined in oxygen (1.7). This protection was less prominent for irradiation in nitrogen or nitrous oxide; tertiary butanol and nitrous oxide were most protective (3.7 relative to oxygen alone). In addition to changes in terminal slope (D_0), we also observed changes in extrapolation number. In general, this parameter was in the range 1.4–2.3 for all irradiation conditions except oxygen alone (15.7). The implications of these data are 1) the OH° radical is important in the registration of radiation damage in mammalian cells only when oxygen is present; 2) of the initial damage registered in the absence of oxygen, ~75% was due to direct effect, and 3) OH° radical interactions in the presence of oxygen may produce a biochemical lesion which, in part, determines the width of the shoulder of the mammalian cell survival curve. (Supported by USPHS grant CA12662-03.)

B-6-4 *Radioprotective and Cytotoxic Actions of Cysteamine in HeLa S_3 Cells: What Causes the Paradoxical Toxicity?* MIKIO SHIKITA, YOSHINARI TAKAGI, TOYOZO TERASIMA, AND SANYA AKABOSHI, Natl. Inst. Radiol. Sci., Chiba-shi, Japan 280.

Cysteamine (MEA) at 0.5 to 5 mM powerfully kills HeLa S_3 cells, while it is much less toxic at higher concentrations. The toxicity increases gradually with time as H_2O_2 generates in the

medium ($2RSH + O_2 = RSSR + H_2O_2$). Addition of catalase to the culture abolishes the toxicity. In higher concentrations, MEA itself reacts with the formed peroxide and protects the cells ($H_2O_2 + 2RSH = RSSR + 2H_2O$). The radioprotective effect of MEA develops rapidly and the magnitude of dose reduction factor (Y) is a linear function of logarithm of the concentration (X) of MEA ($Y = 1.4 \log X + 1.2$). It is to be noted that there is a minimum effective concentration at about 0.7 m*M* and that the protective effect levels off at about 30 m*M*.

B-6-5 *Changes in Sensitivity to Ionising Radiation and to Bleomycin Occuring During the Life History of EMT6/M/CC Cells Growing in Monolayer Culture.* PETER R. TWENTYMAN AND NORMAN M. BLEEHEN, Middlesex Hospital Medical School, London, England.

EMT6/M/CC mouse tumour cells grown in monolayer culture pass from exponential growth into a 'plateau phase' which may be further subdivided on the basis of age and kinetic parameters.

The dose response to ionizing radiation is reduced in early plateau phase from that seen during exponential growth, mainly due to a change in D_0. At a later stage of plateau phase growth, the radiation sensitivity becomes closer to that seen in exponential phase. There is, however, no loss of shoulder even after several days almost complete cessation of proliferation.

The sensitivity to bleomycin (BLM) is also reduced during early plateau phase due to a loss of the initial fall in dose response curve seen for exponentially growing cells in late plateau phase. However, the sensitivity to BLM is greatly increased above that seen for exponential phase cells.

The response of these cells *in vitro* will be compared with results for the same cells growing as a solid tumour *in vivo*.

B-6-6 *Radiation Effects on Synchronized Radiosensitive Mutant L5178Y Cells; Cell Killing Division Delay. Chromosome Aberration.* HATSUMI NAGASAWA, D. F. PETERSEN,* AND J. T. LETT, Department of Radiology and Radiation Biology, Colorado State University, Fort Collins, Colorado 80521.

L5178Y S/S murine leukemic lymphoblasts have been shown to be extremely sensitive to x-irradiation as indicated by cell killing (Do; 38 rads), cell division delay, and giant cell formation.

The cells were isolated according to size; small (early S-phase), medium (mid S-phase), and large (late S-phase) by sucrose gradient centrifugation (2–10%). DNA fluorescence patterns and 50% cumulated mitotic indices with synchronized cells showed long division delay resulted from long G_2 delay (8–15 hr/100R).

Although cell size and protein content per cell were increased after x-irradiation, all cells had a diploid chromosome set in the first cell division which was very often an abnormal division.

Early S cells are the most sensitive and late S cells the least sensitive to cell killing, which correlates well with chromosome aberrations per cell in those phases. (This research is sponsored by NIH Grant No. CA10714.)

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B-6-7 *Modification Effects of the Substance Derived from Mouse Thymus on Radiosensitivity of Cultured Mammalian Cells.* TAKASHI AOYAMA, KURIKO KIHARA, AND KAZUMI NISHIGUCHI, Atomic Disease Institute, Nagasaki University School of Medicine, Nagasaki 852, Japan.

There was observed that the supernatant obtained by the centrifugation (15,000 × g, 60 min) of thymus suspension of mice contained a substance or substances which exhibited dual cytological actions on cultured mammalian cells. One of them was the stimulating action on the colony forming ability of the mammalian cells at concentrations from 10 to 20 μg/ml as a substance positive for Lowry's test and the other was the inhibitory one at concentrations higher than 100 μg/ml.

When the supernatant was added to the culture of mammalian cells irradiated with x-rays, it increased surviving fraction of the irradiated cells at the low concentration and decreased it at the high concentration. The former fact means that the substance possibly made a kind of recovery in the irradiated cells.

The activity of the substance was decreased by trypsin treatment and its molecular weight was estimated about 50,000. The purification of the active substance or substances and determination of its chemical structure are now under way.

B-6-8 *Survival of Synkaryons and of the Two Parental Strains Following X-Ray Irradiation.* A. Lievens, V. Heilporn, S. Limbosch, and F. Zampetti. Laboratoire d'Embryologie et de Cytologie Moléculaires, Université de Bruxelles, Bruxelles, Belgium.

Several synkaryons obtained by fusion of two mutant strains of chinese hamster fibroblasts (WG3H and A23) has been isolated. One of the parental strain (WG3H) is deficient for guanine-phosphoribosyltransferase and the other one (A23) is deficient for thymidine kinase. After x-irradiation, the survival of the synkaryons and of the parental cells has been established by the determination of cell ability to proliferate and form a macroscopic colony.

To obtain D_0 and n values which are the characteristics of the cell radiosensitivity, the experimental curves obtained from clone counting have been computed and compared. The significance of the results will be discussed in relation with the influence of chromosome number on cell radiosensitivity and with a possible interaction between the two parental genomes. (This work was supported by the European Community (Contract Euratom-ULB 099/72 BIAB).)

B-6-9 *Effect of Various Radiosensitizers on Survival and DNA Breaks in Mammalian Cells Irradiated in Cell Pellets.* Branko Palcic, David A. Agnew, and Lloyd D. Skarsgard. B.C. Cancer Institute and University of B.C., Vancouver, British Columbia, Canada.

The effect of chemical radiosensitizers on the survival of mammalian cells exposed to ionizing radiation (γ-rays) has been studied. It was previously reported (D. A. Agnew and L. D. Skarsgard, Radiat. Res. **57**, January 1974) that at least some drugs which sensitize anoxic cells in dilute suspension lose their sensitizing effect if the cells are irradiated while in close contact (cell pellets). Recently, at least two radiosensitizers have been investigated, both nitroimidazoles, which exhibit no loss of sensitizing effect in cell pellets.

The production and the repair of DNA single-strand breaks have been studied in sensitized cells in dilute suspensions and in cell pellets. The objective of these studies is to gain an understanding of the mode of action of various radiosensitizers as well as to examine some of the possible mechanisms responsible for the loss of sensitizing effect of most sensitizers in cell pellets. (This work has been supported by the National Cancer Institute of Canada.)

B-6-10 *The Effect of Post-Irradiation Hypoxia on Radiation Survival of Mammalian Cells.* R. J. Schulz and D. Roberts, Yale University, New Haven, Conn. 06510, USA.

It has been determined that post-irradiation hypoxia reduces the survival of HeLa and Chinese hamster cells, presumably by interfering with oxygen-dependent repair processes. The nature and extent of the increased mortality depends upon the oxygen tension of the cells during irradiation and their temperature during the experimental period. Preliminary experiments established that about five hours of post-irradiation hypoxia are required to achieve maximum reduction in survival of cells irradiated in air. When HeLa cells were maintained at 37°C, survival was reduced by one-half, when the dose was 600 rads. When the temperature was 22°C, during the experimental period, survival was reduced by one tenth. For cells irradiated in air, post-irradiation hypoxia reduces the extrapolation number (n) of the survival curve but has no effect upon the mean-lethal dose (D_0). When hypoxic cells are irradiated, post-irradiation hypoxia reduces D_0 but leaves n unchanged (n was always found to decrease from about 7 to 2 when HeLa cells were irradiated in hypoxia). These results imply that two types of radiation damage are produced in cells, one type that is permanent and another that is subject to repair in the presence of oxygen, and that the proportions depend upon the oxygen tension of the cells during irradiation.

B-7-1 *Studies on the Oxygen Enhancement of the Yield of DNA Single-Strand Breaks in Mammalian Cells.* Ruth Roots and Kendric C. Smith, Department of Radiology, Stanford University School of Medicine, Stanford, California 94305, USA.

The DNA single-strand breakage efficiency was examined under air or N_2 x-irradiations in

Chinese hamster ovary cells. An oxygen/nitrogen strand-breaks ratio (ONBR) of about 4 was obtained. After cellular inactivation by heat, the ONBR diminished greatly, the degree of diminution depending on the quality of hypoxia, such that under relatively poor hypoxia, the ONBR approaches one. In a previous report [Radiat. Res. Soc. Meeting Abstract 55, 520 (1973)], we interpreted that the near abolishment of the ONBR after cellular inactivation by heat or a sulfhydryl binding agent was due to repair enzyme inactivation; however, further studies with sulfhydryl compounds (R-SH) and $Na_2S_2O_4$ (an oxygen scavenger) now indicate that the O_2:R-SH balance contributes significantly to the ONBR. Based on these data, we suggest that chemical restoration of radiation injury to DNA by cellular sulfhydryl compounds can account for the major part of the O_2/N_2 DNA single-strand breaks ratio observed immediately after x-irradiation.

B-7-2 *The Role of the Hydroxyl Radical in Strand-Break Induction and Biological Inactivation of DNA by Ionizing Radiation.* PHILLIP ACHEY AND HOLLIS DURYEA, Radiation Biology Laboratory, Department of Radiology, 403 NSC, University of Florida, Gainesville, Florida 32611, USA.

A large fraction of the radiation damage expressed as strand breaks and loss of biological activity delivered to DNA in aqueous solution by gamma-rays results from "indirect action," as demonstrated by the large change in radiosensitivity when DNA is exposed in the dry state or the wet state (W. Ginoza, Ann. Rev. Microbiol., 1967). We have used the circular covalently closed double-stranded replicative intermediate of ϕX174 synthesis (RFI) to ask which water radicals might be responsible for this "indirect action." Our results indicate that the hydroxyl radical is the major species involved in secondary radiation damage. 37% survival doses (D_{37}) from strandbreak induction of 32 krads in the presence of .05 *M* potassium iodide (an efficient hydroxyl radical scavenger) and 0.8 krads in the absence of KI are observed for irradiation in 0.01 *M* phosphate buffer under anoxic conditions. Potassium ferrocyanide, which is equally as reactive with the hydroxyl radical as potassium iodide, was equally effective in protection of RFI DNA from radiation-induced strand breaks. Biological inactivation by irradiation was also protected against by the hydroxyl radical scavenger. The D_{37} for survival from biological inactivation was 30 krads in the presence of KI and 1.4 krads in the absence of KI. On the other hand, potassium nitrate (an efficient electron scavenger) afforded little protection from radiation damage. (Work was supported by NIH Grant CA 12447-03. One of us (P. A.) is supported by Career Development Award CA 70267-02 from the Cancer Institute of the NIH.)

B-7-3 *Repair of X-Ray Damage to the DNA in Asynchronous Chinese Hamster Ovary Cells: The Effect of 5-Bromodeoxyuridine.* TIEN-SHIH WANG AND J. T. LETT, Department of Radiology and Radiation Biology, Colorado State University, Fort Collins, Colorado 80521, USA.

Chinese Hamster Ovary (CHO) Cells were cultured in McCoy 5A medium containing 1×10^{-6} *M* 5-bromodeoxyuridine (BudR). Under these experimental conditions, 5-bromodeoxyuridine partially replaces thymidine in the DNA of CHO cells. Cells cultured in BudR grew exponentially without measurable change in cell size, shape, or in generation time. However, aerobically x-ray irradiated single-strand breaking efficiency increased in the BudR-labelled cells as measured by alkaline sucrose sedimentation techniques. After two hours of post-irradiated incubation under normal growing conditions, fragmented BudR-labelled DNA were rejoined to a size of 165S. After longer incubation time more rapidly sedimenting species began to appear in the gradient. Present indications suggest that incorporated BudR does not interfere with the ability to repair strand breaks and partially reconstruct DNA chromosomal structure. In this regard, the mammalian cell parallels the bacteria *Micrococcus radiodurans*. (This research is sponsored by NIH Grant No. CA10714.)

B-7-4 *Double-Strand Breaks Resulting from Proximate Single-Strand Breaks in Mammalian DNA.* PETER M. CORRY, The Univ. of Texas System Cancer Center, M. D. Anderson Hosp. and Tumor Inst., Houston, Texas 77025, USA.

Mammalian DNA was irradiated both inside the cell and in solution subsequent to isolation. These irradiations were carried out over the dose range of 500 rads to 2 megarads and the result-

ing molecular weight distributions as well as the frequency of double (DSB) and single (SSB) strand breaks were determined by analytical and sucrose density gradient centrifugation. A mathematical model was then applied to determine the frequency of double strand breaks arising as a result of closely spaced single strand breaks. The results demonstrate that for DNA irradiated in solution the majority (~98%) of DSB arise due to proximate SSB, probably as a result of indirect action. However for irradiation of the DNA within the living cell a maximum of 0.3 percent of the DSB arise from this process at a dose level of 1 kilorad and 7 percent at 25 kilorads. As the dose increases an increasing proportion of the DSB arise by this process. These data imply that the vast majority of DSB are not related to SSB induction in the mammalian cell at biologically significant doses. Implications as to the presence and size of radiosensitive regions of the DNA are also made. (Work supported by AEC contract AT-(40-1)-2832.)

B-7-5 *Modification of DNA Radiation Damage by Low Concentrations of Some Metal Ions.* IGOR A. SERGEANT, Institute of Plant Physiology, Ukrainian Academy of Sciences, Kiev 252 627, USSR.

The possibility of DNA radiation damage modification at the free radical (FR) level was studied by ESR method. Co^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+} ions were introduced into DNA preparations in concentrations corresponding to 1 metal ion per 5–1000 nucleotide pairs (1:5–1:000). The lyophilized preparations were in vacuo irradiated by Co^{60} in 5–10 Mrad dose. High metal ions concentrations lead to decrease in primary FR radiation yields for all the ions studied. The concentrations below 1:50 increase FR recombination rate. The usual dependence of FR radiation yields upon metal ion concentration was registered in the case of Cu^{2+}, Fe^{2+}, Fe^{3+} ions: radiation yield decreases when concentration increases. An anomalous concentration dependence is found in the case of Co^{2+} ion in the concentration range 1:20–1:200: the sensitizing effect. The maximum value of sensitivity coefficient is 3. As a result of these experiments we can speak about the existence of two different mechanisms of ionic influence upon FR yields in irradiated DNA. The DNA radiosensitivity depends upon metal ions binding to DNA molecule, the concentration dependence of this effect being rather complex and depends upon nature of ion. These effects are used to the elucidation of possible mechanisms of DNA radiation damage *in vivo* and to the elucidation of radioprotective and radiosensitizing action of metal ions.

B-7-6 *Single-Strand Breaks and Terminal End Groups in DNA of Irradiated Thymocytes.* THÉRÈSE COQUERELLE, MANFRED LENNARTZ, AND ULRICH HAGEN, Institut für Strahlenbiologie, Kernforschungszentrum Karlsruhe, D 75 Karlsruhe 1, Postfach 3640, West Germany.

Rat thymocytes suspended in Hank's solution were irradiated with fast electrons under oxygen and under anaerobic conditions. The DNA was isolated and the number of single strand breaks were determined in the analytical ultracentrifuge after denaturation with alkali as well as by denaturation by heating at 75°C in the presence of formaldehyde. In this way, the actual single strand breaks as well as the alkali labile bonds in the nucleotide strand can be evaluated. There is an oxygen effect for the total number of strand breaks by a factor of 3.8, whereby this effect is mostly due to an increase of the number of actual strand breaks. Furtheron, the cells were incubated at 37°C for various times after irradiation. For the actual strand breaks, a repair will be observed within the first 15 min, whereas the alkali labile bonds are not repaired in this time. The 3′- and 5′-terminal endgroups of the strand breaks, obtained under the various experimental conditions, will be characterized by means of various enzymes (terminal nucleotide transferase, DNA-polymerase I and polynucleotide kinase). The results allow a discussion of the enzymatic processes involved in the early repair of strand breaks.

B-7-7 *Radiation Effects on the DNA of Chromatin Extracted from Cultured Mammalian Cells.* L. K. Mee and S. J. Adelstein, Department of Radiology, Harvard Medical School, Boston, MA 02115, USA.

We are studying the radiochemical consequences of the *in vitro* irradiation of chromatin extracted from cells which have been well-characterized with regard to their radiobiological responses. Chromatin was isolated from nuclei of Chinese hamster cells. Chemical analyses give a composition in the ratio DNA:RNA:histone:non-histone protein of 1.0:0.2:1.4:0.6. The DNA in the chromatin has been examined by pre-labeling the DNA, layering the extracted chromatin onto alkaline sucrose gradients and observing the sedimentation behavior with ultracentrifugation. The DNA of unirradiated chromatin sediments in a single band corresponding to a peak molecular weight of 4×10^7. X-irradiation shifts the profile to that of lower mean molecular weight. From changes in the sedimentation pattern of chromatin irradiated with varied doses a G-value of 0.02 is obtained at a DNA concentration of 1 mg/ml. If direct action in DNA alone is assumed, a value of 5 eV per single strand break can be calculated for chromatin irradiated *in vitro* as opposed to a value of 100 eV per single strand break for chromatin extracted from irradiated cells. (Supported by USPHS Grant No. AMO 4219.)

B-7-8 *The Primary Structure of DNA and Its Changes in the Irradiated Organism.* G. A. Kritsky and S. V. Alexandrov. Bach Institute of Biochemistry, USSR Academy of Sciences, Moscow, USSR.

The cluster structure of DNA has been studied in connection with the radiosensitivity of the organism and with cancerogenesis. It is shown that animals whose DNA contains the relatively higher percent of the pyrimidine nucleotide sequences are characterized by higher radiosensitivity. The similarity in the changes appearing in DNA of blood forming system at the development of leucaemia and after the organism irradiation was pointed out. The ionizing radiation as a cancerogenic factor changes the quantitative ratio of the pyrimidine nucleotide sequences in the DNA of blood forming system in the same manner that is characteristic for DNA of the malignant cells. The results obtained may be useful for revealing the molecular processes at the radiation damage and understanding the mechanism of cancerogenesis.

B-7-9 *Effect of Radiation Sensitizers on the Radiolysis of Nucleic Acid Bases.* A. J. Varghese and G. F. Whitmore, The Ontario Cancer Institute, 500 Sherbourne Street, Toronto, Ontario, M4X 1K9, Canada.

The increased use of chemical compounds for the sensitization of hypoxic tumor cells to ionizing radiation suggests the desirability of understanding on a molecular level their mechanism of action. To attain this objective, we have undertaken a study of the effect of a number of sensitizers on the radiolysis of nucleic acid bases. The bases were irradiated with x-rays in aerated and deaerated aqueous solutions in the presence and the absence of the sensitizer. The products were separated, chemically identified and the yields determined. The results showed that with *p*-nitroacetophenone or its mannich base as the sensitizer the G-values for the loss of thymine and uracil were markedly enhanced when the irradiations were carried out in deaerated aqueous solutions. The major pyrimidine products were the respective *cis* glycols. In aerated solution such a difference was not observed. Neither sensitizer had any significant effect on the G-value for the loss of adenine in aerated or deaerated solutions even though a sensitizer-adenine adduct was formed. Under the same experimental conditions, metronidazole showed a slight radioprotective effect with all three bases.

B-8-1 *A Continuing Study on the Somatic Effects of a Low-Level, Fractional MPC Body Burden of Ra-226.* JACK J. GABAY,* CHARLES D. BROWN,† AND IAN H. PORTER.‡

A continuing study has been made of an individual known to have been occupationally exposed to Ra-226 twenty-two years ago.

The existence of a Ra-226 body burden has been established by whole-body counting and expired breath radon measurements made at several different facilities over the past 13–20 years.

Possible somatic effects have been studied in terms of general health, changes in skeletal structure, examination of excised bone fragments, clinical blood chemistry and assessment of chromosomal damage in peripheral blood and bone marrow.

The possibility of genetic effects in the subject's four children will be discussed briefly.

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B-8-2 *Factors Influencing the Intestinal Absorption of Radiostrontium in Man.* HERTA SPENCER, CLEMONTAIN NORRIS, AND GERALDINE BELL, Metabolic Section, VA Hospital, Hines, Illinois 60141, USA.

The effect of several compounds on decreasing the intestinal absorption of radiostrontium has been investigated in man under strictly controlled dietary conditions using orally administered tracer doses of ^{85}Sr. Plasma levels, urinary and fecal excretions of ^{85}Sr were assayed and the absorption of ^{85}Sr was determined from the fecal ^{85}Sr excretions. The study conditions were found to be of greatest importance in influencing the changes of the absorption of ^{85}Sr. The presence or absence of food greatly affected the absorption of the tracer. The absorption of ^{85}Sr was very high in the absence of food and could be decreased by a factor of 2 to 3 by the intake of either an entire breakfast meal or by the ingestion of the small amounts of calcium or of phosphorus contained in the breakfast meal. The daily intake of relatively large amounts of calcium, given in divided doses with food, had little effect on the absorption of ^{85}Sr and the daily intake of phosphate given in the same manner decreased the absorption of ^{85}Sr by 20–25%. In contrast, a single relatively large dose of 0.5 to 1 gm calcium or a single dose of 1.2 gm phosphate given either with or without food decreased the absorption of ^{85}Sr distinctly. However, this effect was greater when these amounts of calcium or phosphorus were given in the absence of food. Aluminum phosphate gel was most effective in decreasing the absorption of ^{85}Sr, by an average of 87%, while aluminum alone given as $Al(OH)_3$ was about 50% as effective as aluminum phosphate gel. Orally administered stable strontium decreased markedly the total body retention of ^{85}Sr but this decrease was not due to a decrease of the intestinal absorption of ^{85}Sr but to an increase of the urinary ^{85}Sr excretion. (Supported by USAEC Contract AT(11-1)-1231-96.)

B-8-3 *Metabolism of ^{35}S Sodium Sulfate in Man.* KEITH S. PENTLOW, HELEN Q. WOODARD, KLAUS MAYER, JOHN S. LAUGHLIN, AND RALPH C. MARCOVE, Memorial Sloan-Kettering Cencer Center, New York, New York 10021, USA.

Data on ^{35}S in tissues were obtained from biopsies and autopsies made during, and up to several months after therapy of chondrosarcoma and chordoma. From 70% to 90% of an I.V. dose was excreted in the urine during the first three days. Initial uptakes in tumors and red bone marrow were about ten times that in muscle and were nearly equal, but retentions differed greatly. The biological halftimes of the major components of ^{35}S in tissue were: chondrosarcoma, about 60 days; chordoma, 18 days; marrow, 9 days; blood, 0.25 days; muscle, 3 days. There were minor components with longer T(biol) in several tissues. Uptake in epiphyseal cartilage was comparable to

that in chondrosarcoma; uptake in other types of cartilage was lower. For an administered dose of 1 mCi/kg body weight the integrated radiation doses were about 155 rads for chondrosarcoma, 85 rads for chordoma, 45 rads for red marrow, 2 rads for blood. (Supported in part by grants from AEC and NCI.)

B-8-4 *Internal Dose Calculation for ^{177}Lu-Chloride.* DIETER M. H. GLAUBITT, HANS DETLEV ROEDLER, AND RENATE S. MARX, Institut für Nuklearmedizin, Städtische Krankenanstalten, D-415 Krefeld, West Germany, and Nuklearmedizinische Abteilung, Klinikum Steglitz der FU, 1 Berlin, West Germany.

The use of ^{177}Lu for bone scanning has been proposed several times. This radionuclide decays with a physical half life of 6.8 days. In order to calculate the radiation dose we performed kinetic studies with ^{177}Lu-chloride in male Wistar rats. We determined the distribution factors and the effective half-times in the total body and in 14 organs. The results permitted the estimation of the absorbed dose according to the concept of absorbed fractions.

Two days after intravenous injection of carrier-free ^{177}Lu-chloride 52.0% of the radioactivity is found in the total skeleton. The absorbed dose in the total skeleton amounts to 5.2 mrad/μCi ^{177}Lu; it shows almost half the value in the kidneys and a quarter of the value in the liver. If the absorbed doses calculated for the rats are corrected for the different distribution factors for the organs in the rat and in man, the absorbed dose for ^{177}Lu in the total skeleton is 6.9 mrad/μCi ^{177}Lu. In kidneys, liver, testes, and several other organs the corrected absorbed doses are relatively lower than the uncorrected values.

B-8-5 *Low Dose In Vivo Human Calcium Measurement by $^{48}Ca(n, \alpha)^{37}Ar$ Reaction.* RODNEY E. BIGLER AND JOHN S. LAUGHLIN, Memorial Sloan-Kettering Cancer Center, New York, New York 10021, AND RAYMOND DAVIS, JR. AND JOHN C. EVANS, Brookhaven National Laboratory, Upton, New York 11973, USA.

An experiment, designed to determine requirements for whole body calcium assays *in vivo* in humans using the $^{40}Ca(n, \alpha)^{37}Ar$ reaction, has been carried out. Low background counting techniques (0.5 cts/day) were applied to assay breath samples containing as little as 0.01 dpm_{EOB}. Six samples were taken up to 12.5 hours following a two minute bilateral fast neutron exposure [cyclotron—$^{9}Be(^{3}He, n)$] with less than 2 mrad absorbed dose. Total body production of ^{37}Ar amounted to 5.2 $\pm$ 0.5 dpm_{EOB}. Breath analysis yielded a total fractional release of 0.6, over 90% of which is released within 5 hours. Radon release from humans with long term radium burdens indicates this fractional release is similar in magnitude and should be stable in adults. Dose requirements for a five hour sample collection time and one week analysis would be 0.6 mrads. (This work supported in part by AEC(11-1)-3521, NCI 08748-08B and the Atomic Energy Commission.)

B-8-6 *Excretion Rates of ^{226}Ra for Radium-Burdened People 10 to 55 Years after Exposure.* R. B. HOLTZMAN, D. R. KUCHTA, AND J. Y. SHA, Center for Human Radiobiology, Argonne National Laboratory, Argonne, Illinois 60439, USA.

The rates of excretion of ^{226}Ra have been determined for 30 subjects with residual body burdens ranging from 16 to 8500 nCi ^{226}Ra. The time since their last exposure ranged from 10 to 55 years, and their ages at the time of last measurement were from 50 to 75 years. The excretion rates, expressed as a percentage of ^{226}Ra body content (total coefficient of excretion) and corrected for normal environmental levels of ^{226}Ra, ranged from 0.5% to 8% per year. The ratio of urinary to fecal excretion rates was 0.017 $\pm$ 0.002 (standard error, 25 subjects). The relative constancy of this ratio permits the more easily measured urinary excretion rate to be used to estimate the total excretion rate. This is particularly useful where fecal collections are not available or are unreliable. Results on several of the subjects at different times several years apart showed expected decreases in excretion rates with time. Factors which may affect the coefficient of excretion such as age, exposure type, time since last exposure, and body content, will be discussed.

B-8-7 *The Excretion Rate of Plutonium 10,000 Days after Acquisition.* J. RUNDO, P. M. STARZYK, AND J. SEDLET, Argonne National Laboratory, Argonne, Illinois 60439, USA.

For periods of 8–14 days, complete collections of urine and feces were made on a metabolic ward, from three persons who had received known amounts of plutonium in 1945–47. The 24-hour urine samples were analyzed for plutonium to determine the mean daily excretion at about 10,000 days after acquisition. Two subjects who had received ^{239}Pu(IV) citrate, were excreting daily $1.4 \times 10^{-3}\%$ and $2.5 \times 10^{-3}\%$ of the initial dose; Langham's equation for average daily urinary excretion predicts a rate of $2.9 \times 10^{-4}\%$ of the initial dose at 10,000 days. The third subject received ^{238}Pu(VI) nitrate and his urinary excretion rate at 9474 days was very approximately $10^{-4}\%$ of the initial dose, with a large uncertainty due to inadequate information on the early systemic burden, and to the very small amounts of activity in the urine.

Preliminary results of analyses for ^{239}Pu in the feces of the first two subjects indicate that the excretion rate by this route may have approached 50% of the urinary excretion rate. (Work performed under the auspices of the U.S. Atomic Energy Commission.)

B-9-1 *Some Aspects of Airborne Particles and Radiation in the Atmosphere.* G. M. HIDY, Rockwell International Science Center, Thousand Oaks, CA, USA.

There are two major ways that thermal radiation may interact with airborne particles in the Earth's atmosphere. The first is a classical problem in which the radiation balance is influenced by scattering and absorption from haze or aerosol layers in the atmosphere. Absorption is generally believed to have a minor effect on attenuation of radiation compared with scattering. In the visible and infrared, scattering by submicron sized particles can have a substantial influence on the balance of radiation in the atmosphere. Considerable interest in this question has developed recently with the assessment of the global impact of air pollution in the lower atmosphere and of exhaust emissions from aircraft flying in the stratosphere. In the first part of this review, the physics of atmospheric aerosol scattering is summarized, and the current status of observational knowledge is examined to identify areas of greatest uncertainty.

The second way the radiation is involved in aerosols lies in the production in the atmosphere. Until recently, evidence for airborne particle production by atmospheric photochemistry was quite ambiguous. However, with the advent of results from several new field experiments the role of photochemistry in the generation of aerosol precursors from traces of such gases as sulfur dioxide, nitrogen oxides, and olefinic hydrocarbons is much better understood. The remaining part of this paper is devoted to the discussion of several new observations that indicate the complicated nature of photochemical aerosol formation in the polluted and non-polluted atmosphere.

B-9-2 *The Interdependence of Ozone and the Oxides of Nitrogen in Urban Air, the General Troposphere, and the Stratosphere.* HAROLD S. JOHNSTON, Department of Chemistry, University of California, Berkeley, California 94720, USA.

In the stratosphere, ozone is formed from solar ultraviolet radiation and oxygen, and it is destroyed by a number of processes in decreasing order of importance: fast catalytic cycles involving the oxides of nitrogen (NO_x), direct reactions of oxygen atoms and ozone (O_x), fast catalytic cycles involving free radicals derived from water (HO_x), and transport to the troposphere. In urban air basins, ozone is rapidly formed by NO_x-catalyzed, photochemical oxidation of olefins and other active hydrocarbons (HC); ozone is destroyed by NO_x, HO_x, HC, and by contact with surfaces. The dual role of the oxides of nitrogen, both forming and destroying ozone, in urban air pollution (smog) causes these reactions to be complex and non-linear. In the global troposphere, ozone is slowly formed from the "smog" reactions involving natural methane and nitrogen oxides, and ozone is transported into the troposphere from the stratosphere by air motions. General tropospheric ozone is destroyed both by chemical reactions in the gas phase (HO_x) and (NO_x) and by collision with surfaces at ground level.

B-9-3 *Gas-to-Particle Conversion in the Atmospheric Environment by Radiation-Induced and Photochemical Reactions.* K. G. VOHRA, Bhabha Atomic Research Centre, Bombay, 400085, India.

During the last few years a fascinating new area of research involving ionizing radiations and photochemistry in gas-to-particle conversion in the atmosphere has been developing at a rapid pace. Two problems of major interest and concern in which this is of paramount importance are: (1) radiation induced and photochemical aerosol formation in the stratosphere and, (2) role of radiations and photochemistry in smog formation. The peak in cosmic ray intensity and significant

solar UV flux in the stratosphere lead to complex variety of reactions involving major and trace constituents in this region of the atmosphere, and some of these reactions are of vital importance in aerosol formation. The problem is of great current interest because the pollutant gases from industrial sources and future SST operations entering the stratosphere could increase the aerosol burden in the stratosphere and affect the solar energy input of the troposphere with consequent ecological and climatic changes. On the other hand, in the nuclear era, the atmospheric releases from reactors and processing plants could lead to changes in the cloud nucleation behaviour of the environment and possible increase in smog formation in the areas with significant levels of radiations and conventional pollutants. A review of the earlier work, current status of the problem, and some recent results of the experiments conducted in the author's laboratory are presented. The possible mechanisms of gas-to-particle conversion in the atmosphere have been explained.

B-9-4 *Effects of Air Pollution on Ecological Processes.* N. R. GLASS, U.S. Environmental Protection Agency, Corvallis, Oregon, USA.

Photochemical oxidants are produced by atmospheric chemical reactions which are initiated by the absorption of sunlight by an atom, molecule, free radical, or ion. The most common photochemical oxidants are ozone, peroxyacetyl nitrate (PAN), and nitrogen dioxide. In addition to these common air pollutants, there are also significant effects associated with sulfur dioxide, fluorides, ethylene, pesticides, chlorine, heavy metals, acid aerosols, ammonia, aldehydes, hydrogen chloride, hydrogen sulfide, and particulates. Of the above list, ozone and sulfur dioxide are the most widely distributed and also the most abundant in the U.S. In the eastern United States, the predominating air pollutants of wide distribution are the oxidant air pollutants and sulfur dioxide. In the western United States, the predominating problems are associated with oxidant air pollutants.

National dollar loss estimates from air pollution damage due to crops and other agricultural commodities have been projected from statewide surveys utilizing visible plant injury as a loss criterion. One state, Pennsylvania, has estimated their losses in recent years at 11.5 million dollars (Lacasse, *et al.*, 1970). Nationally, gross estimates of the damage and losses to agriculture, which are related to air pollution, have varied between 500 million and 1 billion dollars annually. These losses include only the effects of air pollutants on crop plants. It is clear that when reductions in growth and yield plus the additional effects on ornamental plants, forest production, wild life, soils, and esthetic values are considered, these gross estimates are much too low.

The effects of the predominating air pollutants, sulfur dioxide and ozone, are discussed below. The effects on individual animals, animal populations, individual plant species, and plant species populations and communities, and the effects on soil processes are discussed as well. The impact of these air pollutants on soil nutrient release rates, soil chemistry, soil litter decomposition rates, and soil fertility can be considerable. Direct and indirect effects on plant photosynthetic rates, primary production, and plant species composition, plus the animal populations both via direct mechanisms and because of the influence on plant communities, these air pollutants exert a substantial ecological impact.

B-10-1 *Introduction.* MITIO INOKUTI, Argonne National Laboratory, Argonne, Illinois 60439, USA.

We devote the Symposium to the memory of the late Professor R. L. Platzman, who contributed outstandingly to our topic, i.e., the nature of ionization and the behavior of gaseous ions. The Symposium will summarize the current understanding and bring out its implications to radiation actions. The morning session covers basic aspects. Discussion then will include many details and intricacies such as pre-ionization and the sharing of excess energy between ions and electrons. The afternoon session starts with two lectures on less usual classes of ionization processes. Energy transfer from excited species exemplifies a mode by which energy initially deposited gets used later to cause a new species (ions)—a question of continuing interest to the radiation chemist. The interpenetration of electron shells upon slow ion-atom collisions has recently attracted much effort of atomic physicists, and promises to become significant to radiation research, especially with the advent of heavy-ion accelerators. The last two lectures dealing with the behavior of gaseous ions will offer a logical link between radiation physics and chemistry. (Work performed under the auspices of the U.S. Atomic Energy Commission.)

B-10-2 *Photoionization.*† J. BERKOWITZ, Argonne National Laboratory, Argonne, Illinois 60439, USA.

The first order interaction of electromagnetic radiation with gaseous matter is photoabsorption. When photoabsorption occurs at photon energies larger than the ionization threshold, a large fraction (though not all) of the photoabsorption leads to ionization for molecular systems. The intensive study of this photoionization process during the past ~15 years by photoionization mass spectrometry and photoelectron spectroscopy has revealed the existence of numerous processes which would be obscured if one were measuring total ionization cross sections. Photoelectron spectroscopy provides us with a clear picture of the orbital structure of electrons in molecules, and in addition shows us how ionization proceeds by removing single electrons (primarily) from successive orbitals. The ions formed can thereby be left in various states of excitation. An active study at the present time is the measurement of the partial cross sections for formation of these various states, and their dependence on photon energy. Photoionization mass spectrometry provides some clues about the subsequent life history of these ionic states—their relative probability of survival or decomposition into fragments. The autoionization and Auger processes and their influence on mass spectra will be discussed. The relationship of these studies to those involving irradiation with fast charged particles will be covered by other speakers.[1,2]

† Work performed under the auspices of the U.S. Atomic Energy Commission.

[1] M. J. van der Wiel.

[2] Y.-K. Kim.

B-10-3 *Electron Energy Deposition in Matter at the Molecular Level.* M. J. VAN DER WIEL, F.O.M. Institute for Atomic & Molecular Physics, Amsterdam, The Netherlands.

For the understanding of radiation mechanisms it is essential to have information on the energy degradation of electrons, being the product of any type of ionizing radiation but also as primary agent. Recent experimental work with slow electrons on isolated molecules is of interest mainly because of the progress made in studying two aspects of dissociation phenomena: the excitation state and kinetic energy of fragments, both of which play an important role in the evolution of radiation action.

Most of the energy deposition of fast electrons is governed by Bethe's expression for the cross section. Prediction of absolute yields hinges on the knowledge of generalized oscillator strengths (GOS), for a measurement of which experimental techniques are readily available at present. For many applications it sufficies to have only the dipole term of the GOS and its spectral distribution. It will be shown that such distributions, normally the result of photoabsorption studies, can also be obtained reliably from electron impact experiments. These experiments cover a variety of aspects, ranging from total absorption or ionization to production of particular electronic continuum states or of particular fragments. Inner shell processes are included in the discussion for two reasons. They lead to production of Auger electrons with energies near the cross section maximum, while also the highly excited hole states frequently decay to strongly repulsive states of the molecule, which are accessible from the ground state with only small probability.

B-10-4 *Secondary Electron Spectra.** YONG-KI KIM, Argonne National Laboratory, Argonne, Illinois 60439, USA.

Recent developments both in theory and experiment on secondary electrons ejected from free atoms and molecules by energetic charged particles are reviewed. The consistency of experimental data is checked against the qualitative and quantitative features expected from theory. For instance, slow secondary electrons are ejected mostly by glancing collisions, and the data on photoionization[1] and various partial cross sections[2] provide an excellent check for the consistency of experimental data on slow secondary electrons. On the other hand, fast secondary electrons are mostly ejected either by knock-on collisions or by the Auger process following inner-shell ionizations. I shall suggest some desirable experiments and discuss the sensitivity of yields such as the W value on the accuracy of various cross sections, including those for secondary electrons.

* Work performed under the auspices of the U.S. Atomic Commission.

[1] J. Berkowitz, this symposium.

[2] M. J. van der Wiel, this symposium

B-11-1 *X-Ray Induction of Leukemia Virus in Vitro.* ALAIN DECLÈVE, EDWARD GELMANN, OHTSURA NIWA, AND HENRY S. KAPLAN, Stanford University School of Medicine, Stanford, California 94305, USA.

Whole-body X-irradiation has been shown to induce the replication of a leukemogenic virus and the development of lymphomas in C57BL mice (Lieberman and Kaplan, *Science* 130, 387, 1959; Kaplan, *Cancer Res.* **27**, 1325, 1967). The complexity of the *in vivo* system has made it difficult to study the mechanism by which radiation triggers viral activation. *In vitro* induction of leukemia virus by halogenated pyrimidines has been shown to require incorporation of the chemical agent into cellular DNA. Rowe and co-workers have been able to induce low levels of leukemia virus from nonproducer cell lines of AKR mice with X rays. We have been able to detect large amounts of infectious virus in C57BL, NIH, and BALB/c secondary mouse embryo fibroblast cultures after treatment of these cells with either 20 μg/ml of IUdR or 750 rads X-irradiation. Overlays of established C57BL or NIH fibroblast lines or a normal rat kidney (NRK) cell line were added. Subcultures revealed the initial appearance of viral antigens in the cytoplasm of all mouse cell cultures after approximately 9 cell generations. Infection thereafter developed rapidly to reach plateau levels at which 70 to 90% of cells were positive for viral antigen by indirect immunofluorescence, and 40–50% of cells had acquired plaque-forming ability on XC cells. We were also able to detect infectious virus by overlay of NRK cells on IUdR-treated or on irradiated BALB/c cells. Viral infection in the rat cells progressed more slowly than that in murine cells. Quantitative studies of viral induction as a function of X-ray dose will be presented. (These studies were supported by grants CA 03352 and CA 10372 from the National Cancer Institute, Bethesda, Maryland.)

B-11-2 *Attempts to Recognize an Oncogenic Virus in ^{224}Ra-Induced Murine Osteosarcoma.* VOLKER F. ERFLE, ARNE LUZ, ILSE-DORE ADLER, AND KARL-HORST MARQUART, Institut für Biologie, Gesellschaft für Strahlen- und Umweltforschung, D-8042 Neuherberg, West Germany.

Cell lines from a ^{224}Ra-induced osteosarcoma of a (C3H × 101)F_1 hybrid mouse were established. These cells were tumorogenic in newborn mice and gave rise to undifferentiated sarcomas. Caryotyping revealed a metacentric marker chromosome.

Treatment with cell culture supernatants induced no tumors in newborn and weanling mice and no transformation in 3T3 mouse cells and in embryonic rat cells. Treatment of newborn mice with cell-free extracts of primary ^{224}Ra osteosarcomas induced no sarcomas during 10 months.

Isopycnic centrifugation of supernatants from ^{3}H-uridine labelled cells in sucrose gradients demonstrated the synthesis and release of viral particles. The radioactivity showed a peak in the density range (1.15–1.16 g/ml) of RNA leukemia-sarcoma viruses. Electronmicroscopy revealed type C virus particles. It is assumed that the RNA sarcoma virus genome is present in ^{224}Ra osteosarcoma cells and is expressed as a "defective" particle. (Work perfumed in association with EURATOM.)

B-11-3 *Virus Particles in ^{224}Ra-Induced Murine Osteosarcoma.* K.-H. MARQUART, A. LUZ, W. GÖSSNER, AND V. ERFLE, Depart. of General and Experimental Pathology, Inst. of Biology, Gesellschaft für Strahlen- und Umweltforschung, Munich, West Germany.

Electron microscopic investigation of primary osteosarcomas from NMRI and (C3H × 101)F_1 hybrid mice, induced by the incorporation of ^{224}Ra, revealed the presence of intracisternal type A virus particles. Particles morphologically identical to type C viruses were found in three cell lines established from a ^{224}Ra-induced osteosarcoma of a (C3H × 101)F_1 mouse. The cell cultures contained abundant immature and mature extracellular type C particles. Budding of virus particles from cell membranes was frequently seen. Additionally, both immature and mature particles occurred intracellularly in membrane-bound vacuoles resembling phagolysosomes. Particles observed within these cytoplasmic structures, sometimes together with myelin-like material, showed various stages of disintegration. Budding, immature and mature type C particles were also detected in undifferentiated sarcomas of (101 × C3H)F_1 mice induced by the intramuscular inoculation of cultured cells. No relation can be seen between the intracisternal type A particles, found in primary ^{224}Ra osteosarcomas, and the type C particles occurring in cell lines of a ^{224}Ra osteosarcoma and in undifferentiated sarcomas induced by cultured cells. (Work performed in association with EURATOM.)

B-11-4 *Transformation in Vitro by X-Rays and Neutrons.* CARMIA BOREK AND ERIC J. HALL, Columbia University, New York, NY 10032, USA.

Transformation by single acute doses of x-rays has been studied in detail over the range 1 to 600 rads, using fresh explants of hamster embryo cells. As the dose is increased, the % of cells transformed increases up to a maximum of about 1% at 150 rads. There is a broad plateau between 150 and 300 rads, after which the proportion of transformed clones falls for a larger dose of 600 rads.

Experiments in which the x-ray dose was split into two halves, separated by a time interval of 5 hours, indicate an *increase* in the incidence of transformed colonies.

Preliminary experiments involving 430 KeV monoenergetic neutrons indicate a large RBE for this high LET radiation for the induction of transformations *in vitro*.

B-11-5 *Evidence against a Direct Carcinogenic Effect of X-Rays in Vitro.* J. C. KLEIN, Radiobiological Institute TNO, 151 Lange Kleiweg, Rijswijk Z. H., The Netherlands.

The carcinogenic effect of a single 300 rad dose of X-rays on cells of nonmalignant established mouse cell lines was investigated. Malignant transformation was induced in some lines under certain conditions; e.g., low viability and high cell concentration resulting in close contact between viable and nonviable cells and cell debris.

The degree of cell damage produced by the radiation was induced mechanically and this resulted in a comparable incidence of malignant transformation.

These findings support the hypothesis that the carcinogenic effect of radiation on cells is in some way related to the presence of dead and damaged cells in close contact with viable cells; this argues against a direct carcinogenic effect of radiation on cells.

B-11-6 *The Increasing Susceptibility of Hematopoietic Stem-Cells to Friend Leukemia Virus after X-Irradiation.* K. HIRASHIMA AND T. KUMATORI, National Institute of Radiological Sciences, Chiba, Japan.

The target cells of Friend Leukemia Virus (FV) were hematopoietic stem-cells expressed as colony-forming cells (CFU-s) of C3H/He mice by the transplantation method of Till & McCulloch. The changes of susceptibility to FV of CFU-s were estimated by measuring the splenic FV-transformed tumor colony-forming units of BC3F1 mice which were grafted by spleen cells of C3H FV inoculated 1 or 3 days previously.

According to the time course studies of CFU-s in bone marrow, spleen and peripheral blood after 150 R total body irradiation of C3H/He mice, an 'overshoot' of CFU-s in spleen and blood was observed 3 weeks after irradiation. At this time, the increased susceptibility to FV of CFU-s was observed to be nearly three times greater than the unirradiated control. This increased susceptibility was temporal and returned to control value up to 4 weeks after 150 R. However, after 300 R irradiation, this effect persisted more than 6 weeks.

According to the 3H-thymidine 'suicide' experiments of CFU-s in marrow after irradiation, a significant increase of CFU-s in cell cycle was significantly observed in these FV-sensitive periods. To summarize our experimental results, the target cells (CFU-s) are sensitized to FV in the state of migration from the marrow and in active cell cycle phase depending on the doses of single total body irradiation.

B-12-1 *Transitional Cell Carcinoma of Bladder—A Study in Tumor Response/Dose.* ROBERT MORRISON, Hammersmith Hospital and Royal Postgraduate School, DuCane Road, London, W12 OHS, England, Great Britain.

This paper is based on the findings from a controlled clinical trial in the treatment of approximately 450 patients with carcinoma of the bladder. The patients were placed in similar groups according to the stage of the disease, the histology and the naked-eye tumor type. Radiation treatment was given at 4 different dose levels which were decided by random method but the same fractionation regime was used in each.

The local tumor control rate as determined by repeated cystoscopic examinations is related to the tumor dose and the findings are expressed as a tumor control curve. The findings will also be compared with dose response curves derived from treatment of squamous cell tumors of the oral cavity and pharynx.

The major complication rates in the bladder series will also be given for the 4 dose levels and shown in relation to the tumor control rates. An optimum dose level can be derived from this particular treatment schedule.

B-12-2 *Repair and Reoxygenation of the Hypoxic Cells in the KHT Sarcoma.* R. P. HILL AND R. S. BUSH, Ontario Cancer Institute incorporating the Princess Margaret Hospital, 500 Sherbourne Street, Toronto, M4X 1K9, Canada.

Combined HN_2 and radiation studies have indicated that *in vivo* treatment of the KHT sarcoma with a certain dose of HN_2 can kill all the oxygenated cells in the tumor while killing few if any of the hypoxic cells. This finding has allowed HN_2 treatment to be used as a way of removing the oxygenated cells in the tumor so that the radiation response of the hypoxic cells can be studied directly. Using the HN_2 technique the hypoxic fraction is found to be 30% as compared to a value of 10% obtained using the more conventional technique of comparing anoxic and air-breathing survival data following the same dose of radiation. Further studies have found that the time course of reoxygenation is also different using the two techniques. The likely explanation for this difference is in the finding that the repair of sublethal damage in the hypoxic cells of the tumor following 470 rads is 3–4 times slower than that observed for the oxygenated cells.

B-12-3 *The Effect of Anticoagulant Treatment on Radiation Response (Hypoxic Cell Fraction and Reoxygenation) of the EMT6 Tumor.* ROBERT F. KALLMAN AND LINDA J. JARDINE, Stanford University, Stanford, California 94305, USA.

Starting 10–12 days after intradermal inoculation of EMT6 tumor cells, the host mice (BALB/c) were given drinking water containing 5–7.5 mg/liter warfarin. On day 14–16 the tumors were irradiated (250 kV x-rays, 900–2100 rads) *in situ* either in air-breathing mice or 5 min after asphyxiation of the host animals with nitrogen. To study reoxygenation, the test radiation doses were administered at 3 or 6 hours after 1,000 rads conditioning irradiation. To study the effects of warfarin on cellular radiosensitivity, cell suspensions were prepared by trypsinization of solid tumors grown in warfarinized mice, and were irradiated in the presence or absence of oxygen. As determined by the paired survival curve method, the fraction of hypoxic cells in the tumors of warfarinized mice was reduced to approximately half the level characteristic of this tumor. This effect could not be attributed to warfarin-dependent changes in cell radiosensitivity, i.e., there was no effect on D_0, extrapolation number, or OER. The rate of reoxygenation was only slightly, if at all, altered in tumors of warfarin-treated mice. These data suggest that anticoagulant treatment may increase net tumor radiosensitivity by permitting improved blood perfusion and therefore oxygenation. The reoxygenation data provide some insight into the mechanism responsible for this phenomenon. (Supported by USPHS Grant CA-03353.)

B-12-4 *Tumor Control and Regrowth Probability after a Single-Dose Irradiation of Animal Tumors.* MUNEYASU URANO, NOBUO FUKUDA, AND KOICHI ANDO, National Institute of Radiological Sciences, Chiba 280, Japan.

TCD-50, 50% tumor control dose of various size of C3H mouse mammary carcinoma after a single dose irradiation was examined. Cellular radiation sensitivity of the tumor cells was assayed by the end point dilution method and number of tumor cells contained in the tumor was determined by counting tumor cell nuclei. A mathematical analysis of TCP, tumor control probability, was made on the basis of these results and a formula was proposed in which cell population kinetics was taken into account, i.e.,

$$\mathrm{TCP} = D_0\{\ln m + \ln M(2\mathrm{Pd} - 1)(2\mathrm{Pd}_x - 1) - \ln \ln P^{-1}\}$$

where Pd and Pd_x are division probabilities of non-irradiated and irradiated tumor cells and M is number of total tumor cells in the tumor. An analysis of regrowth of tumors irradiated with an x-ray dose was made which indicated that regrowth time was lineally related with radiation dose within limited doses and that tumors recurred after a dose of TCD-50 might initiate rapid regrowth after a long lag or a long period of slow growth with reduced division probability.

B-12-5 *The Response of Mouse Skin and Mammary Tumors to Neutrons: Influence of Nonconventional Fractionation Schemes on RBE.* JANET S. R. NELSON AND RITA E. CARPENTER, Division of Radiation Oncology, University of Washington, Seattle, WA 98195, USA.

Feet of BALB/cJ mice and C3HBA mammary adenocarcinomas of the C3H/HeJ mouse were irradiated with 250 kVp X-rays or 8 MeV cyclotron neutrons. Skin response was scored on a numerical scale which assigns increasing values to redness, dry and moist desquamation. Tumor endpoint was growth delay determined from tumor volume measurements. The fractionation schemes used for skin and tumor studies included 1 fraction of neutrons or X-rays, 2 fractions 24 or 96 hours apart, and 2 fractions of neutrons plus 3 fractions of X-rays in 5 days, distributed as n-x-x-x-n or n-n-x-x-x. Additionally, feet were irradiated with 5 fractions of X-rays or neutrons in 5 days. The neutron RBE's for early skin response, relative to a total X-ray dose of 1900 rads were: for 1 fraction, 1.8; for 2 fractions in 24 hours, 2.3; for 2 fractions in 96 hours, 2.5; for 5 fractions, 2.6. RBE's of 2.5 and 2.9 for 2 fractions of neutrons used with 3 fractions of X-rays have been found. For tumor growth delay relative to 1800 rads total X-ray dose, the RBE's are: for 1 fraction, 4.0; 2 fractions in 24 hours, 1.8; 2 fractions in 96 hours, 6.0, and in the mixed neutron-photon irradiation, RBE's up to 9.0 have been measured for the neutron component.

B-12-6 *Changes in Cellular Characteristics and Growth of Two Tumors During Serial Transplantation and Long-Term Propagation of Derived Cell Cultures.* B. F. Deys and G. W. Barendsen, Laboratory for Radiobiology, Univ. of Amsterdam, Amsterdam, The Netherlands.

In order to evaluate the significance of changes which can occur during transplantation or long-term propagation of cell cultures derived from tumors, 2 spontaneous animal tumors, a keratin producing bladder carcinoma of the inbred Bn/Bi rat strain and an osteosarcoma of the inbred strain of Balb/c mice, producing bone structures, were studied.

The bladder tumor has been serially transplanted for 7 generations in syngeneic rats over a period of 11 months and has a volume doubling time of 8 days. During a culture time of 10 months, cells derived from the 2nd tumor generation and now in their 40th passage were regularly assayed for changes in growth pattern, chromosomal constitution and radiosensitivity. A D_0 = 150 rad and D_q = 450 rad for early passages and a D_0 = 140 rad and D_q = 200 rad for a tumor-derived sub-line were measured. Keratin formation diminished gradually in tumors.

The osteosarcoma, serially transplanted for 4 generations during 9 months, has a volume doubling time of 10 days. Cultured tumor cells from first and second transplants, in their 30th passage and growing since 5 months, were inoculated monthly; radiation responses and histology remained nearly unchanged.

B-12-7 *Effects of Multifractionated Irradiation with Fast Neutrons or X-Rays on C3H/He Mammary Carcinoma.* Hiroshi Tsunemoto and Koichi Ando, National Institute of Radiological Sciences, Anagawa, 9-4-1, Chiba, Japan.

It is well known that the reoxygenation of hypoxic tumor cells is an important factor to control solid tumors. This experiment was carried out to estimate effects of multifractionated irradiation with x-rays or fast neutrons on solid tumors. The experimental tumors used were first generation isotransplants of C3H/He mammary carcinoma. Applying a delay-time of the tumor regrowth after irradiation, the effect of irradiation was estimated. The time interval for irradiation was selected at 48 hours in this experiment, when the reoxygenation would be maximum in x-ray experiments, and delay-times for single or fractionated irradiations were compared.

The delay-times of tumors irradiated with fractionated doses of fast neutrons were longer than that of single dose. On the other hand, the delay-times of fractionated doses of x-rays did not reached to that of single dose.

This experiment showed that fast neutrons were more effective to destroy the hypoxic cells in solid tumors, so that the reoxygenation would occur more easily with fast neutrons than x-rays.

It is suggested that fast neutron therapy is an effective modality to manage malignant tumors.

B-12-8 *Cure, Regression and Cell Survival: A Comparison of Common Radiobiological End-points with an in Vitro Tumor Model.* Ralph E. Durand, University of Wisconsin Medical School, Department of Radiology, Madison, Wisconsin 53706, USA.

Multicell spheroids of non-malignant V79 Chinese hamster cells grown in suspension culture display many of the characteristics of solid tumors *in vivo*, and thus can be used as an *in vitro* tumor model. Spheroids are unique in that numerous radiation effects can be observed and quanti-

fied: (1) they can be reduced to single cells by trypsinization, and cell viability then assayed by colony formation; (2) "cure" of individual spheroids can be defined by lack of growth when the entire spheroid is plated in a petri dish; and (3) volume changes of the population of spheroids can be monitored with respect to time and treatment. When these endpoints were compared, regression did not correspond with dose or lethality. However, a correlation was observed between "cure" and single cell survival, and it was possible to demonstrate that every cell had to be killed to obtain a "cure." This result has significant therapeutic implications, since it demonstrates that internal, chronically hypoxic cells are capable of proliferation even in a previously nonpermissive environment. (Supported by ACS Institutional Grant IN-35M and NIH Center Grant CA-06295.)

B-12-9 *Mammary Adenocarcinoma Explants Grown in Organ Culture: Biochemical Parameters and Radiation Effects.* J. Keary Moran, John Shima, H. Vermund, Judith Harrison, and Geraldine L. Dettman, University of California, Irvine, California College of Medicine, Department of Radiological Sciences, Irvine, California 92664, USA.

Mouse mammary adenocarcinoma explants were maintained in organ culture using different growth media. The mitotic index of the explants decreased over a 28 hour period in all cultures observed, although the rate of decrease depended upon the media used. On the other hand, the rate of DNA and protein synthesis did not decrease with time, but increased during the first four hours in culture and then remained at a plateau level. In a gas phase of 95% air/5% CO_2, many cells were necrotic after 24–48 hours in culture; whereas, explants grown in a gas phase of 95% O_2/5% CO_2 and in a rich media were free of necrotic cells after 36 hours in culture. The rates of DNA and protein synthesis were much higher in cultures incubated with 95% O_2/5% CO_2 vs. those grown in 95% air/5% CO_2. Insulin was shown to promote maintenance of the cultures and also to stimulate protein and DNA synthesis. This system is being used to study the effects of irradiation on the rate and total amount of DNA and protein synthesis and the effects of combined irradiation and chemotherapeutic agents on DNA and protein synthesis.

B-13-1 *X-Ray-Stimulated Incorporation of [^3H]Thymidine Triphosphate into DNA of Toluenized* Bacillus subtilis *and Its Inhibition by Nicotinamide Adenine Dinucleotide.* Daniel Billen, University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences, Oak Ridge, Tennessee 37830, USA.

X-ray exposure of *B. subtilis* strains, made permeable to nucleoside triphosphates by toluene treatment, stimulates a repair-type DNA synthesis. Semiconservative DNA synthesis in toluenized cells is reduced by exposure to x-rays. The x-ray stimulated repair-type synthesis is due mostly to the product of the *pol*A gene since a pol$^-$ mutant showed a greatly reduced response. The observed x-ray response requires the presence of the four deoxyribonucleoside triphosphates and ATP for full expression. The DNA synthesized in irradiated pol$^+$ cells shows size heterogeneity whereas in pol$^-$ cells less size heterogeneity was observed. Nicotinamide adenine dinucleotide (NAD) has been found to inhibit the x-ray induced repair-type synthesis. These data are interpreted as showing a competition between ligase and DNA polymerase I for the resulting 3′ terminal hydroxyl group.

B-13-2 *Sensitization of Bacteria to Ionizing Radiation by Pre-Treatment with Near Ultraviolet (365 nm) Radiation.* Rex M. Tyrrell, MRC Experimental Radiopathology Unit, London, England, Great Britain.

Exposure of wildtype bacterial cells to a dose of 10^6 Jm^{-2} of 365 nm radiation (which kills only 35% of the cells) immediately before X-ray treatment increased the slope of the X-ray survival curves by a factor of approximately 4. A *pol*A strain showed 75 percent of this synergistic interaction but no synergism was observed in a *rec*A *rec*B strain. After a similar dose of 365 nm radiation both the *rec*A (type III) and *pol*A (type II) dependent repair of X-ray induced single-strand breaks were initially inhibited. However, a limited amount of repair occurred in both the wildtype and *rec*A *rec*B strains (but not in the *pol*A strain) during extended incubation in full medium after the combined radiation treatments. It is concluded that only the disruption of the *rec*A pathway (which is effectively irreversible under the set of conditions employed) ultimately affects the ability of a cell to reproduce and form a colony. The results suggest that recovery of polymerase 1 activity occurs in full medium.

B-13-3 *X-Ray-Induced Dominant Lethality in Radiosensitive Strains of* Saccharomyces cerevisiae. KAREN S. Y. HO AND ROBERT K. MORTIMER, The Group in Biophysics and Medical Physics, Donner Laboratory, University of California, Berkeley, California 94720, USA.

Yeast strains carrying the *rad52* mutation are more susceptible than wild type to X-ray induced dominant lethal damage. The relationships between zygote survival and X-ray dose for the crosses ($\pm \times \pm$, *rad52* $\times$ *rad52*, $\pm \times$ *rad52*, *rad52* $\times \pm$) in which only one parent was irradiated are similar except for *rad52* $\times$ *rad52*. In this cross a significantly higher frequency of dominant lethal damage is observed. This observation indicates that the *rad52* mutant lacks a repair mechanism for X-ray damage and is consistent with the proposal that dominant lethality is a result of unrepaired chromosome breaks. Additional evidence that *rad52* strains lack a repair system for X-ray damage is provided by the X-ray survival response of tetraploids carrying 0, 1, 2, 3 or 4 *rad52* alleles. Preliminary experiments for determination of frequencies of X-ray induced dominant lethal damage at permissive and restrictive temperatures in *rad54* and *rad55* strains suggest these strains also possess a defective repair enzyme for X-ray damage. Studies on the molecular nature of the X-ray induced lesions leading to dominant lethality are in progress.

B-13-4 *Split-Dose Recovery Controlled by XS1 Gene in Yeast.* TETSUYA SAEKI, ISAMU MACHIDA, AND SAYAKA NAKAI, National Institute of Radiological Sciences, Chiba 280, Japan.

The split-dose recovery of yeast after ionizing radiation was manifested with diploid cells but not haploid. Thus, one can assume that the recovery is mediated by recombination-like mechanisms, for which diploid state is required. To test this hypothesis, we have done several experiments using a X-ray sensitive mutant, xs1, isolated in our laboratory, which is defective in both gene conversion and recombination induced by radiation in mitotic cells. It is demonstrated that the xs1 cells show almost no recovery after γ-rays even in the diploid state and that the recovery factor of xs1 is about 0.7–5% of that of wild type. Furthermore, unlike stationary phase, the exponentially growing haploid cells are able to show the recovery in wild type but not in xs1 mutant. Based on these results and other evidence, we propose a model that chromosomal recombination mechanism controlled by XS1 gene plays an essential role in the split-dose recovery of yeast.

B-13-5 *Effect of the Protein Synthesis Inhibitor Cycloheximide on X-Ray Split-Dose Recovery in Diploid Yeasts of Different Radiosensitivities.* ISOLDE WIENHARD UND JÜRGEN KIEFER, Strahlenzentrum der Justus Liebig-Universität, Giessen, West Germany.

In the experiments cycloheximide was present in nontoxic concentrations during the incubation period between the two dose fractions. For the wild-type strain the colony forming ability of the cells irradiated with the conditioning dose increases with incubation time in the presence of the inhibitor, whereas the maximum amount of liquid holding recovery decreases. The sparing effect of dose fractionation was inhibited.

For an x-ray-sensitive mutant no increase of the colony forming ability of the incubated cells is observed but the sparing effect is also inhibited.

B-13-6 *The Repair of Double-Strand Breaks in the Nuclear DNA of Yeast.* MICHAEL A. RESNICK AND PATRICIA MARTIN, University of Rochester, Department of Radiation Biology and Biophysics, Rochester, New York 14642, USA.

The genetic control of ionizing radiation sensitivity in diploid *Saccharomyces cerevisiae* appears to involve two pathways such that the survival of the wild type exhibits a large shoulder and a D_{37} of 15 krad, while a double mutant (*rad* 18 and *rad* 52, corresponding to each pathway) has no shoulder and a D_{37} of only 1 krad. The role of repair of double-strand breaks in log phase wild type cells has been investigated after low doses, corresponding to 50% survival, and found to be very efficient. Utilizing neutral sucrose gradient techniques, we have determined in the wild type strain that approximately 0.7 double-strand breaks are induced (10^{10} M.W.) krad. This would correspond to one double-strand break per diploid cell in the radiation-sensitive double mutant. Repair of these breaks in the wild type is fast at low doses in that 75% are repaired in 3 hours; this result contrasts with high doses in that little or no repair is observed. Our results thus indicate that the radiation-sensitivity of the double mutant is due to an inability to repair double-strand

breaks in nuclear DNA. The role of protein synthesis and other proposed repair inhibitors will be discussed. (This paper is based on work performed under contract with the U.S. Atomic Energy Commission at the University of Rochester Atomic Energy Project and has been assigned Report No. UR-3490-466.)

B-13-7 *Comparative Sensitivities of Sea Urchin Eggs, Sperm and Zygotes to Radiation-Induced Mitotic Delay.* RONALD C. RUSTAD AND ROSALIND GOLDMAN, Case Western Reserve University, Cleveland, Ohio 44106, USA.

Arbacia eggs and sperm appear identically sensitive to 8 kR or less of γ-irradiation. The separate irradiation of both the eggs and sperm with a unit dose leads to the same postfertilization mitotic delay as twice that dose applied to either eggs or sperm. Exposure of newly fertilized zygotes to a unit dose yields the same mitotic delay as exposure of either eggs or sperm to twice that dose. If 1 and 2 indicate doses, and e, s, and z denote irradiation of eggs, sperm, and zygotes, the comparative effects are:

$$1_e + 1_s = 2_e = 2_s = 1_z$$

Even at 4 kR/min some recovery could occur in eggs but not sperm during radiation exposure. Compact male pronuclei might be poorly equipped for the repair until fusion with female pronuclei. Higher doses cause excessive delay in eggs or zygotes but not sperm. Thus, equal numbers of γ-rays passing through equal fractions of nuclear DNA appear to cause identical mitotic delays at low doses, while higher doses may damage the egg's repair machinery.

B-13-8 *Repair of Nuclear and Mitochondrial DNAs in* Tetrahymena pyriformis. M. S. NETRAWALI, KARPAGAM PASUPATHY, D. S. PRADHAN, AND A. SREENIVASAN, Biochemistry & Food Technology Division, Bhabha Atomic Research Centre, Bombay 85, India.

Like in prokaryotes, repair of radiation-induced single-strand scissions in eukaryotes is reported but the studies have been confined so far to whole cells. In the present investigation, evidence has been obtained to suggest that in *Tetrahymena pyriformis* both nuclei and mitochondria are autonomous in DNA repair. Nuclei and mitochondria exposed to gamma-irradiation (200 krad) *in vitro* could rejoin single-strand scissions in their DNAs when incubated in isotonic buffered media. Further studies indicate that DNA repairs in these organelles may be associated with their membranes. EDTA (1×10^{-3} M) inhibited rejoining of DNA strand scissions by both nuclei and mitochondria. Dinitrophenol (5×10^{-5} M), which inhibited oxidative phosphorylation in mitochondria only partially, suppressed mitochondrial DNA repair completely but not nuclear DNA repair. The two organelles also exhibit rapid repair, since greater number of strand breaks were observed in their DNAs when EDTA (1×10^{-3} M) was present during irradiation. Significance of these findings will be discussed.

B-14-1 *Photosensitized Monomerization of Uracil Dimers by Indoles and Lysozyme.* JAMES CHEN, L. HINMAN, C. W. HUANG, D. A. DERANLEAU, AND M. P. GORDON, Department of Biochemistry, University of Washington, Seattle, Washington 98195, USA.

Indoles with electron-donor property are able to split ^{14}C-labeled uracil dimer when irradiated at wavelengths greater than 300 nm. Among the indole derivatives tested, 5-hydroxyltryptophan and skatole are the most effective, followed by indole-acetic acid, 2-methyl-indole, indole and tryptophan, in that order. Indole-3-aldehyde and oxindole are inactive in the system. Lysozyme, which displays one solvent-available tryptophanyl residue, also causes the splitting of the dimer under the same conditions. Its efficiency of monomerization is about 20–30% that of free tryptophan. N-bromosuccinimide oxidized lysozyme and ribonuclease, a protein void of tryptophan, failed to exhibit the photosensitized monomerization of the pyrimidine dimers. It is concluded that both free and protein tryptophan can serve as photosensitizers in the system. Simple indoles and proteins containing solvent-available tryptophan may play a role in the photorepair of the UV-damaged RNA by interacting with pyrimidine dimers in the molecule. (This research was supported by funds from the U.S. Atomic Energy Commission (contract No. AT(45-1)2225) and the National Science Foundation (grant No. GB 24024).)

B-14-2 *Studies in γ-Endonuclease Activity of* Micrococcus luteus. ANNEMARIE BOPP AND INGEBORG HÄDRICH, Institut für Strahlenbiologie, Kernforschungszentrum Karlsruhe, D 75 Karlsruhe 1, Postfach 3640, West Germany.

It was shown by G. Stephan in our laboratory that a system in *E. coli* hcr$^+$ spheroplasts is able to repair base damage induced by γ-irradiation in covalently closed circular RF I-DNA of φX 174 phage. It will be demonstrated that one of the two UV-endonucleases from M. luteus recognizes a part of this kind of damage. The enzyme was isolated from *M. luteus* according to the methods of Nakayama *et al.* For the first purification steps, UV-irradiated DNA as substrate has been used. To test γ-endonuclease activity in the active fractions, φX174-RF I-DNA, labelled with ^{3}H-thymidine, was γ-irradiated in dilute solution. The RF I-molecules containing all types of γ-ray induced damage without strand breaks were separated from the broken ones by sucrose gradient sedimentation. They were incubated with the isolated fractions from *M. luteus* containing UV endonuclease activity. Single-strand breaks, formed by this treatment, were detected by means of an exonuclease activity in an extract from *M. luteus* uvr$_7^-$ removing radioactive acid soluble material from the labelled RF-molecules. It was found that some fractions of the UV endonuclease of *M. luteus* do show also a γ-endonuclease activity. Furthermore, the yield for the sites sensitive for γ-endonuclease in γ-irradiated RF-DNA was compared with that for the strand breakage in these molecules.

B-14-3 *Endonuclease Activities in Extracts of* M. luteus *that Act on Irradiated DNAs.* W. L. CARRIER AND R. B. SETLOW, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn. 37830, USA.

Many cells are able rapidly to repair several kinds of radiation damage to their DNA. For example, γ-ray induced base damage, UV-induced pyrimidine dimers, and UV alterations to BrUra-substituted DNAs disappear rapidly *in vivo*. The first enzymic step in these repair sequences is thought to be an endonucleolytic attack on the altered DNA. To assay for endonuclease activity we measure, by sedimentation in alkaline sucrose, the numbers of breaks introduced into radioactive DNAs that contain several enzyme-sensitive sites in 5×10^6 daltons. We have identified 3 activities, in extracts of *M. luteus*, that act on irradiated DNA and have separated them into 4 components by chromatography on DEAE cellulose. 1) The activity toward BrUra-containing DNA that was uv-irradiated in the presence of cysteamine (so as to inhibit the formation of alkali labile bonds) seems to be specific for Ura-residues arising as a result of debromination. The active column fractions degrade extensively PBS2 DNA—a DNA that naturally contains Ura in place of Thy. 2) The activities toward DNA containing pyrimidine dimers is found in two distinct chromatographic peaks, one of which seems to represent a breakdown product or subunit of the other. 3) A third distinct chromatographic fraction, eluting at a higher salt concentration than the dimer endonuclease, acts on γ-irradiated DNA. (For assay we normally use DNA irradiated anoxically *in vitro*.) The existence of these three separable endonuclease activities is evidence for the generality of excision repair and for the existence of parallel pathways capable of coping with different types of damage. (Research sponsored by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.)

B-14-4 *Radiation and DNA-DNA Hybridization: the Influence of Reaction Kinetics.* G. V. DALRYMPLE, A. J. MOSS, JR., MAX L. BAKER, AND K. P. WILKINSON, University of Arkansas Medical Center, Little Rock, Arkansas 72201, USA.

We have studied the influence of radiation upon the ability of DNA to compete in a DNA-DNA hybridization system (using a nitrocellulose filter method). Doses of 5000 rads (or greater) destroy the ability of DNA (irradiated *in vivo* or *in vitro*) to hybridize. The results, broadly, may be interpreted two ways: 1) Radiation produces extensive base damage which alters hybridization capability or 2) the reaction rates are significantly depressed secondary to a reduction in DNA molecular weight (Wetmur and Davidson, J. Mol. Biol. **31**, 349 (1968) showed the reaction rate to be proportional to the square root of the DNA molecular weight).

We will report the results of experiments in which the influence of 1) radiation and 2) DNA molecular weight upon the ability of mammalian cell DNA to hybridize in a DNA-DNA hybridization system will be contrasted. The results will be interpreted with regard to the possible importance

of biological damage manifested by a change in the ability of DNA to recognize itself in a hybridization system.

B-14-5 *Excision of X-Irradiated DNA; A Possible First Step in the Repair of X-Ray-Induced Damages.* GARY F. STRNISTE AND SUSAN S. WALLACE, Lehman College, City University of New York, Bronx, New York 10468, USA.

Radiation can induce a variety of changes in the genetic material of a cell, including structural breakage of the nucleic acid molecule and modification of the bases comprising the genetic information. Survival of the organism is directly related to the maintenance of the integrity of this genetic information. Therefore, the ability of a cell to sustain radiation induced damages must be related, at least in part, to the repair capacities existing in the cell. A great deal of information has been catalogued concerning the repair of UV-induced damages to DNA bases, yet little is known about the possible enzymatic repair of base damages induced by ionizing radiation. We have examined extracts of *Escherichia coli* B/r in an attempt to determine if there exists endonuclease activity that specifically reacts with x-irradiated DNA. Using the conversion of tritiated, x-irradiated ϕX-174 RFI to RFII as an assay system, it appears that there exists in this DNA approximately 0.7 to 1.0 site sensitive to endonuclease for every single stranded break induced by x-irradiation. Furthermore, it appears that the x-ray excision process is independent from UV excision repair, since x-irradiated DNA competes in this reaction while UV-irradiated DNA does not. Extracts prepared from a radiation sensitive strain of *E. coli*, B_{s-1} also contain this x-ray specific endonuclease activity. We are presently attempting to purify this x-ray specific endonuclease. (Supported by PHS Grant CA12693 from the National Cancer Institute and CUNY Research Grant 10171.)

B-14-6 *A Thymine Dimer Excision Nuclease in Extracts of Human Cells.* ERROL C. FRIEDBERG, JAMES DUNCAN, AND JAMES E. CLEAVER.* Stanford University, Stanford, California, 94305.

Crude extracts of a number of human cell lines and cell strains have been prepared by sonication of harvested washed cells. When extracts of cells previously stored at $-20°C$ are incubated with UV-irradiated ^{3}H-labeled T7 or *E. coli* DNA, no reduction in the ratio of thymine dimers/thymine monomer in the acid-precipitable fraction of the DNA is observed. If the UV-irradiated DNA is previously incised with a pyrimidine dimer-specific endonuclease from phage T4, a time and protein concentration-dependent selective loss of dimers from acid-precipitable DNA occurs. This activity is dependent on the presence of Mg^{++} or Mn^{++} ions, but has a significant preference for the former. The enzyme has a pH optimum at about 8.0 and is sensitive to SH-group inhibitors. Dimers are excised in the $5' \rightarrow 3'$ direction, since the T4 UV endonuclease creates phosphodiester breaks on the 5′ side of the dimers. This activity is present in extracts of WI-38 cells (human diploid fibroblast strain). This enzyme is possibly required for excision of thymine dimers in human cells *in vivo*, and cells from patients with xeroderma pigmentosum are currently being screened for possible defectiveness in this activity.

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B-14-7 *"Dark Repair" Activity and Photoreactivation in Extracts of Potoroo Cells.* C. D. LYTLE AND S. P. LAI,* Bureau of Radiological Health, Rockville, Maryland 20852, USA.

"Repair" activity in extracts of cultured Potoroo cells was quantitated by incubating UV-exposed replicative form (RF) DNA of bacteriophage ϕX174 with nuclear and cytoplasmic preparations and subsequently assaying the infectivity of the DNA in spheroplasts of *E. coli* C_s(*hcr*$^-$). Incubation of control (unexposed) DNA resulted in loss of infectivity, presumably from degradation by nucleolytic activity in both extracts. The surviving fraction of DNA (ratio of infectivities of UV-exposed DNA to control DNA) incubated with the nuclear preparation increased approximately 3-fold, indicating "dark repair" during incubation with the nuclear preparation but not the cytoplasmic one. Illumination of both DNA-extract incubation mixtures with near UV light (~365 nm) provided higher survival levels (~3-fold above incubation in dark), indicative of photoreactivation for both the nuclear and cytoplasmic preparations. Thus,

in this assay system, the nuclear extract of cultured Potoroo cells contained "dark repair" activity and photoreactivation activity, while the cytoplasmic extract contained only photoreactivation.

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B-15-1 *Base Substitutions Induced by X-Radiations in Synchronized Cells of* Saccaromyces cerevisiae. GIOVANNI E. MAGNI, SILVIO SORA, AND LUCIA PANZERI, Instituto di Genetica, Milano, Italy.

Synchronisation of cell division as judged by DNA synthesis and by cell doubling is accompained in *Saccharomyces cerevisiae* by synchronous variations of sensitivity to x-radiations. By our method of synchronisations cell populations were obtained with more than 95% of cells fully sensitive or showing the maximum degree of radiation resistance. Molecular specificity of point mutations induced by x-radiation was investigated on samples of cells all showing the same sensitivity to x-radiations. The method is based on transitions from a nonsense mutation of *ochre* type to nonsense of *amber* type and viceversa and on reversion from nonsense to sense, always using the same mutated codon, according to the following scheme:

Mutations investigated	Mutational event
UAA → UAG	= Transitions AT → GC
UAA → wild type	= Transitions AT → GC + transversions
UAG → UAA	= Transitions GC → AT
UAG → wild type	= Transitions AT → GC + transversions

Relative frequencies of transitions and transversions induced by a vaste range of x-rays doses will be reported.

B-15-2 *Evidence for Misrepair Mutagenesis in* Saccharomyces cerevisiae. SÁNDOR IGALI AND R. C. VON BORSTEL, Department of Microbiology, "F. Joliot Curie" National Research Institute for Radiation Biology and Radiation Hygiene, Budapest, Hungary, and Department of Genetics, University of Alberta, Edmonton, T6G 2E9 Canada.

The *lys1-1* mutant in yeast is a super-suppressible mutant of the ochre variety. Therefore, backmutations of this mutant could be manifestations of a thymine dimer directly upon the transcription site in the DNA for the second and third letters of the codon. This premutational lesion should be, and is, photoreversible. Over 99 per cent of the reversions of *lys1-1* induced at suppressor loci are by mutation of genes encoding ^{tyr}tRNA, presumably at the third letter of the anticodon. The base sequence around and including the anticodon region of ^{tyr}tRNA is $-ACUG\Psi A^{1}_{A}A\Psi-$ [Madison, Everett, and Kung, Science **153**, 531 (1966)]. Consequently, pyrimidine dimers cannot be formed at the transcription site in the DNA for the wobble position of the anticodon. Experiments by ourselves and Resnick [Mutation Research **7**, 315 (1969)] indicate that premutational lesions of the genes transcribing ^{tyr}tRNA are photoreversible. Also, the spontaneous mutation rate of genes transcribing the eight ^{tyr}tRNA are almost an order of magnitude higher than the spontaneous mutation rate of backmutations of the locus itself, whereas nearly the reverse is true after ultraviolet irradiation. Assuming that only pyrimidine dimers are photoreversible, a case can be made that mutational lesions can be induced at a place other than the site of the premutational lesions and the induction efficiency falls off steeply as a function of the distance between them.

B-15-3 *Mutation and Recombination Behavior of Three UV-Sensitive Saccharomyces Mutants.* F. ECKARDT, S. KOWALSKI, AND W. LASKOWSKI, Zentralinstitut für Biochemie und Biophysik, Freie Universität Berlin, West Germany.

Spontaneous and UV-induced mutation rates were determined for haploid, UV-sensitive *Saccharomyces* strains, mutated in either the Rad 2, $R_1{}^s$ or $R_2{}^s$ locus. Mutation from adenine and lysine dependence to prototrophy (locus and suppressor mutations) was compared with that of wild type. Inter- and intragenic mitotic recombination rates were also compared, using appropriate diploids.

The induced mutation rates of the haploid $r_1{}^s$ and $r_2{}^s$ strains are identical with those of the wild type, whereas those of the Rad 2-haploid differ characteristically. Results obtained with

the r_1^s Rad 2 doublemutant allow the interpretation that the R_1^s as well as the Rad 2 gene product are involved in excision repair. $r_1^s r_1^s$ diploids show a highly increased recombination rate compared with the wild type, whereas $r_2^s r_2^s$ diploids show no mitotic recombination at all. The mutational process in haploids thus seems to be independent of recombinational events. Current experiments on induced mutation rates in the corresponding diploids, however, point to involvement of mitotic recombination in the mutational process in diploids.

B-15-4 *Mutator Activity of Excision-Repair Mutants of Yeast.* Teresa Brynchcy and R. C. von Borstel. Department of Genetics, University of Alberta, Edmonton, Alberta, T6G 2E9 Canada.

The excision-repair pathway in *Saccharomyces cerevisiae* involves the genes *rad1*, *rad2*, *rad3*, and *rad4* [Game and Cox, Mutation Research **16**, 353 (1972)]. The genes *rev1*, *rev2* (*rad5*), and *rev3* [Lemontt, Genetics **68**, 21 (1971)] appear to branch from this pathway. An analysis was made of the spontaneous mutation rates of the first five *rad* mutants using the thousand-compartment method of von Borstel, Cain, and Steinberg [Genetics **69**, 17 (1971)]. The assay system was for reversion of *lys1-1*, a super-suppressible mutant of the ochre variety, and reversion of *his1-7*, a missense mutant. It was found that *rad3-12* and *rad5-1* both demonstrate considerable mutator activity. The mutants *rad2-2*, *rad4-3* and *rad1-1* do not differ significantly from the controls in their spontaneous mutation rates. It is of interest to note that *rad3* is the first gene of the excision-repair pathway (Game and Cox, *op. cit.*). Therefore, it seems that a mutation of this gene (and possibly *rad5*) causes the spontaneous *premutational* lesions to be repaired via another, error-prone repair pathway, or, alternatively, *rad3* and/or *rad5* may encode endonucleases which now cannot efficiently recognize the spontaneous *mutational* lesions which are normally removed in wild-type strains.

B-15-5 *Ultraviolet-Sensitive Mutants of* Saccharomyces cerevisiae. R. K. Vashishat and S. N. Kakar, Department of Genetics, Haryana Agricultural University, Hissar (Haryana), India.

Single gene mutants affecting sensitivity to UV-light and constituting 7 complementation groups have been isolated. Some of the mutants were cytoplasmic or nuclear petites. All the mutants were recessive and showed increase in UV-survival after photo-reactivation.

The wild-type and some mutants showed decrease in survival in the presence of caffeine while other strains were not affected.

When tested for cross-sensitivity to x-ray, nitrous acid, ethyl-methanesulfonate and N-methyl-N′-nitro-N-nitrosoguanidine (NG); some mutants were sensitive to all the mutagens while others were resistant to x-ray and NG, but sensitive to other mutagens. One mutant was resistant to x-ray, NG and nitrous acid. The UV-sensitive mutants of yeast could be classified into three groups comparable to HCR, REC and EXR mutants of *E. coli*.

Studies on UV-induced reversion to prototrophy have shown that the frequency of reversion increased as compared to wild type in some strains while in others it decreased or was not affected. Photoreactivation decreased the frequency of revertants in all these strains.

These studies suggest that UV-induced mutations arise as (a) errors in recombination repairs of single strand gaps and (b) the repair mechanisms and the mechanisms of UV-induced mutation in yeast are similar to those in *E. coli*. Implications of above findings will be discussed.

B-15-6 *Mutations in Cultured Mammalian Cells in Vitro and in Vivo Condition.* N. Suzuki and S. Okada, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan.

Ala 32, one of alanine-requiring mutants of L5178Y cells, was induced with MNNG and isolated by the starvation-BUdR-Light technique of Kao and Puck. The optimum concentration of L-α-alanine for growth of Ala 32 cells was 10^{-3} *M* and reversion of alanine auxotroph to prototroph was used to estimate mutation frequency. The maximal mutation frequency was expressed on the 2nd or 3rd day after UV or MNNG treatment. Dose response curves were linear in MNNG, exponential in γ-ray, and curvilinear in UV. Mutation frequency was dependent on dose rates (0.2 r/min-50 r/min) of γ-ray. Pretreatment with 10^{-6} *M* BUdR for 15 h resulted in doubling of mutation frequency at 200 r level.

In *in vivo* experiment, cells were irradiated on the 9th day after intraperitoneal transplantation to BDF_1 mice. The *in vivo* experiments required a longer expression time, showed higher radioresistancy for cell killing, and lower mutation frequency than those *in vitro*.

B-15-7 *Studies on Radiation-Induced Mutation in Cultured Somatic Mammalian Cells in Vitro.* FUMIO SUZUKI, SADAYUKI BAN, AND MASAKATSU HORIKAWA, Kanazawa University, Kanazawa 920, Japan.

Recently, we have established a technique for a simpler and more suitable replica plating method for cultured mammalian cells, by improving the technique described by Goldsby and Zipser (1969). This procedure should be useful for the isolation and characterization of mutant cells, for the purification of the mutants, and for the investigation of mutagenesis in somatic mammalian cells. We have obtained a prototrophic clone (alanine-, asparagine-, proline-, aspartic acid-, hypoxanthine-, and glutamic acid-sufficient clone) and an auxotrophic clone (thymidine-deficient clone) from the cultured Chinese hamster *hai* cells by this replica plating method. In this paper, we will discuss the experimental results of the investigations on forward and reverse mutation induced by x-radiation and ultraviolet light in cultured Chinese hamster *hai* cells, in the prototrophic- and auxotrophic-cells and furthermore, in 8-azaguanine-resistant and -sensitive cells obtained in the authors laboratory.

B-15-8 *X-Ray-Induced Mutations Resistant to 8-Azaguanine in Synchronous Chinese Hamster Cells in Vitro.* J. H. CARVER AND W. C. DEWEY, Department of Radiology and Radiation Biology, Colorado State University, Fort Collins, Colorado 80521, USA.

Synchronous cells (mitotic selection) were irradiated in G_1 or S, and colony survival in Alpha medium with dialyzed serum was determined with and without 15 μg/ml 8-Azaguanine (8-AG). Plating efficiency of isolated mutants in 8-AG and HAT varied, but 50% of mutants tested exhibited resistance to the selecting drug. Fluctuation tests indicated that 8-AG did not induce mutations. Spontaneous frequencies varied from 10^{-6} to 10^{-5}. Induced mutants/locus/rad (10^{-8}–10^{-6}) was dose dependent and similar to that reported for human diploid fibroblasts. S phase was possibly more sensitive than G_1 at doses above 400 rads. These results are questionable, however, because observed mutations increased with the number of cell cycles following the mutational event (phenotypic expression) and decreased when cell densities prior to selection (metabolic cooperation) exceeded 2–4 $\times$ 10^4 cells/cm^2. This number, observed in asynchronous cells for spontaneous and induced mutants, appeared to increase when doubling time lengthened. When maximum density was kept below 4 $\times$ 10^4 cells/cm^2, however, frequencies increased to a constant value at about 3 generations, with no decrease seen after longer expression times. This "leveling off" suggests a valid use of the system in age response experiments which will be reported.

B-15-9 *Heritable Damages Induced by Radiation to Mouse Leukaemic Cells.* JANUSZ Z. BEER, EWA BUDZICKA, AND IRENA SZUMIEL, Department of Radiobiology and Health Protection, Institute of Nuclear Research, 03-195 Warszawa, Poland.

X-rays induce in L5178Y-S cells changes of proliferative activity transmissible to progenial cell generations. The fate of such changes has been an object of these studies. The composition of cell populations which survive irradiation was examined on the basis of growth kinetics changes and by cloning. It was found that cell populations which proliferate after irradiation contain up to 90% of slowly growing cells. Assays performed on sublines derived from single cells in order to study the nature of the inhibiting growth lesions concerned among others: 1) cloning efficiency, 2) mitotic and labelling indices, 3) DNA content per cell, 4) PLM analysis of the cell cycle. The results indicate that numerous cells in slowly growing populations are arrested in G2 phase (or R2 compartment). Observations on the sublines showed that slow growth often proceeded over many tens of cell generations although all slow clones examined were capable of late recovery. The recovery was observed at various stages of the post-irradiation development, in some cases as late as after 200 cell doublings. (These studies were supported by IAEA Contract 917/RB.)

B-16-1 *Carcinogenic Hazards of Irradiation During Embryonal Development in Mice.* STAN D. VESSELINOVITCH, ERIC SIMMONS, KANDALA V. N. RAO, AND NIKOLA MIHAILOVICH, Franklin McLean Memorial Research Institute,* Chicago, Illinois 60637, USA.

Transplacental, neonatal, and infant carcinogenic risks are being assessed in our laboratory

in relationship to adult age groups. Our earlier studies on prenatal chemical carcinogenesis have shown that certain periods of fetal development are especially sensitive for tumor induction. We were therefore interested in evaluating the carcinogenic risks from external (x-rays) or internal (radionuclide) ionizing irradiation during embryonal development.

Mice were exposed between the 12th and 18th day of gestation to a variety of single or multiple doses of x-irradiation ranging from 10 to 80 R. In addition, one group of pregnant mice was injected intraperitoneally with tritiated thymidine (8 μCi/g) on the 16th day after conception. In both series the offspring were kept for observation 90 weeks, at which time the survivors were sacrified and the tumor response was assessed.

An enhancement of lung, liver, and kidney carcinogenesis was observed in male mice exposed to 80 R on the 16th or 18th days of pregnancy. In general, this age period of development was more sensitive to tumor induction than was the 12 or 14 day age. Male and female mice that received tritiated thymidine on the 16th day of gestation showed an accelerated development of hepatomas. In addition, epilation and premature greying of hair was noted in tritiated thymidine treated mice. These studies are being correlated with those in which chemical carcinogens were administered prenatally.

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* Operated by the Univ. of Chicago for the U.S. Atomic Energy Commission.

B-16-2 *Carcinogenicity of ^{131}I Relative to Age at Exposure.* MELVIN R. SIKOV AND D. DENNIS MAHLUM, Biology Department, Battelle, Pacific Northwest Laboratory, Richland, Washington 99352, USA.

The carcinogenicity of ^{131}I relative to age at exposure has been studied by exposing adult, weanling, newborn, and prenatal rats (17 days of gestation) to graded doses of ^{131}I on five consecutive days. All animals were followed until death or 20 months of age, necropsied, and histologic sections prepared and examined. Radiation dose estimates were made for each age-dose group based on radioanalysis of additional rats sacrificed during and following exposure. Radiation doses in the range of hundreds of rads to the thyroid appreciably increased the incidence of thyroid tumors in all age groups. Groups exposed to 2000 rads or greater had less than peak incidence except for the group in which exposure was started at 17 days of gestation where the high and low doses both produced a high incidence of thyroid tumors. The incidence of pituitary tumors was elevated above control levels only in the rats exposed prenatally to the two highest dose levels. Mammary tumor incidence was increased by postnatal but not by prenatal exposure to ^{131}I. (Research performed under Contract AT(45-1)-1830 between the United States Atomic Energy Commission and Battelle Memorial Institute.)

B-16-3 *Tumors and Transitory Hyperplasia in Irradiated Fetal Mice.* B. OSTERTAG, AND U. HEINZMANN, Institut für Hirnforschung, Tübingen u. Gesellschaft für Strahlenforschung, Neuherberg, West Germany.

Actual meningioma, tumor-like hyperplasia into the pallium, and transitory hyperplasia were found in fetal mice, which had been treated with 180 R intrauterine radiation. The latter, often referred to as rosettes because of their appearance in cross-section pictures, more correctly designated, however, as "germballs," are not causal homogeneous structures. Their pathogenesis must therefore be investigated, whether their origin lie in the ependymal matrix or subependymal germinal layer.

Those originating in the primordial ependyma always show disruption of the ependyma, permeate the entire pallium with monotone medullo-epithelioma cell construction, and are quite unstable structures perishing intrauterinely.

Those from the subependymal germinal layer demonstrate a branching construction, similar to that of the embryonal neural canal, consisting of more or less primitive "spongioblasts". Indeed, no differentiation from nerve cells can be proven. Nevertheless they do contain germinal matter for the cerebral cortex. The cerebral cortex above these hyperplasiae is always reduced.

The general pathological significance is discussed with regard to morphology, distinct determinative phases, as well as the same result from different causes in mind (important for general pathology) the origin of hyperplasiae and tumors.

B-16-4 *A Comparison of Rat Mammary Carcinogenesis Following Total-Body Irradiation at Different Ages.* CLAIRE J. SHELLABARGER, Medical Department, Brookhaven National Laboratory, Upton, New York 11973, USA.

When female, Sprague-Dawley rats were given 350 R of 250 kVp x-ray, total body irradiation on either the 42*nd*, 84*th* or 225*th* day of age, in groups of 30, and studied for 10 months post-exposure, the incidence of rats with one or more mammary adenocarcinomas was 30 to 40% per group, the total number of mammary adenocarcinomas was 12–13 per group, the incidence of rats with one or more mammary fibroadenomas was 63%, and the total number of mammary fibroadenomas was 33–35 per group. The time-related development of mammary neoplasia was not different among the groups irradiated at 42, 84, or 225 days of age. Twenty-nine rats exposed on the 24*th* day of age showed a similar incidence of mammary fibroadenomas, 52%, perhaps a smaller number of fibroadenomas, 19, and a smaller incidence, 7%, and fewer adenocarcinomas, 2. Only 3 of 30 controls developed a total of 4 mammary fibroadenomas. The smaller mammary neoplastic response, particularly in regard to adenocarcinomas, when young rats were irradiated may be due to either a smaller absolute amount of mammary tissue being exposed and/or a relatively high ovarian radiation sensitivity. (Research carried out at Brookhaven National Laboratory under contract with the U.S. Atomic Energy Commission.)

B-16-5 *X-Rays, Influenza and Leukemia: An Examination of the Oxford Study of Childhood Cancers.* EMANUEL LANDAU, Food and Drug Administration, Rockville, Maryland 20852, USA, AND ALICE STEWARD AND JOHN F. BITHELL, Oxford University, Oxford, England.

The reduced incidence of leukemia in children under 5 in Connecticut and Alameda County, California, has been attributed to reduced prenatal irradiation or reduced influenza infection during pregnancy. In the United States, the only available measure of prenatal irradiation may show an increase between 1964 and 1970. However, the Oxford Survey demonstrated that the mean fetal dose per single x-ray film declined significantly since 1940.

It has also shown a positive relationship between maternal influenza, rubella and chickenpox and childhood cancer, and permitted the calculation of relative risk. Estimates of relative risk of cancer have also been made for children whose mothers received prenatal irradiation. An unexpected finding of the Survey is the wide difference in age at onset of childhood myeloid leukemia between radiogenic and non-radiogenic cases. The estimated mean interval from birth to onset was 98 months for the former as compared to 53 months for the latter.

These items represent significant contributions to the field of carcinogenesis.

B-16-6 *Salivary Gland Tumors and Atomic Bomb Exposure, Hiroshima, Japan.* NOBUO TAKEICHI, FUMIO HIROSE, AND HARUO EZAKI, Department of Cancer Research, Research Institute for Nuclear Medicine and Biology, Hiroshima University, Hiroshima, Japan.

During the period 1950 to 1971, 211 cases of benign and malignant salivary gland tumors were identified and confirmed in Hiroshima. Of these 62 had been exposed to the A-bomb and 149 had not been exposed.

Among residents of Hiroshima City, the incidence of the tumors for the period 1953–1971 among exposed persons was 3.2 times greater than that among non-exposed persons for all cases and 11.0 times greater when only malignant tumors were considered.

The ratios of observed to expected cases for both total cases and malignant cases alone were also significantly higher ($P < .001$) among those exposed within 1500 meters from the hypocenter compared to those nonexposed. The relative risk among those exposed within 1500 meters for all salivary gland tumors was 6.23 and for malignant types alone 18.59 when compared to those who had not been irradiated.

The tumorigenic action of A-bomb irradiation on the salivary gland will be further discussed with respect to sex, age at exposure, tumor latency and tumor histology.

B-17-1 *Flash Photolysis and Pulse Radiolysis of Aqueous Solutions of Sulfhydryl Compounds.* JOHN A. STONE AND TZU-LIN TUNG, Queen's University, Kingston, Ontario, Canada.

Flash photolysis of aqueous solutions of cysteine (CySH) or pulse radiolysis of these solutions saturated with N_2O produces the cysteinyl radical which, in the presence of CyS^- is in

equilibrium with the disulfide radical anion ($CySSCy^-$). At concentrations below 6×10^{-7} M $CySSCy^-$ decays by a first order process ($k = 2 \times 10^3$ sec^{-1}) independent of pH and cysteine concentration. At higher concentrations the decay is second order ($k \sim 10^9$ M^{-1} sec^{-1}) and dependent on pH and cysteine concentration. The radical anion reacts with cystine and the disulfide of glutathione ($k \sim 10^6$ M^{-1} sec^{-1}) but not with penicillamine disulfide. In the presence of olefinic compounds (allyl alcohol and acrylic acid) the initial radical anion yield is reduced in the flash photolysis system but not in pulse radiolysis while the subsequent decay is little changed in either case. A transient formed by flash photolysis of dithiothreitol decays at all concentrations by a second order process. The initial yield of this transient is also suppressed by the presence of olefinic materials suggesting that the transient is a cyclic disulfide radical anion.

B-17-2 *Interactions of Drugs Including Radiation Modifiers with Biological Macromolecules as Studied by Pulse Radiolysis.* CLIVE L. GREENSTOCK AND IAN DUNLOP, Medical Biophysics Branch, Atomic Energy of Canada Limited, Whiteshell Nuclear Research Establishment, Pinawa, Manitoba, ROE 1L0 Canada.

The interactions of small molecules with biopolymers may be involved in a wide variety of effects, including mutagenesis, carcinogenesis, antibiotic and antitumor activity, radiation protection and sensitization. A study of these interactions in simple model systems, and an evaluation of binding specificities and optimization, may be helpful in understanding the causes of these biological effects, and useful in general drug screening.

Both ionic binding, and intercalation or solubilization of carcinogens, dyes, antibiotics and radiation modifiers in biological macromolecules, have been investigated by pulse radiolysis. Ionic binding, unlike other types of association, shows little site specificity, and is eliminated at high salt concentration. Complex formation is indicated by changes in the apparent rate constant for reaction of the primary species e_{aq}^- with a particular drug, in the presence and absence of a macromolecule. Specific drug interactions have been observed with nucleic acids, proteins, lipid, and their analogues. Non-dissociative binding reduces the apparent e_{aq}^- reactivity of a drug. In certain cases, for drugs of low e_{aq}^- reactivity, an apparent increase in e_{aq}^- reactivity is observed which is attributed to an unfolding of the macromolecule, resulting in a greater cross-section for e_{aq}^- capture by the denatured macromolecule.

B-17-3 *Pulse Radiolysis Study of Carboxy- and Carbamoyl-Pyridinyl Radicals.* P. NETA AND L. K. PATTERSON, Radiation Research Laboratories, Center for Special Studies, Mellon Institute of Science, Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213, USA.

One-electron reduction of pyridines or N-alkyl pyridinium ions produces pyridinyl radicals. Carboxy and carbamoyl substituted pyridinyl radicals have been produced in irradiated aqueous solutions using e_{aq}^- or $(CH_3)_2\dot{C}OH$ as the reductant. Transient absorption spectra of the radicals and their decay kinetics have been monitored by spectrophotometric pulse radiolysis. From the effect of pH on the spectra, dissociation constants for acid-base equilibria in the radicals have been determined. With carbamoylpyridinyl radicals protonation of the type

$$R\text{-}\dot{N}C_5H_4CONH_2 + H^+ \rightleftarrows R\text{-}\dot{N}C_5H_4\dot{C}(OH)NH_2$$

takes place at pH 1–2. With the carboxy derivatives two equilibria have been observed, one at about the neutral region and another in strong acid similar to the case of the amides. The decay rates strongly depend on the position of the functional group relative to the nitrogen, on the state of protonation, and on the probability of cross reaction between the two forms of the radical. Electron transfer rate constants have also been studied.

B-17-4 *Pulse Radiolysis of Haemiproteins.* M. JOAN PEARSON AND G. A. SALMON, University of Leeds, Leeds LS16 6QB, Great Britain.

The attack of e_{aq}^-, OH and H on met-myoglobin and met-haemoglobin have been studied by pulse radiolysis. For both proteins attack of e_{aq}^- results in a shift of the Soret band from 406 to 430 nm corresponding to reduction of the ferric porphyrin group and the dependence of G(reduction) on protein concentration have been measured. Rates of reaction of e_{aq}^- and rates of reduction of the haem-group have been measured and found to be comparable. However spectral changes in the u.v. indicate some attack of e_{aq}^- on amino-acid residues.

Transient absorption spectra resulting from attack of OH on the proteins have been characterised, but complex post-irradiation changes resulting in some reduction of the haem-group occur over periods of a few milliseconds. The rates of attack of OH on the proteins were established by the carbonate/bicarbonate competition method.

Preliminary studies on the haem-containing enzymes peroxidase and catalase indicate that, whereas attack of e_{aq}^- on the former leads to similar spectral changes to those observed for met-myoglobin, for catalase an entirely different spectrum is observed. (We acknowledge the M.R.C. for financial support.)

B-17-5 *Photodimerization of Cytosine and Its Nucleosides.* Bo-Sup Hahn, Hiroyasu Taguchi, and Shih Yi Wang, Department of Biochemistry, The Johns Hopkins University, Baltimore, Maryland 21205, USA.

Although cyclobutane photodimers and photohydrates have been reported to be products when Cyt and its derivatives are irradiated with ultraviolet light, the nature of these photoproducts is not well established. This is due to the fact that these labile compounds are difficult to isolate in the pure state and their identifications were mainly based on UV absorption spectra and other indirect evidence. To better understand the photobiology of nucleic acids, we have isolated the cyclobutane dimers of Cyt, Cyd, and dCyd, in crystalline state from the irradiation of the respective monomers in frozen aqueous solutions and/or photosensitized reactions. We are able to present the UV, IR, NMR, and mass spectra of these dimers. Also, their properties will be described, such as their reactivities toward acid, base, heat, and light. (A similar study with four methyl derivatives of Cyt will be presented at the 2nd Annual Meeting of American Society for Photobiology.) (This research is supported by U.S. Atomic Energy Commission Contract AT(11-1)-3276 and is identified as No. C00-3276-11 (73).)

B-17-6 *Radiation-Induced Optical Changes in Aqueous DNA Solutions.* G. M. Meaburn and J. L. Hosszu, Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20014, USA.

Changes in optical absorption and turbidity of irradiated DNA solutions have been studied by the methods of kinetic spectroscopy and light scattering photometry. Dilute aqueous solutions of calf thymus DNA (100 μg/ml) were irradiated with either ^{60}Co-γ or pulsed 40 MeV electron radiation. The spectral region 240–280 nm was examined in solutions absorbing 4.5 krad delivered in a single 0.5 μs pulse. No changes in optical absorption are detected in native DNA up to 0.6 ms after the pulse. However, a decrease in absorption intensity at 259 nm is observed within 5 μs in solutions containing previously irradiated or thermally denatured DNA. The data indicate that this decrease in chromophoric activity is occurring over a time scale comparable to the rate of formation of transient DNA-OH adducts which absorb at wavelengths greater than 300 nm. Conformational changes in radiation-damaged DNA leads to a reduction in hypochromicity; light scattering measurements suggest that loss of structural integrity is a relatively slow process spanning several milliseconds in these dilute solutions.

B-18-1 *Radiolytic Cleavage of Thymine Dimer.* L. I. Grossweiner and J. A. Vigil, Biophysics Laboratory, Physics Department, Illinois Institute of Technology, and R. Santus, Department of Medical Physics, Michael Reese Medical Center, Chicago, Illinois, USA.

Thymine dimer produced by UV irradiation of frozen aqueous thymine is cleaved by ionizing radiation. Co-60 G values indicate that each reacted e_{aq}^- leads to $\sim$1.5 thymines and each reacted OH· gives $\sim$0.5 thymines. The initial reaction deduced from pulse radiolysis is attributed to a short-lived thymine dimer electron adduct (465 nm, 565 nm) which decays to form the thymine anion (325 nm). The first observed products of OH· reaction is the thymine OH-adduct (380 nm). Thymine formation is attributed to oxidation of the dimer followed by hydrolytic splitting. Thymine is not formed in nitrous oxide-saturated solutions containing t-butanol, but is enhanced in nitrogen-saturated solutions with t-butanol, suggesting that the t-butanol radical formed by scavenging of OH· reacts with a product of the electron reaction. The possibility of dimer splitting by oxidation or reduction helps explain the variety of molecular types that can sensitize the photochemical monomerization of thymine dimer. (Supported by the U.S.A.E.C. on Contract No. AT(11-1)-2217.)

B-18-2 *The Effect of Electron-Scavenging Cations on Free Radical Products in γ-Irradiated Diethyl Phosphates.* FOUAD S. EZRA AND WILLIAM A. BERNHARD, Dept. of Radiation Biology & Biophysics, University of Rochester, School of Medicine & Dentistry, Rochester, NY 14642, USA.

The Mg^{++}, K^+, Zn^{++}, Ba^{++}, Ag^+, and Cd^{++} salts of diethyl phosphoric acid, as well as the free acid (HDEP), have been γ-irradiated and then observed by electron spin resonance (ESR) in the temperature range between 77 K and 300 K. All of the above forms of DEP yield the $CH_3\dot{C}HOP(O)_2OC_2H_5^-$ radical. In contrast, the ethyl radical, $CH_3\dot{C}H_2$, is observed only in MgDEP, KDEP, and HDEP. A minor radical product, $C_2H_5O\dot{P}O_2^-$, is produced in similar concentrations in all the above compounds except CdDEP and AgDEP. In CdDEP the yield was one fifth that of the other compounds and in AgDEP no ethyl phosphite radical was observed. The ESR data for AgDEP and CdDEP also indicate that Ag^+ and Cd^{++} scavenge electrons to yield Ag° or Cd^+, respectively. When KDEP is doped with Ag^+, the production of $CH_3\dot{C}H_2$ and $C_2H_5O\dot{P}O_2^-$ is depressed, the yield of $CH_3\dot{C}HOP(O)_2OC_2H_5^-$ is unaffected, and Ag° is observed.

It is concluded that the ethyl and ethylphosphite radicals are formed by dissociative electron attachment and that formation of $CH_3\dot{C}HOP(O)_2OC_2H_5^-$ is independent of electron addition. (Supported by USAEC Contract AT (11-1)3490 and assigned Report No. UR-3490-459.)

B-18-3 *Environmental Effects on the Interaction of Hydrated Electrons with Lysozyme.* MORTON Z. HOFFMAN, Department of Chemistry, Boston University, Boston, Massachusetts 02215, AND E. HAYON, Pioneering Research Laboratory, U.S. Army Natick Laboratories, Natick, Massachusetts 01760, USA.

The reaction of e_{aq}^- with lysozyme, a hydrolytic enzyme containing four disulfide linkages (RSSR), has been studied in aqueous solution by means of pulse radiolysis. The rate constant for the reaction is 1.4×10^{11} M^{-1} sec^{-1} at pH 5.7 and its value decreases continuously with increasing pH to 1.4×10^{10} M^{-1} sec^{-1} at pH 12.3. The value of k is also very sensitive to the ionic strength of the solution, consistent with the high positive charge of the enzyme in neutral solution. The result of the interaction of e_{aq}^- with the enzyme is a transient absorption with λ_{max} 420 nm (apparent ϵ 5.8×10^3 M^{-1} cm^{-1}) which decays via pH-independent first-order kinetics ($k = 1 \times 10^4$ sec^{-1}) and is suggested to be a $RSSR\cdot^-$ type of intermediate. Its decay is independent of the presence of other additives (NaCl, phosphate) revealing a secondary transient with an absorption maximum also at 420 nm. Spectrum II decays very slowly (k = 0.2–70 sec^{-1}) with a rate constant that is extremely sensitive to pH and medium environment. The decay of II reveals Spectrum III which shows a very weak absorbance at 400–420 nm with $t_{1/2} \sim 10$ sec. Although the $(CH_3)_2\dot{C}OH$ radical does not produce any observable transient intermediate, the reaction of $CO_2\cdot^-$ with lysozyme generates the 420 nm absorption with a pH-dependent rate constant. The first-order decay of this intermediate is proportional to $[H^+]$ from pH 4.5 to 8.0; thereafter the value of k is constant at about 15 sec^{-1}. The results are discussed in terms of the different possible sites for electron attack and comparison is made with the behavior of other sulfur-containing enzymes and model compounds.

B-18-4 *Electron Transfer in Photosensitization of Biomolecules by Acridine Dyes.* YVES LION AND ALBERT VAN DE VORST, University of Liege, Liege, Belgium.

It is well known that the photosensitization of biomolecules like amino-acids or DNA nucleotides leads to the formation of free radicals characteristic of these entities. Especially in the case of nucleotides, H-adduct radicals are induced by a multiple step mechanism:

a) biphotonic ionization of the chromophore
b) anionic stage of the substrate
c) protonation of the anion.

On the other hand, recently, it was demonstrated that in γ-irradiated complexes between nucleic acids constituents and proflavine, the final radiation damage was localized in the dye moiety.

This paper tries to explain this discrepancy between the radiolysis and photosensitization. It is shown that the photosensitization of biomolecules by proflavine is directly influenced by

the relative molar concentrations of dye and of substrate. For some values of these concentrations, an ESR singlet associated with a radical form of proflavine is observed. The appearance of this signal is discussed in terms of an electron transfer from an ionized proflavine molecule directly to another neutral dye molecule or via a molecule of substrate as an intermediate. It is shown that this transfer is facilitated by stacking.

B-18-5 *One-Electron Reduction of Vitamin B_{12} and Coenzyme B_{12} in γ-Irradiated Frozen Solutions.* HIROSHI SEKI, TADAMASA SHIDA, AND MASASHI IMAMURA, The Institute of Physical and Chemical Research, Wako, Saitama 351, Japan.

The reduction of vitamin B_{12} and coenzyme B_{12} with electrons has been investigated in γ-irradiated aqueous methanol glasses at 77°K to gain insights into the mechanisms of their biochemical reactions.

Vitamin B_{12} [L_5-Co(III)-CN], when irradiated in the glass, exhibits an ESR signal at $g \approx 2.2$, which is ascribed to its anion [L_5-Co(III)-CN^-] having an unpaired electron on the Co-C bond. Upon slightly warming the signal changes to that of $B_{12}r$ [L_5-Co(II)], resulting from the bond cleavage. Optical studies also support this conversion.

In contrast to vitamin B_{12}, the one-electron reduction of coenzyme B_{12} [L_5-Co(III)-Ad] give rise only to an ESR absorption of $g \approx 2.0$, attributed to an organic radical possibly derived from the adenosyl-moiety. The optical spectrum indicates the formation of $B_{12}s$ (L_5-Co(I), diamagnetic); i.e., the Co-C bond is cleft even at 77°K.

Above results are represented by:

$$L_5\text{-Co(III)-CN} + e^- \xrightarrow{\text{at } 77°K} L_5\text{-Co(III)-CN}^- \xrightarrow{\text{warming}} L_5\text{-Co(II)} + CN^-$$

$$L_5\text{-Co(III)-Ad} + e^- \xrightarrow{\text{at } 77°K} L_5\text{-Co(I)} + Ad$$

(L: ligand with N coordinated to Co; Ad: 5′-deoxyadenosyl)

B-18-6 *Reduction of Ferricytochrome c by Hydrogen Atoms: Evidence for Intramolecular Transfer of Reducing Equivalents.* NORMAN N. LICHTIN, AVIGDOR SHAFFERMAN, AND GABRIEL STEIN, Dept. of Physical Chemistry, The Hebrew University, Jerusalem, Israel.

Direct evidence has been obtained by means of the technique of pulse radiolysis-kinetic spectrometry that, consequent upon reaction of a single H-atom with a single molecule of ferricytochrome *c*, a reducing equivalent can be transmitted intramolecularly via the protein structure to ferriheme. Such transmission accounts for at least 70% of the total reduction of the ferri to the ferro state of cytochrome *c*. Approximately half of the H atoms which add to ferricytochrome *c* reduce it to ferrocytochrome *c*. The remainder apparently react at sites from which reducing equivalents are not transmitted to ferriheme.

The following second order rate constants were determined in solutions of low ionic strength at 20 ± 2°C: k[H + ferricytochrome *c*] = (1.1 ± 0.2) × 10^{10} M^{-1} sec^{-1} at pH 3.0 and 6.7; k[H + ferrocytochrome *c*] = (1.3 ± 0.2) × 10^{10} M^{-1} sec^{-1} at pH 3.0; $k[e_{aq}^-$ + ferrocytochrome *c*] = (1.9 ± 0.4) × 10^{10} M^{-1} sec^{-1} at pH 6.7.

(This research was supported by the U.S. Atomic Energy Commission Division of Biology and Medicine.)

B-18-7 *On the Superoxide-Mediated Oxidation of Epinephrine.* MANFRED SARAN, CHRISTA MICHEL, WOLF BORS, EDMUND LENGFELDER, AND RITA SPÖTTL, Gesellschaft für Strahlen- und Umweltforschung, Institut für Biologie, D-8042 Neuherberg; Strahlenbiologisches Institut der Universität, München, West Germany.

Epinephrine is co-oxidized together with purines by enzymatically generated superoxide anions. The same radical has been postulated to mediate the autoxidation of epinephrine in alkaline solution and to be responsible for the stimulating effect of hydroquinones. Inhibition of the formation of adrenochrome by superoxide dismutase (EC 1.15.1.1) is often used as an assay method for this widely distributed enzyme.

Using electron pulses from a Febetron (2 MeV, 40 nsec) at low dose rates, O_2^- was generated in aqueous oxygenated and in anaerobic hydrogen peroxide solutions. The reactions with epinephrine, adrenalone and leuco-adrenochrome and their respective dependence on pH, concentration of substrate or superoxide, and presence of scavengers were determined by kinetic spectroscopy.

The sequence and the kinetics of the four-electron oxidation to adrenochrome will be discussed. Comparisons will be made with oxygeneration reactions of basic hydroquinones, with particular reference to the postulated generation of O_2^- by these compounds.

B-19-1 *Extinction Coefficients of Triplet Absorption* (ϵ_{tt}), *Quantum Yields of Triplet Formation* (Φ_t) *and Quantum Yields of Isomerisation* (Φ_i) *Measured by Pulse Spectroscopy.* BERNARD J. AMAND,* RENÉ V. BENSASSON,* AND EDWARD J. LAND.**

We shall present

i) a general comparative method of determining ϵ_{tt} via energy transfer by complementary techniques of pulse radiolysis and laser flash spectroscopy;

ii) a general method of determining triplet quantum yields Φ_t (using the ϵ_{tt} thus obtained) or isomerisation quantum yields Φ_i by relative transient absorption measurements. Conditions for the procedure will be outlined;

iii) various applications of these methods to simple aromatic molecules and to some molecules of biological interest (mainly quinones and polyenes).

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B-19-2 *Formation of Methylbenzyl Radicals by Pulse Radiolysis of Methylated Benzenes in Aqueous Solutions.* K. SEHESTED, Danish AEC Research Establishment, Risö, DK-4000 Roskilde, Denmark, H. C. CHRISTENSEN, AB Atomenergi, Fack, S-611 01 Nyköping, Sweden, AND E. J. HART, Argonne National Laboratory, Argonne, Illinois 60439, USA.

Radicals of the benzyl type is formed in aqueous alkaline solutions of methylated benzenes: O^- abstracts an H atom from a methyl group. The rate constants are around 2×10^9 $M^{-1} \times s^{-1}$. Benzyl radicals are formed in acid solutions in a more complex way involving an equilibrium between the OH addition complex and its protonated form and also involving water elimination reactions: When the benzyl formation rate constant is plotted as a function of the proton concentration in a double logarithmic plot S-formed curves are obtained with horizontal parts at high and low pH. OH radicals may also react with methylated benzenes by direct abstraction of an H atom from a methyl group. The proportion of abstraction is directly proportional to the number of methyl groups in the hydrocarbon with rate constants of 4.2×10^8 $M^{-1} \times s^{-1}$ per methyl group.

B-19-3 *Radiolysis of Aqueous Solutions of Semifunctional Organic Compounds.* HELEN M. NANOBASHVILI, SOPHIE E. GVILAVA, AND SVETLANA G. IGNATISHVILI, Institute of Inorganic Chemistry and Electrochemistry of the Georgian SSR Academy of Sciences, Tbilisi 380093, USSR.

Radiolysis of aqueous solutions of some semifunctional organic compounds: thiourea, thoacetamide, urea, cysteine, etc., has been studied and principal regularities of their radiation-chemical transformation established. The intermediate and stable products of the radiation-chemical transformations of the above compounds have been identified. A reciprocal effect of different functional groups: –S–, –SH, –O–, $-NH_2$, –COOH, etc. has been shown during radiolysis of compounds under investigation. A comparison of radiation-chemical transformation of sulphur- and oxygen-containing compounds is given. The constants of reaction rate of compounds in question have been calculated in relation to the active products of radiolysis of water. The protective influence of sulphurcontaining functional groups has been ascertained both in a sulphurcontaining compound by itself and in a mixture with other compounds. A relative comparison of reactivity of compounds in question has been made and the mechanism of their radiation-chemical transformation proposed.

B-19-4 *Structural Correlation of Reactivity of Hydrated Electrons with Perfluorocarbons.* LALITHA J. MITTAL AND JAI P. MITTAL, Chemistry Division, Bhabha Atomic Research Centre, Trombay, Bombay 400085, India.

The reactions of hydrated electrons with perfluoro hydrocarbons have been investigated in aqueous solutions and their reaction rate constants are obtained. An attempt is made to correlate the reactivities of these organic compounds towards e_{aq}^- with molecular structural details. The electron reactivity seems to be very much dependent upon the geometric configuration of the molecule. All the cyclic perfluorocarbons are found to react much faster than the corresponding straight chain compounds. It appears that in all the cyclic perfluorocarbons, a kind of electrostatic potential well is generated giving rise to their higher reactivity towards the hydrated electrons.

B-19-5 *Reactions of Thermal Hydrogen Atoms with Low Temperature Unsaturates. III. Effect of Infra Red Heating.* RUSSELL H. JOHNSEN AND ALAN K. E. HAGOPIAN, Florida State University, Department of Chemistry, Tallahassee, Florida 32306, USA.

Thermal hydrogen atoms produced on a hot tungsten filament have been caused to impinge upon various unsaturated compounds in a liquid nitrogen cooled reactor. Evidence is presented which indicates that when these substances undergo moderately rapid reaction the surface temperature of the unsaturate is considerably higher than 77°K due to radiant heating by the filament. When the reactants are actually at 77°K most react very slowly, if at all, with thermal atoms. Much of the anomalous kinetic data relating to hydrogen atom reactions in the literature can be reconciled by these observations.

The significance of these conclusions to radiation chemistry will be discussed.

B-19-6 *Low-Temperature Radiolysis of Binary Systems: Aliphatic Thiocyanates-Alkanes, Dialkyl Sulphides-Alkanes.* HELEN M. NANOBASHVILI, RIMZET G. TUSHURASHVILI, MERAB V. PANCHVIDZE, AND ALEXANDER G. DAPKVIASHVILI, Institute of Inorganic Chemistry and Electrochemistry, Tbilisi 380093, USSR.

γ-Radiolysis of systems $C_nH_{2n+1}SCN$–C_7H_{16} and $(C_nH_{2n+1})_2S$–T–C_8H_{18} with $n = 1$ to 11 and frozen at 77°K has been investigated by the EPR method. When introducing alkylthiocyanates into *n*-heptane the value of the radiation-chemical yield of radicals is less than the additive value, the former being greater than the yields of radicals in the corresponding thiocyanates and lower than in *n*-heptane. A decrease of the quantity of paramagnetic centers formed from isooctane is also observable with lesser adding of dialkyl sulphide introduced into isooctane. In all cases the concentration of radicals is considerably lower than the additive value, whereas with the greater concentration of sulphide the total yield is as good as equal to the yield of radicals in the corresponding sulphides. Some other sulphur-containing compounds also behave analogously. This suggests that the energy transfer from molecules of hydrocarbon to molecules of sulphur-containing compounds takes place in the systems under investigation, leading to the protection of alkanes against the irradiation of sulphurous compounds. The question of the energy transfer from alkane to the sulphur-containing compounds has been considered.

B-20-1 *Observations on the Solvated Electron in n-Hexane in the Presence of Polar Solutes.* JOHN H. BAXENDALE, AND ERIC J. RASBURN, Department of Chemistry, University of Manchester, Manchester, England, Great Britain.

The technique of pulse radiolysis has been used to study the absorption spectrum of the solvated electron in *n*-hexane containing 0 to 0.67 *M* *n*-propanol. No spectral changes were observed below ~15 m*M* *n*-propanol but the rate of decay was increased. Between ~20–100 m*M* *n*-propanol, the absorption spectrum began to peak and shift towards that of the electron in *n*-propanol. The electron lifetime increased but the decay was complex. At 0.67 *M* *n*-propanol, the absorption spectrum and decay were similar to those observed in the pure alcohol. No significant change in the free ion electron yield was observed when studied using pyrene as an electron scavenger in the presence of 0 to 0.67 *M* *n*-propanol. Decreases in the rate constant for the electron + chloroform reaction coincided with the regions spectral changes occurred,

indicating a reduction in the electron mobility. This has been confirmed by a reduction in the electrical conductivity signal in the presence of *n*-propanol. It is concluded that below 20 m*M* *n*-propanol, chemical reaction takes place between the electron and the alcohol before stabilization by dipole coagulation can occur. Up to 50 m*M* *n*-propanol, the electron is localized in the vicinity of alcohol molecules but does not diffuse with the trap. Above 100 m*M* *n*-propanol, the electron resembles that in the pure alcohol. Saturation of *n*-hexane with water (3.6 m*M*) has a greater effect than a similar concentration of *n*-propanol: the intensity of the electron absorption spectrum is increased with a maximum at ~1500 nm and the electron mobility is found to be halved.

B-20-2 *Formation and Decay of Solvated Electrons in Long-Chain Alcohols at Times Greater Than 200 psec.* G. A. KENNEY-WALLACE, Sterling Chemistry Laboratory, Yale University, New Haven, CT 06520, AND C. D. JONAH, Chemistry Division, Argonne National Laboratory, Argonne, Ill. 60439, USA.

We have observed the formation and decay of solvated electrons in a series of alcohols up to 1-decanol and determined the G values relative to water following the picosecond pulse radiolysis of these liquids. Measurements made at times greater than 200 psec show the slow formation of solvated electrons in cyclohexanol, 1-octanol, and 1-decanol over a period of 250 to 2000 psec. There is significant decay of the solvated electron in all alcohols over the initial 30 nsec, exceeding that observed for the hydrated electron in water. The implication of these relative yields, the solvation times, and the fast decays of solvated electrons in alcohols will be discussed in relation to previous picosecond and nanosecond data and spur diffusion theory.

B-20-3 *Experimental Decay of the Hydrated Electron and Hydroxyl Radical in Aqueous Solutions from 100 Picoseconds.* C. D. JONAH, E. J. HART, J. R. MILLER, AND M. S. MATHESON, Chemistry Division, Argonne National Laboratory, Argonne, Illinois 60439, USA.

Using the Argonne Single-Pulse Stroboscopic Pulse Radiolysis system, the decay of the hydrated electron was measured in pure water and in the presence of strong hydrogen ion and OH scavengers. In addition, the form of the decay of the OH radical has been determined. An initial fast decay of the hydrated electron (duration ~400 psec) has been observed as well as the slower decay (~30 nsec) which was originally observed with a sub-nanosecond pulse radiolysis system using laser-fast photodiode detection. The amount of decay of the hydrated electron in the time region of .1–3 nsec is less than that predicted by spur diffusion theory. These results, coupled with previous work seem to imply a greatly different spatial distribution of spur products than has previously been suggested. The implications of these measurements for spur diffusion theory will be discussed. (Work performed under the auspices of the U.S. Atomic Energy Commission.)

B-20-4 *Hydrated Electron Reactions at Picosecond Times.* M. S. MATHESON, E. J. HART, J. R. MILLER, AND C. D. JONAH, Chemistry Division, Argonne National Laboratory, Argonne, Illinois 60439, USA.

We use the Argonne Single-Pulse Stroboscopic Pulse Radiolysis system with time resolution of 50 picoseconds to follow reactions of the hydrated electron with high concentrations of reactive solutes. For diffusion-controlled reactions an increase in rate constant is observed at high concentrations of neutral solutes owing to reactions of initially formed close pairs. In addition ionic strength affects the rate constants of charged solutes, and this effect is less if the hydrated electron reacts before it establishes its equilibrium ionic atmosphere. The reactivities of e^- and H_2O^+ (precursors of e_{aq}^- and of H_3O^+ and OH) toward several solutes were measured. Finally, the OH radical has been directly observed on this time scale and several OH reactions studied at high solute concentrations. This work will utlimately be extended to reactions of e_{aq}^- and OH with solutes more closely related to the constituents of living cells. (Work performed under the auspices of the U.S. Atomic Energy Commission.)

B-20-5 *Spectrophotometric Measurements of Hydrated Electron Formation and Decay in Pulsed Deuteron and Helium Ion Beams.* EDWIN J. HART, K. H. SCHMIDT, AND C. D. JONAH, Chemistry Division, Argonne National Laboratory, Argonne, Illinois 60439, USA.

Pulse radiolysis with electron beams has greatly improved our understanding of the mechanism of irradiated aqueous solutions. We are now extending pulse radiolysis to the relatively feeble 20 MeV d^+ and 40 MeV He^{++}. Motivation for these studies stems from the expectation that transient species identified will help to explain why high LET radiation produce lesions unaltered by chemical radiation modifiers including oxygen. Unlike that of a spherical spur, the infinite-time concentration of free radicals produced in a cylindrical track is zero. Therefore, the measured yieds of e_{aq}^- and other species will depend very strongly on the time scale of the secondary reactions, and the yields under our experimental conditions will be much lower than those previously determined at millimolar scavenger concentrations. After 10^{-6} sec. diffusion from a cylindrical track, we estimate a $G(e_{aq}^-)$ of 1.3 for 20-MeV d^+ and 0.4 for 40-MeV He^{++}. Our kinetic calculations lead to an expected e_{aq}^- concentration of 0.2 to 0.5×10^{-7} M after 10^{-5} sec. irradiation. We intend to use a simulated rotating-sector method with signal averaging for our kinetic studies. Some results on e_{aq}^- formation and decay will be reported. (Work performed under the auspices of the U.S. Atomic Energy Commission.)

B-20-6 *Absorption Spectrum and Primary Yield of the Solvated Electron in Liquid Ammonia at 23°C and Pressures up to 6.7 kbar.* FARHATAZIZ, LEWIS M. PERKEY, AND ROBERT R. HENTZ, Radiation Laboratory, University of Notre Dame, Notre Dame, Indiana 46556, USA.

The absorption spectrum and primary yield of e_{am}^- have been determined by nanosecond pulse radiolysis of liquid ammonia at 23°C. With increase in pressure from 0.009 to 6.7 kbar, the following changes occur: transition energy at the absorption maximum (E_{max}) increases from 0.67 to 0.91 eV; bandwidth increases by 34%; optical density at the absorption maximum decreases by 32%; extinction coefficient at the absorption maximum decreases by 19%; and primary yield decreases from 3.2 to 2.0 solvated electrons per 100 eV. The pressure and temperature dependences of E_{max} are attributed to changes in (1) size of the electron cavity and (2) the *local* dielectric constant associated with the solvation shell of the electron. The results indicate that the cavity of e_{am}^- ($\bar{V}$ = 98 ml mol^{-1}) is more compressible than that of the electron in water ($\bar{V}$ = 7 ml mol^{-1}), ethanol, or methanol. The decrease in primary yield with increase in pressure is attributed to an increase in the probability of neutralization relative to separation of geminate $NH_4^+ - e_{am}^-$ pairs owing to the large negative activation volume of the neutralization reaction as a consequence of the large volume of e_{am}^-.

B-20-7 *Pulse Radiolytic Formation of Solvated Electrons, Ion-Pairs and Alkali Metal Anions in Tetrahydrofuran.* WILLIAM A. SEDDON, G. ARTHUR SALMON, AND J. WALLACE FLETCHER, Atomic Energy of Canada Limited, Chalk River Nuclear Laboratories, Chalk River, Ontario, K0J 1J0, Canada.

Pulse radiolysis of solutions of alkali metal cations in tetrahydrofuran (THF) demonstrates the formation of solvated electrons e_s^-, alkali metal cation-ion pairs (M^+, e_s^-) and alkali metal anions M^-. This paper summarises the spectra, extinction coefficients and radiolytic yields of e_s^-, lithium, sodium, potassium and cesium species in THF. The overall kinetics are complex but principally involve reactions [1] and [2]

$$[1]\quad e_s^- + M^+ \rightleftharpoons (M^+, e_s^-)$$

$$[2]\quad (M^+, e_s^-) + e_s^- \rightarrow M^-$$

and the concomitant disappearance of all three species by reaction with radiolytically produced radicals. The results are compared with data established from studies of blue solutions of alkali metals dissolved in THF.

B-20-8 *Kinetics of Electron Species Formed in the Pulse Radiolysis of Basic Alkali Metal Solutions in Amines and Ethers.* J. WALLACE FLETCHER AND WILLIAM A. SEDDON, Atomic Energy of Canada Limited, Chalk River, Ont., Canada.

Pulse radiolysis of alkali metal alkyl amide or complex metal hydride solutions in amines

or ethers show the formation of three distinct species: the solvated electron (e_s^-), the alkali metal anion (M^-) and a species considered to be the cation-electron pair with stoichiometry M. The three species come to equilibrium in accord with the equations:

$$e_s^- + M^+ \rightleftharpoons (M^+ - e_s^-) \text{ or } M$$
$$M + e_s^- \rightleftharpoons M^-$$

The initial species formed in pulse radiolysis is e_s^-. The absorption spectrum attributed to e_s^- in pulse radiolysis differs from that observed on dissolving tha alkali metal. However the spectra observed for the M^- species are the same.

Rate and equilibrium constants for the formation of M and M^- vary markedly with the nature of the solvent, alkali metal, metal concentration and temperature. Estimates of the yields and extinction coefficients for the various species have been made.

B-21-1 *Variations in Relative Radiosensitivity of Mouse Embryos Irradiated at Selected Times of the Cell Cycle.* RUSSELL J. DUFRAIN AND ALISON P. CASARETT, Department of Physical Biology, New York State Veterinary College, Cornell University, Ithaca, New York, 14850, USA.

Fertilized ova were flushed from superovulated BLU:Ha (ICR) mice 46 hours after HCG injection, placed in culture and observed for cleavage to the 4-cell stage. At various intervals 4-cell embryos were selected and randomly assigned to radiation doses. They were then exposed to doses of 25 to 600 rads using a 280 kVp Picker x-ray machine (80 R/min, ½ mm Cu and Al parabolic filters) at selected times after cleavage. Embryos were maintained in modified Whitten culture medium under 90% N_2:5% CO_2:5% O_2 during and after irradiation.

Developmental progress was recorded at 12-hour intervals after irradiation for a total of 6 days. LD_{50} values for the embryos irradiated at each time were determined for endpoints of initiation of cavitation, blastocyst formation, initiation of hatching and completion of hatching. The values were then related to ^3H-thymidine uptake at analogous times in the 4-cell cell cycle. (Work supported by Environmental Protection Agency Grant No. EP-00679 and Atomic Energy Commission Contract No. AT(11-1)-3167.)

B-21-2 *Concerning the Postnatal Development of Mice Irradiated on the 11th Day of Gestation (clinically and anatomically).* U. HEINZMANN AND B. OSTERTAG, Gesellschaft für Strahlen- und Umweltforschung, Abteilung Nuklearbiologie, Neuherberg/München und Institut für Hirnforschung, Tübingen, West Germany.

The methods of exact irradiation on only one horn of the uterus of pregnant mice are described in this study. For clinical and anatomical research, the irradiated and unirradiated littermates were gathered from the womb (by section), so that optimal material for comparison was available.

Film exposures illustrate the abnormal behaviour of those development disorders caused by irradiation and allow the difference between irradiated and unirradiated littermates to be clearly discerned.

The research of others and our own are confirmed and additional descriptions of the disorders and certain problems of development and misdevelopment have been illustrated with slides.

Difficulties in differential diagnosis receive special consideration, as, for example, specific judgment as to whether the symptomatology is dependent on x-ray damage of the spinal cord, of the cerebellum, or of the cerebrum. This yields newer knowledge concerning development.

B-21-3 *Effects of Continuous Prenatal γ-Irradiation on the Pig.* POLLY G. MARTIN AND BERT H. ERICKSON, UT-AEC Comparative Animal Laboratory, Oak Ridge, Tennessee 37830, USA.

Little data is available on the effects of continuous prenatal irradiation on the long-lived mammal. Since, relative to the rodent, developmental events are protracted in these species the need for a reliable dose-response curve is obvious. In our initial experiments, pregnant gilts were irradiated for 108 days with a dose of 7 rads/22 hour day to the fetus. At all ages studied (birth-150 days) the significant effects were: sterility in both male and female and depression of brain and spleen weights. Other organs which weighed less than controls at birth were: adrenals (69%), heart (84%), kidney (80%), liver (77%) and thyroid (73%) but by 70 days only the adrenal (64%), heart (72%) and thyroid (60%) weighed less. Effects were minimal at 150

days of age. Number of white cells and platelets were lower in irradiated animals at birth but by 70 days control and irradiated values were similar. Other differences were equivocal and were confounded by individual variations apparently due to environmental stresses. (Supported by U.S. AEC under contract No. AT-40-1-GEN-242 with the University of Tennessee.)

B-21-4 *Effects of X-Rays on Preimplantation Mouse Embryos Grown and Irradiated in Vitro.* L. S. GOLDSTEIN AND A. I. SPINDLE, Laboratory of Radiobiology, University of California, San Francisco, California 94143, USA.

Mouse embryos flushed at the two-cell stage from the oviducts of pregnant females were x-irradiated at several preimplantation stages and assayed for their ability to undergo subsequent growth and differentiation *in vitro*. The culture technique eliminated detrimental effects resulting from exposure of maternal tissue and permitted analysis of functional differentiation of the trophoblast and inner cell mass after hatching from the zona pellucida. Irradiation was performed at the two-cell, four-cell, eight-cell, morula, and blastocyst stages. Developmental failure for early cleavage stages was associated with functions occurring between blastocyst formation and early post-implantation. The two-cell embryo was more resistant than succeeding cleavage stages, a finding that agrees with results of other workers. A resistant cell type first emerged at the morula, when determination of inner cell mass and trophoblast is thought to occur and when growth of irradiated embryos may consist entirely of trophoblastic tissue. Blastocysts were more resistant than the cleavage stages. Preliminary evidence indicates that differentiation into endoderm and ectoderm was reduced by 50% after doses of 80–100 rads regardless of the stage irradiated. (Work performed under the auspices of the U.S. Atomic Energy Commission.)

B-21-5 *Dependence of Adult Interzygomatic Distance, Skull Length and Skull Index on Level of Radiation Exposure During Development.* G. M. ANGLETON AND A. C. LEE, Collaborative Radiological Health Laboratory, Colorado State University, Fort Collins, Colorado 80521, USA.

Estimates of parameters were obtained and tests of their significance were performed relative to linear regression relationships on level of radiation exposure for interzygomatic distance, skull length, and skull index of adult beagles. Whole body bilateral exposures to ^{60}Co gamma rays were given at one of six ages: 8, 28, or 55 days postcoitus (dpc) or 2, 70, or 365 days postpartum (dpp). Exposures given at 8 dpc, 70 dpp, or 365 dpp did not appear to influence growth patterns significantly. However, significant reductions in growth were noted for exposures given at 28 dpc, 55 dpc, or 2 dpp. The estimated percent reductions per 100R of exposure were 4.4, 1.7, and 1.9 percent respectively for the interzygomatic distance and 4.6, 2.5, and 2.1 percent for skull length. Smaller changes were detected in skull index for exposures given at 55 dpc, 2 dpp, and 70 dpp. (Research supported by Contract FDA 72-302 with the Bureau of Radiological Health, Department of Health, Education and Welfare.)

B-21-6 *Effect of Radiophosphorus in Mice during Postnatal Development.* P. N. SRIVASTAVA, Radiation Biology Laboratory, University of Rajasthan, Jaipur, India.

Swiss albino mice of different ages (1 day to 4 weeks) were injected with ^{32}P (sodium orthophosphate in isotonic saline containing phosphate buffer) at the dose of 1 μCi/g body weight. No radiation sickness was observed.

Thymus, spleen and bone-marrow show some radiation damage but recovery takes place quickly. There is a temporary depression in the number of pronormoblast, normoblast and lymphocyte in the bone-marrow. The pattern of response of the peripheral blood is typical of mammals. In 2 and 4 week old injected mice, there is a significant elevation in albumin fraction and depression in beta-globulin one week after injection.

The damage in testis is partial and recovery takes place after some time. During postnatal development the testis is most sensitive at 3 to 4 weeks. In ovary, the primordial, secondary and tertiary follicles are totally absent at 6 weeks in the females which are injected up to 3 weeks. The dose of ^{32}P used at these developmental stages produces complete sterility in the females.

(The project was supported by a grant (01-001-3) from the Environmental Protection Agency (USA).)

B-21-7 *The Incorporation of Tritiated Thymidine in Chick Embryos.* BRIAN S. MAWHINNEY, The Royal Free Hospital School of Medicine, London, England.

Tritiated thymidine, if topically applied to the inner shell membrane, is absorbed by chick embryos "in ovo." The amount of isotope absorbed depends on the size of the embryo, the length of time the embryo is exposed to the radioactive solution and the isotope concentration.

Using autoradiography it has been shown that the distribution of isotope within embryos is uniform and does not depend on the length of exposure to the isotope. A cell cycle time of approximately 30 hours has been estimated.

If the radioactive solution is applied to the membrane at the commencement of incubation the degree of lethality and growth inhibition observed after seventeen days is related to isotope concentration. An LD_{50} of about 17 μCi/embryo has been measured.

Effects of X-radiation on the uptake of thymidine by the embryos will be discussed.

B-21-8 *Effect of Solar Radiation on Mineral Contents of* Achyranthes. ASPERA PARMINDAR. S. RATRA AND K. C. MISRA, D. A. V. College, Muzaffar Nagar, India.

Importance of inorganic components in governing the growth, organic productivity and distribution of plant species has been well recognized. Accumulation of the different elements in plant depend upon the climatic conditions. Any change in the environmental condition of plant will reflect upon its nutrient composition. The mineral content studied are N, P, K, Ca and Na. The estimation of K, Ca and Na was done by flame photometer, N by Kjeldahl method and P by colorimeteric method. Plants were cultured in laboratory in open, glasshouse and shade and also subjected to different length of solar radiation i.e., 8, 12 and 16 hours.

Increase in light intensity and duration increased the ash % in shoot, in root it is maximum in moderate light insity and 8 hour duration. Moderate light intensity (glass-house) and longer duration (16 Hour) tend to increase the N content of shoot. Roots show high % of N in full sunlight. Increased light intensity and duration increased the % of P in shoot and root as well. K increased with increase in intensity and duration in shoot, however, in roots K was maximum in 12 hour duration and full sunlight. Ca and Na % is maximum in plants in moderate light intensity and duration of light.

B-22-1 *Studies on Catalase Activity of Radioresistant Strains of* Drosophila nebulosa. M. S. GALIA AND E. K. MARQUES, Department of Genetics, Universida de Federal do Rio Grande do Sul, C. Postal 1953, 90000 Porto Alegre, RS, Brazil.

Radioresistant (R85) and control (C147) strains of *Drosophila nebulosa* that gave different response to Co^{60} radiation in some adaptative value components (Marques, 1973 Mutation Res. 17, 59–72), were studied spectrophotometrically for catalase activity. Whole homogenates of individuals with 72 hrs (2nd instar), and 120 hrs (3rd instar) larvae, prepupae, white pupae (144 hrs), dark pupae (168 hrs), and adults of 1 to 9 days were analyzed with a modified Chance's (1950) method. Quantitative determination in mg of live weight showed a higher catalase activity in the radioresistant strains from larvae up to 3 days old flies. These values are, 72 hrs: 0.14, 0.06; 120 hrs: 0.33, 0.24; prepupae: 0.36, 0.30; white pupae: 0.38, 0.29; dark pupae: 0.40, 0.31; one day: 0.53, 0.33; three days: 0.18, 0.13 for R85 and C147 respectively. These results suggest that the observed radioresistance of R85 may be due to an enzymatic action protecting against the indirect effects of ionizing radiation. (Work supported by CNPq, FAPERGS, CNEN, CEPP-UFRGS, CAPES.)

B-22-2 *Induced Repair—The Mechanism for Increased Lifespan in Irradiated Insects?* HOWARD S. DUCOFF, University of Illinois, Urbana, Illinois 61801, USA.

In contrast to mammals, insects usually exhibit increased lifespan after sublethal X-irradiation. Even survivors of midlethal doses have greater lifespan than controls. The phenomenon has been observed in many different orders, and in both sexes whether or not mated. Our experiments demonstrate that X-irradiation of *Tribolium castaneum* or *T. confusum* late in life, when the

natural mortality rate is increasing, also increases mean lifespan, via an early but temporary decrease in natural mortality. The following hypothesis is suggested: The capacity to repair damaged DNA declines as post-mitotic tissues age; radiation-caused nicks on DNA induce increased levels of repair systems, thereby improving the ability of organisms such as insects, which are primarily dependent on post-mitotic tissues, to survive metabolic and environmental "wear-and-tear"; however, deleterious effects would be predominant in organisms highly dependent on cell turnover, or in insects subjected to irradiation limited to proliferative tissues (e.g., the midgut). (Supported by grant CA 13779, USPHS.)

B-22-3 *Repair of Radiation Damage in* Drosophila melanogaster. R. Wynne Davies, Andrew J. Mill, Jane V. Webb, and Michael J. Hollingsworth, Departments of Radiobiology, Physics and Zoology, Medical College of St. Bartholomew's Hospital, Charterhouse Square, London, EC1M 6BQ, Great Britain.

Repair processes, similar in their expression to those seen in fractionated dose studies in proliferating mammalian cells, have been investigated in adult *Drosophila melanogaster*. These are predominantly post-mitotic organisms.

The lifespan of male *Drosophila melanogaster* was measured following irradiation with single or fractionated doses of 15 MeV electrons. The dose-rate was 1 krad second^{-1} with interfraction times of $\frac{1}{2}$, 2 and 6 hours. Comparison with unirradiated flies showed shortening of lifespan which gave some quantitation of the radiation induced damage.

Various factors affecting the apparent repair between fractions are discussed. The time-scale (of about 2 hours) for maximum repair in these post-mitotic tissues is comparable to that in mitotic mammalian cells, while the quantity of repair is an order of magnitude less.

B-22-4 *Survival of Drosophila Eggs Exposed to Microwave Energy and to Heat.* F. Alan Andersen and Tim L. Pay, Bureau of Radiological Health, FDA, Rockville, Maryland 20852, USA.

Drosophila melanogaster eggs deposited by flies on agar were exposed to 2450 MHz microwave radiation for 3 hours in a waveguide. A flow (38 1/min) of temperature (24°C) and humidity (50% RH) conditioned air is provided through the waveguide. Control eggs were maintained in the same conditioned air outside the waveguide. The ability of the eggs to hatch and produce adult flies (survival), normalized to 100% control survival, was determined as a function of the energy absorbed by the agar plus the eggs. The results indicate that about 90 joules per gram of agar plus eggs is absorbed before an initial decrease in survival can be detected. The normalized survival of eggs deposited on agar and exposed to 30–41°C temperatures for 3 hours in a conventional incubator was also determined. A 3 hour exposure to at least 31°C appears to be needed to yield an initial decrease in survival, with killing complete at 41°C. The role of microwave induced heating in egg death will be discussed in light of the egg survival determined for conventional heating.

B-22-5 *After-Treatment with Tetrahydrofolic Acid (FAH_4) in the Mouse.* Masami Kiga, Hirotsugu Munechika, Yasushi Koga, and Shinichi Hirabayashi, Department of Radiology, School of Medicine, Showa University, Tokyo, Japan.

FAH_4 is known to improve the lethality of wholebody irradiated mouse when given prior to irradiation. This report deals with the results of its post-treatment. In recovery processes involving DNA synthesis which takes place a certain time following irradiation, FAH_4 seems to play its part. Following results were suggestive of these mechanisms. 1) 2 weeks survivors were increased in the group administered FAH_4 6–24 hours after irradiation with 500–750 r. 2) Bone marrow death seemed to be influenced by this treatment, because of the fact that 1000 r. irradiated mouse didn't reveal any improvement by this treatment. 3) ^{125}IUdR (5-iododeoxyuridine) uptake in bone marrow increased 24 hours after 500 r. irradiation.

B-22-6 *Sequestration of ^{65}Zn by Frog Skin Pigment—A Vital Process.* Hyman Guthwin, Lehman College, Bronx, New York 10468 and David L. Willis, Oregon State University, Corvallis, Oregon 97331, USA.

Metal ion uptake by synthetic melanin and melanoma melanin fractions has been attributed to cation exchange (Radiation Research, **32**, 1, 1967) with equilibration in one hour and minimal involvement of protein moieties. We have shown (Radiation Research, **55**, 546, 1973) parenterally administered ^{65}Zn to result in pigment pattern autoradiography; a requirement for formalin fixation suggested a role for protein moieties. Cation exchange alone should allow in vitro uptake by frog skin pigment. Skins from one side were sequentially immersed in (1) 10% formalin in frog Ringer's, (2) Ringer's, (3) Ringer's with ^{65}Zn, and (4) Ringer's; one frog dead for 24 hours is included. For each animal, these served as controls for contralateral skins treated in different ways prior to step 3: (a) Ringer's, (b) skin from the dead frog in Ringer's, (c) Ringer's followed by acetone, (d) Ringer's followed by alcohol, and (e) Ringer's with formalin Ringer's following step 4.

Much higher gamma counts were obtained from acetone treated skin, alcohol treated skin, and both skins from the dead frog.

Autoradiography disclosed no relation between activity and the pigment pattern. Thus, ^{65}Zn sequestration by melanin in intact frogs is not explicable solely on the basis of cation exchange.

B-22-7 *The Immune Response in Rabbits to Cobalt-60 Radiation-Attenuated* Ascaris suum *Larvae.* W. A. Johnson, M. F. Andrews, and J. P. Vacik, North Dakota State University, USA.

Rabbits were given gamma irradiated *Ascaris suum* embryonated ova for immunization against the swine ascarid. The dose level of radiation to the embryonated ova were 30,000, 40,000 and 50,000 rads. Rabbits receiving two immunizations, the first 18 days and the second 8 days prior to challenge, exhibited partial protection against the ascarid at the 5% level of significance. The gross pathology in the lungs from all cases was considerable but gross pathology of the liver was minor.

Progress has been made in the field of active immunization of animals against helminths. Early work with the lungworm, *Dictyocaulus viviparous*, and subsequently with the hookworm, *Ancylostoma caninum*, indicated the possibility of using this method against various other helminths. This study was completed in order to establish guidelines for further research with this helminth. There are other reports in the literature on the irradiation of the ascarid and its response.

In this preliminary study, there were several objectives: the dose range in rads to the ova, the number of ova or larvae necessary for an immune response and an observation of the pathology produced.

B-23-1 *Microdosimetry Parameters for Neutrons.* J. J. Coyne and R. S. Caswell, National Bureau of Standards, Washington, D.C. 20234, USA.

Secondary particles (p, α, C, N, etc.) are generated when neutrons interact with tissue. Using initial spectra and slowing-down spectra for these charged particles, we obtain energy deposition spectra—the spectra of energy depositions by the secondary particles in small tissue volumes. We have calculated energy deposition spectra for many energies of monoenergetic neutrons between 60 keV and 14 MeV using analytic methods previously described. Spectral averages, such as the frequency and dose averages of lineal energy ($\bar{y}_f$, $\bar{y}_D$), or the equivalent quantities for specific energy ($\bar{Z}_1$, ζ) have been calculated as a function of neutron energy and cavity size. Some comparisons with experiment will be presented. (Research supported by the Division of Biomedical and Environmental Research, U.S. Atomic Energy Commission.)

B-23-2 *Event-Size Spectra from a Neutron Radiotherapy Beam.* Keith A. Weaver, Kenneth R. Alvar, Hans Bichsel, Juri Eenmaa, David L. Williams, and Peter Wootton, University of Washington, Seattle, Washington 98195 USA.

Event-size spectra measured with a tissue-equivalent proportional counter in the University of Washington neutron radiotherapy beam will be presented. Spectra were measured for various collimator sizes on the beam axis in air, on the beam axis at several depths in tissue-equivalent fluid, and in the beam penumbra. Gamma dose was separated from neutron dose by a subtractive technique based on measured γ event-size spectra, and neutron dose was separated into proton, α,

and heavy-recoil components. Possible correlations of spectra changes with RBE changes measured by the biology group† will be discussed. (Supported by National Cancer Institute Grant Number CA-12441.)

† J. Geraci, private communication.

B-23-3 *Techniques for Optimizing Proportional Counter Measurements of Event-Size Spectra,* KEITH A. WEAVER, KENNETH R. ALVAR, HANS BICHSEL, JURI EENMAA, DAVID L. WILLIAMS, AND PETER WOOTTON, University of Washington, Seattle, Washington 98195 USA.

Noise levels and gain shifts must be minimized in measurements of event-size spectra with a tissue-equivalent proportional counter. Optimization techniques include reduction of preamp input capacitance, choosing correct amplifier shaping times, and selecting proper bias voltage for reducing gain shift with counting rate. Improved gas multiplication and signal-to-noise ratio obtained with a tissue-equivalent filling gas composed of C_3H_8, CO_2, and N_2 will also be discussed. (Supported by National Cancer Institute Grant Number CA-12441.)

B-23-4 *Proton Energy-Deposition Distributions for Small Sites.* W. E. WILSON, Battelle, Pacific Northwest Laboratory, Richland, Washington 99352, USA.

Energy deposition distributions for 1–4 MeV protons have been calculated using Monte-Carlo techniques[1] for absorber sites having an effective diameter of 250 nm (unit density material) and the results compared with experiment. For proton tracks passing through the absorber site, the calculated mean energy of deposition events agrees quite well with experimental values. Agreement is not as satisfactory for tracks passing near the edge and outside the absorber. The shape of the distributions are in fair agreement; the most probable value of energy deposition for the calculated distribution is smaller than that for the experimental distribution. The reasons for the discrepancies are being investigated and results for absorber sites of different effective diameters will be presented. (This paper is based on work performed under United States Atomic Energy Commission Contract AT(45-1)-1830.)

[1] The collaboration of H. G. Paretzke, München, is gratefully acknowledged.

B-23-5 *Preliminary Studies on Helion Beams (600 MeV kinetic energy) in View of Radiotherapeutic Applications.* Heavy Ions Studies Staff, presented by N. PARMENTIER, Commissariat à l'Energie Atomique, Fontenay aux Roses 92260, France.

Dosimetric preliminary measurements and radiobiological studies have been made in a 600 MeV helions beam, from the synchrocyclotron Saturne in Saclay.

Theoretical studies have been made by the use of a computer program which gives:

—dose distribution and fluence calculation in depth;

—parameter calculations necessary for the determination of an energy sweeper which provides constant doses for irradiating tumors of 5 or 10 centimeters thickness, at a depth of about 15 centimeters.

Dosimetric and microdosimetric measurements have been made with a tank of variable thickness known with a precision of 10 microns. The ratio of dose in Bragg peak to entrance is experimentally about 5, very close to the calculated values. Mean Linear Energy Transfer is about 3 keV per micron at the entrance and 10 keV per micron in the middle of the swept plateau in depth.

The biological samples or animals were irradiated in the same conditions as dosimeters with the same system. Preliminary results, especially the RBE variations with depth doses are given.

B-23-6 *An Approach to Microdosimetry in a π-Meson Beam Using Nuclear Emulsions.* N. F. KEMBER AND F. A. SMITH, St. Bartholomew's Hospital Medical College, London, Great Britain.

In this study 10 μm nuclear emulsions (Ilford K5) are used to measure the incidence of tracks per 10 μm^3 volume of emulsion at various points in a π-meson radiation field. Some discrimination between high density and low density tracks is possible on these short track segments. For the detection of isolated events along low LET tracks the development conditions are critical but useful comparative data can be obtained by these methods.

The system has been used to investigate event distributions in a phantom and also at bone-perspex interfaces using the π-meson beam facility at the Rutherford High Energy Laboratory. (The work is supported by a grant from the Cancer Research Campaign.)

B-23-7 *Proton Stopping Power in Aluminum.* ROBERTA SAXON AND HANS BICHSEL, University of Washington, Seattle, Washington 98195 USA.

Radiation physicists tend to characterize the penetration of matter by fast charged particles in terms of macroscopic quantities such as stopping power and an energy straggling parameter. Although this approach is reasonably satisfactory for, e.g., dosimetry, it will be necessary to consider further details for applications such as microdosimetry. Therefore, we have studied the interaction of charged particles with materials from the point of view of the energy loss spectrum for single collisions. Then stopping power and the straggling parameter are simply related to the first and second moments of such a spectrum. Based on theoretical calculations of atomic collision cross sections, a spectrum is determined for energy losses of protons in solid aluminum. Collective effects of the valence shell are accounted for by a dielectric theory. Hartree-Slater calculations as well as a hydrogenic approximation are used for the inner shells. The comparison with accurate stopping power measurements permits a prediction of total collision cross sections and an accurate assessment of straggling parameters.

(Supported by National Cancer Institute Grant Number CA-12441.)

B-23-8 *The Effect of Site Shape on The Frequency Distributions of Energy Deposition by Charged Particles.* NORMAN A. BAILY AND JOHN E. STEIGERWALT, University of California, San Diego, La Jolla, California 92037, USA.

The influence of site shape on the resulting frequency distribution of energy deposited by an isotropic flux of monoenergetic charged particles traversing such a volume has been investigated. Spheres, and cylinders having a large variation of height-to-diameter ratios, all having the same average pathlength, have been used as model site volumes. Distributions due to particles having large statistical variations in their energy deposition (fast protons) have been compared with those having minimal fluctuations (alpha particles, and heavy ions).

B-23-9 *Measurement of Specific Energy Imparted by Electrons.* L. A. BRABY AND W. C. ROESCH, Battelle, Pacific Northwest Laboratory, Richland, Washington 99352, USA.

The specific energy imparted to a simulated microscopic site has been measured using electron pulses which are short compared to the resolving time of the detector. The mean number of events is varied by changing the amplitude of the 50 nsec 1.5 MeV electron pulses. The microscopic site is simulated by a grid-walled proportional counter 25 mm in diameter inside a 750 mm diameter tank. Specific energy distributions for other mean numbers of events are calculated from a measured distribution by the use of the Fourier transform. The calculated distributions are compared with corresponding measurements. For mean numbers of events substantially less than one, the distribution is essentially equivalent to the single event distribution which is measured using a low current continuous beam of electrons. Comparison of these distributions makes it possible to evaluate the significance of the errors inherent in measuring the very small pulses of the single event distribution.

(This paper is based on work performed under United States Atomic Energy Commission Contract AT(45-1)-1830.)

B-24-1 *Amino Acid-Activating Enzymes in Euglena: Properties of Light-Induced and Constituitive Isoleucyl-tRNA Synthetase Isozymes Inferred from Studies of Chloroplast Development with O-Methylthreonine.* STANLEY SCHER, New College of California, Sausalito CA. 94965, USA.

Previous studies have demonstrated that light induces the formation of a new species of isoleucyl-tRNA synthetase in wild type cells of *Euglena gracilis*. Comparison of light-induced with constituitive species present in wild type dark-grown and mutant cells unable to develop chloroplasts provides evidence that these isoleucine activating enzymes are chromatographically separable and

functionally distinct. Furthermore, the isoleucyl-tRNA synthetase extracted from isolated chloroplasts appears to be identical to the inducible enzyme observed in light-adapted wild-type cells.

O-methylthreonine, an analog of isoleucine, selectively inhibits chloroplast development at levels which do not impede cell division. Wild-type cells grown in the presence of O-methylthreonine lose the ability to produce green colonies when plated on media lacking the analog. Two alternative hypotheses have been proposed to explain the action of O-methylthreonine on chloroplast protein synthesis: 1. the analog interferes with the biosynthesis of isoleucine within the plastid; 2. the analog interferes with the activation of isoleucine mediated by one or more species of isoleucyl-tRNA synthetase. The absence of isoleucine biosynthetic enzymes in isolated chloroplasts of *Euglena* argues against the first hypothesis. Differences in the rate of loss of green colony forming ability in dark-grown and light adapted cells exposed to the analog are consistent with the proposal that the light induced isoleucyl-tRNA synthetase associated with chloroplasts has a higher affinity for O-methylthreonine than the constituitive species.

B-24-2 *Studies on the Effects of High Altitude Cosmic Radiation on Certain Groups of Plants.* J. Nizam, Vidyavati, P. Srivastava, and J. Kaur, Cytological Laboratories, Department of Botany, Osmania University, Hyderabad, India.

Certain members of Desmidiaceae, Chlorococcales and Acanthaceae were exposed to high altitude cosmic radiations, the material hooked to a balloon was maintained at an altitude of 125,000 ft. for a period of seven hours. Attempts have been made to record structural and physiological changes in the cells of Desmidiaceae and Chlorococcales. These changes may be attributed to metabolic breakdown due to the effects of high altitude cosmic radiation. The recorded morphological aberrations and abnormalities were not persistent in several cell generations. In succeeding generations the deleterious effects gradually diminished, and finally after a period of 10–12 weeks cultures recovered and behaved like normal ones indicating a high degree of tolerance to the effects of high altitude cosmic radiation.

Cytological data on the treated seed material of fifteen species of Acanthaceae showed no abnormality either in the mitotic behavior or chromosome morphology. Nevertheless the root tip cells grew to more than twice their normal size. In all fifteen species, the exposure to high altitude cosmic radiation seemed to have induced early flowering.

B-24-3 *Irradiated Wheat Flour. Effects on Rats and EPR Spectra.* Marta Herscovich de Pahissa and Jaime Pahissa Campá, Comisión Nacional de Energía Atómica, Buenos Aires, Argentina.

Radiotoxicological action of irradiated wheat on experimental animals was studied. In the usual feeding, carbohydrates make up 40% of the total. These were replaced by wheat flour. Different doses from 0.02 to 5 Mrad were delivered to the wheat flour and hematological parameters were evaluated, particularly the change in number of lymphocytes.

Trying to elucidate the mechanisms produced by the action of irradiated food on Wistar rats, EPR was employed. The concentration of free radicals induced by irradiation of wheat flour depends on total doses, moisture content and time elapsed after irradiation because the free radicals of high chemical reactivity decrease following an exponential function. Working with the results obtained under different conditions, coefficients of these functions and energy requirements for paramagnetic centers were calculated.

A relation between lymphopenia and free radicals was inferred.

At the usual desinfestation dose of 0.02 Mrad, hematological damage was not detected.

B-24-4 *Radiostimulation (γ, n) of Plant Growth with Emphasis on Chloroplast Development.* George Akoyunoglou and John Sourdis, Nuclear Research Center "Democritos," Athens, Greece.

Experiments with low dose ionizing radiation showed that under certain conditions, irradiation results in stimulating effects. A study of these effects on the cellular and molecular level seems necessary in order to determine the mechanism by which such stimulating phenomena take place. The effect of the preplanting irradiation (γ and n, 50–100 rads) of *Phaseolus vulgaris* seeds, on the growth and development of the plant was studied in the phytotron and the green house. Emphasis

was placed on chloroplast development for two reasons: 1) the steps leading from etioplast to chloroplast are well known and, therefore, such a system can be used as a screening test, and, 2) we hope that this approach will give a clue as to the mechanism by which the low-dose irradiation acts. Our results show a significant acceleration of the germination, different patterns of growth and flowering. A greater yield of seeds and green mass, in the case of neutrons was also obtained. As far as the etioplast to chloroplast transformation and the chloroplast development are concerned a pronounced effect was observed in chlorophyll ($a + b$) biosynthesis, ribulose diphosphate carboxylase formation and the total chloroplast protein.

B-24-5 *Protection Effect of Induced Inhibitors in Irradiated Plants.* L. M. KRYUKOVA, Institute of Biological Physics, Acad. Sci. USSR, Pushchino, Moscow Region, USSR.

In a normally functioning plant cell there is a certain balance between regulators of growth processes: activators and inhibitors. In plants exposed to high-dose ionizing radiation inhibitors are induced. Being formed locally at the sites of irradiation, they are distributed over the whole plant, inhibit cell division and growth of the plant. Accumulation of inhibitors in plants under the action of seasonal changes or various extreme factors such as radiation, infection, trauma etc., results in depressed vital activity and increased resistance of the plant. Maize seeds sort "Nagrada" treated with some natural inhibitors such as chlorogenic acid or caffeic acid, scopoletin as well as inhibitors induced by radiation (in the form of extracts from 25 KR-irradiated leaves of Vicia Faba) exhibited an increased radioresistance to subsequent irradiation with a 15 KR dose. No growth depression was observed with the plants grown from those seeds as compared with the control whose seeds were treated with water solutions of plant hormone: gibberellin (50 mg/1) or kinetin (20 mg/1). Thus, natural inhibitors or those induced by radiation are protective substances and decrease radiation damages in plants.

B-24-6 *Effect of Ionizing Radiation on the Synthesis de Novo of Preformed Enzymes: Polyphenoloxidase, Peroxidase.* V. A. KOPYLOV, Institute of Biophysics, Acad. Sci. USSR, Pushchino, USSR.

As reported earlier, the enzymes involved in oxidative processes (polyphenoloxidase, peroxidase) undergo changes under the action of ionizing radiation. Polyphenoloxidase isolated from γ-irradiated potato tubers is shown to change quickly its conformational state under the action of medium ionic strength, hydrogen ion concentration, and temperature, unlike enzymes obtained analogously from non-irradiated objects. It was observed that the enzyme (*in vivo*) subjected to ionizing radiation changes to an insoluble state two-three times as quick as a non-irradiated object, all other things being equal. Intramolecular regulation of the enzymic reaction rate was found to be disturbed by irradiation as well. At the same time the synthesis de novo of the enzymes such as peroxidase in sections of γ-irradiated potato tubers (10 kr) occurs quite differently as compared to the norm. For example, isoenzymes A and B in γ-irradiated samples are formed at a rate slower than those in non-irradiated ones, whereas isoenzyme C is synthesized 198% as high as the norm. Hence, the ionizing radiation damages both performed enzymes and the systems responsible for the enzyme synthesis. This assumption is supported by experiments with incorporation of labelled precursors into peroxidase and application of inhibitors of nucleic acids and proteins. Peroxidase newly synthetized in γ-irradiated potato tubers had a more wide action spectrum (specificity) as compared to the control.

B-25-1 *Alterations at Transcriptional Level in the Rat Liver Following Whole-Body X-Irradiation.* D. S. PRADHAN, M. N. SUBBA RAO, M. S. NETRAWALI, AND A. SREENIVASAN, Biochemistry & Food Technology Division, Bhabha Atomic Research Centre, Bombay 400085, India.

Whole-body exposure of rats to 1000 r x-rays brings about a significant increase in the rate of RNA synthesis in the liver during 4–18 hr post-irradiation. This stimulus has been found to be mainly the consequence of amplification in the template activity of chromatin rather than activation of RNA polymerase (Subba Rao *et al.*, Ind. J. Biochem. Biophys. 8, 257, 1971). During this period post-irradiation, significant stimulation in methylation, acetylation and phosphorylation of acidic chromosomal proteins could be discerned. In addition, rates of syntheses of two chromosomal acidic proteins are selectively enhanced. The observed changes at the transcriptional

level can be suppressed if adrenals are removed from the rat prior to irradiation indicating that these changes may have been elicited by increased output of adrenal steroids known to take place in response to whole-body radiation-exposure.

B-25-2 *Enhanced Synthesis of Low Molecular Weight Nuclear RNAs after Gamma-Irradiation and Hepatectomy.* ANNA FÓNAGY AND E. J. HIDVÉGI, "F. Joliot-Curie" Natl. Res. Inst. Radiobiology, 1775-Budapest, Hungary.

As confirmed by several researchers, in the early phase of regeneration or a few hours after whole-body X-irradiation the synthesis both of liver ribosomal RNAs and of heterodisperse high molecular weight nuclear RNAs increased. Synthesis of rat liver low molecular weight nuclear RNA (LMW nRNA) was studied after partial hepatectomy and gamma-irradiation. Animals were injected with ^{3}H-orotic acid i.v. and killed 1 hr later. The LMW nRNA fraction was isolated and fractionated by polyacrylamide gel electrophoresis. As established, both 6–12 hours after hepatectomy and 6 hours after 2000 rad irradiation the synthesis of each species of LMW nRNA increased. This enhancement is particularly high in 4.5 S, 5 S and approximately 10 S species, resp. Provided irradiation intervened in the early phase of regeneration, i.e. animals were irradiated in the 6th hr after hepatectomy and killed in the 12th hr, the synthesis of the same species of LMW nRNA was even further increased.

B-25-3 *The Influence of Various Radiation Qualities on High Molecular Weight Nuclear and Ribosomal RNA of Novikoff Hepatoma Ascites Cells.* G. SCHMITT, K. P. BRUCKSCH, K. EWEN, G. HUEDEPOHL, AND S. SEEBER, Radiological Centre, Division of Clinical Radiation Physics and Department of Medicine, Tumor Research Division, University of Essen, 4300 Essen, West Germany.

Novikoff hepatoma ascites cells were cultivated under standardized conditions on inbred Holtzman rats. On the 6th day after tumor transplantation the animals were locally irradiated. The following radiation qualities were used: (1) 300 kV x-rays, (2) 60 Co γ-rays, (3) 43 MV photons, (4) 43 MeV electrons, (5) 14 MeV neutrons. 6 hrs after irradiation, high molecular RNA was labeled with ^{32}P-orthophosphate. Nuclear RNA was extracted with the hot phenol-SDS-procedure. The preparation of polyribosomes and the extraction and fractionation of ribosomal RNA followed the conditions given by Quagliarotti and co-workers (1970). Labeling patterns, specific activities and nucleotide compositions of 45 S nuclear RNA as well as 18 S and 28 S ribosomal RNA were determined. Differences of these parameters compared to the control values were found *in vivo* and *in vitro*. Some differences were also noted between samples treated with different radiation qualities. These observations indicate that macromolecular RNA metabolism may be influenced in a specific way by the individual radiation qualities examined.

B-25-4 *Radiation-Induced Alterations in the Synthesis of Ribosomal and Informosomal Particles in Rat Liver.* R. GOUTIER AND W. BAEYENS, SCK-CEN, 2400 Mol and University of Liège, B-4000 Liège, Belgium.

Total-body X-irradiation of rats by 2000 R produces a hormonal induced increase in synthesis of liver RNA. Analyses in Cs Cl gradients of the cytoplasmic 40 S particles reveal that the specific activity of both mRNA and rRNA is increased 6 hrs post-irradiation. This wave of overall stimulation is followed by a phase of depression of synthesis, already detectable 12 hrs after irradiation. Because of the temporarily increased production of ribosomal particles and mRNA immediately after irradiation, and because of the shorter life span of mRNA compared to that of rRNA, a higher ratio ribosomes/mRNA sets in at later times post-irradiation and accounts for the observed increase in the proportion of heavier polyribosomes.

B-25-5 *A Fast Neutron Source for Cultured Cell Irradiation.* R. M. PRIOR, A. J. MOSS, JR., MAX L. BAKER, AND G. V. DALRYMPLE. Little Rock Veterans Administration Hospital and the University of Arkansas Medical Center, Little Rock, Arkansas 72207, AND F. S. WILLIAMSON, Argonne National Laboratory, Argonne, Illinois 60439, USA.

Fast neutrons produced by the ^{9}Be$(d, n)^{10}$B reaction have been used to study the effects of fast neutrons on cultured mammalian cells. The deuterons are produced by a 1.3 MeV Van de Graaff accelerator and strike a water-cooled beryllium target. The cells were irradiated with dose rates

between 20 and 45 rad/min. of fast neutrons. The neutron dose rate was measured with a tissue equivalent ion chamber. The gamma ray contribution was measured with a neutron insensitive ion chamber. The dose rate was monitored with a paraffin moderated BF_3 proportional counter. The neutron spectrum has been measured, and a computer calculation of the LET has been made.

B-25-6 *Fast Neutrons and X-Ray-Induced Single-Strand DNA Breaks in Cultured Mammalian Cells.* A. J. Moss, Jr., Max L. Baker, G. V. Dalrymple, and R. M. Prior. Little Rock Veterans Administration Hospital and The University of Arkansas Medical Center, Little Rock, Arkansas 72201, USA.

The production of DNA single strand breaks was studied using the alkaline sucrose sedimentation technique. L-929 cells, prelabeled with ^{14}C thymidine, were irradiated in plastic culture flasks in the presence of 1×10^{-4} *M* 2,4-dinitrophenol (DNP). Monolayers were irradiated with both 250 kVp X-rays and fast neutrons ($^{9}Be(d, n)^{10}B$ reaction) at a dose rate of approximately 25 rads/min. DNA break analysis was conducted with cells irradiated with doses ranging from 0 to 5000 rads. Parallel measurements of the effects of neutrons and X-rays upon cell survival were also measured. RBE values for both survival and DNA damage will be presented.

B-25-7 *Metabolism of RNA in Rat Liver and Spleen upon γ-Irradiation.* V. I. Tokarskaya, S. R. Umansky, P. A. Nelipovich, O. I. Skotnikova, and Yu. N. Runova, Institute of Biological Physics, Academy of Sciences of the USSR, Pushchino, Moscow Region, USSR.

Incorporation of different RNA precursors (C^{14}-orotic acid, C^{14}-uridine, P^{32}) into nuclear and cytoplasmic RNA and acid-soluble fraction of rat liver and spleen after γ-irradiation at a dose 800 rad has been studied. The first two hours after irradiation specific activity of liver RNA increases whereas that of spleen declines. This effect is largely accounted for by increased uptake of uridine and orotic acid into acid-soluble fraction and their phosphorylation to UTP in liver. These processes are inhibited in spleen. The calculations indicate that after irradiation the total synthesis of RNA in both organs decreases. A rapidly labelled nuclear RNA isolated from the liver of the irradiated rats exhibits a higher content of (poly A)-RNA, increased hybridizability and an increased specificity coefficient measured as a U/C ratio, which points to an increased AU-type RNA/GC-type RNA ratio. After irradiation of rat liver nuclei *in vitro* at doses to 10 krad the activity of Mg^{++}-dependent RNA-polymerase does not change whereas that of Mn^{++}-$(NH_4)_2SO_4$-dependent one increases. Chase-experiments have shown that the degradation and transport of rat liver nuclear RNA do not change for an hour after irradiation and increase by the second hour. Possible mechanisms of radiation disturbance of RNA metabolism are discussed.

B-26-1 *Activity Changes in Lysosomal and Non-Lysosomal Enzymes in the Skin of Mice Caused by Soft X-Ray-Irradiation.* Lajos Soltész, and Edith Báthori, Hungarian Academy of Sciences University Research Team of Medical Radiology, Üllői ut 78, H-1082 Budapest, Hungary.

The changes in activity of acid phosphatase, beta-glucuronidase, cathepsin D, neutral proteinase, alkaline phosphatase and arginase were examined.

Data of irradiation: 29 kV, 25 mA, 0.3 mAl. Focus-skin distance 10 cm, irradiated field 2×3 cm. Applied doses: 100, 500, 1000 R. Except for alcalic phosphatase in case of all the enzymes it was possible to produce an outwashable enzyme-fraction by washing before homogenizing the skin.

The enzyme activity of this washing-solution increased remarkably as the effect of different doses, while in case of homogenate an increase of activity was found only after a 500 R irradiation.

B-26-2 *X-Irradiation-Induced Damages in the Drug Metabolizing Enzyme System in Liver Microsomes during Development of Male Rats.* Osami Yukawa and Tohru Nakazawa. Division of Biology, National Institute of Radiological Sciences, Chiba, Japan.

X-irradiation to the whole body at weaning stage of male rats caused a suppression of increase in the activity of hexobarbital hydroxylation in liver microsomes during development. It was suppressed partially by 200 R of X-ray and completely by 400 R. Activities of microsomal electron transport system were inhibited only slightly at 40 days of age in 400 R-irradiated rats, but both content of cytochrome P-450 and the magnitude of cytochrome P-450 spectral change induced by hexobarbital were suppressed considerably. Kinetic properties of hexobarbital-induced cytochrome P-450 spectral change were also studied after X-irradiation. These results indicate that the sup-

pression of the activity of hexobarbital hydroxylation after X-irradiation may be due to decreases in both content of cytochrome P-450 and binding capacity of cytochrome P-450 for hexobarbital.

B-26-3 *Kinetic Studies on Damages in Microsomal Drug-Metabolizing Enzyme Systems after X-Irradiation of Male and Female Rats.* TOHRU NAKAZAWA, OSAMI YUKAWA, AND SATORU USHIJIMA, Division of Biology, National Institute of Radiological Sciences, Chiba, Japan.

Activities of metabolizing enzymes of drugs, hexobarbital, aminopyrine and aniline, in liver microsomes of rats receiving 750 R of X-ray to the liver region decreased to different extents. These changes in enzyme activities after X-irradiation were based on the decrease of either contents or binding capacities of cytochrome P-450. The affinity of aminopyrine to cytochrome P-450 in male rat liver microsomes was higher than that in female, but the former was suppressed by X-irradiation to the latter lower level. The affinity of aniline which represents another type of binding capacity to cytochrome P-450, was the same in both male and female. These results indicate that kinetic properties of cytochrome P-450 in microsomal electron transfer system were damaged remarkably after partial X-irradiation, resulting in decreases of drug metabolizing enzyme activities.

B-26-4 *The Role of Lysosomes in Autolytic Reactions Developing in the Cell Organelles in Radiation Damage of Animals.* BORIS A. LOMSADZE, Tbilisi State University, Department of Biophysics, Tbilisi, USSR.

Physico-chemical alterations developing in the cells of irradiated organism are associated mainly with damages of cell structures containing proteolytic enzymes. This has been shown in our experiments on the dynamics of postradiation autolysis of nuclear, mitochondrial and lysosomal fractions of rat liver during various forms of radiation damage of animals. Regular phase alterations of autolysis in the process of radiation damage of animals are observed. At the beginning of the damage autolysis in various organs and organelles is activated, while during developing of the disease it is inhibited. Increased activity of cathepsins and acid phosphatase is observed in phases of enhansed autolysis but inhibition of autolysis is accompanied by decrease in the activity of those enzymes. Maximum of autolytic reaction develops earlier with the increase of radiation dose. Of all organelles of the cell lysosomes have the most significant role in developing of autolytic reactions.

B-26-5 *Lysosomal Activation in Cultured Mammalian Cells and Mammary Tumors Following Irradiation.* CLAIRE REYNOLDS AND E. D. WILLS, Medical College of St. Bartholomews Hospital, London, Great Britain.

B-27-1 *Radiobiological Characteristics of 35 MeV Cyclotron-Produced Neutrons.* ERIC J. HALL, Radiological Research Laboratory, College of Physicians and Surgeons of Columbia University, New York, NY 10032, USA.

The 35 MeV cyclotron at the Naval Research Laboratory, Washington, D.C. is now being used for radiotherapy treatments.

As a prelude to clinical use, the oxygen enhancement ratio (OER) was measured using *Vicia* seedlings, while the Relative Biological Effectiveness (RBE) was measured with a variety of biological systems, principally mammalian cells in culture. RBE was measured as a function of dose. Over the intermediate dose range, RBE is a strong function of neutron dose, and decreases with increased dose. RBE's reach constant values for high and low doses.

B-27-2 *Fractionated Irradiation of Cultured Human Cells with Therapeutic Doses of Fast Neutrons.* PAUL TODD, CARTER B. SCHROY, AND R. B. THEUS, The Pennsylvania State University, University Park, Pennsylvania 16802, and Naval Research Laboratory, Washington, D.C. 20375, USA.

Patients undergoing fast neutron therapy usually receive 125 to 200 rads per fraction. Most quantitative *in vivo* and *in vitro* cell survival studies have employed larger doses per fraction. Asynchronous cultured human kidney (T-1) cells were exposed to 1, 2, and 3 fractions of 155 rads of fast neutrons at the Naval Research Laboratory Cyclotron. The following conditions were used: dose rate = 60 rads/min; collimation = 10 × 10 cm; E_d = 25.0 MeV; $\bar{E}_n$ = 17.5 MeV; TSD = 125 cm; HVL = 11 cm T.E. fluid; and depth of exposure = 12.3 cm in T.E. fluid.

Very little recovery could be detected between doses of 155 rads, although recovery was easily demonstrated to occur between two doses of 310 rads and it was maximum in 3 hr. Data have been obtained that indicate that under clinical application of this neutron beam RBE = 2.6 at 145 rads/fraction; the same cellular dose-response relationship applies at all points in tissue-equivalent material; and the exponent of N in the Ellis formula should be 0.04 ± 0.01. (This work was performed under the auspices of the Middle Atlantic Neutron Therapy Association, supported by the Grants Division of the National Cancer Institute.)

B-27-3 *Dosimetry and Oxygen Enhancement Ratios of Neutrons Produced by 70 MeV and 85 MeV Deuterons.* GEORGE H. HARRISON, University of Maryland School of Medicine, Baltimore, Maryland 21201, USA.

The University of Maryland cyclotron is capable of producing high intensity beams of high energy deuterons (70–85 MeV). This facility provides a unique source of therapeutically configured neutron beams of higher energies than generally available—energies which may have different therapeutic properties than conventional neutron beams. Neutrons produced by stopping 70 and 85 MeV deuterons in beryllium have been characterized dosimetrically: beam profiles, depth dose distributions, dose rates, and gamma contamination will be described.

Radiobiological studies have been conducted with the Vicia faba assay system, and with cultured mammalian cells. Preliminary determinations with Vicia Faba yielded an oxygen enhancement ratio of 1.3 ± 0.2. Possible advantages of this high energy beam for radiation therapy will be discussed. (Supported in part by Public Health Service Research Grant CA-06518-11 from the National Cancer Institute.)

B-27-4 *Response of Mammalian Cells in Culture to Fast Neutron Beams at the Texas A & M University Variable Energy Cyclotron.* ROYCE L. GRAGG,* RAYMOND E. MEYN, AND RONALD M. HUMPHREY, The Univ. of Texas System Cancer Cntr., M. D. Anderson Hosp. & Tumor Inst., Dept. of Physics, Houston, Texas 77025, USA.

Chinese hamster ovary cells in monolayer culture were irradiated with one of two cyclotron-produced fast neutron beams, with peak energies of 16 and 50 MeV, under necessary and sufficient conditions for the determination of (1) relative biological effectiveness (RBE), (2) oxygen enhancement ratio (OER), (3) variation of RBE and OER with tissue depth, (4) capacity of cell to recover from radiation damage, and (5) variation of radiation sensitivity throughout the cell cycle.

Survival data from exposure to the neutron beams, as compared with that from cobalt-60 (Co-60) gamma rays indicate the following: (1)the relative biological effectiveness is in the range of 2.0 to 2.8, depending upon the survival level, (2) the OER is decreased from 2.9 to a range of 1.3–1.5, (3) these values do not vary with tissue depth, (4) the capacity for cellular recovery from neutron damage is decreased while neutron-irradiated cells retain the capacity to recover from subsequent Co-60 gamma irradiation, (5) the magnitude of the variation in cell cycle radiation sensitivity is decreased, and (6) little change in any of these parameters occurs with change in peak neutron energy. (This work was supported by Public Health Service Research Grants CA 12542 and 4484 from the National Cancer Institute.)

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* Graduate Trainee, Division of Compliance, Bureau of Radiological Health.

B-27-5 *Relative Biological Effectiveness of Fission Neutrons of 1 MeV Mean Energy for Effects on Normal Tissues in Mice.* J. A. G. DAVIDS, Reactor Centrum Nederland, Petten N.H., The Netherlands.

A converter facility in the Low Flux Reactor at Petten has been used for several years in a comparative study in mice of the biological effects of high-LET fast fission neutrons with the effects of Low-LET X-rays. The conditions of neutron exposure which were kept constant during this study, are: bilateral exposure resulting in a uniform distribution of absorbed dose in the animals, a fast neutron dose rate in the centre-line of 10 rad/min. with a gamma contamination of 1 rad/min. and a mean energy of the incident neutrons of 1.0 MeV. Initially 250 kVp X-rays and subsequently 300 kVp X-rays (HVL 2.1 mmCu) were used, both at a dose rate of 30 rad/min.

The RBE values have been determined for male mice of the same inbred CBA subline. The biological criteria studied include killing of haemopoietic stem cells and haemopoietic death,

killing of intestinal crypt stem cells and intestinal death, damage to the gastric epithelium, damage to the incisors and dental death, and killing of spermatogonial stem cells. Depending on the effect the RBE varies with a factor of about two.

B-27-6 *RBE of NRL Fast Neutron Beams for Lymphocytic Leukemia Cells.*[1] RAYMOND U., DON M. WHELESS, JOHN C. EVANS,[2] AND PATRICK C. CAVANAUGH, Duke University Medical Center and Veterans Administration Hospital, Durham, North Carolina 27710, USA.

The determination of relative biological effectiveness for therapeutically useful fast neutrons produced by the U.S. Naval Research Laboratory Cyclotron (35 MeV deuteron on a thick beryllium target) was investigated by *in vitro* assay after irradiation of BW 5147 lymphocytic leukemia cells *in vivo*. The comparison of dose-effect relationships for NRL fast neutrons (15 MeV mean energy) and 250 kVp x-rays was made for doses ranging from 50 to 1200 rads. The BW5147 lymphocytic leukemia cells were obtained from AKR/J mice, the subcutaneous implant of this cell line being first transplanted as an ascites form. Using standard cell culture techniques, these cells proliferate readily *in vitro* and can be measured for the post irradiation cell population kinetic response patterns by the Coulter cell counter. A plot of the minimal cell surviving fractions against the radiation doses for 15 MeV fast neutrons and 250 kVp x-rays shows relative biological effectiveness for fast neutrons to be 2.2 (up to 300 rads). The 250 kVp x-ray survival curve indicated an extrapolation number about 2, while there appears to be virtual absence of any shoulder for NRL fast neutrons. Also the results of fast neutron irradiation in 3 split doses will be presented.

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[1] Collaborators: Drs. Leon August and Richard B. Theus, U.S. Naval Research Laboratory Cyclotron Branch, Washington, D.C. and Drs. Charles C. Rogers and John Wilson, Biology Committee, Middle Atlantic Neutron Therapy Association, Medical College of Virginia, Richmond, Virginia.

[2] Present Address: West Virginia University Medical Center, Morgantown, West Virginia 26506, USA.

B-27-7 *Iso-Effect Curves for Normal Tissue Damage, Using Fractionated Doses of 14MeV D-T Neutrons.* J. H. HENDRY, I. ROSENBERG, AND J. G. STEWART, Christie Hospital and Holt Radium Institute, Manchester M20 9BX, Great Britain.

In view of the proposed use of 14 MeV neutrons for radiotherapy in Manchester, RBE values for daily fractionated doses of neutrons and X or γ-rays have been obtained. Radiation damage to three normal tissues has been investigated, measuring (a) rat-tail skin reactions, (b) killing of mouse intestinal crypts, (c) killing of bone marrow stem cells. The number of daily fractions was varied from 1 to 4, 8 and 16, to produce a given level of damage, and iso-effect curves are presented with respect to tissue type and level of damage.

B-27-8 *OER Values for Pink Mutations in Tradescantia at Various Neutron Energies and at High and Low X-Ray Doses.* A. G. UNDERBRINK, A. H. SPARROW, AND D. SAUTKULIS, Radiological Research Laboratories, Columbia University, New York, NY 10032 and Biology Department, Brookhaven National Laboratory, Upton, NY 11973, USA.

Cuttings of *Tradescantia* clone 02 were irradiated under aerated and hypoxic conditions and dose-reponse curves were constructed for pink somatic mutations induced in the stamen hairs. The slopes of the dose-response curves under both conditions approached +1. OER values determined thus far using neutrons of 0.43-, 0.68-, 1.02-, 5.8-, and 13.4-MeV were respectively about 1.4, 1.3, 1.5, 1.6 and 1.4. These OER values, which show some variation with neutron energy, will be compared with previously reported OER values obtained from different systems.

The slopes of the x-ray dose-response curves under aerated and hypoxic conditions were parallel and steeper than +1 at doses above 5 rads. The OER was about 3.2. At doses below 5 rads, the aerated dose-response curve becomes linear. Present evidence, although inconclusive, suggests that the hypoxic curve will also become linear at low doses and that the OER will not change. Difficulties in determining OER at very low doses will be discussed. (Supported in part by the U.S. Atomic Energy Commission at Brookhaven and contract no. AT-(11-1)-3243 to Columbia University and Public Health Service Research Grant CA-12546 from the National Cancer Institute.)

B-27-9 *Studies on the Variation of Neutron RBE with Energy.* MICHAEL KEY,* Australian Atomic Energy Commission, Lucas Heights, N.S.W., Australia.

A Van de Graaff accelerator was used to produce beams of low and high energy neutrons by the $^{7}Li(p, n)^{7}Be$ and $^{9}Be(d, n)^{10}B$ reactions respectively. The low energy beams were essentially monoenergetic with energies between about 30 keV and 500 keV. The high energy beam had a spectrum of energies with a maximum at about 6 MeV.

Chinese hamster cells, grown *in vitro*, were irradiated with neutrons or x-rays and the damage was assessed by measuring cell survival and chromosome aberrations, as a function of dose. The pulse-chase technique with ^{3}H-thymidine was used to discriminate between different stages in the cell cycle for the chromosome studies.

The neutron RBE (relative biological effectiveness) showed a clear dependence on energy and was consistently higher for G1 aberrations than for G2 aberrations. As the energy decreased the RBE increased, and went through a maximum at an energy of about 100 keV.

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B-28-1 *Optimization of Monitoring System in Environment of Nuclear Facilities.* ATSUYUKI SUZUKI AND YUTAKA YAMAMOTO, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan.

A model for optimization of monitoring system to detect radioactive wastes released from nuclear facilities is constructed by using Bayesian statistical decision theory. When the maximum permissible dose rate becomes 5 mrem/year, which corresponds to several percent of natural background, it is necessary to analyze statistically the informations obtained from monitoring system in order to verify whether data exceed the limit or not. Multiple sensors are employed for this purpose and an optimal multiple sensors system is determined as a compromise between the value of observed informations and the costs required for operation of the system. In other words, the number of sensors (n), for example, is determined optimally as follows; since the expected cost or average risk of decision is a monotonously decreasing function of n, while the associated costs of operation are described as monotonously increasing function of n, a value of n may exist for which the total average cost (or risk) can be minimized, and it is an optimal n. Several numerical examples are solved to illustrate the approach both conceptually and quantitatively.

B-28-2 *Establishing Acceptable Limits of Non-Radiological Damage in the Environment.* RAJENDRA K. SHARMA AND JOHN D. BUFFINGTON, Argonne National Laboratory, Argonne, Illinois 60439, USA.

The sources of potential non-radiological environmental damage due to nuclear power plant operations have been identified. Although quantitative prediction of the extent and nature of adverse impacts is possible, a baseline criterion of acceptable damage has not been established. In the case of radiological releases it is possible to relate discharge limits to the background levels, although disagreement exists as to the acceptability of these limits. For non-radiological impacts such relationship does not exist, nor is it practical. The lack of guidelines for establishing limits of acceptable damage has resulted in largely subjective judgments regarding significance of impacts and hence in a great deal of controversy. Solutions to the establishment of acceptable thresholds of environmental degradation are proposed in terms of (1) measurability of effect, (2) irreversibility of effect, (3) species population structure, (4) ecosystem structure, (5) relationship of first order and second order effects, (6) identification of socio-economic damage, and (7) intangibles and economic externalities. Limitations of these concepts in terms of state-of-the-art, baseline data, natural fluctuations, and non-power-plant degradation are identified.

B-28-3 *Environmental Radioactivity and Trace Elements Studies in India.* UMESH C. MISHRA AND C. RANGARAJAN, Air Monitoring Section, Bhabha Atomic Research Centre, Bombay-400 085, India.

The paper describes recent results of the countrywide programme of sampling and analysis of environmental samples for their natural and fallout gamma emitting nuclide concentrations as

well as trace element concentrations, based on instrumental neutron-activation analysis methods. These measurements have been conducted over the past several years. Ge(Li) and NaI(Tl) gamma spectrometric methods have been developed and the data processing has been done by digital computers. Some of the interesting features e.g., seasonal variation, countrywide distribution and annual variations etc., have been discussed. The paper also brings out some of the observations which are associated with topographical features of India and their influence of the distribution of radioactivity on a countrywide scale. Variations in trace element concentrations have also been discussed in relation to the amount of radioactive nuclides observed as well as major pollutants present in the environment.

B-28-4 *Data for Determining the Environmental Impact of Nuclear Powered Cardiac Pacemakers.* Bobby I. Griffin and Kenneth A. Gasper, Medtronic, Inc., Minneapolis, Minnesota 55418, USA.

Medtronic, Inc., now has four years of clinical experience with manufacturing, for human use, nuclear powered cardiac pacemakers containing plutonium-238.

From this experience insights have been gained that will aid in the determination of the risk/benefit to society for these devices. Of course, such a study must be done prior to wide-scale use. Specifically, the problems of main concern appear not to be the radiation dose to the bearer or to that portion of the population in intimate contact with the bearers. Rather, the main concerns are control and recovery of the radioactive material contained in the pulse generator.

Dose equivalent rate from a Medtronic Model 9000 pulse generator containing plutonium-238 will be presented as a function of depth into tissue, implantation site and organ location.

A flow chart of the radioactive material from the source of radioactive material to safe return will also be presented.

Finally, that portion of potential pacemaker bearers thought to be candidates for use of extremely long lived nuclear power sources will be identified and discussed.

B-28-5 *The Use of Radiation-Sensitive Bacterial Mutants in the Detection of Deleterious Effects of Environmental Agents.* Ina E. Mattern and B. Roberti, Medical Biological Laboratory TNO, Rijswijk (ZH), The Netherlands.

Living cells posses various enzymatic mechanisms to repair damage, induced into the DNA by physical (e.g., UV and ionizing radiation) or chemical agents. In micro-organisms many mutants are known (e.g., Uvr^- and Rec^-) which are deficient in one or more of these repair mechanisms, resulting in an increased sensitivity to the deleterious effects of these agents.

In the present study various radiation-sensitive mutants of *Escherichia coli* and *Salmonella typhimurium* have been used

1) to identify environmental pollutants that damage DNA. In particular attention was paid to agents with suspected carcinogenic activity. In order to facilitate extrapolation of the results to humans, mammalian liver homogenates were added to the test system to imitate *in vivo* metabolism. [See also Ames *et al.*, PNAS **70** 2281 (1973)].
2) to investigate the effects of 3 GHz microwaves on biological systems, as judged from survival and mutation induction.

B-28-6 *Adsorption Characteristics of Radioactive Strontium in Urine and Sea water.* Shoichi Kawamura, Katsumi Kurotaki, Sadao Shibata, and Hiroshi Takeshita, National Institute of Radiological Sciences, 9-1, 4-chome, Anagawa, Chiba, Japan.

Current techniques for the determination of radioactive strontium in environmental materials involve ion-exchange, precipitation and solvent extraction method, etc. These procedures require tedious and time-consuming chemical separations. To investigate a simple method for determination of radioactive strontium, the adsorption characteristics was determined by adding sodium phosphate solution to urine or sea water either in the presence or absence of copper nitrate solution. When a 120 ml of 0.5 *M* sodium phosphate was added to one liter of urine or sea water, the spiked strontium-85 was coprecipitated quantitatively with the phosphate either in the presence or absence of a 24 ml of 0.5 *M* copper nitrate. Furthermore, the precipitates

are filterable within a short time by suction. Considering the above findings, the technique is quite effective for the adsorption of radioactive strontium in urine or sea water.

B-29-1 *Ionization by Energy Transfer from Excited Species.* A. NIEHAUS, Universital Freiburg, 78 Freiburg, West Germany.

The mechanisms of the different types of exothermic ionization reactions caused by transfer of energy from excited species in single collisions $A \rightarrow B$ will be outlined. The following processes are considered: (1) Penning- and associative Penning ionization, which occur if $E^*(A) > IP(B)$, with E^* and IP being excitation and ionization energies, (2) the Hornbeck-Molnar process, requiring that $E^*(AB) > IP(AB)$ at certain separations of A and B during collision, (3) the ionization of atoms in high Rydberg states, with $E^*(A) - IP(A) \sim 0.1$ eV, in thermal collisions with molecules, and (4) the ionization arising during collisions of particles B with ions carrying enough potential energy, e.g., $A^{++} + B \rightarrow A^+ + B^{++} + e^-$.

A selection of recent experimental results, concerning these processes, will be reported and interpreted in terms of the available theories and models. No complete review will be given. Included are cross section measurements, the angular and energy analysis of ejected electrons, and ion mass- and energy analysis. Where available, results concerning the collision velocity dependence of the various quantities of interest will be reported. In the case of molecules, the population of vibrational states of the molecular ion formed, and the possibility of dissociation reactions competing with ionization will be discussed.

B-29-2 *Ionization by Interpenetration of Electron Shells.* FELIX T. SMITH, DONALD C. LORENTS, AND RONALD E. OLSON, Stanford Research Institute, Menlo Park, California 94025, USA.

Until recently, the prevailing model for ionization by charged particle impact was based on the coulomb interaction of a fast structureless charged particle with the electron cloud of the target. This model is characterized by a very rapid decline in the ionization cross section at low relative velocities (measured by comparison with the electron velocity in the atomic shell being ionized). When higher Z particles with filled electron shells are used, measured cross sections for ionization are often much larger than coulomb cross sections. The reason lies predominantly in the exchange interaction between electrons in interpenetrating shells, which causes high promotion and even ionization of one or more of the electrons involved. This exchange ionization can be predicted from the behavior of quasi-molecular wave-functions for the pair of colliding atoms. The same principles generally apply whether the ionization is from deep inner shells of heavy particles or from outer shells, provided the relative velocity is similar. Typical cross sections may be as large as the electron shells involved, and remain high down to threshold energies determined by the appropriate ionization potential, augmented by the effective internuclear coulomb repulsion energy at the distance involved.

B-29-3 *Spontaneous Break-Up of Gaseous Ions.* CH. OTTINGER, Max-Planck-Institut für Strömungsforschung, Göttingen, West Germany.*

While the majority of fragment ions in a mass spectrum are formed unmeasurably fast, some fragmentations occur after a noticeable time lapse ("metastable ions"). Modern techniques for the observation of metastables will be explained, followed by three sections which describe how metastables are put to use. 1) Chemistry: Metastables delineate decomposition paths of complex organic ions, in particular in conjunction with isotopic labeling. 2) Energetics: Small amounts of kinetic energy released in the decomposition can be measured with great accuracy, permitting ion structures to be inferred. 3) Kinetics: Time-resolved studies today allow the decomposition to be followed from 10^{-12} to 10^{-3} sec. These results, if paralleled with theoretical predictions, can give insight, e.g., into the effect of isolated electronic states on the break-up. Chemistry, energetics and kinetics of spontaneous decompositions all have direct bearing on problems in radiation chemistry.

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B-29-4 *Ion Molecule Reactions.* P. KEBARLE, Chemistry Department, University of Alberta. Edmonton, Alberta, Canada.

The chemical consequences of an ion molecule collision depend on the relative velocity of the interacting pair. Most common and thus important, in radiation chemistry are interactions at thermal velocities. The fate of the persistent reaction complex formed at thermal energies also depends on the occurrence of collisions with third molecules which may remove excess energy. The nature of the products is therefore also dependent on pressure. Apart from collisional deactivation and thermal activation, high pressure leads to long reaction sequences, occurrence of reactions with small rate constants and the achievement of ionic equilibria. The mass spectrometric study of ionic reactions under these conditions has proven extremely rewarding. The field has developed to such an extent that now it produces important information on energetic and stability of ions. This information is of great significance also to outside fields like physical organic and inorganic chemistry and to the theory of ionic solvation.

Thus, the observations on ion solvent molecule complexes answer questions pertaining to the importance of hydrogen bonding by protic solvents and modification of acidity, basicity and reactivity of ions in the presence of solvent molecules.

B-30-1 *Evaluation of Radiation Exposure of General Population in the Environment of Nuclear Installations from Dietary Intake and Whole Body Counting Studies.* I. S. BHAT, Environmental Survey Laboratory, Tarapur Atomic Power Station, Thana Dist., Zone 401 504, India.

Indirect radiation exposure of general public due to the environmental releases from nuclear installations are usually estimated from the evaluation of daily intake of radioactive contaminants through diet and drinking water.

In India for the first time, periodic whole body counting of members of general population in the environment of nuclear installations has been carried out to evaluate the exposure due to body deposited contaminants.

In the environment of Tarapur Atomic Power Station (400 MWe), the population exposures have been evaluated by both, dietary intake and whole body counting methods were in agreement in case of $^{137}Cs + {}^{134}Cs$ but it was differing by a factor of hundred or more in case of 60-Co especially when the intake is through sea food.

B-30-2 *Environmental Studies on Hg and Se Concentration in a Mineralized Area of Italy.* L. CIGNA ROSSI, G. F. CLEMENTE, AND G. P. SANTARONI, Lab. Rad. Amb. CNEN C. P. 2400–00100 Roma, Italy.

In recent time epidemiological, pathological and experimental evidence has been accumulated toward a correlation between Hg and Se environmental concentration and some diseases. In order to get more information about the concentrations of these elements in the environment, in the food-chain and in some human biological samples (blood, urine etc.), a survey has been carried on in the Mt. Amiata area (Toscana, Italy). Such an area was selected for this study owing to its large mineralization due to many elements (e.g., Fe, Cu, Ag, Sb, and particularly Hg). The Se and Hg content in all samples has been determined by non-destructive neutron activation analysis. The knowledge of both the Se and Hg distribution in the environment and their human intake is of particular interest, due to the influence of Se and Hg on the health status of the population. The total intake of these elements by the population of the area under investigation is reported for both the main routes of entrance: inhalation and ingestion. An approximate balance between total intakes, excretions and human contents is also proposed for Hg and Se.

B-30-3 *Ra^{226} Content of Environmental Samples in Some Contaminated Areas in Northern Iran.* B. KHADEMI AND A. MAHDAVI, Tehran Univ. School of Public Health & Institute of Public Health Research P.O. Box 1310, Tehran, Iran.

The Ramsar area along the Caspian Sea has been investigated for natural radioactivity. Some anomalous area has been found. The study has included the measurement of Ra^{226} content in spring and mineral waters, soil and plant samples. Also so the Ra^{226} content of milk samples of contaminated sheep were determined.

The result obtained for spring water is: 0.02–17 pCi/l, mineral water 0.02–10000 pCi/l, soil samples 2.5–3.5 pCi/g soil. Maximum Ra^{226} content for plant samples obtained is: 0.9 pCi/g ash for bean samples, 20 pCi/g ash for orange samples. The maximum uptake by contaminated sheep was 1.1% of total intake of 5000 pCi Ra^{226} per day.

B-30-4 *Sr-90 and Cs-137 in the Pacific.* YASUO MIYAKE, KATSUKO SARUHASHI, TERUKO KANAZAWA, YUKIO KATSURAGI, AND YUKIO SUGIMURA, Meteorological Research Institute, Koenji-kita 4-35-8, Suginami, Tokyo 166, Japan.

The secular changes in Sr-90 and Cs-137 contents in the Pacific waters have been studied since 1957. In surface water Sr-90 and Cs-137 which were mainly derived by the Castle Test in 1954 decreased from a few pico curie per liter in 1957 to 0.2–0.5 pico curie per liter in 1961. The second maximum appeared in 1964 due to the stratospheric fallout originated in 1961–62 tests. The difference in concentration between the west and east in the North Pacific decreased year by year and the same level of concentration was observed in 1964.

In the vertical direction, Sr-90 and Cs-137 reached down to six thousand meters in 1959 which suggested the faster penetration of these nuclides down to the deep. According to recent studies, Sr-90 and Cs-137 reached the surface and deep layers of the South Pacific though concentrations were a little lower than that in North Pacific yet.

B-30-5 *Ingestion and Inhalation Intake of Fallout Plutonium and the Dose to Man.* BURTON G. BENNETT, Health and Safety Laboratory, U.S. Atomic Energy Commission, New York, New York 10014, USA.

The eventual utilization of plutonium fuels in nuclear reactors has focused attention on the consequences of potential inadvertent releases into the environment. Measurement of fallout plutonium provides some direct data on the environmental behavior of plutonium. Weapons testing resulted in an estimated 300 kCi of globally dispersed ^{239}Pu. Transfer to man has been primarily via the inhalation pathway. The ingestion pathway appears to add insignificantly to body burdens. The results to be reported of analyses of plutonium in 19 food items comprising the total diet indicate the ingestion intake in recent years. Body burdens due to inhalation of airborne activity have been computed from the measured and inferred levels of ^{239}Pu in air in New York, utilizing the ICRP Task Group lung model. The inhalation intake is estimated to have been 42 pCi thru 1973. The adult body burden was 4 pCi at maximum in 1964 and is currently estimated to be about 2 pCi. The burdens and doses to lung, lymph glands, liver and bone are specified. This application of the lung model to the fallout data provides results which are in reasonable agreement with autopsy tissue analyses.

B-31-1 *Genetic Variance Components for Sensitivity of Inbred and Hybrid Mice to Chronic Gamma-Irradiation.* S. A. TYLER, G. A. SACHER, AND E. F. STAFFELDT, Argonne National Laboratory, Argonne, Illinois 60439, USA.

A diallel design was set up comprising all 25 matings of 5 inbred mouse strains, A/JAn1, BALB/cJAn1, C57BL/6JAn1, C3H/fJAn1 and DBA/2JAn1. Twelve progeny of each sex from each mating were entered, at age 100 days, into daily ^{60}Co gamma ray exposure at 125 and 43 R/day. Survival time was recorded. Analysis of genetic variance components was performed, following Griffing. The genetic determination of radiosensitivity is complex, with major terms for general combining ability, specific combining ability, inbreds versus hybrids, sex, and sex/dose interaction. Maternal effect and reciprocal cross effect are small. Genetic components, measured as F ratios, tend to be larger at 125 R/day than at 43 R/day.

The same genotypes and diallel design are being used for the analysis of genetic factors governing longevity, aging and disease incidence in normal and cold environments, and of their associations with a number of anatomical, physiological and biochemical parameters. The genetic interrelations between radiation survival and some of these traits will be discussed. (This work supported by the U.S. Atomic Energy Commission.)

B-31-2 *Long-Term Survival and Causes of Death in Chemical Protected and Non-Protected Irradiated Mice.* J. R. MAISIN, G. MATTELIN, M. LAMBIET-COLLIER, Départment de Radiobiologie, CEN, B-2400-Mol, Belgium.

BALB/c and C57Bl male mice 4 or 12 weeks old were exposed to a single x-ray dose from 100 to 2000 R. (200 Kv, 20 mA H.V.L. 2 mm Cu, exposure rate 100 R/min). Half of the mice were treated prior to x-ray exposure with 2-β-aminoethylisothiouronium-Br, HBr (AET), 5-hydroxytryptamine (5-HT) or a mixture of AET, glutathion, 5-HT, cystein and mercaptoethylamine.

The dose effect curves for the survival of protected and not protected mice are not parallel yielding for both strains an optimum dose reduction factor of about 2 after 500 R. For an exposure to low doses of irradiation (175 R) the dose reduction factor is only 1.7.

Mixtures of chemical protectors are very effective against both thymic lymphomas and myeloid leukemias. In protected irradiated mice, the total incidence of epitheliomas is markedly reduced compared to non-protected mice when mice of the same lifespan are compared. In both strains of mice, mixtures of chemical protectors diminish the incidence of glomerulosclerosis. In protected C57Bl mice the incidence of liver diseases (adenoma, angioma and cirrhosis) is significantly lower than in the non-protected mice. The mechanism of action of chemical protectors against late effects of radiation will be discussed.

B-31-3 *Radiation-Induced Life Shortening in the Parabiont Rat.* SHIELDS WARREN, ROSANNA CHUTE, CLARK BROWN, AND MARILYN PORTER, Cancer Research Institute, New England Deaconess Hospital, Boston, Massachusetts 02215, USA.

Parabiosis prior to radiation permits survival from a dose of radiation (1000 R, 250 kVp x-ray) lethal to single rats when one of the partners is shielded. Parabiosis theoretically should favor lengthened lifespan by providing a complete duplicate set of organs immediately available for function in case of emergency. This procedure, however, has handicaps: stress from the operation, hormonal imbalance and slight loss of immunocompatibility. Due to these factors parabiont pairs live an average of 50 days less than single rats. When one of a parabiont pair is irradiated as above, the average pair lives 517 days postradiation longer than do single rats given the same dose. The hormonal imbalance between the members of the pairs is even more marked postradiation than between unirradiated pairs, chiefly due to gonadal damage and loss of pituitary feedback. Much of the mortality of irradiated parabionts is caused by neoplasms, involving chiefly breast, bone and endocrine glands. (This investigation was supported by U.S. Atomic Energy Commission Contract AT(11-1)-3017 with the New England Deaconess Hospital.)

B-31-4 *Trial Evaluation of the Long-Term Survey of Death Causes in Small Population Living in High Background Radiation Area.* KAZUHO MAEDA AND YOSHIYASU KUROKAWA, Faculty of Medicine, University of Tokyo, Tokyo, Japan.

An attempt was made to distinguish the effect of long-term exposure with very low dose rate of ionizing radiation from the effect of natural background levels.

Sample households were randomly selected from the population living in an area adjacent to radiation-rich hot spring where natural background radiation levels are higher than surrounding.

Death cases were collected by interview from the households, which were of those persons who passed their whole lives in their home local area, and they extend over three generations. Control households and death cases were also investigated collecting from the surrounding of the hot spring.

Applying the modified cohort analysis, we tried to evaluate the effect of exposure to very low dose rate of ionizing radiation.

However, authors could not show noticeable differences between sample and control by analyzing the death causes of both groups.

In case we do not find distinct differences, the way of positively evaluating the results from the epidemiological view-point will be discussed.

B-31-5 *Abnormality of Life Span, Reproductive Span and Weight of Thymus in Mice Continuously Administered with ^{90}Sr and ^{137}Cs.* KAZUO NISHIO, Radiation Center of Osaka Prefecture, Shinke-Cho, Sakai, Osaka, Japan.

Mice of NA-2 strain were continuously administered with ^{90}Sr and ^{137}Cs through drinking water and propagated by brother- and sister-mating. Concentration of the isotopes was 0.001 microcurie of ^{90}Sr and 0.004 microcuries of ^{137}Cs per ml in experimental group and zero in control.

The mice were from third to 9th generation after start of internal-irradiation, and abnormality was averaged as follows.

1) Life span of female was lengthened by 55 days than 363 days of control group.

2) Total litter size of one female in her whole life was smaller by 2.8 than 23.2 of control and reproductive span defined as the period from first to last birth was delayed to older age and lengthened. Age of first birth was older by 21 days than 70 days of control and total litter size was smaller till 300 days while total size later than 400 days was larger than control.

3) Thymus weight was smaller than control till 300 days old, but greater later than 400 days.

These results suggest that thymus would play great role in abnormal reproduction and aging in these irradiated mice.

B-31-6 *Long-Term Observations of Mortality of Irradiated Fish at Different Temperatures.* NOBUO EGAMI, Zoological Institute, Faculty of Science, Tokyo University, Tokyo 113, Japan.

Three series of experiments were designed to study late effects of radiation of poikilothermal vertebrates at high (23–28°C) or low (below 15°C) temperatures. The freshwater small teleost, *Oryzias latipes* was used as material. In the first series, embryos were singly irradiated with 10, 25, 50, 100, 250, 500 and 1000 R of x-rays, respectively, and life span of each group was examined. The higher the dose, the higher the age specific mortality rate during the life (Experimental Gerontology, 8, 219). In the second series, aged fish (about 150 week old) were irradiated with 1000 R and kept at high or low temperature. Life shortening effect of radiation as in the first series was demonstrated at both temperatures. In the third series, adult fish were given daily dose of 25, 50, 100 or 200 R under different temperature conditions. Median survival times of fish and accumulated doses for 50 per cent mortality were calculated. Fish could accumulate higher doses before death at the lower temperature. Histopathological examination revealed variety of causes of death in these fish in all series.

B-31-7 *Late Effects in Syngeneic Radiation Chimaera.* P. METALLI, V. COVELLI, G. BRIGANTI, AND G. SILINI, Laboratory of Animal Radiation Biology, C.N.E.N., C.S.N. Casaccia, Casella Postale 2400, Roma, Italy.

Mice whole-body irradiated with 900 rad of x-rays and restored by intravenous injection of isogenic bone-marrow cells showed a specific life-span shortening of only 3% per 100 rad. Data from irradiated animals repopulated by unirradiated marrow have shown that: 1) the frequency of reticulum cell sarcoma was reduced from 60% of controls to about 5%; 2) no other types of leukaemia have been observed; 3) treated animals developed an increased frequency of benign and malignant tumors with shortened latency times; 4) nephrosclerosis and other degenerative diseases were also greatly increased in frequency; 5) none of the observed effects was dependent on the number of marrow cells injected, from 80,000 to 10 million per mouse. Irradiated animals repopulated with irradiated marrow (200 or 400 rad) showed in addition some cases of thymic lymphoma, while intact animals treated with the same doses developed some myeloid leukaemias. These results indicate that the type of leukaemia observed after irradiation of hemopoietic cells may depend on the environment in which the surviving cells are allowed to grow, and that the mouse syngeneic radiation chimaera can be proposed as a potentially useful experimental model for studies on the mechanisms of leukaemia induction at the cellular level.

B-32-1 *Separate and Combined Effects of DTPA and Glucan in Removal of Monomeric and Polymeric Plutonium from the Dog Liver.* ARTHUR LINDENBAUM AND MARCIA W. ROSENTHAL, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439, USA.

Glucan facilitates the removal of polymeric plutonium from intracellular deposition sites in the mouse liver. Because monomeric Pu is long retained in the dog liver, as is polymeric Pu in the mouse liver, the effectiveness of glucan for removing monomeric Pu from the dog liver was tested. Glucan, 15 mg/kg, was injected intravenously into beagle dogs 6, 34, and 57 days after a single intravenous injection of 0.32 μCi/kg of monomeric ^{239}Pu(IV)-citrate. At sacrifice, 90 days after Pu, no significant differences between control and treated dogs were found in the Pu levels of liver, other soft tissues, skeleton, or excreta. The inability of glucan to remove

monomeric Pu indicates that the initial deposition sites of monomeric Pu in the dog liver are mainly extracellular. This evidence is reinforced by previous work in which 24 bi-weekly treatments with $CaNa_3DTPA$, used either alone or with glucan, resulted in nearly complete removal (96%) of monomeric Pu from the dog liver. In contrast, treatment of dogs receiving highly polymeric Pu with either $CaNa_3DTPA$ or glucan or both, begun 6 days post Pu, caused only a small reduction in the hepatic burden by 90 days (85% of injected dose in controls; reduced to 71% by combined therapy). To test whether monomeric Pu, when long retained in the dog liver, might also become resistant to removal, beagles were injected with monomeric Pu and, after 3 months, were given bi-weekly treatments with $CaNa_3DTPA$ for 3 months. The results of this work will be reported, and possible modes of Pu retention in the dog liver will be discussed. (Work supported by the U.S. Atomic Energy Commission.)

B-32-2 *Chelation of ^{241}Am from the Liver and Skeleton of the Adult Baboon.* NORMAN COHEN, RAYMOND GUILMETTE, AND MCDONALD E. WRENN, Institute of Environmental Medicine, New York University Medical Center, New York, New York 10016, USA.

Investigations have been performed to define DTPA action in the removal of ^{241}Am from the adult baboon. By administering therapy at long as well as at short times after single dose i.v. exposures, it was possible to study the efficacy of DTPA therapy as a function of the site of ^{241}Am deposition in the primate, i.e., the skeleton and/or the liver. Measurements of concentration changes effected in the bone and liver were performed by monitoring ^{241}Am in tissue biopsy specimens, *in vivo* scintillation counting and routine radioanalysis of excreta.

In those animals with established bone burdens of ^{241}Am, a total of 8% of the body burden was removed due to DTPA administered over a three-week period, at a therapy schedule duplicating that presently used for man. Approximately 15% of the body burden was removed during the same time period from an animal still having a significant fraction of its burden in the liver. Partition of activity excreted as a result of chelation therapy was monitored by daily analysis of urine and feces.

B-32-3 *DTPA-Induced Decorporation of Am-241.* R. D. LLOYD, SUSAN S. MCFARLAND, G. N. TAYLOR, J. L. WILLIAMS, AND C. W. MAYS, Radiobiology Division, University of Utah, Salt Lake City, Utah 84132, USA.

Chelation treatments using subcutaneous injections of Zn-DTPA were begun two weeks after the i.v. injection of Am-citrate into 6 beagles. Retention of Am in the liver and in non-liver tissue (mainly skeleton) was followed serially in the living dogs by a combination of total-body and partial-body counting. During the first 1 to 5 months of DTPA therapy, roughly the same fraction of deposited Am was removed from 2 dogs by 1 injection each day of 0.034 m moles of DTPA per kg body mass as from 2 other dogs by the same total daily amount given in 5 injections per day. Increasing the daily amount by factors of 10 and 100 in the 2 remaining dogs only slightly increased the relative removal of Am. At 1 month of chelation therapy, Am retention in non-liver tissue was less than $\frac{1}{2}$ and in liver was less than about 1/10 of the respective pretreatment values, while liver Am had correspondingly decreased to about 1/100 of pretreatment retention by 5 months of DTPA administration. (Supported by U.S.A.E.C. Contract AT(11-1)-119.)

B-32-4 *Alternate Methods for Administering Chelating Agents in Decorporation Therapy.* W. STEVENS, D. R. ATHERTON, B. GRUBE, AND F. W. BRUENGER. Department of Anatomy, Division of Radiobiology, Salt Lake City, Utah 84132, USA.

Decorporation therapy with DTPA is partially effective. Such therapy could be improved by providing a means through which the chelator could enter cells. Decorporation of ^{241}Am from the rat has been studied using a variety of chemical forms and/or physical vehicles in the administration of these chelators. At 12 and 22 days, DTPA (Zn) was given either (A) alone (subcutaneously); (B) encapsulated in lecithin (i.v.), or (C) as the ethyl-ester in an emulsion of oil (orally) or as a combination of A + B (i.v.) or A + C (i.v. + oral). At 29 days after injection of ^{241}Am, the effectiveness of the different regimes were compared. The combination of A + C reduced the amount of nuclide retained by the whole body to 55% of that of controls. This

treatment regimen was also most effective in reducing the actual skeletal retention (humerus 68%, femur 67% and lumbar vertebrae 46% of control). Treatments A and (A + B) were most effective in reducing liver retention to 7% and 6.5% of control, respectively. In the kidney, treatment with A + C reduced the retention to 33% of control values. The combination of ZnDTPA-solution with either lecithin encapsulated ZnDTPA or the emulsified ester of DTPA provided a more effective treatment than the liposome encapsulated DTPA or emulsified esters of DTPA alone. (Supported by USAEC Contract AT(11-1)-119.)

B-32-5 *Liposome-Encapsulated DTPA in Plutonium Therapy.* MARCIA W. ROSENTHAL AND YUEH-ERH RAHMAN, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439, USA.

The usefulness of the chelating agents EDTA (ethylenediaminetetraacetic acid) and DTPA (diethylenetriaminepentaacetic acid) in removal of toxic metals is limited by their rapid excretion and their inability to cross cellular membranes. Encapsulation of these chelating agents within lipid spherules, liposomes, has been shown to (1) increase their uptake and retention in mouse tissues; (2) promote their intracellular penetration; (3) remove between 20–40% of the liver Pu not removed by nonencapsulated DTPA; and (4) improve Pu removal from bone and excretion in urine. Further experiments with mice injected with polymeric ^{239}Pu and 3 days later with liposomes (2–5 μm) made with phosphatidylcholine and cholesterol (3:1) have now shown that intraperitoneal injection of liposomal DTPA is as effective as intravenous injection in removal of additional Pu from liver and bone. A single injection of liposomal DTPA (100 mg/kg) given at 24 days is as effective as at 3 days in removal of Pu from liver. Removal of additional Pu from bone appears in part dependent upon the use of highly purified, undegraded phosphatidylcholine in liposomes containing DTPA. Four injections of liposomal DTPA (100 mg/kg), given at weekly intervals from 3 days after injection of Pu, remove 45% of the liver Pu not removed by nonencapsulated DTPA. Preliminary results using liposomes differing in lipid constituents and surface charge to encapsulate DTPA have thus far not given different results for Pu removal. (This work was supported by the U.S. Atomic Energy Commission.)

B-32-6 *Decreased Uptake of Plutonium-239 in Bone of Diphosphonate-Treated Rats.* W. S. S. JEE, R. DELL, S. C. MILLER, AND D. KIMMEL. University of Utah, Salt Lake City, Utah 84132, USA.

The purpose of this experiment was to measure the effect of pretreatment with ethane-1-hydroxy-1,1-diphosphonate (EHDP) on the uptake of plutonium (Pu-239) in bone of adult rats. 300 gram male, Sprague-Dawley rats were injected daily for 5 or 25 days with 0, 0.4, or 10 mg EHDP/kg body weight/day. An additional group was injected for 150 or 180 days with 0, 0.4, 2.0, 4.0, or 10 mg EHDP/kg/day. At the end of treatment, they were injected with 3 μCi plutonium citrate per kg body weight and sacrificed three days later. One tibia was ashed and the amount of radioactivity in it determined. The other tibia was sectioned undecalcified for autoradiographic inspection. From histologic sections, the amount and surface area of trabecular bone in the tibial metaphysis was determined.

After 5 or 25 days of 10 mg EHDP/kg/day, the uptake of Pu-239 in bone was lower. After 150 or 180 days of all doses (0.4, 2.0, 4.0, and 10 mg EHDP/kg/day), the uptake of Pu-239 in the rat tibia was decreased. There was more trabecular bone, both in surface area and quantity with each increasing dose of EHDP after 150 or 180 days of treatment. Autoradiographically, the decrease in Pu-239 uptake was noticeable on bone surfaces.

Five or 25 days pretreatment with 10 mg EHDP/kg/day lowered the uptake of Pu-239 in bone of adult rats. Longer treatment periods (150 or 180 days) at lower dose levels (0.4, 2.0, 4.0 mg/kg/day) showed a decreased uptake of Pu-239. (Supported by USAEC Contract AT(11-1)-119 and NIH Grants DE-151 and GM-958.

B-32-7 *Specific Mobilization of the Heavy Alkaline Earth Metals from the Skeleton.* OSCAR L. J. VANDERBORGHT, Mineral Metabolism Laboratory; Belgian Nuclear Centre, SCK/CEN, B 2400 Mol, Belgium.

A method is presented that allows the mobilization of radiostrontium from an old radiocon-

tamination in the skeleton. The effect of the treatment on the mobility of radiocalcium and radium is compared; and the influence of the age of the Sr-deposit in the skeleton on this mobilizing effect is studied.

The treatment is more effective on Sr than on Ra; and is affecting more a several months old deposit than more recent ones.

B-33-1 *Radiation-Induced Changes in Aorta.* M. YUSUF KHAN, College of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, New Jersey 07103, USA.

This study was undertaken to investigate the nature and pathogenesis of radiation-induced changes in descending thoracic aorta of rabbits subjected to a single aortic radiation dose of 800 and 1000 rads. Radiation was delivered from a source of 250 KeV, (mA 15; HVL 2.2 mm Cu). At light microscopic level, mucinous edema of media, stainable with colloidal iron and Alcian blue stains, was the earliest demonstrable change. This was followed by deposition of discrete calcium granules in the ground substance which progressed to calcium encrustation of tissues limited to either inner or middle third of media. Atherosclerosis was relatively rare. Electron-microscopy showed significant changes in vasa vasorum, progressive smooth muscle cell degeneration, disruption of elastica and calcification of microfibrils. This study indicates that radiation-induced aortic sclero-calcification results from direct radiation injury to smooth muscle cells and compromised vasa vasorum. (Work supported by Monmouth County Chapter of American Heart Association.)

B-33-2 *In Vivo Evaluation of Radiation Late Effects on Brain Circulation in the Rat.* ANDRÉ KEYEUX, Laboratoire de Radiobiologie UCL, Institut du Cancer, Kapucijnenvoer 37, B-3000 Leuven, Belgium.

Late vascular changes appearing in the brain of rats after local irradiation are estimated by studying the variation of indices characterizing the brain blood flow (A), the brain blood volume (V) and the brain circulatory mean transit-time (τ) as well as, the blood brain barrier permeability (P). These indices are calculated on the basis of parameters extracted by computer analysis from the cephalic blood dilution curves of both ^{99m}Tc pertechnetate and ^{131}I antipyrine. These curves were recorded by external counting over the head after a sudden injection of the radiotracers in the femoral vein. This quantitative radioisotope method was applied 6, 12 and 24 months after irradiation of the brain to various doses (500–1000–1500–3000–4000 R) of ^{137}Cs gamma rays. The results obtained at 6, 12 and 24 months after irradiation show a significant reduction in the values of brain blood flow and/or brain blood volume indices at 6 months for the 3000 and 4000 R groups and at 12 months for the 500, 1000 and 1500 R groups. These results and the influence of a late radiation induced change in the blood brain barrier permeability on their interpretation are discussed. (This study was supported by the European Late Effects Project Group.)

B-33-3 *Radiation-Effects on Circulatory Efficiency.* EMILY J. B. CHRISTIAN AND S. PHYLLIS STEARNER, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439, USA.

Long-term microvascular studies on the effects of ionizing radiation include estimation of circulatory efficiency (measurement of capillary blood flow) in selected tissues. Xenon-133 clearance, a rapid and non-destructive technique for determination of capillary flow in localized tissue regions, was adapted for use in the mouse. Repeated determinations have been made on individual animals at intervals up to 30 months after irradiation. The clearance of locally-injected ^{133}Xe from the subcutaneous region is usually adequately fitted by a single exponential function. In 20- to 66-week controls, the mean half-time is 9–10 minutes, with a standard error of about 10%. Somewhat longer clearance times are observed as an aging effect in older controls. After 285–855 rad ^{60}Co gamma irradiation, a 50–70% increase in mean clearance time is apparent at 6 months and is sustained over 104 weeks. The response is more variable after 24-week fractionated exposures. After fission neutron doses of 80–240 rad, individual values are more variable; at 52 weeks, some show a 2-exponential clearance, but there are fewer after 78–104 weeks. The fractionation effect is minimal. Clearance curves from lung, liver and kidney

typically show 2-exponential functions. The faster component from liver and kidney has a half-time of less than 1 minute, while the slower component is similar to that seen for the single clearance time from subcutaneous regions. (Work supported by the U.S. Atomic Energy Commission.)

B-33-4 *Structural Changes in the Microvasculature in the Aging, Irradiated Mouse.* S. Phyllis Stearner, Emily J. B. Christian, and Rosemarie L. Devine, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439, USA.

Blood vessels, essential structures in virtually all tissues, are made up of very slowly dividing cell populations. Radiation effects on the vasculature, therefore, represent a non-cytokinetic injury not associated directly with cell division.

In vivo observation of the microvasculature in the mouse pinna reveals segmental stenosis of arteries and increased tortuosity and irregular dilatation of veins and venules as a radiation effect within 12–24 months after whole-body exposure (285–855 rad ^{60}Co gamma rays; 80–240 rad neutrons). Venular irregularities are apparently enhanced by aging, but segmental arterial stenosis has been observed only in irradiated animals. Prominent ultrastructural effects in irradiated blood vessels include thickening of the basement lamina and increased electron density of interstitial material, especially around capillaries and small veins. Veins frequently show marked attenuation of their walls. Changes in arteries include varying densities in the elastic lamina and multiple vacuoles in smooth muscle cells. Endothelium of all blood vessels shows degenerative changes (e.g., electronluscent cytoplasmic matrix, vacuolization and/or electron-dense cytoplasm, dilated mitochondria). There may be rupture of the endothelial cell membrane, but seldom any apparent disruption of the endothelial cell junction. (Work supported by the U.S. Atomic Energy Commission.)

B-33-5 *Late Effect of Ionizing Radiation on Size and Number of Glomeruli in Murine Kidneys.* Fumiaki Sato, Sosaku Tsuchihashi, and Naoyuki Kawashima. National Institute of Radiological Sciences, Chiba, Japan.

By means of statisco-geometrical methods the glomeruli of murine kidneys have been examined. The average radii of the glomeruli in non-irradiated groups are 26 μ in 10 weeks old mice, 29 μ in 30 weeks old mice and 33 μ in 90 weeks old mice. The increase of the radii seems to be linear with logarithm of the age. The number of glomeruli per unit volume of total kidney in non-irradiated groups are 240 per mm^3 in 10 weeks old mice, 170 per mm^3 in 30 weeks old mice and 130 per mm^3 in 90 weeks old mice. The decrease of the numbers seems to be linear with logarithm of the age. Mice exposed to 400 R at 10 weeks of age were sacrificed at 50 weeks and effects of the irradiation on size and number of glomeruli are not large.

B-33-6 *The Renal Effects of Perinatal Gamma-Irradiation in the Beagle.* Robert D. Phemister, Roger S. Jaenke, and Robert W. Norrdin, Collaborative Radiological Health Laboratory, Colorado State University, Fort Collins, Colorado 80521, USA.

Approximately 100 beagles received sublethal exposures to whole-body ^{60}Co gamma radiation at 55 days *in utero* or 2 days *postpartum* and were studied from 60 days up to 4 years of age. Twenty dogs, including eighteen males, died in chronic renal failure at ages ranging from 8.5 to 45.5 months. This was the only appreciable source of mortality during the period of study. Clinical and morphologic evidence of renal disease was also evident in animals killed according to a predetermined sacrifice schedule at 70 days, 2 years, or 4 years of age. The principal lesion, detected as early as 70 days, was a progressive increase in mesangial cells and matrix leading to severe glomerulosclerosis. These findings emphasize the radiosensitivity of the kidney during its final phase of development. (Research supported by Contract FDA 72-302 with the Bureau of Radiological Health, Department of Health, Education and Welfare.)

B-33-7 *The Tolerance of the Pig Kidney to Fractionated X-Irradiation.* J. W. Hopewell and R. J. Berry, Radiobiology Laboratory, Radiotherapy Department, Churchill Hospital, Oxford, Great Britain.

Changes in renal function following local irradiation of one kidney in the pig with 1, 6 or 30

dose fractions have been studied up to 6 months after treatment by I^{131} Hippuran renography. The doses used were based on 70% of the "skin tolerance" (Nominal Standard Dose) of 1800 rets, and doses above and below this value were given. Dose levels of 1260 rads single dose and 1050 rets for fractionated treatments approximated renal tolerance for single doses and 30f/39d but *not* 6f/18 days. The renal tolerance for the 6 fraction treatment was 30–45% lower than in the other two treatment groups. The marked reduction in renal tolerance with 6 fractions/18 days could be due either to the relatively small number of large fractions or to the short overall treatment time: Preliminary results with treatment given in 14 fractions/18 days suggest that the latter is true.

The widely differing partial tolerance doses observed in this study infer that the NSD system cannot be applied to predict the variation in radiation dose with dose fractionation which produces equivalent damage to the kidney.

B-33-8 *Renal and Adrenal Factors in Radiation Hypertension and Nephrosclerosis.* Alan G. Lurie and George W. Casarett, Univ. of Rochester, Rochester, New York 14642, USA.

The effects of adrenalectomy on the incidence, severity and progression of radiation hypertension and nephrosclerosis in male rats were studied. Adrenalectomized and non-adrenalectomized rats received single exposures of 500 R or 2000 R x-radiation bilaterally to temporarily exteriorized kidneys, or 500 R to the whole body with temporarily exteriorized, shielded kidneys. Rats were sacrificed 5 and 10 weeks after treatment and evaluated for systolic blood pressure, gross pathology and renal histopathology. Plasma renin activity (PRA) was studied in selected rats. Hypertension and arteriolonephrosclerosis (ANS) were produced in all irradiated rats: hypertension occurred prior to or concurrently with the earliest detectable ANS changes. Hypertension and ANS were considerably more severe in adrenalectomized rats: peak systolic pressure was 227 mm Hg in adrenalectomized, whole-body irradiated, renally shielded rats, and the most severe and widespread ANS was in adrenalectomized, renally irradiated (2000 R) rats. PRA was substantially elevated in irradiated adrenalectomized rats. Loss of adrenocortical hormones may exacerbate the progression of radiation hypertension and nephrosclerosis by causing increased renin secretion, increased renal immune complex deposition, and/or severe microvascular damage leading to occlusive changes.

B-34-1 *Survival Curve at Low Doses for Mouse Jejunal Crypt Cells.* Carlos E. deAlmeida, Kathy Mason, and Rodney Withers, The University of Texas System Cancer Center, M. D. Anderson Hospital & Tumor Institute, Houston, Texas 77025, USA.

The cellular dose survival relationship of most interest to radiotherapists covers the dose range of 0 to 300 rads of γ-radiation. It is not possible to determine directly the survival curve for crypt cells at such low doses because surviving cells are too numerous to permit growth of discrete colonies suitable for scoring. To circumvent this problem, mice were exposed to γ-ray doses of 0 to 300 rads and then, 4 hours later, given an "assay" dose of 500 rads of neutrons generated at the TAMVEC cyclotron from bombardment of a beryllium target with 50 MeV deuterons. Assuming that the assay dose produces an equal decrement in crypt cell survival in all animals, the survival relationship measured after the neutron dose should reflect relative cellular survival immediately before the neutron dose. In this manner a curve relating low γ-ray dose to cellular survival has been obtained. It is bending slightly but can be fitted, over the γ-ray dose range of 50 to 200 rads, by a straight line whose slope is described by a D_0 value of about 200 rads. These data, together with the results of fractionating the γ-ray dose will be presented.

This work was supported in part by Neutron Grant CA 12542.

B-34-2 *Effects of X-Irradiation on Isoproterenol-Induced Cell Proliferation and DNA Synthesis in Parotid Gland of the Mouse.* Ikuko Furuno and Hiromichi Matsudaira, National Institute of Radiological Sciences, Chiba, 280, Japan.

Changes in proliferative response to isoproterenol (IPR) were studied in the parotid gland of the mouse previously given local x-irradiation.

X-irradiation produced dose-dependent inhibition of DNA synthesis and cell proliferation induced by the drug. A D_0 of approximately 900 R was obtained from the dose response curve

based on changes in the DNA content of the organ at the end of the mitotic stimulations. Similarly, split-dose experiments showed a slight but significant recovery from the radiation damage within a few hours after irradiation (Radiat. Res., in press).

Several lines of evidence suggest that the initial rise of cAMP following IPR is not a major site of radiation action, although it is important in triggering proliferation of otherwise quiescent acinar cells of the gland. (Supported in part by grants from the Ministry of Education.)

B-34-3 *Increasing Radiosensitivity of Ileum Crypt Cells of Ground Squirrels During Arousal from Hibernation.* BERNARD N. JAROSLOW, KATHERINE M. SUHRBIER, AND R. J. MICHAEL FRY, Biology Division, Argonne National Laboratory, Argonne, Illinois 60439, USA.

Earlier studies (Jaroslow *et al.*, Rad. Res. **38**, 379, 1969) showed that ground squirrels (*Citellus tridecemlineatus*) exposed to ^{60}Co γ-radiation during hibernation survived longer than those exposed when awake. In the present study the survival of the crypt cells in the ileum was assayed after irradiation of animals in hibernation, arousal, and the awake state.

The number of microcolonies was highest in animals irradiated during hibernation and decreased to control levels in those irradiated within seven hours after arousal. The number of mitoses per crypt at various times after arousal suggested that: (1) a majority of crypt cells were in G_1 during hibernation; (2) seven hours after arousal, when crypt cell survival was similar in hibernating and non hibernating animals, the crypt cells were in late G_1 and early S. (Work performed under the auspices of the U.S. Atomic Energy Commission.)

B-34-4 *Persistence of Latent Radiation Damage in Neutron or X-Irradiated Intermitotic Cells of Rat Lens Epithelia.* EDGAR F. RILEY, BRUCE T. AUSTIN, ALICE L. LINDGREN, AND RICHARD C. MILLER, Radiation Research Laboratory, University of Iowa, Iowa City, Iowa 52242, USA.

In response to a mitogenic stimulus resulting from a small puncture wound, the intermitotic cells of the rat lens epithelium enter the cell division cycle. Irradiation 1 hour before wounding caused a delay and a partial suppression of the response. Comparable effects on the wound response and on the ultimate opacification of the lens were produced by 200 rads of fission neutrons or 1000 rads of 250 kVp x-rays. By 30 days post-irradiation, some cellular recovery was evident, i.e., there was no delay and less suppression of the response to wounding. The recovery was not complete, however. At 30 days post-irradiation, the mitotic figures in about 30% of the responding cells were clearly abnormal. Latent radiation damage persisted in the intermitotic cells for 30 days, at least. This persistent latent radiation damage apparently results in abnormal differentiation of lens fibers and a progressive opacification of the lens as the damaged intermitotic cells are "called upon" to divide and differentiate.

B-34-5 *Effect of Combined Radiation and Adriamycin Treatment on the Mouse Intestine.* DENNIS R. BURHOLT, JOHN W. COOPER, AND RONALD F. HAGEMANN, Allegheny General Hospital, Pittsburgh, Pennsylvania 15212, USA.

Adriamycin is an antibiotic which has shown recent promise as an effective antineoplastic agent. In order to ascertain the feasibility of radiation-adriamycin therapy regimens the influence of this combination on the cellular and tissue level of the mouse intestine was assessed. When given alone adriamycin, in a dose up to 10 mg/kg, does not produce gastrointestinal lethality nor affect jejunal crypt survival, however, it does reduce crypt cellularity which elicits a subsequent hyperplasia. If adriamycin is administered immediately after abdominal x-ray exposure the LD_{50} for gastrointestinal lethality is reduced 300–400 R. The combination of 1000 R and drug increased by 24 hours the time interval between exposure and the onset of compensatory cell proliferation. A crypt survival curve for the radiation-drug combination was also obtained. The effect of the changed cell population kinetic parameters will be discussed in relation to cell input from the crypts to the villi and animal survival.

B-34-6 *Increased Gastrointestinal Radiosensitivity in Genetically Anemic W/W^v Mice.* BEVERLY J. TOROK AND SALLIE S. BOGGS, University of Pittsburgh Medical School, Pittsburgh, Pa. 15261, USA.

The W/W^v mouse has deficits in melanoblastic and germinal stem cells expressed during embryogenesis and as adults, a defect of pluripotent hematopoietic stem cells.

Gastrointestinal mucosa is dependent upon an intact stem cell system for recovery from x-irradiation. Recovery of the gut was studied in W/W^v which had been "cured" of their hematopoietic defect by transplantation of normal marrow. "Cured" W/W^v mice and their normal +/+, W/+ and W^v/+ littermates were exposed to 1250 rads of irradiation. Mean survival time of controls was 8.4 ± 0.31 days compared to only 5.85 ± 0.18 days (p = .001) for W/W^v mice. Incorporation of ^{3}H thymidine into mucosal cells of control +/+ mice was minimum two days after exposure and exceeded non-irradiated levels by day 3, while W/W^v mice had values lower than controls on days 2–4 and never significantly exceeded non-irradiated values. Changes in gut weight followed the same pattern as was observed with ^{3}HTdr uptake. These studies suggest that the W/W^v defect involves stem cells of the gut as well as hematopoietic stem cells.

B-34-7 *Wound Response to the Shielded and X-Irradiated Halves of the Rat Lens Epithelia.* Richard C. Miller and Edgar F. Riley, Radiation Research Laboratory, University of Iowa, Iowa City, Iowa 52242, USA.

The complete opacification of a rat lens is prevented if half of the lens is shielded during exposure to 1000 R of x-rays. We are utilizing the ability of intermitotic (G_0) cells in the rat lens epithelium to respond to mechanical wounding as a measure of radiation effect, cellular recovery, and the interaction of shielded and unshielded halves of the lens. In unirradiated lens epithelia, the G_0 cells adjacent to a puncture wound begin DNA synthesis about 14 hours after wounding and begin mitosis about 10 hours later. X-irradiation of the entire lens delays and suppresses the response to wounding. Preliminary results indicate that the response may be delayed about 2 hours in the shielded half of a lens but the cells may respond with greater intensity (a larger peak response) and more synchronously than they do in unirradiated epithelia. In the irradiated half, the response may be suppressed as much, but may continue longer than when the entire lens is irradiated. The suppression of the response in irradiated epithelia is apparently due to a decreased ability of cells to respond rather than a decreased amount of mitogenic stimulus produced by wounding.

B-34-8 *Swine Epidermal Cell Population Changes Following Degraded Fission Neutron Irradiation.* John O. Archambeau and Joseph J. Abata. Nassau County Medical Center, East Meadow, NY 11554, USA.

The number of basal and prickle cells per centimeter were determined at regular intervals up to seven weeks following irradiation. The 10 cm. in diameter fields were irradiated with 750 and 1050 rads of degraded fission neutrons using the patient facility of the Medical Research Reactor at Brookhaven National Laboratory. A single animal was biopsied and sacrificed at each time period. There was a progressive loss of cells which reached a minimum at day 17 to 21. Following this there was regeneration of the epidermal population with overshoot by day 32 and return to control levels at later times. These changes were similar to those found for x-ray field. The dose survival data were obtained by counting the number of regenerating islands occurring per centimeter at 21 days following 600, 750, 900, 1050 and 1200 rads. The D_0 was calculated and the RBE was estimated to be 3.

B-35-1 *Effect of Heat and Radiation on Synchronous Chinese Hamster Cells: Killing and Repair.* Leo E. Gerweck, Edward L. Gillette, and William C. Dewey. Colorado State University, Fort Collins, CO 80521, USA.

Survival was determined between single or split doses of heat or radiation alone or in combination. Unlike the sensitivity to x rays, the sensitivity to heat was at least as great for hypoxic cells as for aerobic. Differences between effects of heat and x-radiation were also seen in studies of repair. Repair of sublethal damage during S-phase as measured with fractionated treatments had a half-time of only 20–30 minutes for x-radiation in contrast to 3 hours for heat. Heat (45.5°C for 7–9 minutes) prior to radiation eliminated both the shoulder of the radiation survival curve and repair of sublethal damage as measured by split doses of radiation. The half-time

of repair of heat damage as measured by the return of the shoulder of the x-ray survival curve was 9 or 16 hours (G_1 or S). This repair occurred in the absence of cell cycle progression (delayed approximately 17 hours). In summary, the elimination of the shoulder of the radiation survival curve indicates that heat and radiation damage a common target. However, the different repair kinetics and effect of hypoxia suggest that the molecular lesion for heat and x rays are not identical.

B-35-2 *Interaction of Radiation and Hyperthermic Damage in CHO Cells.* D. B. Leeper and K. J. Henle, Thomas Jefferson University, Philadelphia, Pa. 19107, USA.

The effects of hyperthermia (45 ± 0.05°C) and x-rays on cell survival and cell division have been determined in Chinese hamster ovary (CHO) fibroblasts in monolayer culture. The asynchronous hyperthermia survival curve was exponential, D_0 = 3.8 min. at 45°C, D_q = 7.9 min., n = 7.4. G_1 was the most resistant phase in the cell cycle with M and S equally sensitive, reflecting a variation of the shoulder of the survival curve. Hyperthermia-induced division delay was completely reversible (28.6 min. of delay per min. at 45°C).

CHO cells accumulate and repair sublethal hyperthermic damage. There exist two components to the exponential recovery curve observed by fractionating two 20 min. doses of hyperthermia at 45°C. The initial repair process was complete by 30 min. post treatment and proceeded at a rate of 0.009 per min. The second repair process proceeded at the rate of 0.003 per min. and was completed by 12 hr. post treatment. When a dose of 400 rads of x-rays was used to challenge sublethal hyperthermic damage the two-dose recovery curve exhibited the same shape but the apparent repair kinetics were 0.0125 per min. initially, followed by a repair rate of 0.015 per min.

Radiation damage eliminated the shoulder on the hyperthermia survival curve and reduced the D_0 by a factor of 2. The shoulder on the hyperthermia survival curve was partially restored within five hours post-irradiation, but the slope remained sensitized. Although the sublethal damage which interacts with a second dose of radiation is completely repaired, some form of damage remains which continues to sensitize CHO cells to hyperthermia. (Supported by NIH Grant No. CA 11602.)

B-35-3 *The Radiosensitization of Hypoxic Tumor Cells by Hyperthermia.* J. H. Kim, S. H. Kim, and E. W. Hahn, Department of Radiation Therapy, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA.

Hypoxic cells in tumors represent a major source of local failure due to their high radioresistance. Various means have been employed to circumvent this problem. Hyperbaric chambers have been used in an attempt to increase the oxygenation of hypoxic cells. High LET radiations are being explored because of their relative independence from the oxygen effect. The present study suggests that heating hypoxic cells is another effective means of reducing the oxygen dependence factor in radiation effects. Dose survival curves for HeLa S-3 cells in culture were determined with and without postirradiation heating, both in the oxic and hypoxic state. Heavily irradiated feeder cells were used to produce anoxia. In this system, the ambient oxygen is removed by the respiratory activity of an excess of irradiated feeder cells. An OER of 1.56 was obtained for hypoxic cells treated with hyperthermia (42°C for 2 hours), while an OER of 3.0 was obtained for irradiation only (gain factor = 1.92). The potential significance of these findings for clinical radiotherapy as well as possible mechanisms that might explain the reduced OER associated with hyperthermia will be discussed. (Supported in part by NIH Grant CA-08748.)

B-35-4 *The Effect of Precooling on the Radiation Sensitivity of Proliferating Hair Follicle.* N. Dubravsky, N. Hunter, and H. R. Withers, Section of Experimental Radiotherapy, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77025, USA.

Newton and Wildy (1959) and Neifakh and Rott (1958) showed that short periods of cooling to temperatures around 3–4°C of He-La cells in culture and fish embryos in the blastula stage, respectively, caused partial synchronization of the culture and the embryos. Sensitivity to radiation *in vitro* and *in vivo* has been shown to be different at the different stages of the cell cycle, G_2, mitosis, and the G_1/S junction being the most radiosensitive. Experiments were done to

examine whether prechilling of the proliferating hair follicle would cause partial synchronization *in vivo*. Two assays were used: ^{3}H-TdR incorporation in the skin and changes in radiosensitivity of the hair follicles as a function of time from the end of cooling. All irradiations were done 72–80 hours after plucking. Cooling of the skin took place at different times between 48–70 hours after plucking. Partial synchronization is demonstrated in the skin. Results of ^{3}H-TdR incorporation and sensitivity of the hair follicle show that cooling arrests cells in mitosis. This is followed by a long G_1 estimated to be around 13 hours, and an S phase which is around 10 hours. The cells are synchronized at mitosis with a radiosensitive period between 0.5–3 hours after the end of the cooling. Survival curves determined 3 and 7 hours after the end of cooling are described by mean D_0 values of 114 and 412 rads, respectively. The D_0 value for the survival curve in control animals at the same time after plucking is 197 rads.

B-35-5 *The Radiation Enhancement Factor (REF) of Hyperthermia in Combination with Fast Neutrons on Local Tumor Response.* Eric W. Hahn, Thomas R. Canada, Alan A. Alfieri, and Joseph C. McDonald. Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA.

Studies in our laboratory have shown that fractionated x-ray in combination with hyperthermia significantly increased local tumor control and the cure rate of mice bearing the Ridgway osteogenic sarcoma (ROS). The REF was estimated to be approximately 4.

In order to examine the mechanisms involved in the radiosensitization of tumor tissues with hyperthermia, male AKR/Jax mice bearing a ROS were exposed to 3 equal fractions (M, W, F) of cyclotron produced fast neutrons ($\bar{E}_n \approx 3$ MeV, 0–100 rads/fraction) alone and in combination with local tumor hyperthermia (42.5 ± 5°C for 15 min). Hyperthermia was achieved with heat lamps.

Hyperthermia alone produced a transitory short-lived retardation in tumor growth. The tumor response for both neutron and the neutron plus heat treatments was approximately equal (REF ≈ 1), suggesting that the large REF observed with the x-ray/hyperthermia treatments may result from heat induced inhibition of the tumor cell repair mechanisms. It would appear that hyperthermia when used in combination with fast neutrons and possibly other high LET radiation offers no added advantage in local tumor control. (Supported in part by NCI CA Grant 08748 and AEC (11-1)-3522).

B-35-6 *Radiation and Hyperthermal Response of Normal Tissue in Situ—3 Fraction Studies.* J. Eugene Robinson, Morris J. Wizenberg, Edgar A. Edelsack, and Welton A. McCready, University of Maryland School of Medicine, Baltimore, Maryland 21201, USA.

As part of an investigation of hyperthermia and ionizing radiation as a combined modality for cancer therapy, we have conducted a study of response of a normal tissue (mouse skin) to a three-fraction regimen combining hyperthermia and ionizing radiation. The combined treatment used equal thermal treatment times and equal radiation doses on three successive days. The elevated temperatures were applied for 20 minutes each day and were maintained pre-, during-, and post-irradiation. Radiation response curves were determined for each treatment temperature. The assay system is based on numerically graded grossly visible responses as described by Brown.[1] Radiation sensitivity of the skin increases treatment temperature, with thermal enhancement ratios of 1.13, 1.33, and 1.49 for elevated temperatures of 41.0, 42.5, and 43.0°C (T.E.R. for 37.5°C is one). The assay system will be described and the results compared with single and three-fraction tumor studies at elevated temperatures. (Supported in part by Public Health Service research grant CA-06518-11 from the National Cancer Institute.)

[1] Brown, J. M., *et al.*, JNC, Fol. 47, pp. 75–89 (1971).

B-36-1 *Radiosensitisation of Hypoxic Cells in Solid Tumours in Air-Breathing C_3H Mice.* John F. Fowler, Peter W. Sheldon, and Lance J. Foster, Gray Laboratory of the Cancer Research Campaign, Mount Vernon Hospital, Northwood, Middlesex, Great Britain.

The doses required for local control of 50% of solid tumours at 150 days after irradiation

was determined for single x-ray doses and certain multi-fraction schedules. The tumours were first-generation transplants from spontaneous mammary tumours into the flank of C_3H/He mice. They contained about 10% of hypoxic cells. X-ray doses were substantially reduced when either a 5- or a 2-nitromidazole drug had been administered to the mouse (0.5–1 mg per gram body weight i.p.) 20 to 60 minutes before irradiation. The Enhancement Ratio (i.e., Dose Reduction Factor) was about 1.4 for the 5-nitroimidazole (Metronidazole) and 1.9 for the 2-nitroimidazole compound. In the latter case the TCD 50 was reduced from 4000 to just over 2000 rads so that few hypoxic cells could have survived.

It seems clear that these radiosensitisers can reach hypoxic cells in the tumours and sensitise them very effectively.

B-36-2 *The Relationship Between Mouse Arterial Partial Pressure of Oxygen (PaO_2) and the Effectiveness of Localized Tumour Irradiation.* DIETMAR W. SIEMANN, MICHAEL J. BRONSKILL, RICHARD P. HILL, AND RAY S. BUSH, The Ontario Cancer Institute Incorporating The Princess Margaret Hospital, Toronto, Ontario, M4X 1K9 Canada.

The effect of localized x-irradiation on the transplantable KHT sarcoma has been investigated under various conditions. Subcutaneous tumours in the right flank of C3H mice anaesthetized with nembutal, urethane, or valium were irradiated while mice breathed gas mixtures ranging from 5% O_2 + 95% N_2 to 100% O_2. Radiation effectiveness was determined by a growth delay assay, specifically, the delay in days for tumours to grow to a mean diameter of 10 mm following single doses of 1500 or 2500 rads. Measurements of PaO_2, $PaCO_2$, and arterial blood pH were made under conditions identical to the irradiation experiments. There exists a clear correlation between PaO_2 and growth delay under all conditions studied. For PaO_2 values <95 mm Hg (air-breathing animals), growth delay decreases linearly with PaO_2. For PaO_2 values >95 mm Hg, tumour delay increases slowly and reaches a plateau at $PaO_2 \simeq 500$ mm Hg (100% O_2-breathing animals). This relationship holds for the two doses (1500 and 2500 rads) and the three anaesthetics studied.

B-36-3 *The Hypoxic Fraction of a Murine Squamous Carcinoma as a Function of Transplantation Site.* LESTER J. PETERS, Gray Laboratory, Northwood, Middx., Great Britain.

An experimental tumor system consisting of *cutaneous* implants of a murine squamous carcinoma in syngeneic mice has been found by TCD_{50} estimations to contain a very low proportion of hypoxic cells. For discoid cutaneous tumors of 40–60 mm^3, the TCD_{50} in air-breathing mice was ~3300 rads, increasing to ~5300 rads, when the tumor blood supply was occluded. Using cellular radiation survival parameters known for this tumor, the hypoxic fraction was calculated to be 0.3%. Selective chemical sensitization of the hypoxic cells resulted in a TCD_{50} of ~1850 rads, which is consistent with a cure probability determined by survival of cells in the *oxic* compartment.

Tumors which had grown subcutaneously had a TCD_{50} of >4900 rads, unclamped, in air-breathing mice.

It is postulated that this difference may be due to the greater demand for new vessel formation in the subcutaneous site compared with the richly vascular corium.

The results go some way towards explaining radiotherapeutic experience which suggests that the cure probability of most human skin cancers is not influenced by radiobiologically hypoxic cells.

B-36-4 *Hypoxic Cell Radiosensitization of Normal and Malignant Cells in Vivo.* JULIE DENEKAMP, BARRY D. MICHAEL, AND S. R. HARRIS, Gray Laboratory, Mount Vernon Hospital, Northwood, Great Britain.

Several electron affinic sensitizers found to be effective specifically on hypoxic cells *in vitro* have now been tested *in vivo*, using both normal tissues made artificially hypoxic and experimental tumors. Results will be presented on one of these compounds, a 2-nitro-imidazole, tested using the *in vivo* skin clone technique and using regrowth of an experimental tumor after irradiation in air or clamped off to make it totally hypoxic. A 100-fold range of concentrations of the drug has been tested with the skin system, so that the sensitizing efficiency relative to the drug dose has been characterized. The maximum dose tested achieved 70% of the sensitizing efficiency of oxygen

(i.e., Enhancement ratio of 2.2). Enhancement ratios of approximately 2.0 have also been obtained from measurements of tumor regrowth both in aerobic and clamped tumors.

The sensitizing action of the drug is restricted to cells irradiated under hypoxic conditions, and the drug must be present at the time of irradiation. The efficiency of this drug will be compared with other electron affinic drugs such as NDPP and metronidazole using the same biological test systems.

B-36-5 *Modification of Radiation Effect on Mouse Tumor and Normal Tissues by Four Cancer Chemotherapeutic Agents.* THEODORE L. PHILLIPS, MOODY D. WHARAM, GLENDA ROSS, AND LAWRENCE KANE, University of California, San Francisco, California 94143, USA.

Four different cancer chemotherapeutic agents and irradiation were administered to Balb/c mice carrying EMT-6 tumors and the tumor response compared to response of esophagus, lung, and intestinal epithelium with radiation and radiation plus the chemical agents. When Actinomycin D was given in a dose of 750 μg/kg a Dose Modifying Factor (D.M.F.) of 1.61 was noted for esophagus, 1.26 for intestine and 1.2 for lung. Tumor D.M.F. values ranged from 2.2 to 1.1 depending on radiation dose. With Bleomycin A_2 the D.M.F. was 1.14 for esophagus, 1.1 for intestine, 1.0 for lung and 2.24 to 1.0 for the tumor. Bischloro Nitrosourea yielded D.M.F.'s of .99 and 1.07 for esophagus and intestine and 2.0 and 1.2 for tumor. Cyclophosphamide produced D.M.F. values of .92 and 1 for esophagus and intestine but 1.9 and 1.2 for tumor. Thus enhancement of the therapeutic ratio is seen with the possible exception of Actinomycin D, suggesting that use of the agents could be valuable in radiation therapy.

B-36-6 *Effects of Metronidazole and Radiation on Tumors and Normal Tissues in Mice in Vivo.* HELEN B. STONE AND H. R. WITHERS, University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77025, USA.

The TCD50 (radiation dose to control the tumor in 50% of the animals) of a C3H mouse mammary carcinoma was reduced by a factor of 1.2 through 30 minutes following i.v. administration of 2.5 mg/mouse of the nitroimidazole, metronidazole. Mice were not anesthetized, and were breathing air during the exposure to ^{137}Cs γ-rays. A similar reduction was observed in the DD50 (radiation dose to produce complete moist desquamation in 50% of the animals) at this drug dose. Tumor growth was not affected by drug treatment alone. Radiation dose response curves for jejunal epithelium diverged at doses greater than 1200 rads suggesting a protective effect on a portion of the crypt cell population.

B-36-7 *Radiosensitization of Anoxic Cells and Tumors with Diamide.* JOHN W. HARRIS, CAMERON KOCH, JUDITH POWER, WILLIAM WARA, AND LAWRENCE KANE, Laboratory of Radiobiology and Experimental Radiation Therapy Laboratory, University of California, San Francisco 94143, USA.

Diamide [diazenedicarboxylic acid bis (N,N-dimethylamide)] is an effective radiosensitizer for anoxic cells (Rad. Res. **56**, 97, 1973). Further studies have now disclosed that the compound decreases the anoxic D_0 and sometimes the extrapolation number, the exact combination of the two depending on the cell line, experimental conditions and sensitizer concentration. In some cells (e.g., CHO) concentrations of diamide as low as 100 μM can yield anoxic survival curves which are superimposable on the euoxic curve. By irradiating diamide-treated cells under various conditions of temperature and substrate availability, and by assaying various cellular components, we have been able to correlate changes in responsiveness to X-ray with cellular redox states. These studies provide information about the mechanism of anoxic radioresistance and support the view that diamide sensitization involves cellular DNA (as shown by measurement of strand breaks and of chromosome aberrations). Diamide, in common with several other sensitizers, does not affect split-dose recovery. Initial attempts to sensitize anoxic tumor cells *in vivo* with this compound have been successful in the case of the P388 murine leukemia growing as an ascites tumor (DMF = 1.8) but unsuccessful in the solid EMT-6 murine carcinoma. (Supported by USAEC and NCI Training Grant CA 05177.)

B-36-8 *The Effect of a Radiosensitizer on Tumor Growth Delay and Cell Survival.* NICOLAS J. MCNALLY, Cancer Research Campaign Gray Laboratory, Mount Vernon Hospital, Northwood, Middlesex, Great Britain.

A comparison has been made of the effects of an hypoxic cell radiosensitizer, a 2-nitro imidazole, on alterations in growth of a murine sarcoma after irradiation and on tumor cell survival assayed *in vitro* after irradiation *in vivo*. Such a study provides information not only on the possible radiotherapeutic benefit of such a drug, but also on the relationship between alterations in tumor growth and cell survival assayed *in vitro*, since it has been found for a rat fibrosarcoma that the sensitizing effect of oxygen is different for the two end-points (McNally 1973).[1] Knowing the serum concentration of the drug at the time of irradiation and the sensitization this should produce *in vitro*, an estimate of the efficiency of the drug *in vivo* can be obtained. Preliminary results indicate that the drug is dose-modifying for survival of the hypoxic cells of the tumor irradiated with animals breathing in air, with an enhancement ratio of about two. Further results on the effect of the drug on the two end-points will be presented.

[1] N. J. McNally, Brit. J. Radiol. **46**, 450 (1973).

B-36-9 *Demonstration of Hypoxic Cell Sensitization in Solid Tumors Using ^{125}IUdR.* ADRIAN C. BEGG, Gray Laboratory, Mount Vernon Hospital, Northwood, Great Britain.

^{125}IUdR has been used to measure cell loss rates in murine tumors both before and after irradiation. Increasing doses of X-rays to solid sarcoma F tumors produced increasing loss rates of ^{125}I radioactivity. Using this as a test of tumor damage, two nitroimidazole compounds were tested for their effectiveness as radiosensitizers of hypoxic cells (a property previously demonstrated *in vitro*) in both normal and fully hypoxic (clamped) conditions. With 1000 rads in air, both compounds showed enhancement ratios of approximately 1.5 to 1.6. With 1000 rads given under hypoxic conditions, enhancement ratios of approximately 1.6 and 2.0 were demonstrated for the 5-nitroimidazole (metronidazole) and the 2-nitroimidazole respectively. These two compounds are therefore efficient radiosensitizers of hypoxic cells in tumors at concentrations which produce little toxicity.

B-37-1 *Comparative Immediate and Long-Term Effects of Gamma-Ray Dose Protraction by Fraction and Continuous Low-Dose-Rate Exposure in Dogs and Monkeys.* L. M. HOLLAND, J. F. SPALDING, J. R. PRINE, AND O. S. JOHNSON, Mammalian Radiobiology Group, Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico 87544, USA.

Two mammalian species having widely different $LD_{50/30}$ values, monkeys (*Macaca mulatta*) and dogs (beagle), were given thirteen 100-rad cobalt-60 gamma-ray exposures at 28-day intervals. The comparative response (injury and recovery) of the hematopoietic system of dogs and monkeys to this dose-rate regime was observed on blood samples taken at 7-day intervals. The magnitude of the long-term radiation lesion of the two species was measured 84 days after the thirteenth exposure as a reduction in mean after-survival time in a continuous gamma-ray environment with a dose rate of 35 rad per 24-hour day. Results of this study, which is in progress, will be reported. (This work is being performed under the auspices of the U.S. Atomic Energy Commission.)

B-37-2 *Life-Shortening in Mice Exposed to Discrete Doses of Gamma-Rays at Low Dose Rates.* J. F. SPALDING, O. S. JOHNSON, AND R. F. ARCHULETA, Mammalian Radiobiology Group, Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico 87544, USA.

Life-shortening has been generally accepted as an expression of the long-term residual lesion from whole-body exposure to ionizing radiation. Radiation-induced life-shortening is reportedly proportional to dose, and low dose-rate exposures are assumed to be less effective than acute exposures. However, investigations on life-shortening effects of discrete doses of gamma rays delivered at low dose rates have not been reported. Strain RF female mice 10.5 months of age were used to determine the life-shortening effects of 11 discrete gamma-ray doses ranging from 457 to 3008 rad given at a dose rate of approximately 66 rad per 23.5-hour day. Under these conditions, radiation exposure was less effective in reducing life expectancy than has been reported

for acute exposures by a factor of approximately 8. Life-shortening was not found to be dose-dependent over the dose range used. (This work was performed under the auspices of the U.S. Atomic Energy Commission.)

B-37-3 *Influence of Dose Rate on Induction of Late Effects by Gamma-Rays in Mice; a Reanalysis.* HARRY E. WALBURG, UT-AEC Comparative Animal Research Laboratory,* Oak Ridge, Tennessee 37830, USA, DAVID G. HOEL, National Institute for Environmental Health Sciences, Research Triangle Park, North Carolina 27709, USA, AND ARTHUR C. UPTON, State University of New York, Stony Brook, New York 11790, USA.

Conclusions on the influence of dose rate on induction of neoplasia have been confounded heretofore by competing probabilities of death. Part of the data from a previously reported study was reanalyzed utilizing necessary adjustments. The reported decrease in effectiveness of low as compared to high dose-rate γ-radiation for life-shortening and induction of thymic lymphoma, myeloid leukemia and ovarian tumors proved to be statistically significant. Significant radiation induction of nonthymic lymphomas, solid tumors other than ovarian, nephrosclerosis and non-neoplastic diseases was not detected and thus there was no dose rate effect for these diseases. The *increased* effectiveness of low dose rate γ-radiation previously reported for non-thymic lymphomas was not detected. A similar effect with lung tumors was limited to only one of the 18 groups studied suggesting limited biological significance.

* Operated by the University of Tennessee for the U.S. A.E.C. under Contract No. AT-40-1-GEN-242.

B-37-4 *Response of the Beagle Dog to Protracted Exposure to ^{60}Co Gamma-Rays.* WILLIAM P. NORRIS AND THOMAS E. FRITZ. Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439, USA.

Purebred, young-adult, beagle dogs have been exposed continuously, until death, to ^{60}Co gamma rays at rates ranging from 5–300 R/22 hour exposure day. An exposure rate of 35 R/day, or more, invariably produced early death with all the usual signs associated with the acute radiation syndrome, except gastro-intestinal damage. As the exposure rate was reduced to 17 R/day, or less, other causes of death (anemia or myeloproliferative disease) appeared in a stepwise, dose-rate dependent, manner.

With similar, but terminated, exposures to 17 R/day, the LD_{50} was ~2000 R (~1500 rad). The LD_{50} total dose was inversely related to dose-rate when the exposure rate was 17 R/day, or greater. At lower exposure rates, however, that allowed for physiologic accommodation to irradiation damage, no meaningful LD_{50} could be obtained and total accumulated doses, when irradiation was continued until death, became as high as 15,000 rad.

The beagle fetus, irradiated *in utero*, responded in a manner similar to that seen in the adult. So long as the exposure rate did not exceed 17 R/day, beagle bitches, irradiated continuously from conception to parturition, delivered normal litters. At the lowest exposure rate tested (5 R/day), however, all female puppies were sterile. Male pups, similarly irradiated *in utero*, were sterilized by 10 R/day, but developed normal sperm counts following exposure to 5 R/day. (Work supported by the U.S. Atomic Energy Commission.)

B-37-5 *Pathologic Responses of the Beagle Dog to Protracted Exposure to ^{60}Co Gamma-Rays.* THOMAS E. FRITZ AND WILLIAM P. NORRIS. Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439, USA.

In beagle dogs exposed continuously to ^{60}Co γ-rays, at rates ranging from 5–300 R/day, there were only three prominent causes of death—septicemia, anemia, and myeloproliferative disorders (MPD)—all of which were related to hematopoietic damage. The exposure rate separating the acutely lethal septicemic response from the other two, slowly developing, syndromes was ~17 R/day. Among dogs exposed to 17 R/day, all three causes of death were seen and survival times in the γ field varied by a factor of 13, indicating large differences in individual capabilities of dogs to response to this stress.

When septicemia was prevented by antibiotic treatment in dogs given 17–35 R/day, they

survived longer and died of anemia. Anemia (most prominent at 10 R/day) was always preceded by profound thrombocytopenia, but there was no associated hemorrhage. MPD (generally as myelogenous leukemia) occurred in more than 75% of the dogs exposed until death at 5 R/day. Dogs dying of MPD in the γ field always showed depression and subsequent recovery of circulating blood cell numbers before the onset of MPD.

Dogs given terminated exposures of 1400–4000 R at rates ranging from 5–35 R/day had subsequent hematologic recovery at rates directly related to exposure rate, indicating that a given total dose of radiation is fundamentally more damaging when given at a lower exposure rate. The early causes of death in dogs given terminated exposures were the same as those in dogs irradiated until death. (Work supported by the U.S. Atomic Energy Commission.)

B-37-6 *Design Analysis for a Low-Level Gamma-Ray Experiment Under Two Alternative Dose-Effect Assumptions.* S. A. TYLER, W. P. NORRIS, AND G. A. SACHER, Argonne National Laboratory, Argonne, Illinois 60439, USA.

The radiation-specific mortality in mouse populations given daily ^{60}Co gamma exposure increases as the square of the daily dose for rates between 18 R/day and 100 R/day, and approximately as the first power for rates below 18 R/day. Several other species show the rate-squared branch, but the first-power trend at low rates has not been seen in any other species. An experiment is planned to determine whether beagle dogs, which follow the rate-squared trend down to 5 R/day, break over to the linear trend at lower rates. We describe a statistical procedure that gives, with prescribed confidence, the dose rate and number of animals required to decide whether the linear or the quadratic assumption is tenable for the beagle irradiated at levels below 5 R/day. The procedure provides a determination of the "price" one must pay for any predetermined exposure levels, and of the limitations imposed if the minimax "costs" are accepted. A description of the procedures is obtainable from the authors. (Work supported by the U.S. Atomic Energy Commission.)

C-1-1 *The Late Effects of Acute External Exposure to Ionizing Radiation in Man.* SEYMOUR JABLON, National Research Council, Division of Medical Sciences, Washington, D.C. 20418, USA.

The principal late somatic effect of radiation on man is cancer induction. Effects on the optic lens occur and chromosomal aberrations can be detected in peripheral leucocytes decades after exposure. Nevertheless, cataracts as a result of radiation are rare, and the significance for health of the chromosomal aberrations is unclear. There is, however, no doubt that ionizing radiation is, for man, not only a potent leukemogenic agent, but a general carcinogen as well. Although there are not very many human studies capable of yielding numerical estimates of risk, the agreement among those that do exist is, in general, sufficiently close to give some confidence in the results.

The fetus apparently is especially sensitive to radiation. After irradiation in the first trimester there is impaired later growth, and especially diminished head size, with associated mental retardation. However, the sensitivity of the fetus to radiation carcinogenesis is unclear: Some investigators find the fetus extremely susceptible, while others cannot confirm this. Those studies which show great susceptibility also find latent periods much shorter than those among children exposed after birth. It is possible that some of the relationships that have been reported reflect, in reality, selection of gravida for X-ray.

C-1-2 *The Risk of Malignancy from Internally-Deposited Radioisotopes.* R. E. ROWLAND, Center for Human Radiobiology, Argonne National Laboratory, Argonne, Illinois 60439 USA.

Extensive experience has shown that a number of radionuclides—including ^{32}P, ^{131}I, ^{222}Rn, ^{224}Ra, ^{226}Ra, ^{228}Ra, and ^{232}Th—and their decay products can induce malignancies when deposited in the human body. However, the relationship between the quantity of isotope deposited and the probability of subsequent induction of a malignancy is less clear. For some of these radionuclides, we do not have accurate estimates of the body contents of the exposed populations (and hence of the dose), and in no case do the body burdens or total doses extend over more than a small portion of the range of interest to us.

The hypothesis that the risk is linear with dose, independent of the dose rate, may be defensible in obtaining conservative estimates for use in guiding public policy. Verification of linearity, how-

ever, should be sought by studying only populations whose doses ranged over several orders of magnitude—e.g., from studies of radium in man. Current ^{224}Ra results can be described by a risk proportional to either the first or the second power of dose, but a first-power law does not adequately describe the current ^{226}Ra-^{228}Ra results. Thus these studies cast doubt on the validity of linear risk estimates for bone-seeking alpha-emitting radionuclides in man. (Work performed under the auspices of the U.S. Atomic Energy Commission.)

C-2-1 *A Comparison of the Mutagenic Effects of Chemicals and Ionizing Radiation. Chairman's Remarks.* F. H. SOBELS, Department of Radiation Genetics and Chemical Mutagenesis, University of Leiden, The Netherlands.

A number of differences between the effects of mutagenic chemicals and that of ionizing irradiation will be described. Chemical damage to the genetic material often remains latent for many cell generations. As a result of the fact that chromosome breaks open up in different cell cycles, a relative shortage of chromosome rearrangements, in comparison to gene mutations, is observed with many chemical mutagens. The quantitative relationship with which mutations and chromosome aberrations are induced, varies greatly from one chemical to the other. Thus some chemicals fail to produce any chromosome aberrations, in spite of pronounced mutagenic activity. Specificity of response is another factor which makes it difficult to decide to what extent a given chemical may be mutagenic to man. Irradiation produces genetic effects in all types of cells; certain chemicals exhibit extreme cell or stage specificity. For the evaluation of genetic radiation hazards, the mutation spectrum and the dose effect relationship have been of great importance, but very little is known about these factors for most chemical agents.

C-2-2 *Factors Which Determine the Mutagenic Specificity of Chemical and Physical Agents in Micro-organisms.* B. J. KILBEY, Department of Genetics, University of Edinburgh, Great Britain.

Early successes in the typing of mutants by means of their response to supposedly specific chemicals caused much emphasis to be laid on the importance of the DNA-mutagen interaction as the source of mutagen specificity. Now, even at this basic level, it is becoming clear that the picture is far from simple. The same base pair, for example, in the same position in the same triplet may react differently to the same mutagen when the triplet occupies different locations within the gene. To the complexity at this level may be added the effects of cellular physiology on the realization of mutant phenotypes: The response of the same two genes to the same mutagenic treatment may be very different in different strains and at different times in the age of the culture. Repair processes may act differently on premutational lesions of different types and locations. These effects may be transient, produced by the mutagenic agent itself, and fading with time after treatment. The present contribution will attempt to review some of the recent results which have a bearing on the mutation process laying special emphasis on the relative importance of cellular processes in mutagenesis by chemical and physical agents in micro-organisms.

C-2-3 *Comparison of the Mutagenic Effects of Chemicals and Ionizing Radiation Using Drosophila Melanogaster Test Systems.* WILLIAM R. LEE, Department of Zoology, Louisiana State University, Baton Rouge, LA, USA.

The first chemical mutagens were detected by using the same genetic test with *Drosophila melanogaster* that had been used to show that ionizing radiation is mutagenic. Initial comparisons emphasized the similarity between chemical and ionizing radiations as mutagens; however, soon quantitative differences and later qualitative differences became apparent. Different germ cell stages vary in sensitivity to chemical mutagens as they do to ionizing radiation, but the magnitude of differences is much larger for chemical mutagens. In some cases only one germ cell stage can be shown to be sensitive to certain chemical mutagens, and the most sensitive stage is not the same for all chemicals. Chemical agents are relatively more effective in producing mosaics while ionizing radiation is relatively more effective in inducing chromosome breaks. Therefore, the spectrum of mutational effects differs among chemical mutagens and between most chemical mutagens and ionizing radiation. Some chemical agents like caffeine have been shown to affect repair mechanisms in the Drosophila egg and thereby alter both the quantity and mutational spectrum of ionizing

radiation. The greater variation in mutational spectrum and in sensitivity among germ cell stages to chemical agents in contrast to ionizing radiation must be taken into account in assaying chemicals for genetic hazards.

C-2-4 *Comparison of the Mutagenic Effects of Chemicals and Ionizing Radiation in the Spermatogenetic Cells of the Mouse.* B. M. CATTANACH, Medical Research Council, Harwell, Didcot, Oxfordshire OX11 ORD, Great Britain.

Mutation studies with ionizing radiation have shown that both point mutations and chromosome damage can be induced and recovered from all male germ cell stages. Stage sensitivity differences exist, although for spermatogonia, cell selection processes play a large part in reducing the yield of chromosome rearrangements. Chemical mutagens can also produce point mutations and chromosome damage but there are far greater specificities of action than with radiation and, in this respect, there are great differences between chemicals. Some proven chemical mutagens are almost totally ineffective in post-meiotic cells, others are ineffective in spermatogonial stem cells. Generally, there appears to be a correlation between the induction of point mutations and of chromosome damage in any one cell stage, but this may be an artefact and there are notable exceptions. There also appears to be a correlation between the induction of genetic damage in spermatogonial cells, and of a delayed sterility which, for X rays, results from stem cell killing. However, again there are notable exceptions and it also seems possible that the chemically-induced sterility may not always be due to cell killing. Specificity of action may also be indicated by the observations that the spectrum of point mutations and the sites of chromosome breakage also appear to be different with chemical mutagens. The interpretations offered to account for these observations will be discussed.

C-3-1 *Changes in the Rate of Proliferation in Normal Tissues after Irradiation.* JULIE DENEKAMP, Gray Laboratory, Mount Vernon Hospital, Northwood, Great Britain.

Changes in the proliferation rate in normal tissues after irradiation are important both for understanding homeostatic mechanisms and also for calculating acceptable doses in radiotherapy when treatment times are altered. The time at which more rapid proliferation occurs in compensation for radiation damage depends upon the time of expression of that damage. This is related to the normal turnover of the tissue. Constantly renewing tissues e.g., skin and small intestine may respond within the treatment time, whereas slowly dividing tissues respond slowly and in these proliferation will be less important in determining acceptable doses than their capacity to repair sublethal injury.

The proliferation characteristics of several normal tissues (particularly skin) have been studied using tracer techniques with tritiated thymidine, and using split dose experiments with varying intervals between the fractions. The rate of repopulation is not constant through an extended course of treatment, it becomes more important (i.e., faster proliferation) as more damage is registered. The response of renewing epithelial tissues will be compared with slowly dividing tissues such as lung and kidney.

C-3-2 *The Control of Cell Proliferation in Haemopoietic Tissue.* B. I. LORD, Paterson Laboratories Christie Hospital & Holt Radium Institute, Manchester, Great Britain.

Many of the direct effects of irradiation and/or drug administration on the haemopoietic tissues are now well recognized and have, in recent years, been well reported. It is proposed therefore to concentrate on some of the more recent developments in the field of experimental haematology in which various drug or irradiation systems are being used to learn more about normal proliferation and the manipulation of their proliferative potentials.

In the earliest stages of haempoiesis, the pluripotential stem cell, it has been found that irradiation can be used to uncover a degree of "potential stemness" which can be realized when live thymus cells are made available: "potential" stem cells are induced to proliferate. It will be shown how this may affect the radiation dose response of stem cells. Differences in the proliferative capacity of stem cells will be demonstrated by the use of isopropyl-methane-sulphonate and manipulation of the degree of proliferation with steroid hormones will be described.

Further along the maturation sequence of haemopoietic cell populations, detailed measurements of cell proliferation parameters have been described. Current investigations into the use of cell specific proliferation inhibitors will be described and their significance in the control of proliferation will be discussed.

C-3-3 *The Importance of the Proliferation Kinetics and the Clonogenicity of Tumor Cells in Relation to Clinical Radiation Effects of Experimental Tumors.* A. F. HERMENS AND G. W. BARENDSEN, Radiobiological Institute TNO, 151 Lange Kleiweg, Rijswijk (ZH), The Netherlands.

In order to interpret changes in growth rate and of tumor volume after irradiation with the aim to design optimal treatment schedules, a large number of factors have to be analyzed before, during and after treatment, namely; 1, changes in the fractions of proliferative, P, cells and of nonproliferative, Q, cells if they are clonogenic; 2, changes in the cell kinetic parameters of clonogenic P cells; 3, changes in the cell kinetic parameters of nonclonogenic P cells; 4, the rates of recruitment of clonogenic and nonclonogenic Q cells into P cells; 5, changes in the fractions of P and of Q cells, which are hypoxic; and, 6, changes in the kinetics of cell death and removal.

Several of these factors have been studied for the R-1 rhabdomyosarcoma which is transplantable in the inbred strain of WAG/Rij rats, using different techniques applied to irradiated and non-irradiated tumors. Fractions of clonogenic cells could be measured by plating isolated tumor cells in Petri dishes and with this technique it was shown that the tumors were repopulated rapidly with colonogenic cells at a minimum doubling time of about 24 hr. By the ^{3}H-TdR pulse labelling technique and autoradiography it was shown that this rapid repopulation is due to an increased production of clonogenic P cells. By a combination of both techniques it could be demonstrated that both P and Q cells do show clonogenic capacity *in vitro* and evidence has been obtained that a fraction of both the P and Q cell populations in the tumor are hypoxic. These results will be compared with data from other tumors.

C-3-4 *Cell Proliferation Kinetics and Growth Rate of the Irradiated Human Tumors.* E. P. MALAISE, Institut Gustave-Roussy 94800, Villejuif, France.

A lot of information on kinetics of irradiated experimental tumors are actually available; very little is known about the influence of irradiation on the kinetics of human solid tumors.

Work performed on lung metastases suggest that since the tumor attains its minimal volume after irradiation, growth restarts with an accelerated rate for a short period; then the growth rate becomes the same as that observed before irradiation. Maybe an accelerated repopulation whose biological meaning is not well understood exists in lung metastases of human tumors.

For primary tumors, the only available observations concern cell multiplication after labeling with 3H-TdR. After single dose irradiation of 330 rad, and also after 2 doses of 850 rad, generally a significant and persistent decrease in labeling index is observed; this result argues against an accelerated repopulation. However, at the time of recurrence, some results suggest a shortened cycle time with an increased growth fraction.

These results appear to be insufficient to estimate the role of repopulation in the radiosensitivity of human tumors.

C-4-1 *Studies on the Effects of Cosmic HZE-Particles on Different Biological Systems in the Biostack Experiments I and II Flown on Board Apollo 16 and 17.* H. BÜCKER AND G. HORNECK, Arbeitsgruppe für Biophysikalische Weltraumforschung, Frankfurt, West Germany.

The Biostack experiments I and II were exposed to the bombardment of HZE-particles of cosmic radiation for a period of 226 hrs during the Apollo 16 lunar mission or of 305 hrs during the Apollo 17 flight. They are part of an investigative program, which aims

(1) to determine the biological effectiveness of single HZE-particles and the influence of space-flight environmental factors on this phenomenon,
(2) to yield the information required for establishing radiation protection guidelines for manned long-duration spaceflights.

The two Biostack hardwares housed seven selected biological systems in resting state (unicellular organisms, plant seeds and animal eggs), which, in monolayers, were sandwiched between

visual track detectors. By this arrangement, it was possible to identify each hit biological object and to correlate the biological injury with the charge and energy loss of the responsible HZE-particle. The experiments were performed in collaboration of 12 European Research groups. It was found, that the seven biological representatives responded quite differently to the penetration of an HZE-particle. The mosaic eggs of *Artemia saline* had the highest sensitivity, showing a high frequency of developmental disturbances. In general, the intensity of biological response to HZE-particle bombardment turned out to depend on the replaceability of the hit cell or cellular component.

C-4-2 *Effects of Cosmic Heavy Particles on Artemia Egg Development.* H. PLANEL, Y. BLANQUET, J. P. SOLEILHAVOUP, AND R. KAISER, Laboratorie de Biologie—Faculté de Médecine, Toulouse et Laboratoire de Physique Corpusculaire, Strasbourg, France.

Biological effects of cosmic heavy particles are still unknown. Heavy cosmic ions are very high LET particles. But their flux is low (1% of all cosmic particles), so, every research on their biological effects requires an experimental method which provides a good correlation between biological objects and particles tracks.

In order to obtain this result, two experiments have been carried out during Apollo XVI and XVII flights (Biostack 1 and 2, Dr. Bücker principal investigator). Artemia eggs, embedded in polyvinyl alcohol, were sandwiched with visual detectors (nuclear emulsions or plastic).

After recovery, individual development has been studied for hit eggs by heavy ions, non hit eggs and earth control eggs.

Hit egg developmental ability was decreased by a factor of ten, compared with control eggs. A lower inhibition was observed with in-flight non-hit eggs.

Hit egg inhibition is explained by large biological damages induced by heavy ions. Non-hit egg inhibition is not due to dynamic flight factors (vibrations and accelerations); cosmic background irradiation may be involved in this phenomena. Irradiation experiments are in progress to confirm this assumption.

C-4-3 *Radiobiological Effects of Accelerated Boron-, Carbon-, and Neon Ions in Mammalian Cells and Tissues.* YU. G. GRIGORIEV, N. I. RYZHOV, V. I. POPOV, E. I. KUDRYASHOV, S. V. VOROZHTSOVA, R. D. GOVORUN, E. A. KRASAVIN, V. N. GERASIMENKO, AND L. A. KOSHCHEEVA, Institute of Medical and Biological Problems, Ministry of Public Health, Moscow, USSR.

Cytologic and cytogenetic effects were studied on cornea epithelium cells of mice, liver and brain cells of rats, and human lymphocytes, exposed to B^{10}, B^{11}, C^{12}, Ne^{20}, and Ne^{22} ions accelerated at the cyclotron in Dubna to energies of 7.5–9.5 MeV per nucleon.

The results obtained reveal a relation of the biological effects to dose LET, radial distribution of energy absorbed, the type of biological samples, and the duration of the tests.

The estimated RBE values for the ions studied did increase with the decrease of the dose, and the increase of duration of the test. A difference of the RBE values has been found depending on the type of biological samples and the endpoints chosen.

A long term persistence of radiation damage in cornea epithelium and liver cells of mammals was shown, which is considered to be related to a low ability to recover after heavy ions exposure. It should be emphasized that pretreatment with some radioprotectors had no effect.

The use of these results for better understanding the regularities and mechanisms of densely ionizing radiations action on living systems, and for solving problems of radiation protection in manned space flights or of radiation therapy will be discussed.

C-4-4 *Some Studies on Visual Perception and Pathologic Effects of Accelerated Heavy Ions.* CORNELIUS A. TOBIAS, THOMAS F. BUDINGER, JOHN T. LEITH, JOHN T. LYMAN, ABDEL M. MAMOON, AND T. C. H. YANG, Donner Laboratory/Lawrence Berkeley Laboratory, Berkeley, CA 94720, USA.

Nonrelativistic, individual accelerated particles, including helium, nitrogen, oxygen, and neon ions, as well as secondaries from fast neutrons, produce light sensations of stars and streaks in the dark-adapted human eye. The particles usually do not leave a visible track if their rate of energy loss is less than 10 keV per micron. Many similar events have been seen by astronauts on American

lunar and orbital flights. X rays down to a dose of 90 microrads or less also produce light flashes, but these are diffuse light phenomena different in character from events seen by astronauts. The efficiency of perception of heavy-ion-initiated events is related to the physiological state of the individual, such as dark adaptation, and also to the particle energy deposited in and near the retina.

The pathologic effects of accelerated nitrogen and oxygen ions on the rabbit and rat retina appear earlier and are more severe than those produced by comparable X-ray doses. These include degeneration of the outer segments of visual rods and other nerve tissues, as revealed by scanning electron microscopy. Degeneration of the rods appears to be related to derangement of protein synthesis in the inner segment as studied by autoradiography. Accelerated heavy ions have an immediate effect on membranous structures of neurons in culture, which is evidenced by osmotic imbalance and swelling of the cells. Heavy-ion injury to differentiated neurons manifests itself in reduction of life-span and eventual lysis. We are searching for the sites of primary injury and the energy thresholds for irreversible central nervous system cell damage. (This work is supported by the U.S. Atomic Energy Commission and NASA.)

C-5-1 *Blood and Bone Marrow Effects of ^{253}Einsteinium in Miniature Swine.* H. A. RAGAN, B. J. MCCLANAHAN, AND P. L. HACKETT, Biology Dept., Battelle, Pacific Northwest Laboratories, Richland, Washington 99352, USA.

A number of chloromas were noted in rats exposed to ^{253}Es at this laboratory. Because of this finding a similar study was initiated in miniature swine to compare the metabolic results to those observed in rats, and especially to determine if ^{253}Es might be leukemogenic in a large mammalian species. Twenty-two weanling (~6 weeks of age) miniature swine were injected intravenously with 3 μCi/kg of ^{253}Es citrate and placed in metabolism cages to obtain excretion data. Ten animals were killed at intervals following injection to determine Es tissue distribution and retention. Most of the ^{253}Es was deposited in the skeleton, as reflected by femur concentration, and in the liver. Twenty-four hours after injection the liver contained 15% and femur 3.4% of the injected ^{253}Es. Only about 6% was excreted in 7 days (2.4% in urine, 3.3% in feces). By day 10 the major excretory pathway was via urine. Blood and bone marrow effects in the remaining 12 animals have been periodically evaluated. Hematologic effects of Es injection were manifested as an early, absolute neutropenia and thrombocytopenia of about 3 months duration, and a significant ($P < 0.01$) depression in the bone marrow myeloid to erythroid ratios over the same period. Of particular interest are the significantly elevated bone marrow mitotic indices observed during the 18 month period since Es injection. No consistent serum biochemistry changes have been noted. (Research performed under Contract AT(45-1)-1830 between the United States Atomic Energy Commission and Battelle Memorial Institute.)

C-5-2 *Relative Toxicity to Blood Cells of Transuranium Nuclides as Measured by Dose-Response Curves.* JEAN H. DOUGHERTY AND LOWELL A. WOODBURY, University of Utah, Salt Lake City, Utah, 84132, USA.

A large body of hematological data has been accumulated on beagles injected with graded doses of bone-seeking radionuclides. A summary of the comparative effects of several transuranium nuclides (^{239}Pu, ^{241}Am, ^{249}Cf, ^{252}Cf) for at least 2 years post-injection has been made by determining dose-response curves at various times post-injection. These curves were derived by two methods: (a) probit of the percent drop in blood count; (b) a time weighted average of the blood cells over a given time post-injection (6, 12, 18 and 24 months). Both were plotted against the logarithm of the injected dose to yield dose-response curves. From these curves relative hematologic toxicity values were obtained. Whereas both methods yielded useful results, the time weighted average technique was easier to compute and was more sensitive in detecting small changes. Toxic effects, which were primarily observed in the leukocytes, varied with the type of nuclide, the dose and the time post-injection. (Supported by USAEC Contract AT(11-1)-119.)

C-5-3 *The Effect of Polymeric Plutonium-239 on the Red Cell Life Span of Mice.* HISAMASA JOSHIMA, MASATOSHI KASHIMA, AND OSAMU MATSUOKA, National Institute of Radiological Sciences, Chiba, Japan.

Since both erythropoietic system and reticuloendothelial system are continuously irradiated with γ ray when polymeric plutonium are injected intravenously, the change in red cell life span, hemoglobin, hematocrit, red cell count and reticulocyte count were examined in CF #1 male mice during 7 to 125 days following a single intravenous injection of polymeric ^{239}Pu at the dose level of 10 μCi/Kg and 5 μCi/Kg.

In red cell life span study, red cells from normal donor mice which had been labeled with ^{51}Cr were transfused into normal recipient or ^{239}Pu injected mice (control and extracorpuscular defect), and in turn ^{51}Cr labeled red cells from ^{239}Pu injected mice were transfused into normal recipient or ^{239}Pu injected mice (intracorpuscular and combined defect). The red cell life span of mice was shortened both due to extracorpuscular and intracorpuscular defect, but combined effect could not be observed with extracorpuscular defect and intracorpuscular defect. Since the life span of red cell following external irradiation changes little, these results might be considered the specific effect of ^{239}Pu to the red cells.

C-5-4 *Magnesium Transport in Sheep: Fitting a Compartmental Model to Data from Multiple Compartments with Multiple Routes of Injection.* GARY E. SPALDING, FRED MADSEN, JAMES K. MILLER, AND SAM L. HANSARD, UT-AEC Comparative Animal Research Laboratory, Oak Ridge, Tennessee 37830, USA.

To model the transport of substances in a whole organism it is necessary to be able to fit the model to data from several compartments simultaneously. Further, it is desirable to incorporate data obtained using differing initial conditions in order to obtain a model with maximum likelihood of being unique. This can be achieved by using differing routes of injection of the substance being studied.

Blood concentrations, and urine and feces contents of ^{28}Mg were measured serially for 106 hours following oral administration to 10 sheep. Six weeks later the experiment was repeated using intravenously administered ^{28}Mg. To normalize the data from the various compartments, so that they could be simultaneously fit by the model, results were expressed in percent-of-dose. To stabilize variances, a logarithmic transform was applied before regressing the model to the data.

An 8-compartment model (including excreta compartments) was fit to the data using a least-squares technique. (Supported by U.S. A.E.C. under contract No. AT-40-1-GEN-242 with University of Tennessee, and Animal Science Department, University of Tennessee.)

C-5-5 *Internal Emitter Data Retrieval Systems—A New Approach.* GERHARD R. EISELE AND HARRY E. WALBURG, UT-AEC Comparative Animal Research Laboratory, Oak Ridge, Tennessee 37830, USA.

While the number and use of computer data bases are increasing rapidly, the data often do not serve the needs of research biologists. The identification of published data by species, age and sex of animal or by route of injection and end points observed are often not available. Use of key words in classifying data does not ensure that every report is keyed for every required category. Seven data bases were queried to locate data on the dosimetry of plutonium in various categories such as species, age, sex of animal as well as by chemical or physical state and route of injection of plutonium. All failed to provide an adequate catalog of reports by these fields. Therefore, we developed a data base containing over 1,000 articles on biological aspects of plutonium and classified under specific fields of importance to the research scientists. In cooperation with other Oak Ridge information groups we are currently working toward a uniform format for research biologists interested in the dosimetry and toxicology of environmental pollutants. (Supported by U.S. A.E.C. under contract No. AT-40-1-GEN-242 with University of Tennessee.)

C-5-6 *Alpha and Beta Radioassay Using Liquid Scintillation with Energy or Pulse Shape Discrimination.* JOHN W. MCKLVEEN, Arizona State University, Tempe, Arizona USA.

The assets of high counting efficiency and low self absorption, which have established liquid scintillation as the attractive technique for detecting low-energy beta emitters, are also applicable to the counting of short-ranged alpha particles. There is an added advantage from the mono-energetic decay of alpha particles, generally in the 4 to 6 MeV range. Techniques may be adapted

to rapid, low level, mass sample, alpha-beta assay of numerous sample types in either controlled laboratory research or practical environmental monitoring situations.

Commercial liquid scintillation counting systems, using energy discrimination, provide alpha particle counting efficiencies up to 100%, and possess detection thresholds on the order of 0.2 pCi. However, in samples containing mixed radionuclides the investigator must realize that the poor fluorescence conversion efficiency for alpha emitters may hamper efforts to identify the respective type of ionizing radiation by energy discrimination. Consequently, accuracy of alpha-beta analysis depends upon precision of the empirically derived correction curves, necessitating prior knowledge of the beta energy spectrum as well as the degree of scintillator quench.

Pulse shape analysis schemes permit positive identification of alpha or beta emitters by discriminating the characteristic light shapes produced in the scintillator. Discrimination appears to be influenced by the presence of oxygen, but is not affected by moderate amounts of other quenching agents. Detection thresholds for alpha particles appear to be on the order of 0.02 pCi, or lower.

C-6-1 *Variation in Somatic Mutation Rates Induced by X-Rays, DBE and EMS in Several Tradescantia Species and Hybrids.* RAFAEL VILLALOBOS-PIETRINI, ARNOLD H. SPARROW, LLOYD A. SCHAIRER, AND RHODA C. SPARROW. Brookhaven National Laboratory, Upton, New York 11973, USA.

Somatic mutation rates induced by 250-kVp x-rays and the chemical mutagens 1,2-dibromoethane (DBE) and ethyl methanesulfonate (EMS) have been determined for several *Tradescantia* species or hybrids heterozygous for flower color. Radiation-induced pink and colorless mutations appear in flowers maturing about 9 days after exposure, reach a peak between 11–15 days and decrease thereafter, whereas chemical-induced peak mutation rates have similar patterns of response but occur 2–3 days earlier. X-ray induced pink mutation response varied among 13 clones by up to a factor of 7.5 and for colorless (among 9 clones) up to a factor of 10. These differences are attributable mainly to chromosome volume, ploidy and normal biological variability. Dose response curves for pink and colorless rates induced by gaseous exposures of EMS and DBE showed slopes similar to those for radiation exposures. However, sensitivity differences to chemical mutagens as great as a factor of about 10 were observed between two diploid clones (4430 and 02) which have very similar radiosensitivities. The reason for the differences among clones in their comparative sensitivities to chemical and physical mutagens is not known; differences in amount of heterochromatin, number of SAT-chromosomes, and effectiveness of repair systems are suggested possible factors. Timing of mutation responses and dose response curves for radiation and chemically-induced somatic mutations will be presented. (Research supported in part by the U.S. Atomic Energy Commission and in part by the National Institute of Environmental Health Sciences.)

C-6-2 *Patterns of Response to Varying Exposure Rates and Total Exposures for Mutation, Survival and Growth Inhibition End Points.* A. H. SPARROW, C. H. NAUMAN, P. J. BOTTINO, AND V. P. BOND, Biology Department, Brookhaven National Laboratory, Upton, New York 11973, USA.

Single exposures of x and gamma rays have been used to demonstrate the influence of varying exposure rate on somatic mutation rate in *Tradescantia* stamen hairs and in *Tulipa* petals and sepals. Somatic mutation rate in these two species is a linear function of the logarithm of the exposure over five and three orders of magnitude, respectively. A similar relationship was found for growth inhibition in barley seedlings over three orders of magnitude.

Data from experiments in which a wide range of both exposure rates and total exposures were used to study growth inhibition in barley (30–24,000 R/hr) and survival in wheat (12–3400 R/hr) indicate the presence of two or three ranges of exposure rate where alternating trends of response may be observed. The observance of similar response configurations for these two end points will be discussed with regard to the possible causes involved and their relation to current dose rate theory. (Research supported by the U.S. Atomic Energy Commission.)

C-6-3 *Comparative Effects of Ionizing Radiation and Gaseous Chemical Mutagens on Mutation Induction in a Mutable Clone of Tradescantia.* CHARLES H. NAUMAN, ARNOLD H. SPARROW,

LLOYD A. SCHAIRER, AND E. ERIC KLUG, Biology Department, Brookhaven National Laboratory, Upton, New York 11973, USA.

The x-ray dose responses of mutable clone #0106 of *Tradescantia* (mutable for blue to pink), and the parent clone (clone 02) have been determined for pink and colorless mutations in stamen hair cells. These responses are compared to the dose response curves for the same two clones following exposure to the gaseous phase of the mutagen 1,2-dibromoethane (DBE).

X irradiation induces pink and colorless mutation rates in mutable clone 6 which are only slightly different from the rates induced in clone 02. In both clones pink mutations occur more frequently than colorless mutations at lower doses; however, colorless mutant events saturate at higher doses than do pink events.

The response curves for pink and colorless mutations following exposure to a series of gaseous concentrations of DBE are qualitatively similar to those following x-ray exposures, however, DBE-induced mutant events saturate at a frequency 30 times lower than x-ray induced events. These results will be compared to responses from other chemical mutagens and to preliminary data from other mutable clones. (Research supported in part by the U.S. Atomic Energy Commission and in part by the National Institute of Environmental Health Sciences.)

C-6-4 *Comparative Mutagenicity of Beta, Fission Neutron and Gamma-Radiation in Barley.* MILTON J. CONSTANTIN AND BOB V. CONGER, UT-AEC Comparative Animal Research Laboratory, Oak Ridge, Tennessee 37830, USA.

Limited experimental data concur with the theory that relative biological effectiveness (RBE) of beta versus gamma radiation is one. However, no data exist for mutation induction via external beta irradiation of seeds. In contrast, high RBE values are reported for fission neutrons. An experiment was conducted with seeds of Atlas-57 barky to compare the mutagenicity of these different kinds of radiations.

Seeds, with 12.4% water, received 8–40 krads of ^{90}Sr-^{90}Y beta radiation (Biology Department, BNL) and ^{60}Co gamma radiation (Variable Dose Rate Irradiation Facility, UT-AEC CARL) and 140–700 rads of fission neutrons (Health Physics Research Reactor, ORNL). M_1 plants were grown to maturity in the field at the University of Arizona, Tucson, and the M_2 generation was scored in the greenhouse for chlorophyll deficient mutants.

Results indicate an RBE of one for beta and >100 for fission neutrons compared to gamma radiation on the basis of M_2 seedling survival per spike and mutation frequency (mutants/100 M_2 seedlings ÷ dose in rads). Mutation spectrum was similar for each kind of radiation. (Supported by U.S. A.E.C. under contract No. AT-40-1-GEN-242 with University of Tennessee.)

C-6-5 *Combined Mutagenic Effects of X-rays and Chemicals in Pulse Legumes.* MANAS K. JANA, Applied Botany Section, Indian Institute of Technology, Kharagpur, India.

Seeds of *Phaseolus mungo*, *Vigna sinensis* and *Lens esculenta* adjusted to different water content were treated singly and in combination with varying doses of X-rays (1–40 kR), ethyl methanesulphonate and hydroxylamine (0.1–0.5%) for different durations. Both EMS and X-rays were highly mutagenic whereas the mutation rate of HA was very low. When combined, as post-treatment of the chemicals after X-rays, the extent of interaction (statistically estimated from the yield of mutation and/or chromosome aberration) greatly depended on the doses of the mutagens and the synergistic effect often observed at lower doses could be absent at higher doses. The results in detail including the effects of hydration will be presented and discussed.

C-6-6 *Radiation-Induced Phenol Colour Mutation in Dwarf Wheat.* B. L. DHONUKSHE AND J. G. BHOWAL, Division of Genetics, Indian Agricultural Research Institute, New Delhi, India.

Most of the dwarf wheats of Norin origin give dark colored "Chapaties," a kind of unleavened pan baked bread made of whole wheat meal. The enzyme tyrosinase present in the bran oxidizes the substrate phenol to melanin to give the dark color of dough and "Chapati." Indian consumers are very sensitive to this darkening of "Chapaties."

Induced mutation studies were conducted with three dwarf varieties, viz., Sonalika, Choti Lerma and Hira with a view to induce mutants with low or little tyrosinase activity in these varieties. Dry seeds were treated with 20 kr and 25 kr acute doses of gamma rays. By screening a

large population of more than nine thousand M_2 plants in these varieties, with the help of phenol color reaction technique, a few mutants with low reaction were isolated.

One mutant in Choti Lerma showed almost negative reaction. Ten other mutants having light reaction have been isolated in the three varieties. From the progenies of these mutants, M_3 lines having different grades of reaction have been established in the three varieties.

C-6-7 *On the Interpretation of Some Regularities of Radiation Mutagenesis.* N. V. Luchnik, Research Institute of Medical Radiology, Academy of Medical Sciences of USSR, Obninsk, USSR.

Many radiation geneticists accept a hypothesis that shape of time-effect curves is connected with different sensitivity of cell cycle stages, and the type of aberrations is determined by chromosome duplication. Our experiments on *Crepis capillaris* give convincing evidence that these views are not well grounded. After pulse-labelling at the time of irradiation identical time-effect curves for labelled and unlabelled cells are obtained, thus their form is connected with time elapsing after irradiation rather than with a time of DNA synthesis. Aberration spectra are also identical in labelled and unlabelled cells, in particular relative frequencies of chromosome and chromatid aberrations are the same. It follows that the types of aberrations are not connected with chromosome duplication.

Fine analysis of time-effect curves and pattern of action of DNA inhibitors suggest that two critical periods exist in a cell cycle; an intermolecular proof associated with repair and amplification of primary injuries take place at these periods. It is ascertained that some types of aberrations are formed instead of others depending on the degree of injury or on influence of inhibitors.

The following hypothesis is discussed. The main factor which determines the shape of time-effect (stage-effect) curves is the degree of repair. The type of aberrations arising is connected with a degree of amplification of primary injury in chromosome crossection, which depends on the damage of the mechanism of proof connected with dose, time, and concomitant factors.

C-7-1 *Radiation-Induced Lethal Chemical Events in Bacterial Spores Suspended in Organic Liquids.* Ian J. Stratford and Alan Tallentire, Department of Pharmacy, University of Manchester, Manchester, M13 9PL, Great Britain.

There is now appreciable information about products and transient species formed in a variety of irradiated liquids. Transients formed from water are known to act, in part, in the radiation inactivation of aqueous cells, a fact amply justifying investigation of the potential damaging effects in cells of radiolysis products of non-aqueous liquids.

Bacillus megaterium spores (5×10^{-4} Torr H_2O equilibrium vapor pressure) were suspended in dried ethanol, acetone, dioxane, ethyl acetate, *n*-hexane or benzene for Co60 irradiation. Inactivation rate constants for anoxic spores (k_I) decrease in the order

$$k_{I(H_2O)} > k_{I\ (\text{organic liquids except EtOH})} \equiv k_{I\ (\text{dried})} > k_{I\ (\text{EtOH})}$$

This indicates that generally the species produced by radiolysis of organic liquids (electrons, ions and radicals) are not effective in the induction of lethal damage in cells. On the contrary, it seems that EtOH prevents certain damaging species from exerting their effect. For spores irradiated with O_2 present (conditions giving the total inactivation rate constant $k_I + k_{II} + k_{III}$), rates of inactivation are markedly different for the different suspending liquids. No obvious relationship exists between inactivation rates and the nature and yields of the products or transients formed in oxygenated liquids. Present evidence is that the liquids affect differentially the lifetimes of potentially lethal species that require the presence of oxygen for their damaging action.

C-7-2 *Mechanisms of Oxidative Radiation Sensitization of Cells in Aqueous Suspensions.* M. Simic and E. L. Powers, Laboratory of Radiation Biology, Zoology Department, University of Texas, Austin, Texas 78712, USA.

Some aspects of radiation sensitization based on oxidative free radical mechanism will be presented.

Conversion of the extremely reactive OH radicals with less reactive ones of lower redox potential and more selectivity towards the sites of attack leads to higher radiation sensitivity as in the case

of $\cdot Br_2^-$ and Mn(III). From the known reactivity of sensitizing free radicals and various molecular components the approximate "target" sites in cells can be deduced.

Free radicals resulting from the reaction of OH radicals with DNA can be oxidized by a number of radiation sensitizers if certain requirements are satisfied. This type of sensitization is less efficient (50% of the oxygen effect) unless some additional reactions take place, e.g., increase in local pH as in the case of $Co(NH_3)_6^{3+}$.

The above conclusions are based on the radiation response of *B. megaterium* spores in aqueous suspensions. Other cellular organisms are expected to show similar responses.

C-7-3 *Interactions Between p-Nitroacetophenone and 0.8% Oxygen During Radiation Sensitization of Bacterial Spores.* David Ewing, Laboratory of Radiation Biology, University of Texas, Austin, TX 78712, USA.

The combined effects of PNAP (*p*-nitroacetophenone) and 0.8% oxygen (10^{-5} *M* dissolved in solution) on the radiation sensitivity of *B. megaterium* spores have been studied. This low oxygen concentration was used to decrease the scavenging efficiencies of oxygen for radiation-induced radicals and make them more nearly equal to those of PNAP. The results of these experiments show an important interaction between these two sensitizers. When sufficient PNAP is present, at least part of the sensitization from 0.8% oxygen is eliminated; the overall sensitivity is reduced and becomes equivalent to that seen when PNAP alone is tested under anoxic conditions. These results are unexpected and seem contrary to earlier proposals that PNAP and oxygen had partly similar mechanisms of action. However, a model has been developed for sensitization of bacterial spores by oxygen and by PNAP which allows PNAP to have a protective action while still assigning a common sensitizing role for the two.

C-7-4 *Reduction of Radiosensitization in Bacterial Spores by High Concentrations of Ketonic Agents.* Geoffrey P. Jacobs and Alan Tallentire, Department of Pharmacy, University of Manchester, Manchester M13 9PL, Great Britain.

The OH radical is suspected as a damaging species in anoxic radiosensitization by certain ketonic agents. The scavenging of $\cdot OH$ by diacetyl and acetone has been suggested as being responsible for reductions in the extent of sensitization at high concentrations of these agents (E. L. Powers, Israel J. Chemistry, **10**, 1199, 1972). Tests of their effective $\cdot OH$ scavenging abilities have been done with suspensions of *B. megaterium* spores in which the $\cdot OH$ concentration has been increased by flowing N_2O through suspensions during γ-irradiation. The enhanced sensitivity due to N_2O presence was prevented by either SCN^- or NO_2^-, two agents that react readily with $\cdot OH$ and are therefore useful reference scavengers. Diacetyl at concentrations that removed completely sensitization in degassed suspensions prevented wholly sensitization by N_2O, and acetone reduced but did not totally prevent N_2O sensitization. A notable feature with acetone is that the reduction in N_2O sensitization occurred over a concentration range below that over which it acts as a sensitizer in degassed suspensions. Moreover, *p*-nitro-3-dimethylamino-propiophenone (NDPP), another ketonic radiosensitizer, reduced N_2O sensitization at concentrations which show maximal effectiveness in degassed spore suspensions.

C-7-5 *Fast Kinetics of Cellular Radiation Damage Measured Using a Gas Explosion Technique.* Barry D. Michael, Gerald E. Adams, and Margaret E. Watts, Cancer Research Campaign, Gray Laboratory, Mount Vernon Hospital, Northwood, Middlesex, Great Britain.

A fast response gas explosion technique has been used to measure lifetimes of oxygen-dependent radiation damage in bacteria. Cells mounted on a surface are exposed to a high velocity shot of oxygen before, during, or after a short pulse of electrons from a linear accelerator, (Michael, Adams, Hewitt, Jones and Watts, Rad Res., **54**(2), 239, 1973). The technique has now been adapted for use on a 600 keV Febetron electron pulser, permitting the study of more resistant organisms for comparison with earlier data for *Serratia marcescens*. The time scale of dose modification effects of oxygen, other sensitizers and protective compounds including H_2S has been studied. The technique has also been adapted for the observation of similar effects in mammalian cells and preliminary data for these systems will be presented.

C-7-6 *Iodinated Radiological Contrast Media as Radiosensitizers.* M. QUINTILIANI, Lab. F.R.A.E. (C.N.R.), Bologna, Italy; P. MISITI-DORELLO AND O. SAPORA, Istituto Superiore di Sanita, Roma, Italy; K. C. GEORGE, M. A. SHENOY, AND B. B. SINGH, Bhabha Atomic Research Centre, Bombay, India.

Isothalamic acid (ITA), (Angio-Conray, Bracco S. p. A), has been shown to be a powerful radiation sensitizer in some single cell systems. The systems and end points which have been tested include: cell survival in *E. coli* B/r and *M. radiophilus; in vitro* survival of Yoshida sarcoma ascites cells and of rat thymocytes, scored by the erythrosin-B exclusion test; and loss of intracellular K^+ from rabbit erythrocytes *in vitro*. In general sensitization by ITA was stronger in oxic than in anoxic conditions. In aerated buffer (pH 7), with ITA concentrations from 10 to 25 mM, the DMF's were: 0.13 and 0.2 for 1% survival of *E. coli* and *M. radiophilus* respectively. Post irradiation lethality of thymocytes and Yoshida cells and K^+ loss in erythrocytes were also enhanced on irradiation in presence of ITA. In protein containing media the sensitizing activity of the above concentrations of ITA was more or less completely inhibited, however it could be substantially restored by increasing the ITA concentration. ITA solutions in buffer pH 5.6 developed on irradiation a long lasting bactericidal activity which could not be detected at pH 7. The very low toxicity and metabolic inertness of iodinated contrast media seem to justify further studies of their radiosensitizing ability in order to explore their potential interest in tumor radiation therapy. (These investigations were partially supported by International Atomic Energy Agency (Contract no: 1396/RB).)

C-7-7 *Modification of Radiosensitivity of* E. coli *by treatment with Reductone.* R. ALCANTARA GOMES, A. C. LEITAO, AND L. R. CALDAS, Faculdade de Ciências Médicas da Universidade do Estado da Guanabara and Instituto de Biofísica da Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

It has been shown that reductone blocks one or more steps of repair processes in UV-irradiated bacteria, probably by its interaction with pyrimidine dimers, thus preventing excision. This model is consistent with the absence of reductone effect on X- or gamma-irradiated bacteria as well as on cells treated with alkylating agents (nitrosoguanidine or nitrogen mustard) or submitted to ^{32}P decay. The reductone effect is observed in bacteria submitted to photodynamic treatment with methylene blue as sensitizer, but, in this case, it seems that the effect of reductone is located elsewhere. The reductone effect has also been studied on different mutants including *pol A* mutants, and on starved bacteria. Some *"in vitro"* experiments aiming to study the interaction of reductone with dimers have been performed. Results are consistent with the hypothesis of reductone action on repair processes. (This work is part of the joint research program of the "Universidade do Estado da Guanabara" and "Universidade Federal do Rio de Janeiro" and has been supported by both, by "Comissão Nacional de Energia Nuclear," "Banco Nacional do Desenvolvimento Econômico" (Grants FUNTEC-74 and 143) and "Associação Brasileira de Industria Farmaceutica.")

C-7-8 *Radiosensitization by Membrane Specific Drugs.* B. B. SINGH, M. A. SHENOY, K. C. GEORGE, AND A. R. GOPAL-AYENGAR, Biomedical Group, Bhabha Atomic Research Centre, Trombay, Bombay 400 085, India.

Procaine hydrochloride, a local anaesthetic, is known to interact with the lipid moiety of cellular membranes. It has been found to enhance the radiation lethality of *E. coli* B/r cells when present during anoxic irradiation at various non-toxic concentrations between 0.25 and 25 m*M* with corresponding DMF varying from 0.54 to 0.42. Since scavengers of e_{aq}^- and OH do not modify its sensitizing effect it can be inferred that short-lived radiolytic transients of water do not play any significant role. It is further supported by the fact that enhanced lethality is noticed if bacterial cells irradiated with gamma-rays under anoxia are exposed to unirradiated procaine HCl. In addition to the lethality of *E. coli* B/r measured in terms of colony forming ability, the dye exclusion test using erythrocin B revealed that the post irradiation death of Yoshida sarcoma ascites cells and rat thymocytes is accelerated if procaine HCl is present during anoxic irradiation of cells with gamma-rays. On the basis of these results a model will be proposed which incorporates the concept of membrane damage, its repair and its implications in modification of radiation lethality.

(These investigations were partly supported by International Atomic Energy Agency (contract No. RB/1396).)

C-7-9 *The role of Cell Thiols in Variation of Natural Radioresistance of the Cell.* MARINA M. KONSTANTINOVA, Institute of Developmental Biology Academy of Sciences of the USSR, USSR.

The experiments were carried out on bacteria *E. coli* B and on the eggs of sea urchin *Strongylocentrotus nudus*. The study of dependence of the radioresistance on the content of cell thiols was performed both by determination of correlation between these parameters and by their modification with a radioprotector or radiosensitizer. The change of radioresistance of *E. coli* B with age and of developing sea urchin eggs during the cell cycle correlates directly with the changes of the thiol content. The natural level of endogenous thiols determines the efficiency of radioprotection and radiosensitization. The protective effect of MEA is more pronounced when the content of cell thiols is the lowest, i.e., at logarithmic phase for *E. coli* B population and at the telophase for sea urchin eggs. On the contrary, higher extent of sensitization by NEM was established in the stationary phase of *E. coli* B population when the level of thiols was the highest.

These data suggest that variations of radioresistance which occur in normal development of the cell population of bacteria and in the cell cycle of sea urchin eggs depend on the change of the thiol content in the cells.

C-8-1 *Sensitisation of Hypoxic Mammalian Cells* in vitro *by Electron-Affinic Agents.* J. C. ASQUITH, M. E. WATTS, AND G. E. ADAMS, C. R. C. Gray Laboratory, Mont Vernon Hospital, Northwood, Middlesex, Great Britain.

Many electron-affinic sensitisers of hypoxic cells which are active *in vitro* show little activity *in vivo* due to rapid metabolism. Exceptions include the nitroimidazoles, which are relatively non-toxic *in vivo* and are only slowly metabolised. Following the demonstration of the sensitising properties *in vivo* of the 5-nitro derivative, Metronidazole,[1] a 2-nitro substituted imidazole has been found which is considerably more effective, both *in vitro* and *in vivo*, including solid tumours in animals. This paper summarises the *in vitro* data.

The drug sensitises hypoxic cells, but not oxic cells, at concentrations above 10^{-4} *M* and gives an enhancement ratio of 2.6 at 4×10^{-3} *M*. This concentration range is well below the cytotoxic concentrations (50% cell killing for 2 hours' contact at 8×10^{-2} *M*.

Fast-flow rapid-mixing studies and investigations with synchronised cultures provide further evidence that this drug, like other electron-affinic sensitisers, mimics the oxygen effect.

Experiments using a mixture of this sensitiser with another sensitiser, Nifurpipone, will be described. Nifurpipone is a soluble nitrofuran which, in addition to decreasing D_0, suppresses the shoulder of the hypoxic survival curve.

[1] A. C. Begg, P. W. Sheldon, and J. L. Foster, Brit. J. Radiol. in press.

C-8-2 In vitro *Studies of Hypoxic Cell Sensitizers.* GORDON F. WHITMORE AND SANDOR GULYAS, Ontario Cancer Institute, Toronto, Ontario M4X 1K9, Canada.

A wide variety of electron-affinic compounds are capable of sensitizing hypoxic cells to the lethal action of ionizing radiation. To date, however, there appears to be no model which adequately accounts for the wide variation of sensitizer concentrations required, the strong dependence of the sensitizing ability of some compounds on cell density, and the apparent inability of many of the sensitizers to produce the full oxygen enhancement ratio. Data will be presented from a variety of sensitizers including NDPP, Flagyl, Tinidazole, 0582, etc., which seems to suggest that at least two mechanisms of action are involved, that these two mechanisms may require very different sensitizer concentrations and that some sensitizers may not be capable of both activities.

C-8-3 *Redox Reactions of Anoxic Radiosensitizers.* JOHN E. BIAGLOW AND ODDVAR F. NYGAARD, Case Western Reserve University, Cleveland, Ohio 44106, USA, AND CLIVE L. GREENSTOCK, Whiteshell Nuclear Establishment, Pinawa, Manitoba, Canada.

Many of the anoxic radiosensitizers currently being studied affect electron transfer reactions within the cells. We have measured several cellular redox reactions to establish the interference by

various sensitizers as well as to delineate the metabolism of the sensitizers themselves. The classes of anoxic sensitizers tested were: 1. nitrofurans; 2. quinones; 3. redox dyes (tetrazoliums); 4. stabilized free radicals (e.g., TAN); 5. nitrated heterocyclics; and 6. azo compounds (e.g., diamide). Groups 1, 2, 3, and 6 reacted with FMN· (semiquinone) and $FMNH_2$ in solution, and also altered the cellular NAD(P)H. Groups 1, 2, and 3, when reduced, reacted with dissolved oxygen. These latter groups also stimulated, whereas groups 5 and 6 inhibited cellular oxygen utilization. The cellular effects depended upon the supply of reducing equivalents (including glucose) that might eventually react with oxygen. Groups 1 and 2 also abolished glucose-mediated inhibition of oxidation. We conclude that since most of the anoxic radiosensitizers interfere with cellular metabolism they may affect cell survival in ways other than by direct interaction with radiation-induced radicals in essential cellular targets. Conversely, the metabolic reduction of a sensitizer may limit its potential usefulness.

C-8-4 *Metabolic Limitations in the Use of Anoxic Radiosensitizers.* Oddvar F. Nygaard and John E. Biaglow, Case Western Reserve University, Cleveland, Ohio 44106, USA.

The metabolism of anoxic radiosensitizers, believed to mimic oxygen for electron accepting ability, may limit their usefulness *in vivo*. Some anoxic sensitizers, such as the nitrofurans, are readily reduced under anaerobic conditions which means that they may be efficiently removed from the anoxic centers of tumors, their intended site of action. The intracellular concentration of a sensitizer obviously depends on such factors as the rate of its reduction, the content of reducing equivalents in the cell, the extracellular concentration of sensitizer, and the volume of the extracellular space. Using dense suspensions of Ehrlich ascites tumor cells, made anaerobic by metabolic consumption of oxygen, we have determined the cellular reduction of many of the currently used anoxic sensitizers (e.g., nitrofurans and quinones). Prior removal of intracellular reducing substrates (e.g., by diamide) inhibited, whereas the addition of glucose or vitamin C increased the rate of reduction of the sensitizers. Preliminary experiments indicate that the study of metabolism of anoxic radiosensitizers in dense cell suspensions may be of predictive value for the use of these compounds in radiotherapy of solid tumors.

C-8-5 *Sensitisation of Hypoxic Mammalian Cells* in vitro *by Some Unusual Nitroxyl Compounds.* Barbara C. Cooke and Margaret Johnson, Physics Department, Institute of Cancer Research, Clifton Avenue, Sutton SM2 5PX, Surrey, Great Britain.

A number of nitroxyl compounds have been investigated for their potential to sensitise hypoxic mammalian cells, Chinese hamster line V-79. One particular compound Bis(2,2,6,6-tetramethyl-1-oxyl-4-piperidyl) succinate behaved differently from the others viz. tetramethyl-4-piperidinol N-oxyl and nor-pseudo-pelletierine N-oxyl in that it markedly reduced the shoulder of survival curves. Furthermore this compound was more effective on a molar basis with regard to the number of functional groups than either of the other compounds. Results comparing these three compounds will be presented and discussed together with some pharmacological data.

C-8-6 *Radiosensitization of Chronically Hypoxic Cells in Multicellular Spheroids.* Robert M. Sutherland and John Richardson, Ontario Cancer Treatment and Research Foundation, Victoria Hospital and Dept. of Biophysics, University of Western Ontario, London, Ontario N6A 4G5, Canada.

Multicellular spheroids of Chinese hamster V79-171b cells provide a useful *in vitro* tumor model to test: 1) the ability of chemicals to diffuse to and sensitize centrally-located, chronically hypoxic cells, 2) the effect on the chemicals of the outer layers of actively metabolizing cells and 3) the influence of the central tumor-like environment, including necrosis, on radiosensitization. Experiments have been carried out to compare chemicals from various classes of sensitizers (nitroxyl free radicals, nitrobenzenes, nitrofurans and nitroimidazoles) and to determine conditions for maximal sensitization. Spheroids of 350–450 μ diameter (11–18 days of growth) were treated with different concentrations of sensitizers for various periods at 37°C prior to irradiation in air with Co^{60}. For most sensitizers, short pretreatment times (<30 min) produced sensitization which increased with increasing drug concentration; however, after longer pretreatment (up to 3 hrs) sensitization was lost at low concentrations and increased with time at high concentrations pro-

ducing cell killing comparable with that which could be obtained by exposing the hypoxic cells to oxygen. These results demonstrate some of the difficulties involved in choosing optimal sensitizer concentrations and pretreatment exposure times for use in radiotherapy. We have begun detailed studies with metronidazole, which shows the most promise for clinical use. (Supported by the Ontario Cancer Treatment and Research Foundation, Grant No. 227).

C-8-7 *Effect of Hypoxia on the Response of Synchronous HeLa Cells Exposed to Low LET Radiations: Comparison of Two Methods of Oxygen Removal.* MICHAEL D. SAPOZINK AND BOZIDAR DJORDJEVIC, Biophysics Laboratory, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA.

HeLa S3 cells were synchronized by the mitotic selection method and rendered hypoxic either by co-incubation with an excess of heavily irradiated oxygen scavenging cells, or by passing 5% CO_2 in nitrogen over cells attached to 0.4 mm thick Permanox dishes. With both methods of deoxygenation with x and gamma irradiation a D_0 ratio for hypoxic and oxygenated cells of about 3 was obtained for interphase cells. For cells in or close to mitosis a consistently lower ratio was obtained, particularly when dense cultures were used. This finding is in contrast to a report where no fluctuations in the oxygen effect were observed during the entire division cycle of chinese hamster cells [Rad. Research, **37**, 161–172 (1969)]. In view of the physiological means of achieving hypoxia in a dense culture, the decreased oxygen effect in mitotic HeLa cells may be relevant to *in vivo* situations and for tumor therapy. (Supported in part by AEC Grant AT(11)-3521 and NCI Grant (CA 08748).)

C-8-8 *Action of Some Chemotherapeutic Agents and X-Irradiation on Cells in Tissue Culture.* K. B. DAWSON AND J. H. PEACOCK. Radiotherapy Research Unit, Institute of Cancer Research, Royal Cancer Hospital, Downs Road, Sutton, Surrey, Great Britain.

A search was made for possible synergism or potentiation between some of the drugs in current use as cytotoxic agents and X-radiation using cells from established cell lines and tumour cells in primary cultures, both in regard to the particular type of agent and the time between dosing with the agent and irradiation.

C-8-9 *Reappearance of Chemosensitivity to Hydroxyurea or 5-Fluorouracil Following Radiation or Alkylation.* YOSH MARUYAMA, University of Kentucky, Lexington, Kentucky 40506, USA.

Current concepts of radiation and drug effect regard response and sensitivity as a characteristics of the system under study. Recent experiments using combinations of radiation and drugs have been undertaken using agents exhibiting phase-specific activity against DNA synthesis. The LSA Ascites Lymphoma shows a characteristic pattern of chemosensitivity to DNA antimetabolites during growth. These correlate with determinations of DNA-S activity as measured by H^3-TdR uptake and DNA per cell content. When tumor was exposed to a alkylating agent such as HN_2 during plateau phase, an increase in sensitivity to the antimetabolite hydroxyurea followed.

The tumor collects in G_1 in plateau phase. These indicate a shift of the age distribution of cells present to a younger composition with a larger number of antimetabolite sensitive cells present. Using endogenous spleen CFU as a model of response, it was found that hydroxyurea of 5-FU given before as opposed to after 550 rads of whole body radiation alters the number of CFU observed. Since the sensitivity following irradiation or alkylation was greater, the data indicated that the cycling or non-cycling status of tumor or hematopoietic cells is important to sensitivity. With greater cycling activity, increased chemosensitivity to anti DNA-S agents occurred.

C-9-1 *Some Results of Studies of the Kinetics of Exchange and Biological Effects in Rodents of Plutonium and Other Transuranium Elements.* YU. I., MOSKALEV, V. K. LEMBERG, T. I. LEVDIK, E. R. LUBCHANSKY, N. A. KOSHURNIKOVA, A. P. NIFATOV, I. A. TSEVELEVA, L. G. PHILIPPOVA, R. A. EROKHIN, A. G. SURINA, M. G. POPLYKO, AND I. A. TERNOVSKY, Institute of Biophysics, Ministry of Public Health, Moscow, USSR.

The report will give results of studies of the kinetics of exchange and biological effects in rodents of plutonium and other transuranium elements at various modes of their entry into the body.

After being inhaled, transuranium elements are non-uniformly distributed within the lungs. The degree of this non-uniformity depends on the duration of inhalation and on the period of observation. After being resorbed from the lungs, Pu and Np are deposited selectively within the skeleton while Am is deposited within the liver and the skeleton. The rate and amount of Np and Pu accumulation within organs depend essentially on the chemical form of the elements.

Pneumosclerosis, cancer of the lungs and osteosarcomas are the most serious late effects of damage by transuranium elements. The type, frequency and the rate of development of the corresponding pathology depends on the dose, on the form of injected compound and on the mode of radioisotope entry.

After being inhaled, soluble compounds of transuranium elements have one and the same sclerogenous effect. Alpha-irradiation causes lung tissues to develop early changes in metabolic processes. The amount of collagenous proteins and glycosoaminoglycanes is increased due to activation of their synthesis. The newly-formed connective tissue has an increased relative amount of gialuronic acid and a decreased amount of oxyproline and collagen.

The report will analyse data on the optimum and minimum carcinogenic dose levels, on dose- and time-effect curves for various forms of late pathology; comparison is given of the time necessary for carcinogenic effects to develop and of the natural duration of life of animals.

C-9-2 *Late Effects of Inhaled Plutonium in Dogs.* JAMES F. PARK, Biology Department, Battelle, Pacific Northwest Laboratory, Richland, Washington 99352, U.S.A.

Pulmonary neoplasia was the primary cause of death in beagle dogs 5 to 10 years after inhalation of $^{239}PuO_2$ depositing 0.2 to 2.0 μCi in the lungs (3 to 22 nCi/g lung). Approximately 10% of the alveolar deposited plutonium was retained in the lungs 8 to 10 years postexposure with an estimated accumulated average radiation dose to the lungs of 2000 to 12000 rads. Forty to fifty percent of the plutonium was translocated to the tracheobronchial and mediastinal lymph nodes, 10 to 15% to the liver, 5% to the skeleton and 5% to the abdominal lymph nodes. The highest plutonium concentration occurred in the lymph nodes followed in descending order by lungs, liver and skeleton. Bone neoplasia was the primary cause of death in dogs 5 to 6 years after inhalation of $^{238}PuO_2$, depositing 2 to 5 μCi in the lungs (30 to 70 nCi/g lung). Twenty to fifty percent of the alveolar deposited plutonium was in the skeleton 5 to 6 years postexposure with an estimated accumulated average radiation dose to the skeleton of 200 to 500 rads. Four to thirty percent of the Pu was in the lungs, 15 to 30% in the liver and 4 to 15% in the tracheobronchial and mediastinal lymph nodes. The highest plutonium concentration occurred in the lymph nodes followed in descending order by liver, lung and skeleton. After inhalation of $^{238}PuO_2$ or $^{239}PuO_2$ at these low levels, lymphopenia was the earliest observed effect, occurring 1 to 2 years after deposition of more than 20 nCi Pu in the lungs. (This work was performed under United States Atomic Energy Commission Contract AT(45-1)-1830.)

C-9-3 *Current Status of Information Obtained from Plutonium Contaminated People.* C. R. RICHMOND. Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico 87544, USA.

There has been no recorded incidence of cancer of serious biological damage in man from internally deposited plutonium. However, we must consider the relatively short time since exposure in some cases. Because of industrial accidents and early Manhattan Project exposures, a number of individuals have been exposed to plutonium. Some have maintained multiples of the maximum permissible body burden (MPBB) for almost three decades. Although the exact levels of contamination are uncertain, it is clear that this body of information represents a valuable resource for study. Data have been reported for specific groups (Manhattan Project workers), and other studies are accruing data for future use as these populations age. Another source of particularly valuable information, 18 people who received plutonium during the late 1940's, provided the basis of the human excretion equations derived by Langham. The injected levels ranged from $\sim$0.25 to $\sim$5.8 μCi and, in one case was retained for 21 years. Other information exists in the form of virtually the entire world's population which has accumulated plutonium, although somewhat inefficiently, from detonations of nuclear weapons and burnup of one thermoelectric generator (plutonium-238) in the atmosphere. Information obtained from these and other sources will be

summarized. (This work is being performed under the auspices of the U. S. Atomic Energy Commission.)

C-9-4 *Dose-Response Relationships for Beagles Injected with $^{239}Pu(IV)$ or $^{241}Am(III)$.* BETSY J. STOVER, Department of Pharmacology, University of North Carolina, Chapel Hill, NC 27514, and Division of Radiobiology, Department of Anatomy, University of Utah, Salt Lake City, Utah 84132, USA.

The long-term toxic effects of ^{239}Pu(IV) and of ^{241}Am(III) in the beagle are being extensively investigated at the University of Utah. Each radionuclide is injected intravenously in approximately 9 ml 0.08M citrate buffer of pH 3.5. The occurrence of osteosarcoma was increased in beagles at the initial six dose levels of ^{239}Pu which range from 0.016 to 2.9 μCi/kg. Consequently additional beagles were added at five dose levels that range from 0.00064 to 0.016 μCi/kg. The osteosarcoma is also a critical response in beagles at eight dose levels of ^{241}Am which range from 0.0018 to 2.8 μCi/kg. The results of these continuing long-term studies as of March 31, 1974 will be summarized. Special emphasis will be given the lowest dose levels for which data are available and the relationship between skeletal distribution of the radionuclides and the sites at which tumors arise. (Supported by the U.S. Atomic Energy Commission.)

C-9-5 *Summary and Speculative Interpretation Relative to Exposure Limits.* ROY C. THOMPSON, Biology Department, Battelle Pacific Northwest Laboratory, Richland, Washington 99352, USA.

The preceding papers in this symposium deal primarily with the facts of transuranium element toxicity as these facts are currently understood. This paper will summarize these facts, consider their limitations, and speculate on their implications with regard to hazards to humans in an era of expanding nuclear technology. Special attention will be given to the problem of exposure limits, appropriate to both workers in the nuclear industry and to general populations. Specific conclusions will depend upon input from the preceding papers, not available at the time of preparing this abstract. (This work was performed under United States Atomic Energy Commission Contract AT(45-1)-1830.)

C-10-1 *Rapid-Mixing Studies on the Time Scale of Radiation Damage in Bacteria and Mammalian Cells.* G. E. ADAMS AND B. D. MICHAEL, C. R. C. Gray Laboratory, Mount Vernon Hospital, Northwood, Middx. HA6 2RN, Great Britain.

Two fast-response techniques are used in our laboratory for the study of the time-scale for interaction of cellular damage with oxygen, hypoxic cell sensitisers and other substances which modify the overall cellular response.

One technique involves exposing cells, mounted on a surface, to an explosion of oxygen from a high pressure chamber. The time resolution of the techniques is 10^{-4} second and this permits the direct measurement of the life-times of oxygen-dependent damage.

The second technique involves fast-mixing of cell suspensions with solutions of oxygen or sensitisers in a liquid flow apparatus either just before or after irradiation with an electron beam. The time resolution of this technique is about 2 milliseconds.

Application of these techniques has shown, for example, that the oxygen effect in mammalian cells is resolved in time into two components. Time-resolved effects are also observed with the electron-affinic sensitisers which mimic, at least in part, the oxygen effect. The results illustrate how the technique can provide information on the mechanism of sensitisation by oxygen and other chemical compounds, particularly those mechanisms which involve fast free radical processes.

C-10-2 *Application of Fast Polarography in Pulse Radiolysis.* MICHAEL GRAETZEL, KRISHAN M. BANSAL, AND A. HENGLEIN, Hahn Meitner Institute, Berlin, West Germany.

The principle of this method consists in the detection of short lived intermediates formed by pulse irradiation of liquids on the basis of their redox behavior at a mercury electrode. Electrokinetic analysis of the oscillographically recorded current-time curves yields information about the rate of electron transfer from the electrode to the electroactive transient and the rate constant for the disappearance of the transient in the solution bulk. Short time polarograms, i.e., plots of

the current shortly after the pulse as a function of the applied potential, have been measured for numerous radicals and may now be used for their identification. The technique has been employed to investigate the electrochemical behavior of intermediates in biological redox systems such as NADH and ascorbic acid.

C-10-3 *Laser Photolysis and Pulsed Radiolysis in Micelles and Biological Membranes.* J. K. THOMAS, Chemistry Department and Radiation Laboratory, University of Notre Dame, Notre Dame, Indiana 46556, USA.

The kinetic motion into micelles of species such as hydrogen atoms and solvated electrons and factors like ionic charge on the micelle which influences their entry have been investigated by pulsed radiolysis techniques. Also the kinetic motion of such stable probes as iodide ion, triethylamine, and various arenes have been studied entering within, and leaving the micelle. Pulsed laser techniques have been used to specifically excite regions of the micelle, including micelles composed of lecithin and cholesterol. In particular, the role of cholesterol in making the micelle more rigid is discussed. This work extends to inner and outer membrane vesicles from *E. coli* where phase changes are clearly illustrated and show a distinct difference in the permeability of the inner and outer membrane. The effect of various additives such as basic anesthetics and ionic species on the degree and function of permeability are also discussed.

C-10-4 *The Picosecond Reactions of Electrons with Biologically Important Molecules.* KIT Y. LAM AND JOHN W. HUNT, The Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, Ontario, M4X 1K9, Canada.

The picosecond pulse radiolysis system has been used to study reactions of the hydrated electron, e_{aq}^-, with high concentrations of scavengers. Reaction rates of many scavengers observed in the picosecond time scale are quite different from that observed in the dilute solution. Moreover, the yield of e_{aq}^- are drastically reduced in the presence of many scavengers. There is a direct relationship between the reaction rate constant, k, measured in picosecond time region and the efficiency, E, of the scavenger to reduce the initial yield of e_{aq}^-. The hydrated proton $H_3O_{aq}^+$ is an exception to the above correlation. For many ionic dissociable compounds including many amino acids and nucleotides both the k and E vary with the pH of the solution. However, at different pH's, the same relationship still holds between the k and E values. This suggests that the e_{aq}^- and it's precursor are attacking the same sites on many of these scavengers. These results suggest that studies in dilute solutions may not have direct bearing on the radiation chemistry of a living cell.

D-1-1 *Applied Industrial Radiation Chemistry of Monomers and Polymers.* ALLAN S. HOFFMAN, University of Washington, Seattle, Washington, USA.

The latest trends in the application of radiation from radioisotopes or electron accelerators to the chemical processing of monomers and polymers will be highlighted. The chemical reactions involved in these processes are all free radical in nature and include monomer-monomer reactions (homo and co-polymerization), monomer-polymer reactions (graft copolymerization), and polymer-polymer reactions (crosslinking and chain scission). Processes and products to be covered are: wire and cable, foam, shrinkable film and molded objects, coatings, textiles, wood-plastic combinations, wood chips, solution-degraded polymers, and specialty grafted-polymer products as new biocompatible materials, enzyme reactors, membranes, etc.

D-1-2 *Radiation and Plant Breeding—a Glance Back and a Look Forward.* ÅKE GUSTAFSSON, Institute of Genetics, Lund University, 223 62 Lund, Sweden.

The damaging effect of X-radiation known at the time of Röntgen and were analysed by early studies of radiation effects on mitosis and chromosomes. In 1946 H. J. Muller obtained the Nobel Prize in medicine and physiology for his insistent warnings concerning the misuse of radiation in relation to widely increased mutation rates. Although his first results primarily dealt with lethal mutations in Drosophila, he outlined in his pioneer article of 1927 the possible value of induced mutations in plant breeding.

Russian scientists such as Delone, Sapehin, and Didus soon afterwards emphasized the useful-

ness of induced mutations in aestivum and durum wheat, barley and other crop plants, a line of research also taken up by Nilsson-Ehle and Gustafsson in Sweden, Baur, Stubbe, and Schick in Germany. The hesitative view of Stadler was advisable around 1930 but it led for many years to a negative attitude from the side of American agronomists and plant breeders.

Since then an enormous number of junk mutations have been isolated in crop plants, useful no doubt in theoretical analysis, but of relatively little practical value. There is indeed a necessity of coordinating mutation induction and active plant breeding more intimately in future work. A "revolution" of plant breeding cannot be expected by such a cooperative program but rather a continuous "evolution" of the crop plants, combining methods and tools of modern genetics and biochemistry with practical aspects of agriculture and breeders' dexterity.

D-1-3 *The Use of Ionizing Radiation for Preservation of Food and Feed Products.* EDWARD S. JOSEPHSON, ARI BRYNJOLFSSON, AND EUGEN WIERBICKI, U.S. Army Natick Laboratories, Natick, Mass. USA.

Two decades of research have established the basis for a variety of uses of ionizing radiation to preserve food and feed products.

Radiation sterilizing (radappertizing) doses (1 to 6 Mrad) provide tasty nutritious flesh foods even after two years non-refrigeration storage. Hospital patients requiring sterile diets have eaten only radappertized meals during treatment. Radappertized diets are now fed to germ-free animals.

Radiation pasteurizing doses (0.1 to 0.8 Mrad), by destroying non-spore forming bacteria, can provide more disease-free nutritious food through larger marketing radii because of longer refrigerated storage life. Radiation pasteurization eliminates salmonellae in poultry and animal feed.

Radiation doses below 0.1 Mrad insect disinfest grain and fruit, prevent losses of bulbs and tubers caused by sprouting during storage, extend the marketing life of mushrooms and some fruits by delaying ripening, and eradicate animal parasites, such as trichinae, in fresh foods.

Although fourteen countries have approved at least one of seventeen irradiated foods, the major research effort is now directed to obtain the scientific evidence confirming that all irradiated foods and animal feeds are safe to eat.

D-1-4 *Radiation Sterilisation–An Industrial Process.* F. J. LEY, Irradiated Products Ltd., Elgin Drive, Swindon, Wiltshire, Great Britain.

A total of between 10 and 20 million curies of cobalt 60 is currently in use in a variety of gamma radiation sterilisation plants throughout the world. Various types of electron machine are also installed in industrial premises for the same purpose. The process has played an important role in the introduction of pre-packed disposable medical supplies which have had a considerable impact in control of cross infection. Apart from the sterilisation of such products as plastic syringes, catheters and rubber gloves, radiation is also applied to massive reels of packaging materials, to complex apparatus used in operating theatres and even to meals for patients being nursed under 'germ-free' conditions.

National health authorities have approved a minimum dose of 2.5 Mrad for sterilisation purposes. This choice is based on basic information made available from the general field of radiation microbiology with applied studies superimposed. These latter studies have attempted to recognise the importance of such parameters as population levels, environmental conditions and inherent resistance of contaminating species. Microbiological quality control procedures relevant to the process and methods of dosimetry appropriate to rountine industrial operation are now recognised internationally. An I.A.E.A. Recommended Code of Practice on Radiation Sterilisation is constantly under review. More information on the effects of radiation on materials and methods of avoiding radiation induced degradation could broaden the range of practical application.

D-2-1 *Mammalian Cell Sensitization, Repair and the Cell Cycle.* WARREN K. SINCLAIR, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois, USA.

Synchronous, fully oxygenated mammalian cells are sensitized to X radiation by N-ethylmaleimide and some similar agents present during, just before, or just after irradiation. The

sensitization is differential in magnitude during the cell cycle, being greatest at the end of S and in G_1 (in the latter case especially, in cells with a long G_1, e.g., HeLa.) The effect is greater on the slope than on the shoulder of the survival curve. Results in both Chinese hamster cells and HeLa cells are consistent with the notion that NEM interferes with repair and that the kinetics of the repair process are quite different in G_1 and late S cells.

Earlier studies with hydroxyurea showed that when the agent is added in G_1, cells are sensitized to a minimum survival level about equal to that of cells in mitosis; and if NEM is added also, this level of survival is maintained throughout the rest of the "cell cycle."

These data suggest a preliminary model for survival during the cell cycle, namely that interphase cells become as sensitive as mitotic cells (which have no repair capability) when prevented from developing repair capacity by hydroxyurea and when postirradiation repair is suppressed by NEM. (Work supported by the U.S. Atomic Energy Commission.)

D-2-2 *Chemical Radiosensitization Studies with Mammalian Cells Growing* in vitro. J. D. CHAPMAN, D. L. DUGLE, A. P. REUVERS, C. J. GILLESPIE, AND J. BORSA, Medical Biophysics Branch, Whiteshell Nuclear Research Establishment, Atomic Energy of Canada Limited, Pinawa, Manitoba, Canada.

Chinese hamster fibroblasts have been used 1) in the selection of chemicals which selectively radiosensitize in hypoxia and 2) in the elucidation of mechanisms of radiosensitization. Early studies showed that a threshold electron-affinity, near to that of nitrobenzene, was required by a structure to effect radiosensitization in hypoxic hamster cells. Several nitroheterocyclic structures have now been shown to have excellent radiosensitizing activity and are good base structures for the design of clinical radiosensitizers. Most electron-affinic (EA) compounds, like molecular oxygen, sensitize hamster cells through the indirect action of OH ($\sim$75%) and an additional component ($\sim$25%], attributed to direct effect. Neutral radicals in cellular target material, thus generated, can be oxidized by O_2 and EA compounds resulting in stable addition products and electron transfers. This reaction competes with a radical reduction process which can restore the cellular target to a nonlethal form. Alkaline sucrose gradient analyses of the DNA from hamster cells irradiated under a variety of conditions of chemical radiosensitization and radioprotection, indicate remarkable agreement between the rate of DNA strand breakage and the rate of cell inactivation. Kinetic analyses of these rates suggest that radiation chemical damage in cellular DNA results in cellular reproductive death.

D-2-3 In vivo *Testing of Hypoxic Cell Radiosensitizers.* ANDREW M. RAUTH, Physics Division, Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada.

On the assumption that hypoxic cells are significant in determining human tumor control there has been considerable interest in ways of specifically sensitizing such cells to ionizing radiation. Recent experiments using *in vitro* cultures of mammalian cells have identified a large variety of compounds which do this. It is important to see if these compounds are able to work *in vivo* where additional problems of drug delivery, metabolism and toxicity exist. A number of tumor and normal cell assay systems are available in the mouse for testing the effects of these compounds. We have used the KHT transplantable solid tumor of C3H mice which normally contain 10–20% hypoxic cells. A number of nitro-benzene, -imidazole and -furans have been tested for their ability to radiosensitize hypoxic KHT tumor cells *in vivo*. Single dose radiation survival curves were determined using the *in vivo* lung colony to measure the percent of viable cells remaining after treatment. One class of these compounds, the nitroimidazoles, tested in collaboration with the Gray Laboratory, London, have given positive results *in vivo*. For example, metronidazole (a 5-nitroimidazole) gave an enhancement ratio of $\sim$1.5 for hypoxic cells *in vivo*. Other 2, 4, and 5 nitroimidazoles are being tested. These compounds are characterized by a low acute toxicity, greater than 1.5 grams/Kg, a persistance in the plasma, $t_{\frac{1}{2}}$ greater than 1 hour, and a resistance to metabolic degradation. Representatives of this class of compounds are presently used as antibiotics in humans albeit at lower concentrations. The possibility of using these compounds as adjuncts to normal radiotherapy procedures will be discussed.

D-3-1 *The Stem Cells, Radiobiological Quirk?* PATRICIA J. LINDOP, Department of Radiobiology, Medical College at St. Bartholomew's Hospital, Charterhouse Square, London, EC1M 6BQ, Great Britain (*see page 316*).

D-3-2 *Concept of Human Hemopoietic Stem Cell Kinetics.* EUGENE P. CRONKITE, Medical Department, Brookhaven National Laboratory, Upton, N. Y. 11973, U.S.A.

Knowledge of stem cell kinetics and radiosensitivity derives from experiments in mice. Does this apply to man? From human red blood cell and granulocyte turnover rates and structure of human bone marrow (BM) one can work backwards and compute the minimum stem cell influx into erythro- and granulopoiesis. If the pluripotent (PSC) and committed stem cell pools (CSC) have the same DNA synthesis time as granulopoiesis and erythropoiesis one can estimate the size of PSC and CSC pools. The CSC is estimated at 4% of total marrow cells and hence larger in size than *in vitro* colony forming cells (CFC) in the marrow ($1–5/10^4$). If 4% is a fair estimate for CSC then either the CFC is not the CSC or *in vitro* culture grossly underestimates the abundance.

If the CSC really constitutes 4% of the bone marrow the "lymphocytic" pool is the most reasonable place for them to reside.

Others have found the D_0 of CFC's to be circa 100 rads. The limiting dilution assay growing BM cells in diffusion chambers has been used by Carsten et al. to estimate radiosensitivity of human CSC (granulocytic precursor) with comparable results.

Whether stem cells have a finite or infinite capacity to undergo mitosis is fundamentally and clinically important. ^{55}Fe erythroid cytocide (Reincke et al.) has been introduced as a potential method for testing their mitotic capability. Preliminary results will be presented.

D-3-3 *Response of the Stem Cell System to Whole Body and Partial Body Irradiation.* JULIA GIDÁLI, Frédéric Joliot-Curie National Research Institute for Radiobiology and Radiohygiene, P.O.B. 101, 1775-Budapest, Hungary.

The pluripotential hemopoietic stem cells possess not only the capacity of extensive self-replication and differentiation but can also migrate from the bone marrow to repopulate the other hemopoietic organs. It is also wellknown that stem cells of a certain area respond to the damage of similar cells elsewhere in the body.

The response of stem cells to irradiation is not only dose-dependent but is determined also by: a) Dose rate (continuous irradiation, dose-fractionation) b) size of irradiated part (i.e., as to whether part of the body was shielded. The attention, therefore, will be focussed on 1) regeneration kinetics of pluripotential and committed stem cells after acute or continuous irradiation, 2) regeneration kinetics of stem cells in partially irradiated animals concerning both the regeneration of stem cells in partially irradiated areas (as a function of local stem cell concentration, circulating stem cells, neutropenia) and the migration of stem cells out of the shielded part as a function of demand. These data will also be discussed as the possible local or abscopal regulation of stem cell population size.

D-3-4 *Characteristics of the Stem Cell Population Surviving a Sublethal Exposure to Ionizing Radiation.* J. F. DUPLAN, Unité Inserm 117, Fondation Bergonié, 33076 Bordeaux, France.

There is accumulating evidence that the stem cell population surviving a sublethal exposure to ionizing radiation shows characteristic changes. The number of cycling CFUs as well as their rate of replication of is seen to increase, whereas their radiosensitivity decreases, and their differenciation is directed towards the granulocytic series. Consequently, the therapeutic efficiency, which depends on several of the preceeding parameters is also apparently increased. The problem therefore is to investigate into whether these changes are a consequence of a) disturbances in the hormonal, humoral and environmental control of hemopoiesis, b) a selection based on the differential radiosensitivity of the subpopulations which constitute the stem cell pool, or c) a combination of both. On the one hand one might consider that the existence of microcolonies and the increased production of granulocytic clones is responsible for a lack of correlation between the number of spleen macroscopic nodules and the production of both stem cells and differentiated elements. On the other, a unifying concept would be that irradiation selects a stem cell population of low radiosensitivity which is preferentially sensitive to a factor (s) which trigger (s) the ganulocytic differentiation.

D-4-1 *Induction of Immune Tolerance in Rabbits after Gamma-Irradiation: Interference of Hydrocortisone.* DAVID NACHTIGAL, ISRAEL ZAN-BAR, AND MICHAEL FELDMAN, Department of Cell Biology, The Weizmann Institute of Science, Rehovot, Israel.

Immune tolerance can be induced in adult rabbits by antigen stimulation combined with certain types of immunosuppression, as gamma irradiation or treatment with the immunosuppressive drugs 6-mercaptopurine or cyclophosphamide. It was demonstrated that each of these drugs will act synergistically with irradiation in predisposing to the induction of tolerance to human serum albumin (HSA). Hydrocortisone acetate, however, which for itself was shown to be distinctly immunosuppressive, when combined with irradiation will antagonize the induction of immune tolerance. Confirmation of this interference was found in mice. Lethally irradiated mice can be made tolerant to HSA after reconstitution with a mixture of normal syngeneic thymus and bone marrow cells. However, when the thymus cells come from cortisone pretreated donors, the recipients cannot be made tolerant. This suggests that induction of tolerance in this case depends on an active mechanism which requires an intact T cell population, rather than on the elimination of specific immunocompetent clones. Since corticosteroids are employed as immunosuppressants in the management of transplantation cases, the possibility whether they do not interfere in the establishment of lasting tolerance to the grafted tissue should be explored.

D-4-2 *Combined Radiotherapy and Immunotherapy of Spontaneous Primary C3H Mammary Tumors.* DARRELL Q. BROWN, H. GUNTER SEYDEL, AND RICHMOND T. PREHN, Fox Chase Center for Cancer and Medical Sciences, Philadelphia, Pennsylvania 19111, USA.

The purpose of this study was to determine whether the effectiveness of radiotherapy of spontaneous primary tumors can be improved by combined immunotherapy with BCG (Bacillus Calmette-Guerin). Aged female C3H mice with spontaneously occurring tumors were selected to begin treatment when the mean tumor diameter was 5.5 to 7.0 mm. Treatment groups were: local x-ray therapy with 3000 rads (group R), 3000 rads plus 3 weekly intratumoral injections of 2×10^6 live BCG bacteria beginning a week before radiotherapy (RI − 1), a week after radiotherapy (RI + 1), or 3 weeks after radiotherapy (RI + 3). One control group (NT) received neither radiotherapy nor BCG, and the other controls (group I) received 3 weekly intratumoral injections of BCG. Tumors were measured weekly with calipers. Although no tumors were cured, the relative increase in tumor volume in 60 days after start of treatment was significantly less (t-test, $p \leq .01$) in all treated groups as compared with non-treated controls. Radiotherapy alone was significantly more effective ($p \leq .05$) than immunotherapy alone. There appeared to be no significant advantage in adding BCG therapy to radiotherapy for these spontaneous primary tumors.

D-4-3 *Protection Against Radiation-Induced Enhancement of Tumor Metastases in Lung of Mice by Treatment with* C. granulosum. IVAN BASIC, LUKA MILAS, NANCY HUNTER, AND H. RODNEY WITHERS, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77025, USA.

Fibrosarcoma cells injected intravenously into syngeneic C_3Hf/Bu mice generate tumor nodules (metastases) on pleural surfaces of the lung that can be easily quantitated 14 days after injection. Treatment of mice with *Corynebacterium granulosum* (CG), a potent nonspecific stimulant of the reticuloendothelial system, greatly reduced the number of metastatic nodules. In contrast, the number of pulmonary metastases was markedly increased in animals irradiated to the whole body with 200 to 600 rads of γ-rays one day prior to tumor cell inoculation. Treatment of mice with CG before irradiation not only abolished this effect of irradiation, but produced an antitumor response equal to that in mice treated with the immunostimulant alone. CG protected the recipients against radiation-induced enhancement of lung metastases if given within 2, but not at 4 weeks prior to irradiation. However, if animals were first irradiated and then 1 or 2 days later treated with CG, the radiation effect on formation of pulmonary metastases was not affected. The inability of irradiation to promote formation of lung metastases in CG treated mice seems to depend upon the tumoricidal activity of radioresistant macrophages.

D-4-4 *Combination of Radiotherapy and Nonspecific Immunotherapy in Treatment of a Mouse Fibrosarcoma.* LUKA MILAS, NANCY HUNTER, AND H. RODNEY WITHERS, M.D., Anderson Hospital and Tumor Institute, Houston, Texas, 77025, USA.

We studied whether the effectiveness of local irradiation of a syngeneic fibrosarcoma in C_3Hf/Bu mice could be increased by treating the tumor hosts with *Corynebacterium granulosum* (CG), a very potent immunostimulant. Tumor cells were inoculated into the right thigh of mice and the developing tumors were irradiated with 2500 rads when they had grown to 8 mm in diameter. CG (0.5 mg i.p.) was injected into the mice 3 to 4 days before irradiation, or at 3 hours, 2, 7 or 14 days after irradiation. The irradiation alone induced permanent regression of only 18% of tumors. When combined with nonspecific immunotherapy between 27 and 50% of animals were cured, depending upon the time of CG treatment. CG alone given to mice when their tumors were 8 mm in diameter slightly slowed the growth of some tumors. More recently, we studied the effect of this combined therapy on TCD50 values (radiation dose that cures 50% of recipients) for fibrosarcoma and the data so far indicate that the TCD50 is lowered by this treatment. It appears, therefore, that immunotherapy with CG can improve the efficiency of local radiotherapy.

D-4-5 *Radiotherapy and Immunotherapy of Fibrosarcoma of C3H Mice.* C. W. SONG, S. K. S. SO, R. L. SIMMONS, AND S. H. LEVITT, University of Minnesota Medical School, Minneapolis, Minnesota 55455, USA.

The combined effect of radiotherapy and immunotherapy with VCN (Vibrio cholerae neuraminidase) treated syngeneic tumor cells has been studied using a 3-methylcholanthrene induced fibrosarcoma of C3H/HeJ mice. Local irradiation of tumors growing in a thigh with 2000 rads of x-rays in a single exposure induced a significant retardation of tumor growth and a regression of 6 out of 16 tumors. Subcutaneous injections of 10^6 tumor cells treated with VCN and mitomycin every other day for 6 treatments resulted in a slight retardation of tumor growth and a regression of 1 out of 16 tumors. Of the 12 tumors treated with 2000 rads and VCN-treated cells, 7 tumors regressed completely. It was concluded that the combination of radiotherapy and immunotherapy is more effective than with either of these alone. Results of different radiation doses and timing of immunotherapy will also be presented. (Supported by a research grant from the Minnesota Division of American Cancer Society.)

D-4-6 *Stimulation of Immune Response by Adjuvant During Radiotherapy of a Mouse Fibrosarcoma.* HAROLD MOROSON AND MARVIN Z. ROTMAN, Department of Radiology, New York Medical College, New York, N.Y. 10029, USA.

Destruction of lymphocytes in regional lymph nodes during tumor radiotherapy can decrease the cell mediated component of immune resistance to that tumor. A means has been sought by adjuvant RES stimulation to compensate for this loss in total number of immune active cells. Injection i.p. of the immune adjuvant BCG or *C. parvum* in the C57/BL mouse prior to local fractionated x-radiation ($1000\ r \times 4$) of a transplanted methycholanthrene induced syngeneic fibrosarcoma, is found to increase host survival over that of animals receiving only x-radiation. These adjuvants alone had little effect on host survival, though they were effective if administered prophylactically. We presume this result is due to immunological stimulation of host response during radiotherapy. A valid conclusion is impossible however until challenge tumor growth in tumor host cured by this means and by radiation alone are compared.

Local x-radiation of the mouse hip was found to enhance the humoral response to SRBC antigen as determined by Jerne plaque assay. Immune adjuvant also enhanced the humoral response, which was not further increased however when combined with x-radiation. (Supported by Research Grant CA 14374 from the National Cancer Institute, NIH.)

D-4-7 *Alteration of the Immune Response to Transplantation by Beta-Irradiation of Circulating Blood.* JAMES S. WOLF, Department of Surgery, Medical College of Virginia, and McGuire Veterans Administration Hospital, Richmond, Virginia 23298, USA.

The development of transplant immunity and destruction of a transplanted organ are related to lymphocyte activity. The marked radiosensitivity of the lymphocyte both *in vitro* and *in vivo*

make the application of radiation to the lymphocyte a method of immunosuppression. Our studies of the effect of chronic irradiation of the extracorporeally circulating blood have examined this modality in animals and man, and have examined the effect on allografted organ survival. The effects of lymphopenic response in animals and man has been examined with applicators using Sr^{90} as an encapsulated source, and efficiency of several applicators of varying geometry have been compared.

The studies conclude that extracorporeal irradiation alone can produce significant immunosuppression in animals, and, in man, can favorably affect the early post-transplant period, when patient and graft are at highest risk.

D-4-8 *Chronic Irradiation of Blood with a Portable Irradiator.* F. P. HUNGATE, W. R. REIMATH, L. R. BUNNELL AND M. F. GILLIS, Biology Department, Battelle Pacific Northwest Laboratory, Richland, Washington 99352, USA.

Periodic acute irradiation of blood pre- and postimplantation has been shown to suppress rejection of renal transplants, and there is limited evidence that chronic blood irradiation may be even more effective. A lightweight portable irradiator has not been available for more extensive clinical evaluation of chronic blood irradiation; however, we are now developing such a device. The cylindrical irradiator is fabricated of vitreous carbon. Advantages are its low Z, potential blood compatibility and resistance to radiation damage and neutron activation. The unit contains $^{169}Tm_2O_3$ microdispersed in a thin cylindrical region near the irradiator lumen. This composite is neutron activated to produce ^{170}Tm which decays by emission of a 0.96 MeV beta ($T_{\frac{1}{2}}$ = 125d). Weighting but 2 g, the total device weight (after addition of bremsstrahlung shielding) is less than 200 g. Transit dose (at 100 ml/min blood flow rate) was 21 rads in the first prototype. The first prototype was applied to an arteriovenous shunt (common carotid artery and external jugular vein) of a 20 kg goat. Small lymphocytes dropped to 15% preirradiation level within 7 days and the unit was removed at 11 days. Lymphocyte recovery is being followed. A reciprocal skin allograft performed immediately postirradiation survived twice as long (24 days) on the irradiated animal as on its nonirradiated control (12 days). Studies of lymphocyte response and allograft survival using other dose regimens are in progress. (Research performed under Contract AT(45-1)-1830 between the United States Atomic Energy Commission and Battelle Memorial Institute.)

D-4-9 *Restoration by Immunocompetent Cells of the Chemotherapy Efficiency Abolished by Total Irradiation in Experimental Trypanosomiasis of R and Wistar Rats.* TRAIAN T. ANDRIAN, OCTAV H. COSTĂCHEL, AND I. CORNECI, Oncological Institute, Bucharest 12, Romania.

In a previous paper we showed the negative influence of the ionizing radiations on the therapeutic efficiency of neosalvarsan in the experimental rat trypanosomiasis. The efficiency of neosalvarsan is significantly reduced due to inhibition of immunological reactivity in the irradiated animals.

In order to increase the immune competence of the whole body irradiated (700 rads) rats infested with *Trypanosoma brucei* and treated with neosalvarsan (30 mg/kg), we added to chemotherapy an immunologic treatment consisting of intravenous and intraperitoneal administration of lymphnodes and/or peritoneal syngeneic and allogeneic cells in various doses (25×10^6–50×10^8/animal) simultaneous with the neosalvarsan (in single or more sequential administrations). This chemo-immunological treatment has produced an increase of the survival rate of totally irradiated and infested rats, emphasizing the importance of the immune mechanisms in increasing the therapeutic efficiency of various drugs administered to the irradiated organisms.

Our results suggest a new therapeutic schedule for the irradiated and immunorepressed animals. The necessity of some simultaneous immunotherapeutic methods for the reestablishment of the immune reactivity is obvious and could be attempted.

D-5-1 *Biokinetics and Dosimetry of ^{113}Sn and ^{119m}Sn.* HANS DETLEV ROEDLER, DIETER M. H. GLAUBITT, AND TRAUTE VOGELSANG, Nuklearmedizinische Abteilung, Klinikum Steglitz der

FU, 1 Berlin (West), and Institut für Nuklearmedizin, Städtische Krankenanstalten, D-415 Krefeld, West Germany.

As ^{113}Sn contamination of eluates from ^{113m}In generators may occur under certain circumstances, the radiation dose caused by ^{113}Sn was of interest to us. In order to obtain the necessary biokinetic data distribution studies in animals were performed after intravenous administration of ^{119m}Sn hexachlorostannate (IV). ^{119m}Sn was chosen instead of ^{113}Sn for technical reasons with regard to the measurement of radioactivity. Based on studies using ^{119m}Sn the absorbed dose both for ^{119m}Sn and ^{113}Sn in the total body and 11 organs was calculated according to the concept of absorbed fractions.

The absorbed doses from ^{119m}Sn and ^{113}Sn are highest in the liver (210 mrad/μCi ^{119m}Sn and 240 mrad/μCi ^{113}Sn) whereas in the kidneys about one third of these values is estimated. The absorbed dose in the total body amounts to 4,6 mrad/μCi ^{119m}Sn and 6,6 mrad/μC ^{113}Sn.

If the distribution factors are corrected for the different proportions of the mass of the organs to the mass of the total body in the rat and in man, the resulting doses in liver and kidneys are approximately half as high as the uncorrected values.

D-5-2 *The Distribution of Bismuth-206 in Normal Rats.* GERALD A. RUSS, ROY S. TILBURY, RODNEY E. BIGLER, HELEN Q. WOODARD, AND JOHN S. LAUGHLIN. Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA.

A study was made of the organ and tissue distribution and of the kinetics of retention and excretion of Bi-206 citrate and chloride in normal rats. Large female rats of an inbred strain were injected I.P. with 180 μCi. of Bi-206 citrate or chloride and the animals were immobilized and counted over a NaI crystal at selected times. Groups of animals were sacrificed at designated times and eighteen organs and tissues were excised and counted. The whole body clearance of Bi-206 was found to have two components. About 70% of the injected dose was cleared with a biological half time HT(b) of about one day. The HT(b) of the other component was about one week. The highest concentration was found in the kidneys, followed by the liver and spleen in which the HT(b) were about 3 days, 8 days and 36 days respectively. The equivalent integrated radiation dose to the kidney was estimated to be 8.0 rads/100 μCi/70 kgm man. (Research supported in part by AEC-(11-1)-3521 and by NCI 08748-08B.)

D-5-3 *Studies on the Radioactive Ratio of Yttrium-90 to Stontium-90 in the Rat Skeleton after Incorporation of Strontium-90.* A. F. G. STEVENSON, Unité Inserm, 117 Bordeaux, France.

Radiobiological studies on the problem of ^{90}Sr and ^{90}Y have often been conducted independently. Any attempt to make an evaluation of the radiation burden must take into account both the mother and daughter isotopes because of their Secula Equilibrium.

As Yttrium and Strontium each follow different metabolic pathways, the distribution of the two radioisotopes within the organism is not equal. In this study, attempt has been made to throw some light on different factors that influence the biological behavior of these two radioisotopes.

D-5-4 *The Radioisotope Concentration Effect: Differences of Biological Behaviours Between Stable and Radioactive Cesium Chlorides.* P. GUILLOT, C. MYTTENAERE, AND J. M. MOUSNY, Commission of European Communities, Environmental Research Programme, Bruxelles, Belgium.

Previous experiments indicated differences of biological retentions between stable cesium and radioactive cesium, in various conditions. Particular experiments with radioactive xenon inhaled by people and with radioactive cesium absorbed by tomato plants showed an influence of the radioisotope concentration in the medium on the biological absorption or retention of the same radioisotope: this radioisotope concentration effect has important consequences.

New experiments were planned to avoid systematic errors, ensuring: physico-chemical equilibrium between radioactive and stable cesium chlorides—double method of analysing stable cesium, by radioactivation and spectrophotometry—constancy of the nutrient medium during the whole culture.

Factorial analysis of variance of results studies the influence of three parameters, including the radioactive cesium concentrations and the stable cesium concentration, on the dry weights of plants, the 137 Cs concentration factors and the specific activities of plants. The radioactive concentration influence on all these variables is noticeable.

In most of the conditions, the radioactive cesium chloride does not trace the stable cesium chloride.

D-5-5 *Autoradiographic Studies on the Relationship between the Translocation of Polymeric Plutonium-239 and Iron Deposition in Mice.* MASATOSHI KASHIMA, HISAMASA JOSHIMA, AND OSAMU MATSUOKA, National Institute of Radiological Sciences, Chiba, Japan.

It seems reasonable to assume that translocation of polymeric Pu caused by death of Pu containing cells and incorporation of Pu into iron deposited cells is one of the factors for evaluation of α-radiation response in cells. Whole body and micro autoradiography of mice injected with polymeric Pu at the concentration levels 15 μCi and 30 μCi/kg demonstrated that: in the liver, polymeric Pu deposited initially at perilobular parts, diffused to central veins with time and, was found increase by incorporation within Kupffecells deposited iron. Significant increase of iron deposition in hepatocytes near around the portal veins was observed in moribund mice at 30 μCi/kg level.

In the spleen, initial deposition site of polymeric Pu was the marginal zone of white pulp which showed low iron storage. Pu particles were seen to migrate to the outer, higher iron containing red pulp and to be incorporated with the deposited iron.

In this presentation Pu particles in blood and fate of macrophages deposited polymeric Pu will be discussed with the relation to above results.

D-5-6 *Plutonium Metabolism in Newborn and Weanling Pigs.* BEATRICE J. MCCLANAHAN, HARVEY A. RAGAN, AND D. DENNIS MAHLUM, Biology Department, Battelle, Pacific Northwest Laboratories, Richland, WA 99352, USA.

Monomeric plutonium was injected intravenously into newborn and weanling miniature pigs to study its metabolism. As reflected by the femur, the skeletal deposition and retention of ^{239}Pu was the same for the two age groups. However, initial liver deposition in the newborn animals was only about one-fourth of that found in the weanling liver. At the end of the 28-day experiment the ^{239}Pu content of the newborn liver had increased three fold to 11% while the Pu level of the weanling liver increased from an initial deposition of 14% to 20% of the administered plutonium over the same time interval. No major differences between the two age groups were seen in the other tissues sample. At the end of the first seven days the newborn pigs had excreted 1.4% (1.1% fecal, 0.3% urinary) of the plutonium and the weanling pigs had excreted 1.6% (1.1% fecal, 0.5% urinary). (This paper is based on research performed under United States Atomic Energy Commission Contract AT(45-1)-1830.)

D-5-7 *Influence of Age on the Late Effects of Plutonium-239 in Rats.* D. D. MAHLUM AND M. R. SIKOV, Biology Department, Battelle Pacific Northwest Laboratories, Richland, WA 99352, USA.

This study was designed to examine the influence of age at the time of exposure on the late effects, particularly tumorigenesis, produced by the administration of plutonium-239 to Wistar rats. Rats were injected with monomeric ^{239}Pu at dose levels selected to deliver radiation doses of approximately 7, 23, or 70 rads to the femur during the first 10 days postexposure. Adults and weanlings were exposed by intravenous, and newborns by intracardiac injection, while the prenatal animals were exposed by intravenous injection of the dam. The cumulative radiation doses to bone for the prenatal and newborn groups, as well as their chronological ages for any given time at risk, were markedly different from these of the weanlings and adults. In general, there was a dose-dependent reduction in life spans in the adult and weanling groups. This was less pronounced in the newborns while the groups exposed prenatally showed no reduction. In the adults, the bone tumor incidence increased with increasing dose. The incidences of bone tumors in the weanlings and newborns, but not in those exposed prenatally, were comparable to those for the adults when cumulative radiation doses to bone and chronological age are considered. The anatomical distribution of bone tumors was also influenced by age at time of exposure. (Research performed under Contract AT(45-1)-1830 between the United States Atomic Energy Commission and Battelle Memorial Institute.)

D-6-1 *Evaluation of Potential Dosages to Man on Enewetak Atoll.* Y. C. Ng, W. L. Robison, and D. W. Wilson, Biomedical and Environmental Research Program, Lawrence Livermore Laboratory, Livermore, California 94550, USA.

A comprehensive radiological survey of Enewetak Atoll was carried out by the U.S. Atomic Energy Commission in 1973. It purpose was to obtain data to guide the U.S. plans for clean-up and rehabilitation of this former nuclear weapons test site to insure safe return of the Enewetakese. The Lawrence Livermore Laboratory, in cooperation with a number of additional university and government laboratories had responsibility for development and implementation of the survey and evaluation.

This presentation covers the overall design of the survey, summarizes survey data, and emphasizes the results of dose evaluations made for several exposure pathways: external gamma-radiation, inhalation, terrestrial food chains, and marine food chains. As a result of a wide range of radionuclide concentrations from island to island, potential dosages are dependent upon expected living patterns. The contribution of the four pathways to the population dose in descending order are: terrestrial food chain, external gamma, marine food chain and inhalation pathway. Plutonium is the primary potential contributor through the inhalation pathway. Dosage via the marine food chain is largely insensitive to catch location and species and is largely due to 137Cs and 90Sr. Cesium-137 and 60Co contribute most of the dose via the external gamma pathway. The highest potential dose results from 90Sr and 137Cs through the terrestrial food chains. Population dosages can be minimized by planning in areas such as clean-up, location of homes, agricultural development, and dietary considerations. (This work was performed under the auspices of the United States Atomic Energy Commission.)

D-6-2 *Turnover of Radionuclides in Oysters in the Vicinity of a Nuclear Power Plant.* Florence L. Harrison, Robert E. Heft, and Kai M. Wong, Biomedical Division, Univ. of California, Lawrence Livermore Laboratory, Livermore, California 94550, USA.

^{54}Mn, ^{60}Co, ^{65}Zn, and ^{137}Cs were followed in oysters introduced into a discharge canal receiving waste from a boiling water reactor. Waste was released at irregular intervals and contained radionuclides in variable concentrations. Radionuclides were determined in the animals after a single release (6 hr), a group of releases (1 month), or a long series of releases (18 months). Radionuclides were determined also in the water and suspended and settled particulates. Stable Mn, Co, and Zn were analyzed in the oysters and selected samples of water and particulates.

In the oysters, radionuclide concentrations reflected those in the water and particulates. In the canal water, concentrations changed rapidly during a release, reaching peak values within 30 minutes. Oysters held in filtered canal water had radionuclide concentrations lower than oysters in nonfiltered water; ^{60}Co concentrations decreased the greatest.

During the 18-month period, concentrations of Co, Mn, and Zn per gram ash in oysters varied a factor of about two. Concentration factors (animal/water) were highest for Zn and lowest for Co. (This work was performed under the auspices of the U.S. Atomic Energy Commission.)

D-6-3 *A Stochastic Model for the Concentration Process of Radioactive Substances.* I. Aoyama, M. Yamamoto, and Y. Inoue, Kyoto University, Yoshida Honmachi, Sakyoku, Kyoto, Japan.

The purpose of this report is to consider the problem of the distribution of radioactive concentration in aquatic organisms on the concentration process. From this point of view, an attempt is made to explain the radioactive contamination of organisms as a time series using a birth-death process, which is one of the stochastic processes.

The existing probability $P_i(t)$ that the concentration step of radioactive substances in aquatic organisms is in the concentration step "i" at an arbitrary time t is given by the Poisson distribution and the parameter of the distribution is equal to the mean concentration step obtained from an exponential model. The experiments are performed to verify the stochastic model by using fresh water fishes, top minnows. From the experimental results, it is found that the theory can be applied generally to both cases in which the radioactive concentration in the environmental water is maintained constant and in which the concentration varies with time t.

This analytical method will be applied to the concentration process of radionuclides through a food web system.

D-6-4 *Lethal and Teratogenic Effects of Plutonium-239 on Carp* (Cyprinus carpio) *Embryos.* JOHN R. TRABALKA AND L. DEAN EYMAN, Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

There is a singular lack of information on the effects of Pu-239 on aquatic biota. Since the vertebrate embryo represents the most radiosensitive stage in the life cycle, data on the effects of Pu-239 on the fish embryo is essential to aid in assessment of plutonium releases. Carp gametes were mixed in silicone-treated dishes containing a range of concentrations of plutonium solutions (5×10^{-2} to 5×10^{-5} μCi/cc). Samples of eggs were collected at a number of intervals during incubation to determine the distribution and concentration of Pu-239 within the eggs. Subsamples of eggs were fixed and prepared for α-counting and autoradiography. Several replicates, exposed to either Pu-239 polymer or citrate, were scored for percentage hatch, malformation frequency, at 72 hrs post-fertilization (hatching time at 26°C). Effects of Pu-239 on the fish embryo are compared to reported effects of other types of radiation exposure. (Research sponsored by the U.S. Atomic Energy Commission under contract with Union Carbide Corporation.)

D-6-5 *Gastrointestinal Absorption and Retention of Chelated Plutonium by Channel Catfish* (Ictalurus punctatus). L. DEAN EYMAN AND JOHN R. TRABALKA, Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

Environmental chemical (chelation) transformations of plutonium are expected to occur in moist soils, forest litter, and aquatic ecosystems. The effects of such transformations on absorption and retention of plutonium ingested by aquatic biota are poorly understood. Channel catfish, maintained at 24°C in a flow-through system, were divided into six treatment groups. Each treatment group was dosed, by gavage, with 1 μCi of Pu-237 ($T_{1/2}$ = 45.6d, electron capture 99+%, α .003%) in one of the following chemical forms (oxide, nitrate, citrate, humic acid complex, protein complex, and phenolic derivative). Doses were mixed with diatomaceous earth and enclosed in gelatin capsules. After a 90-day period of repeated live counting, individuals were sacrificed in order to determine tissue distribution of retained Pu-237. Biological absorption characteristics of complex and non-complexed plutonium are compared. Differences in uptake and retention between fish and other animals are discussed. (Research sponsored by the U.S. Atomic Energy Commission under contract with Union Carbide Corporation.)

D-6-6 *The Metabolism of Iron-59 in the Rough-Skinned Newt,* Taricha granulosa. DAVID L. WILLIS AND DAVID J. KORWIN, Oregon State University, Corvallis, Oregon 97331, USA.

The whole-body retention of ^{59}Fe in the newt (*Taricha granulosa*) was determined over periods of 4–5 months at 10° and 23°C following a single intraperitoneal injection. These results were subsequently compared to the retention pattern after a single feeding of ^{59}Fe-labeled worms at each temperature indicated. The longer, and ecologically more significant, loss components were clearly affected by temperature. For newts injected with ^{59}Fe the long-term loss components showed T_b values of 1,104 and 796 days for the 10° and 23°C temperature groups, respectively. Approximately 80% of the administered ^{59}Fe was involved in this component. Only 4–8% of the ^{59}Fe administered via the labeled worms appeared in the long-term loss components. T_b values were much shorter.

The pattern of ^{59}Fe distribution in twelve tissues and organs of the newt was traced for 100 days following injection at 10°C. Most tissues showed gradually decreasing concentrations of ^{59}Fe with time. At 100 days the kidneys accounted for 42%, the total circulating blood for 34%, and the liver for 11%, of the body burden. The radioecological significance of these results are considered.

D-6-7 *Studies on Cd^{115m} Uptake in Rat Tissues.* KULDIP C. KANWAR AND SUBHASH KAUSHAL, Biophysics Department, Panjab Univ., Chandigarh, India.

Uptake of orally administered Cadmium^{115m}—an industrial pollutant, and effective mammalian male sterilant, has been studied in various rat tissues.

Male albino rats, each weighing 240–290 gms, were administered single oral dose (0.2 uci/gm body wt.) of Cd^{115m}. The animals were sacrificed and Cd^{115m} levels, were determined in blood,

testes, epididymes, seminal vesicles, prostate, adrenals, spleen, kidneys, liver, ileum, duodenum and muscles.

The peak Cd^{115m} levels in liver, spleen, adrenals, duodenum and ileum were touched before 48 hrs post-treatment, whereas in kidney and muscles peak was attained after 7th day.

The highest Cd^{115m} level was initially encountered in the duodenum and ileum. Liver and kidney retained most of the Cd^{115m} and exhibited low turn over rate. Cd^{115m} levels of post peak intervals in liver and kidney however markedly varied. Cd^{115m} uptake in the spleen, adrenals and muscles is significantly low while in testes, epididymes, seminal vesicles and prostate no detectable activity could be recorded.

The differential Cd^{115m} uptake in nuclear and cytoplasmic fractions, as well proteinous/non-proteinous fractions of liver and kidney were also studied.

Studies reveal that more than 70% of the orally administered Cd^{115m} is excreted through the faeces by 5th day of oral dosing.

Cd^{115m} uptake after oral dosing is meagre when compared with parenteral administration.

Physiological significance of the data shall be discussed.

D-7-1 *International Intercomparison of Neutron Dosimetry.* R. S. CASWELL, National Bureau of Standards, Washington, D.C. 20234, L. J. GOODMAN AND R. D. COLVETT, Radiological Research Laboratories, College of Physicians and Surgeons, Columbia University, New York, New York 10032, USA.*

An International Neutron Dosimetry Intercomparison (INDI) has been carried out at the Radiological Research Accelerator Facility (RARAF) at Brookhaven National Laboratory under the sponsorship of the International Commission on Radiation Units and Measurements. The objective was to compare results obtained by different groups performing fast neutron dosimetry in situations approximating those generally encountered in radiotherapy and radiobiology. Twelve groups representing six countries participated. Intercomparison energies for tissue kerma in free air were 15.5, 5.5, 2.1, and 0.63 MeV and the ^{252}Cf fission neutron spectrum. In addition, depth dose measurements in a phantom were made at the two highest energies. The experimental arrangements will be described, and preliminary results of the intercomparison given.

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D-7-2 *Dosimetric Parameters and Terminology Relevant to Radiobiology.* R. OLIVER, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 OHS, Great Britain.

ICRU has set up a small Working Party to consider the rewriting and expansion of Publication No. 10e on Radiobiological Dosimetry (1962). This paper is to be taken as an opportunity to refer to proposals in this connection and to obtain some discussion from workers in the field. Consideration of the concepts of biological dosimetry and of physical parameters such as absorbed dose, specific energy, linear energy transfer and radiation quality will be followed by some discussion of the possibilities for the calculation and measurement of absorbed dose with different types of radiation under the conditions of experimental radiobiology. To allow intercomparison of results, it is felt to be important to encourage standard methods of specification of irradiation conditions and dose. Equally, some standardisation of terminology is proposed in the description of dose response relationships, the factors on which they depend and the ratios (such as RBE and OER) used in their comparison. Reference will be made to the particular difficulties in the interpretation of such ratios in the clinical situation at which much radiobiology research is aimed.

D-7-3 *Microdosimetry of Auger Electrons.* Y. FEIGE AND A. GAVRON, Soreq Nuclear Research Center, Yavne, Israel.

Due to the extremely short range of the Auger electrons produced by electron capture, the local energy deposited in cells or subcellular structures may exceed by far the average dose as calculated by conventional radiation dosimetry. For these cases therefore microdosimetry

concepts seem more appropriate. The use of radionuclides which decay by electron capture, such as ^{55}Fe, ^{76}Ga, ^{75}Se, ^{123}I, ^{125}I, ^{197}Hg and many others, is being extended rapidly in medicine and radiobiologic research. Iodine-125 has been extensively used in the study of different and unrelated biological systems. Its effect on DNA molecules as well as on thyroid glands of humans and experimental animals will be reviewed. Most of the apparent discrepancies regarding the relative biological effectiveness of ^{125}I as compared to ^{131}I or with other radionuclides may be reconciled by the microdosimetric approach. The possible biological significance of molecular disruptions, resulting from the multiple charged ^{125}Te ion left after the shake off of several electrons will also be discussed. A deeper understanding of biophysical interactions in relation to microstructure is needed to derive satisfactory dose-response relationships at the macromolecular and cellular levels.

D-7-4 *Particle Dosimetry by Track Etching, with Applications to Apollo Astronauts.* ROBERT L. FLEISCHER, General Electric Research Laboratory, Schenectady, New York 12301, USA.

The passage of heavily ionizing, nuclear particles through most insulating solids creates narrow paths of intense damage. These damage tracks may be revealed and made visible in an ordinary optical microscope by treatment with a properly chosen chemical reagent that rapidly and preferentially attacks the damaged material. The holes that are made in this manner may be counted individually or by various integrating methods. Track etching has been applied widely for dosimetry of neutrons, high energy particles, and heavy ions. The major techniques may be divided into two categories. Most frequently particle detection has been indirect, the particles of interest not being registered but making themselves known by induced reactions that give detectable particles. A simple example is the observation of fission fragments from ^{235}U (n, fission) reactions to measure the thermal neutron flux. By using different activated nuclides, neutron energy spectra can be found. The second, more direct form of dosimetry is that in which the particles of interest are recorded directly. For example particles that individually are cytologically damaging can be recorded in polycarbonate plastic such as has been used for Apollo space helmets. Their tracks give a measure of the radiation hazard from heavy ions to which the astronauts were exposed.

D-8-1 *Dissipation of UV Energy in Nucleic Acids and Nucleoproteins.* J. W. LONGWORTH, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830, USA.

A fluorescence and phosphorescence is found from all of the heterocyclic bases of nucleic acids, and this permits an investigation of the channels of energy dissipation in nucleic acids. The luminescence is subject to thermal activated quenching and is only readily detected at low temperatures. The separate heterocyclic bases have individual features allowing characterization in complex heteropolymers, particularly by the phosphorescence, spectra and triplet decay times. The series nucleotide, dinucleotide, homopolymer and heteropolymer (random and seqeuntial) has been studied to follow the perturbation imposed when residues become incorporated into nucleic acids. Two striking features are present in dinucleotides which also apply to polymers. The fluorescence of many dinucleotides is subject to a significant red shift and is featureless. This is termed an exciplex fluorescence and results from contiguity of heteroaromatic rings. The phosphorescence originates in a singlet base, and is the base with the lowest energy triplet state. Excitation spectra show both bases contribute to this phosphorescence, there has been a transfer of electronic energy. DNA in both the duplex and the single stranded stacked conformation exclusively phosphoresces from thymidylyl residues (T). A phage DNA, PBS2, which replaces T with uridylyl (U) exclusively phosphoresces from U. The absence of emission from the other bases is attributed to transfer and this is supported by excitation spectral studies. The fluorescence lifetime of duplex DNA is 10 ns at 77°K, reflecting the exciplex nature. This is an unusually long lifetime and suggests that considerable electronic transfer would occur within a polymer. Anisotropy of fluorescence of polyA is depolarized, but only to the same extent as ApA, indicating that there is only a small degree of energy migration. Exogenous ligands which quench the singlet and triplet show there is only a small amount of electronic energy transfer in DNA at singlet and triplet level. The predominant process of energy dissipation in nucleic acids is radiationless and thermally activated. To derive information about DNA

at 300°K it is necessary to study DNA at much lower temperatures and extrapolate. The internal quenching of the fluorescence of heterocyclic bases occurs through a temperature independent and a temperature dependent channel. The temperature dependent internal conversion is subject to a compensation law, the pre-exponential rate linearly correlates with the activation energy. Intersystem crossing to the triplet is a relatively small process at 77°K and for several of the bases is significantly increased at 300°K. Photochemical reactions proceed from both the singlet and triplet levels and occur in C, T and A in homopolymers. In DNA T and C are photochemically reactive. The limited studies on nucleoprotein complexes have not disclosed any significant interaction of the excited states of nucleic acids and protein. (Research sponsored by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.)

D-8-2 *Genetic Effects of UV on* Escherichia coli*—a Model for Prokaryotes.* B. A. Bridges, MRC Cell Mutation Unit, University of Sussex, Falmer, Brighton, BN1 9QG, Sussex, Great Britain.

The induction of base pair substitution mutations by UV in excision-deficient *E. coli* occurs during the operation of DNA repair processes dependent upon the *exrA*$^+$ (= *lex*$^+$), *exrB*$^+$, and *recA*$^+$ genes. These genes are also involved in mutation induction after ionizing irradiation but whereas a linear dose response curve is obtained with ionizing irradiation, UV almost always gives a curve with approximately dose-squared kinetics. Two explanations have been proposed for the UV curve. One states that the mutagenic repair system is inducible (similar to the system involved in the induction of prophage λ), such that increasing UV doses both produce more premutagenic lesions (pyrimidine dimers) and also progressively induces the errorprone repair system. An alternative hypothesis is that two lesions are involved in mutagenesis and that one of these lesions is nonphotoreversible and promotes the mutagenicity of photoreversible pyrimidine dimers. The dose-square response curve on this hypothesis would reflect the increasing probability with increasing dose that a pyrimidine dimer at an appropriate site will be accompanied by a promoting lesion. The author will discuss the mechanisms of UV mutagenesis in the light of these hypotheses and of recent data.

D-8-3 *The Present Status of DNA Repair Mechanism in UV Irradiated Yeast.* E. Moustacchi, Fondation Curie-Institut du Radium, Biologie, Orsay (91), France.

Yeast cells can be taken as a model eucaryotic system for studying the physiological and genetical control of radiation or chemically induced DNA damages. A variety of repair-related phenomena have been analysed in these cells: photoreactivation and photoprotection, post-irradiation dark holding, mitotic and meiotic inter and intra genic recombination, induced nuclear and mitochondrial mutations, "diploid repair." Radiosensitive mutants affected in their response to radiation induced lethality and/or nuclear and cytoplasmic mutations or recombination, have been isolated and genetically characterized.

A part from nuclear and mitochondrial photoreactivation only excision repair of UV-induced pyrimidine dimers in yeast nuclear DNA has so far been demonstrated at the molecular level. Such damages do not appear to be selectively removed from mitochondrial DNA on dark liquid holding and extensive post-UV degradation of this type of DNA is observed. Nevertheless biological data on the cytoplasmic "petite" induction do show that the respiratory character is not irrevocably determined after the UV treatment. Hence the possible repair of genetic mitochondrial damages should involve degradation and recombinational events.

Data concerning periodic fluctuations in UV sensitivity to lethal and cytoplasmic mutation of wild type and radiosensitive mutants synchronized populations will be presented. They show that when variations occur they are mainly related to changes in repair abilities within the cell cycle. The respective contribution of the different repair pathways at the various stages will be discussed.

D-8-4 *Genetic Effects of UV on Phages—a Model for Viruses.* M. Radman, Belgium.

D-9-1 *Complete Excision of Ultraviolet-Induced Pyrimidine Dimers from the DNA of Human Cells.* James D. Regan and W. L. Carrier, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

In 1968 we reported the excision of ultraviolet (UV)-induced pyrimidine dimers from the DNA of human cells (Biophys. J. 8, 319, 1968). Those experiments employed UV doses from 50–150 ergs/mm² and showed that about 50% of the dimers induced in these cells were excised. With refinement of techniques and scintillation counters of higher efficiency, we can now assay dimer excision at doses of 25 ergs/mm². We find, under these conditions that dimers are completely excised to control levels in human cells and that the rate of dimer excision is approximately the dimer equivalent of 5 ergs/mm²/hr, or, about 40,000 dimers/hr. The data suggest that real physiological ranges for dimer excision in human cells is probably 0–30 ergs/mm² and that the same absolute amount of dimers is excised no matter what the dose; previous studies at higher doses extrapolate to 100% excision at lower doses. (Cleaver and Trosko, Photochem. Photobiol. 11, 547, 1970; Setlow *et al.*, Proc. Nat. Acad. Sci. 64, 1035, 1969; Setlow *et al.*, Biophys. Soc. Abst. 16, 19a, 1972). This would explain why some workers (Klimek, Studia Biophys. 19, 243, 1970) failed to observe any appreciable dimer excision in cells receiving 1000–3000 ergs/mm², a dose equivalent to an initial dimer induction level of 0.5–1.3% dimers. (Research jointly sponsored by the National Cancer Institute, and by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.)

D-9-2 *Excision of Damaged Thymine from γ-Irradiated Polyd(A-T) by Isolated Nuclei from Cultured Human Cells.* J. Remsen, M. Mattern and P. A. Cerutti, Dept. of Biochemistry, University of Florida, Gainesville, FL. 32601, USA.

We have studied the removal of damaged thymine residues of the 5,6-dihydroxydihydrothymine type (t′) from γ-irradiated or OsO_4-oxidized polyd(A-T) by isolated nuclei from HeLa cells and lungfibroblasts WI 38. The t′-content in polyd(A-T) was determined by the alkali-acid degradation assay of Hariharan and Cerutti (Biochemistry, in press). Within 30 min. incubation at 37°, WI 38 nuclei had excised 25% of t′ from acid precipitable polyd(A-T) which had been irradiated with 22 Krads of ^{137}Cs-γ-rays under nonprotective, aerobic conditions. During the same time period, only 6% undamaged thymine residues had been rendered acid soluble. A similar result was obtained with HeLa nuclei using OsO_4-oxidized polyd(A-T) containing a low level of 5,6-dihydroxy-dihydrothymine but no adenine damage or γ-ray induced strand breaks.

It is concluded that nuclei from human cells possess an excision repair system which accomplishes the selective removal of ring-damaged thymine residues in the presence or absence of radiation-induced strand breaks. (Work supported by the U.S. Public Health Service and U.S. Atomic Energy Commission.)

D-9-3 *Postirradiation Repair in Mammalian Cells Induced by Viral DNA.* Danilo Petrović and Ana Ferle-Vidović, Institute "Ruder Bošković," 41001 Zagreb, Yugoslavia.

Postirradiation treatment by exogenous DNA or by its precursors can increase the survival of mammalian cells. Relevant data suggest that this effect is due to the action of these materials on the disturbed DNA metabolism in the irradiated cells. Improved DNA synthesis could support repair processes which manifest themselves in increased survival. In the present communication a new approach to this problem will be presented. It will be shown that similar effects can be produced if irradiated cells were infected either by viral DNA or by living viruses. The mechanism of this restorative effect is essentially different than that mentioned previously, and this will be discussed in more detail.

D-9-4 *Excision Repair Capacity in Cultured Mammalian Cells in Relation to Carcinogenesis in Xeroderma Pigmentosum.* Hiraku Takebe, Osaka University, Kita-ku, Osaka 530, Japan.

Most strains of xeroderma pigmentosum (XP) cells have been characterized as having high UV sensitivity for colony forming ability, no unscheduled DNA synthesis coupled with no excision of pyrimidine dimers from DNA after UV irradiation, and reduced host cell reactivation (HCR) of UV irradiated virus such as herpes simplex. Although these characteristics generally appear in parallel in XP cells, comparative studies on excision repair in several mammalian cell lines demonstrated that it is necessary to examine more than two parameters of repair to compare the efficiency of the excision repair among different species. For comparison among normal

human and different XP cells, HCR seems to be the most practical reliable measure for the relative efficiency of excision repair. There are XP patients with skin cancer whose cells show apparently normal or intermediate excision repair capacity. Survey of the relationship between the efficiency of excision repair and the age of cancer development suggests that the lower the level of excision repair in XP patients, the more likely that they will develop skin cancer early in life.

D-9-5 *Repair of DNA Damage Induced in Human Skin by Sunlight.* R. J. WILKINS, Department of Microbiology, University of Otago Medical School, Dunedin, New Zealand.

Cellular DNA repair systems are often assumed to offer protection against the DNA damage induced in human skin by the ultraviolet (UV) component of sunlight. However, detailed calculations show that such an assumption is only valid for weak sunlight. Moderate and intense sunlight produces DNA damage appreciably faster than it can be repaired with the result that significant damage accumulates in cells. For example, exposure to moderate sunlight for 1 h typically produces the same amount of DNA damage at the surface of Caucasian skin as would 120 erg/mm^{-2} of 254 nm UV light. The corresponding dose rate to the upper dermis of 22 erg/mm^{-2}/h^{-1} produces DNA damage some three-fold faster than it can be removed by cellular repair mechanisms. Thus it is clear that DNA damage will accumulate in the upper dermal layer of skin during prolonged exposure to moderate sunlight. Furthermore, a significant fraction of this damage remains unrepaired even when the cells are allowed to recover, in the absence of UV light, for 24 h. This unrepaired DNA damage may play a role in solar carcinogenesis. (My work is supported by the Cancer Society of New Zealand.)

D-9-6 *Investigation of Radiation Enhanced Survival of Nuclear and Cytoplasmic Replicating Mammalian Viruses.* LARRY BOCKSTAHLER, C. DAVID LYTLE, JULIA STAFFORD, AND LYNDA KRAMER, Bureau of Radiological Health, Rockville, Maryland 20852, USA.

Survival enhancement of UV-irradiated nuclear replicating simian virus 40(SV_{40}) and two cytoplasmic replicating viruses, vaccinia and polio, by exposure of host monkey kidney cell monolayers to UV light or x-rays before virus assay has been investigated. Capacity of the cells to support unirradiated virus growth (plaque formation) decreased following UV exposure to the cells for SV_{40} and vaccinia, but remained constant for polio. Survival enhancement of UV-irradiated SV_{40} following cell UV exposure was similar to that previously reported for herpes (2–3 fold) in the same cells. The enhancement was relatively long-lived and persisted for at least a 4 day interval between cell exposure and virus assay. Pre-irradiation of cells with x-rays produced a 6–10 fold enhancement in SV_{40} survival, greater than that for herpes (2–3 fold), and was also long-lived. No UV or x-ray enhanced survival was observed with either vaccinia or polio (for both immediate and delayed infection). Thus radiation enhanced survival occurs for two UV-irradiated nuclear replicating viruses, but not for two cytoplasmic viruses.

D-9-7 *A Correlation between DNA Repair and Life Span Among a Number of Mammalian Species.* R. W. HART AND R. B. SETLOW, Oak Ridge Natl. Lab., Oak Ridge, Tenn. 37830, USA.

The ability of fibroblasts to perform unscheduled DNA synthesis (a measure of excision repair) following UV-irradiation was measured radioautographically for seven species at several times following several UV fluences. Both the initial rate and the maximum incorporation of ^{3}HdT increased with the life span of the species (shrew, mouse, rat, hamster, cow, elephant, man). Unscheduled DNA synthesis was approximately proportional to the logarithm of life span. Unscheduled DNA synthesis in human fibroblast cultures of different cellular ages was measured following both physical and chemical insult. Both the initial rate and maximum incorporation of ^{3}HdT decreased in Phase 3 cultures. However, the rate of decrease for unscheduled DNA synthesis was less than that for scheduled DNA synthesis. The number of cells unable to perform DNA synthesis were proportional to those that showed a decrease ability to preform unscheduled DNA synthesis. Since this reduction did not occur until late in the culture's life span we conclude that excision repair may not be the basis for *in vitro* aging in human cultures, however differences in excision capacity between species does seem to relate to life span. (Research

jointly sponsored by the National Cancer Institute and by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.)

D-9-8 *Repair of UV Induced Alkaline Labile DNA Damage in Human Adenovirus.* ANDREW J. RAINBOW, Department of Radiology, McMaster University, Hamilton, Ontario, Canada.

A repair of UV-induced alkaline labile lesions has been detected in irradiated adenovirus following the infection of human cells. Human adenovirus type 2 was irradiated with various doses of UV and subsequently used to infect human KB cells in tissue culture at approximately 100 pfu (original) per cell. Before, and at various times after infection, the viral DNA was examined on alkaline sucrose gradients. Irradiated free virus DNA showed a dose dependent decrease in molecular weight compared to unirradiated virus DNA, indicating the presence of UV-induced alkaline labile lesions. Furthermore, an increase in the molecular weight of the irradiated virus DNA was found after infection indicating that alkaline labile lesions were removed from the viral DNA by a host mediated repair mechanism. After infection, the molecular weight of the irradiated virus DNA reached a maximum of between 90 and 100% that of unirradiated virus DNA for all the UV doses studied. The largest UV doses resulted only in a greater time after infection required to reach this maximum. Incubation of the infected cells in the presence of caffeine was found to inhibit the repair of UV-induced alkaline labile lesions. (Supported by the National Cancer Institute of Canada.)

D-10-1 *Gamma-Ray Induced DNA Single Strand Breaks and Their Rejoining in Mouse Testis in vivo.* TETSUYA ONO AND S. OKADA, University of Tokyo, Hongo, Bunkyo-ku, Tokyo, 113, Japan.

The DNA single strand breaks in mouse testis *in vivo* was estimated by an alkaline sucrose gradient centrifugation combined with fluorometric DNA analysis (T. Ono and S. Okada, J. Radiat. Res. **14**, 204 (1973)). The number of DNA breaks was increased linearly with dose and its efficiency was 0.29 breaks/10^{12} daltons DNA/rad. The efficiency was significantly lower than those (0.6–0.7) of liver and thymus *in vivo*. This is likely to be attributed to a hypoxic state of mouse testis *in vivo*. The DNA scissions were rejoined quickly with a half time of 18 minutes in testis. However, the breaks were hardly rejoined in the cauda spididymal sperms *in vivo*.

D-10-2 *Do Irradiated Cells Rejoin DNA Strand Breaks Correctly?* C. SUN, C. M. SU, AND J. T. LETT, Department of Radiology and Radiation Biology, Colorado State University, Fort Collins, Colorado 80521, USA.

Mammalian cells possess the ability to rejoin strand breaks induced in their DNA by ionizing radiation. Many workers have shown that the rejoining processes appear to proceed to completion. A serious unresolved question, however, is whether the breaks are rejoined correctly. We are attempting to probe that question by investigating DNA damage at very long post-irradiation intervals following the exposure of non-dividing cells to moderate doses of γ-rays *in situ*.

The retinas of New Zealand White rabbits were irradiated *in situ* with Cobalt 60 γ-rays—primarily 1000 rads. Rabbits were sacrificed periodically after irradiation and the DNA from their retinal photoreceptor cells was analyzed by alkaline sucrose gradients. Following irradiation strand break rejoining appears to proceed rapidly to completion in the sense that the DNA sedimentation profiles from irradiated retinas were similar to those of controls. But as the post-irradiation interval extended into weeks and then months, the photoreceptor DNA from irradiated animals began to exhibit symptoms of damage. At the time of writing (35 wks.), differences had appeared in the DNA profiles between control and irradiated animals. (Supported by NIH Grant No. NS08491.)

D-10-3 *The DNA Precursor Pools of Cells which Differ in Their Capacity for Unscheduled DNA Synthesis.* R. VINCENT AND T. MERZ, Radiological Science Department, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland 21205, USA.

Cells that exhibited x-ray-induced unscheduled DNA synthesis (UDS) incorporated tritium label from exogenously-supplied [^{3}H-methyl]TdR into autoradiographically detectible repair-associated precursor pools. Cells which did not exhibit UDS (Rad. Res. **47**, 426 1971) did not incorporate label into detectible pools.

The tritium labeled DNA precursor pools associated with scheduled DNA synthesis in cells which do and cells which do not exhibit UDS were also examined after exposure to x-rays. The total labeled pools of cells which do not exhibit UDS varied following x-irradiation, while the pools of cells that exhibit UDS remained nearly constant. The precursor pools of cells not exhibiting UDS also contained a greater percentage of [^{3}H]TdR, [^{3}H]TMP, and [^{3}H]T than the other cells. The percentage composition of [^{3}H]T was also greater than that of [^{3}H]TdR in the former cells, but the reverse was observed in the latter. An inverse relationship between the percentages of [^{3}H]TTP and the percentage of [^{3}H]TdR and [^{3}H]T was demonstrated in the cells not exhibiting UDS.

D-10-4 *Radiation Effects on Initiation of DNA Replication.* Ikuo Watanabe, National Institute of Radiological Sciences, Chiba 280, Japan.

In the previous report (4th ICRR) it was shown by the author that DNA chain growth was radioresistant while DNA synthetic rate was highly sensitive. To dissolve the discrepancy, M-band experiment (Tremblay *et al.*, 1969) was carried out. Exponentially growing L5178Y cells, preliminarily labeled with ^{14}C-TdR for 45 hrs, were X-rayed and pulsely labeled with ^{3}H-TdR. The cells were lysed with sarkosyl, mixed with Mg-acetate and sheared. Resulted sarkosyl-Mg complex containing 4–8% of total DNA was sedimented in sucrose gradient. The rate of ^{3}H-TdR incorporation into acid insoluble fraction of the complex was determined. The results indicated that (1) decrease in the rate was dose-dependent (Do = 375 rads) and (2) it reached to the minimum within 30 min and started to recover at 80 min after irradiation. In nonirradiated cells preliminary experiments showed that the initiation points might be condensed in the complex whereas growing points remained in supernatant. Accordingly radiosensitive component might be attributed to the initiation process involved in DNA replication.

D-10-5 *Relative Biological Effectiveness (RBE), Split Dose Recovery, and Oxygen Enhancement Ratio (OER) for Low Energy Electron Irradiation of CHO Cells.* Arthur Cole, William T. Tobleman, Francis J. Shonka, and W. Grant Cooper, Univ. of Texas, M.D. Anderson Hospital, Houston, Texas 77025, USA.

Low energy electron beams (~10 KeV) which stop in the nuclear periphery of a cell appear to be three to ten times more efficient in producing killing and DNA breakage than fully penetrating beams (~50 KeV). This suggests either that radiosensitive sites are concentrated at the nuclear periphery or that electron trackends exhibit large RBE values approaching 10. Comparison of DNA sedimentation profiles for short range electron irradiation with those for penetrating radiations implied RBE values for double strand DNA breakage of only about 2. Other responses for 10-KeV beams followed those expected for penetrating high LET radiation; the killing response was shifted to single-hit dependence, split dose cell recovery was absent and the OER was greatly reduced. The results suggest (1) radiosensitive sites are concentrated at the nuclear periphery and (2) energy deposited by electron track-ends induces irrepairable damage. The latter implies that no true threshold dose occurs for any form of ionizing radiation.

D-10-6 *Oxygen and Repair of Radiation Injury.* L. Révész, Radiobiology Unit, Department of Tumor Biology, Karolinska Institutet, 104 01 Stockholm, Sweden.

Experiments will be reviewed which were performed to study the effect of oxygen on the repair of different types of radiation injury in mammalian cells. Data will be presented which indicate that repair of single strand DNA breaks as well as recovery from sublethal damage are promoted by oxygen. Enhancement of the cellular radiosensitivity to varying degrees at different survival levels will be considered as a major consequence of the oxygen dependent recovery. Experiments performed to test this consequence will be described, and the therapeutic aspects will be discussed which concern the size of the radiation dose fraction in connection with the use of oxygen or oxygen mimicking chemicals as sensitizers of anoxic tumor cells.

D-10-7 *Mechanisms of Radiosensitization by DNA Binding Antibiotics.* Young C. Lee, Paul Y. M. Chan, Fimi M. Kulhanian, and John E. Byfield. Laboratory of Nuclear Medicine and Radiation Biology, UCLA, Los Angeles, California, 90024, USA.

The interaction of DNA-binding antibiotics with x-ray repair mechanisms have been studied in murine leukemia (L-1210) and recently explanted human squamous cell carcinoma cells. Antinomycin D, Adriamycin, Daunomycin and Bleomycin were employed at *in vitro* concentrations below, equal to, and above those obtained *in vivo* during cancer chemotherapy. All antibiotics radiosensitized the squamous cells *in vitro*, reducing the D_0 and D_q of radiation survival curves. All antibiotics blocked repair replication in the leukemia cells when studied in alkaline CsCl gradients by the BudR substitution method. Repair replication could not be demonstrated in the squamous cells by methods which clearly yielded extensive repair to HeLa cells. The effect of each antibiotic on the induction of DNA strand breaks (no radiation) and the rejoining of x-ray induced strand breaks was also examined using both standard 5–20% alkaline sucrose gradients and modified (10–30%) gradients employed to study low dose strand break repair. High antibiotic concentrations induced DNA degradation in either gradient, this effect being more prominent in the 10–30% gradients. Each antibiotic similarly inhibited the rejoining of x-ray induced strand breaks under either gradient condition and this effect was demonstrable at antibiotic concentrations which produced none or minimal primary breakage (no x-rays). The results suggest that radiosensitization by DNA-binding antibiotics most probably relates to their effects on the integrity of the DNA polydeoxyribose-phosphate backbone. Mechanistically it is not possible to distinguish between accelerated breakage or inhibition of strand break repair using the sucrose gradient approach. (Supported by Grant CA 12691, by Kaiser Research Funds and by A.E.C. Contract GEN-12.)

D-10-8 *Repair Processes in Plateau Phase Cells: Effect of Dose-Rate and DNP.* JOHN B. LITTLE AND JERRY R. WILLIAMS, Laboratory of Radiobiology, Harvard University, School of Public Health, Boston, Mass., USA.

Exposure of plateau phase cells to $5 \times 10^{-4} M$ dinitrophenol (DNP) during x-irradiation delivered at 80 rads/min greatly enhanced their survival. The Do for H_4 (mouse hepatoma) cells was increased from 140 rads to 260 rads, and for αRST (a mouse-rat hybrid strain) from 260 rads to 375 rads. Survival was further increased if the cells were allowed to repair potentially lethal damage after irradiation by delaying subculture. Exposure to DNP under identical conditions (time and concentration) had no influence on survival, however, following gamma-irradiation delivered at 90–220 rads/sec, though the cells were still capable of repairing potentially lethal damage. Preliminary experiments suggest that split-dose recovery is very rapid in plateau phase cells irradiated at the high dose-rates; a 3-fold enhancement in survival occurred with intervals of 2–3 minutes between doses. No significant recovery occurred within this interval in exponentially growing cells. These results suggest that events which occur during or very shortly after irradiation may have considerable influence on the eventual survival of plateau phase cells.

D-10-9 *Spontaneous and X-Ray-Induced Chromosomal Aberrations in Progeria.* MICHAEL A BENDER AND JACK M. RARY, The Johns Hopkins University, Baltimore, Maryland 21205, USA.

Epstein, Williams and Little (Proc. Natl. Acad. Sci. U.S. **70**, 997–981, 1973) have reported that cells from a patient with the Hutchinson-Gilford progeria syndrome were deficient in their ability to repair single polynucleotide strand breaks induced in their DNA by ionizing radiation. Bender, Bedford and Mitchell (Mutation Res. **20**, 403–416, 1973) have shown that polynucleotide strand breaks induced in postsynthetic chromosomes give rise to chromosomal aberrations at the next metaphase. We have determined spontaneous chromosomal aberration frequencies in lymphocyte and fibroblast cultures and radiation-induced aberration frequencies in lymphocyte cultures from the same patient studied by Epstein, Williams and Little, as well as in lymphocytes from clinically normal subjects. No elevation in spontaneous aberration frequencies was seen in the progeria cells, nor was there a significantly higher aberration yield in G_2-irradiated lymphocytes from the patient than in similarly-treated control cells. (This research was supported by the U.S. Atomic Energy Commission, contract AT (11-1)-2382.)

D-11-1 *Growth Characteristics and Cell Proliferation of X-Irradiated Solid Tumors.* CHARLES J. KOVACS, ELIZABETH HAMILTON, AND WILLIAM B. LOONEY, Division of Radiobiology and

Biophysics, University of Virginia, School of Medicine, Charlottesville, Virginia 22901, USA.

The radiation response of a rapidly growing (3924A) and a slow growing (16) hepatoma has been investigated as part of a continuing study to develop quantitative solid tumor model systems. The tumors were exposed to x-rays when their mean volume was 250 mm^3 ($L \times W \times H \times 0.5$). Over the dose range studied (375–2250 R), hepatoma 3924A responded within 48 hours. Fifteen days post irradiation the mean tumor volumes were 45 percent (375 R), 25 percent (750 R), 6 percent (1500 R), and 5 percent (2250 R) of control. The mean growth delay was 0.012 days/R. Unlike 3924A, the slowly growing hepatoma 16 responded to 1500 R 22 days after irradiation and tumor regression continued over an additional 60 days.

Cell proliferation kinetics have been analyzed before radiation, following radiation when growth ceases, and during regrowth. The results of autoradiographic and liquid scintillation analyses, as well as changes in tissue histology will be reported. Discussion of these observations will include an attempt to develop a mathematical model to analyze perturbations of tumor growth. (Supported by USPHS grants CA-12758, CA-13102 and CA-107-29 from the National Cancer Institute.)

D-11-2 *Effects of Hydroxyurea and Radiation on the Survival and Proliferation of EMT6 Tumor Cells in vivo and in vitro.* SARA ROCKWELL, EMILIA FRINDEL, AND MAURICE TUBIANA, Stanford University, Stanford, Calif. 94305, USA, and Institut Gustave Roussy, Villejuif, France.

Treatment of EMT6 cell cultures or tumors with hydroxyurea (HU) was shown to kill selectively the cells in S phase. This observation formed the basis for the development of a technique for determining the proportion of the clonogenic tumor cell population in S phase. In this technique the solid tumors were converted to single cell suspensions, the cells were plated in cell cultures, and colony formation was compared in replicate cultures which had or had not been exposed to HU during the first 90 minutes after plating. When tumors *in situ* were treated with either HU (5 mg/mouse injected IP) or local irradiation (300 rads of 220 kV x-rays) and subsequently tested by this technique, evidence was obtained which suggests that the surviving clonogenic tumor cells progressed through the cell cycle in a state of partial synchrony. These findings were compared with data on the DNA specific activities, labeling indices, and mitotic indices in the tumors after the same treatments, and were analyzed in terms of the age-specific actions of the two agents and the cell population kinetics of the tumors.

D-11-3 *The Effects of 1500 Rads on DNA Synthesis in a Fast-Growing (S102F) and a Slow-Growing (Slow) C3H Mouse Mammary Tumor.* L. A. DETHLEFSEN, R. WILLIAMS, AND J. R. STEWART, Department of Radiology, University of Utah Medical Center, Salt Lake City, Utah 84132, USA.

This is a preliminary report on a series of studies designed to evaluate the importance of growth kinetics in the interpretation of tumor response to fractionated radiation. Specifically, this study is a prelude to measuring cell loss after irradiation. Tumor fragments were transplanted into the left flank of young mice, allowed to grow to a diameter of 8–10 mm, and irradiated with a single dose of 250 Kv x-rays (148 S102F and 122 Slow tumors were used). At selected times post-irradiation, the mice were injected with 20 μCi ^{3}H-TdR, killed one hour later, and the tumors removed and homogenized. The DNA was extracted and measured by UV absorption, and the ^{3}H-activity determined in both the DNA and acid-soluble fractions. There is no significant change in the DNA content (μg DNA/mg tumor) or the ^{3}H-activity in the acid-soluble fraction as a function of time post-irradiation in either line. In the S102F line, ^{3}H-incorporation (DPM/μg DNA) is inhibited as early as 5 min. (68% of control). It then drops to 11% at 8 hrs and stays in this range until 25 hrs post-irradiation. The level increases to 67% at 41 hrs but drops again to 10% at 48 hrs before rising to 111% of control at 72 hrs. In the Slow line, there is also inhibition at 5 min. (46%). Incorporation continues to drop until 16 hrs (7%), then increases to 26% at 22 hrs and stays in this range until 45 hours. It then rises to 44% at 72 hrs and 72% at 112 hrs. The cell cycle times (hrs) are S102F: 17.3 and Slow: 33.4. The initial recovery in each line is distinctive. The period of depressed DNA synthesis is long in both lines but related to the specific cell cycle. Also, there is a small degree of x-ray induced

synchrony only in the S102F line. However, early recruitment of cells from the non-proliferating fraction is not evident in either line.

D-11-4 *Rates of Loss of Lethally-Irradiated (LI) Tumor Cells Following Intraperitoneal Implantation into Normal and Immune Mice.* K. M. Porteous, N. Ie, R. Cabrera, and D. D. Porteous, Department of Radiology, Downstate Medical Center, Brooklyn, NY 11203, USA.

The rate at which LI tumor cells are lost is of interest to both the radiation therapist and the tumor immunologist.

BP8 ascites sarcoma cells were labeled *in vivo* with I-125-labeled iododeoxyuridine (IUdR) while growing in exponential phase. Whole body gamma counts were made at intervals after intraperitoneal injection of measured numbers of cells and loss of radioactivity was equated with tumor cell elimination. When 10 million labeled tumor cells were injected into syngeneic C3H mice cells were lost at a rate of about 10%/day. If the cells were previously irradiated with 5000 rads (LI cells) they disappeared with a half-time ($T_{1/2}$) of 48 hours. When varying numbers of LI cells in excess of 10 million were injected the $T_{1/2}$ increased to as much as 170 hours.

When labeled cells were injected into hyperimmunized C57B1 mice the $T_{1/2}$ of both normal and LI cells was 8 hours. Increases of $T_{1/2}$ up to 50 hours were observed when numbers larger than 10 million were injected. It is suggested that the rate of elimination is not proportional to the number of tumor cells present. (Supported by Grant CA 14117-01, NCI, NIH.)

D-11-5 *Effects of Fractionation on Synchronization and Radiosensitivity of Mouse Tumors.* Titus C. Evans, Sharyn Eklund, and Jose Cuevas, Radiation Research Laboratory, University of Iowa, Iowa City, Iowa 52242, USA.

Fractionation of x-ray exposures partially synchronized ascites tumor cells (S-180 and Ehrlich) so that subsequently they could be irradiated with no cells in division or with three times the usual in mitosis. Radiosensitivity was reduced when there were no cells in division, and when the division rate was increased there was an increase in percentage cells killed by a given dose of radiation. Attempts to demonstrate this in solid tumors were only partially successfull. Irradiation at frequent intervals, when cells were not in mitosis, resulted in less growth initially than in those irradiated at longer intervals when many cells were dividing. For a time, there was more reduction in growth of those irradiated when cell division rates were higher. However, for the high total dose usually required, the eventual effects were somewhat similar regardless of regimen used. Cell kinetics of solid tumors have been followed after irradiation in hopes of distinguishing between radiation injury associated with cell division and that related to host resistance, nutrient supply, etc. Other possible factors are being investigated. (Supported in part by grant ET-37L, American Cancer Society.)

D-11-6 *Effect of Fractionated Irradiation on the Ehrlich Ascites Tumor.* Jacob J. Clement and Donald G. Willhoit, Dept. of Ther. Radiol., U. of M. Hospitals; Minneapolis, Minn., and Dept. of Envr. Sci. and Eng., U. of NC, Chapel Hill, NC, USA.

Cell survival and cell progression were examined following single-dose and fractionated irradiation. Cyclic fluctuations in cell survival following 2-dose irradiation was interpreted in light of mitotic activity and DNA synthesis patterns as representing repair of sublethal injury and progression of a semisynchronous cell population from S-phase into sensitive G_2, M, and G_1 stages. The effect of 2-dose irradiation on cell progression and cell synchrony were found to depend on fractionation interval. Intervals of 5 and 15 hours each produced results interpreted as representing the independent progression of two age cohorts, while a fractionation interval of 9 hours appeared to result in a partial loss of cell synchrony produced by the second dose. Predicted fluctuations in radiosensitivity following 2-dose irradiation were substantiated by 3-dose survival studies. The data are consistent with the theory that the effect of each dose fraction on the degree of synchrony is an important factor in planning effective multidose cell killing regimes designed after consideration of cyclic cell radiosensitivity.

D-11-7 *Post-Irradiation Proliferation Kinetics in a C3H Mouse Adenocarcinoma.* Lore v. Szczepanski and Klaus R. Trott, Institut für Biologie der GSF, D-8041 Neuherberg, West Germany.

The post-irradiation proliferation kinetics of a serially transplanted C3H mouse adenocarcinoma was analyzed at daily intervals during the first week after 600 rad and 1,200 rad by various methods including repeated labelling with 3HTdR and p.l.m. curves. The combined application of several methods at the same time allowed the identification and exact timing also of transient phenomena as e.g., the post-irradiation wave of synchronization. The mean generation times of the tumour cells were always longer after irradiation and showed a larger spread than in unirradiated tumours. 3 days after 600 rad and 4 days after 1,200 rad the growth fraction increased to twice the normal values but returned to the normal levels a few days later. Evidence is presented that the increase in growth fraction is due to a triggering of resting cells into cycle.

D-11-8 *Radiation-Induced Synchronization of the Walker-Carcinoma in Vivo.* WALFRIED A. LINDEN, FRIEDRICH ZYWIETZ, HEINZ BAISCH, AND JÖRN SKIBA, Institut für Biophysik und Strahlenbiologie der Universität Hamburg, Hamburg, West Germany.

Synchronization of tumor cell populations *in vivo* has become of interest for phase specific treatment with ionizing radiation and cytostatic drugs. Rats with an exponentially growing tumor were irradiated with single and fractionated doses at a 200 kVp x-ray unit. The influence of the irradiation on the cell cycle of the carcinoma was studied with an impulsecytophotometer. By this method DNA distribution patterns of the tumor cell population are obtained.

The mathematical analysis of these DNA distributions using a computer program gives the percentage of cells in the various phases of the cell cycle. The results show a partial synchronization of the cells in the G_2-phase, which is dose-dependent. Under proper timing the synchronized fraction of G_2-cells can be increased considerably by fractionated irradiation.

D-11-9 *Kinetic Studies in Irradiated Tumors of Diverse Growth Rates: Light and Electron Microscopic Observations.* ANNA GOLDFEDER AND MILAN POTMESIL, Cancer & Radiobiological Research Laboratory, Dept. of Health & Hospitals, and NY University, New York, NY 10032, USA.

Research in this laboratory has been concerned with establishing a relationship between growth rate, metabolic activity and radiosensitivity of neoplasms. This study was performed on a spindle cell tumor rapidly growing in isogenic X/Gf mice. The tumor consists of bundles and whorls of spindle cells forming solid sheets. Tumors were exposed to 100–3000 rads of x-rays. At specific time intervals after irradiation, tumors were processed for light and electron microscope studies. Light microscope studies revealed the absence of mitosis within 4 hr. p.r at any dose level used. Recurrence of mitosis followed within 24 hrs depending upon the dose. After 1,500 rads at 24 hrs, dilatation and breakage of blood capillaries and cell death were prominent. This process progressed with time and increased with radiation dose. Electron microscope studies revealed dilatation of plasma membranes and swelling of mitochondria as first discernible events after 500 rads. The frequency of degenerative changes increased with dose and time. (Supported by NIH Grant No. CA-12076-03.)

D-11-10 *Proliferation Kinetics in Irradiated Tumors: Cell Transition from the Nonproliferating to the Proliferating Pool in Regenerating Tumors.* MILAN POTMESIL AND ANNA GOLDFEDER, Cancer and Radiobiological Research Laboratory, New York University and Department of Health and Hospitals, New York 10032, USA.

A model of tumor regression and regrowth after low L.E.T. irradiation has been developed, in which quantitative changes of morphologically defined cell subpopulations and their kinetic parameters are utilized in studies on the role of nonproliferating but potentially clonogenic cells in regenerating tumors. The study of transplantable mouse mammary adenocarcinoma DBAH was based on three types of experiments: 1) estimation of cellularity per tumor; 2) differential cell-counts and determination of $S \rightarrow G_2$ phase cell fluxes; 3) assay of cell clonogenicity. There were three dose levels used (470, 940 and 2250 rads), and tumors were assayed at various time-intervals after a single dose of radiation. The experimental data were analysed introducing a mathematical three-compartmental model with feedback terms. Regrowth of irradiated tumors seems to have two stages: a) regeneration starts with intercompartmental cell transit within

the nonproliferating pool, followed by cell transit to the proliferating pool; b) both depleted compartments belonging to the nonproliferating pool are replenished from the proliferating pool. (Supported by NIH Grant No. CA-12076-03.)

D-12-1 *Tumor Response to a Three-Fraction Regimen Combining Hyperthermia and X-Radiation.* J. Eugene Robinson, Morris J. Wizenberg, Welton A. McCready and Edgar A. Edelsack, University of Maryland School of Medicine, Baltimore, Maryland 21201, USA.

As part of an investigation of hyperthermia and ionizing radiation as a combined modality for cancer therapy, we have conducted a study of the response of C3H mammary tumors to a three-fraction regimen combining hyperthermia and ionizing radiation. The combined treatment used equal thermal treatment times, and equal radiation doses on three successive days. The elevated temperatures were applied for 20 minutes each day, and were maintained pre-, during-, and post-irradiation. Radiation response curves were determined for each treatment temperature. The assay system is derived from tumor regrowth times following treatment. The sensitivities of the tumors increased with treatment temperature, yielding thermal enhancement ratios of 1.05, 1.46, and 2.60 for treatment temperatures of 41.0, 42.5, and 43.0°D (T.E.R. for 37.5°C = 1). The assay system will be described and the results compared with single dose studies with the same tumor at elevated temperatures.[1] Relevance to clinical radiation therapy will also be discussed. (Supported in part by Public Health Service Research Grant CA-06518-11 from the National Cancer Institute.)

[1] J. E. Robinson *et al.*: Digest 3rd International Congress of Medical Physics, Goteborg, Sweden, July 30–August 4, 1972, 39.5.

D-12-2 *Changes in the Radiation Response of C_3H Mammary Carcinoma by Precooling.* N. Dubravsky, K. Mason, and H. R. Withers, Section of Experimental Radiotherapy, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77025, USA.

S. Inoue showed that cold and colchicine caused similar changes in the birefringence of the mitotic spindle. Cold caused disintegration of the microtubuli and colchicine combines with the protein tubulin to inhibit the formation of microtubuli. Both arrest cell progression in prometaphase, an effect which is reversible after changing the temperature or washing out the colchicine.

Dewey *et al.* showed that cultured Chinese hamster lung cells (Don) synchronized using colchicine and mitotic selection are more sensitive to irradiation after washing out the colchicine than mitotic cells synchronized by mitotic selection only. Our results with prechilling of the plucked skin show a radiosensitive stage half an hour after the end of cooling, which is attributed to the partial synchrony of cells in the radiosensitive prometaphase stage. Experiments were performed to examine whether transplanted mammary carcinoma in the mice would show the same phenomenon. The results show that total body irradiation of the animals with 800 or 1000 rads 30 minutes after the end of cooling causes elongation of the doubling time of the tumor as compared to irradiation without cooling. Tumor volumes were reduced more in animals irradiated with two doses of 600 rads each 20 and 30 minutes after the end of the cooling than in controls irradiated without cooling. A single dose of 1200 rads had the most effect when given 30 minutes after the end of the cooling.

D-12-3 *Combination of Heat and Ionizing Radiation on the C3H Mouse Mammary Adenocarcinoma in vivo: Effect of Variable Heat Doses and Repair of Heat Damage.* Edward L. Gillette and Donald E. Thrall, Colorado State University, Fort Collins, CO 80521, USA.

Eight millimeter average diameter tumors were heated for 0, 8, 15 or 30 minutes by immersion in 44.5°C water. Immediately after heating the tumors were irradiated under hypoxic conditions. The radiation dose required to control 50% of the tumors (TCD_{50}) was reduced by the various heat treatments by an amount equivalent to 66 rads per minute immersion. The results best fit the linear equation $TCD_{50} = [-66$ (minutes immersion in 44.5°C water $+ 6282]$ rads, $r^2 = 0.99$.

A heat treatment consisting of a 15 minute immersion in 44.5°C water administered to 8 mm average diameter tumors was followed at 6, 12, 24, 48 and 72 hours by irradiation under hypoxic

conditions. The TCD_{50} values increased in the first 12 hours and stabilized at the value equal to that for hypoxic irradiation alone. This indicated complete repair of the heat damage in the first 12 hours.

D-12-4 *Combination of Heat and Ionizing Radiation on C3H Mouse Skin and the C3H Mouse Mammary Adenocarcinoma in vivo: Significance of the Order of Application and Quantitation of the Heat Effect.* DONALD E. THRALL AND EDWARD L. GILLETTE, Colorado State University, Fort Collins, CO 80521, USA.

Combining heat (15 minute immersion in 44.5°C water) with radiation, administered under hyperbaric O_2 (O), ambient (A) or hypoxic (H) conditions, on C3H mouse skin significantly lowered the radiation dose required to produce moist desquamation in 50% of the irradiated mice (DD_{50}). The order of application of the two modalities was not significant when irradiation was conducted under O or H conditions. Heating prior to irradiation under ambient conditions sensitized mouse skin to the effects of the radiation.

When the same protocols were used to treat 8 mm C3H mouse mammary adenocarcinomas in C3H/Mai mice, different trends were observed. In all cases the dose of radiation required to produce tumor cure in 50% of the mice (TCD_{50}) was significantly reduced by the heat, however, the order of application of the modalities was significant in all cases with radiation followed by heat being most effective.

These results indicate that heat should follow irradiation to produce maximal tumor damage in relation to skin damage.

D-12-5 *The Effect of Radiation and Hyperthermia on Growing Bone.* ERIC W. HAHN, STEPHEN M. FEINGOLD, AND JAE HO KIM, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA.

The local application of heat to tumors immediately before or after fractionated x-ray significantly increased local tumor control and subsequent cure rates in mice. This study was carried out to determine the effect of radiation and hyperthermia on growing bone. One leg of 21 day old male BLU-LE rats was x-irradiated with single (500 R) or fractionated doses (333 R $\times$ 3, M, W, F) with or without hyperthermia (42.5 $\pm$ 5°C for 15 min.). Tibia length, measured from x-ray films, were used to calculate absolute growth and length ratios of the treated *vs.* untreated tibiae.

Heat alone did not decrease bone growth or change tibial length ratios. All radiation and radiation plus heat treatments significantly retarded bone growth with the combined treatment having the greatest effect. Two months after treatment bone growth was retarded by approximately 10% following 500 R *vs.* 13.5% for 500 R + heat ($P \leq .01$) and 18% following 333 R $\times$ 3 *vs.* 22% for 33 R $\times$ 3 + heat ($P \leq .01$).

In conclusion, radiation in combination with heat compared to radiation alone had an increased effect on bone growth retardation. The estimated Radiation Enhancement Factor (REF $\approx$ 1.04–1.08) was considerably less than the REF $\approx$ 4 estimated for tumors in prior studies. (Supported in part by NCI grant CA 08748.)

D-13-1 *X-Ray Induced G_2 Block in Chinese Hamster Cells in vitro.* D. P. HIGHFIELD AND W. C. DEWEY, Department of Radiology and Radiation Biology, Colorado State University, Fort Collins, Colorado 80521, USA.

In monolayer culture, the mitotic selection technique was used to monitor the number of cells entering mitosis. Transition points beyond which the progression of cells towards mitosis was unaffected by a particular treatment, were as follows: x-ray ($\geq$ 100 rads) at 10 min. prior to prophase, cycloheximide (CH of 5–50 μg/ml) at 25 min., and actinomycin D (AMD of 5 μg/ml) at 38 min. With lower x-ray doses (30–60 rads), the transition point increased to 20 min., but when CH was applied concurrently with radiation, the point shifted back to 10 min. The AMD-S/G_2 cells, i.e., those located between the AMD point and S/G_2 (92 min.), sustained the greatest delay. After recovering from a delay in leaving S or in crossing the AMD point, cells were blocked, i.e., sustained additional delay, in the CH-AMD interval of 13 min. However, when AMD and x-rays were applied concurrently, the transition point increased from 10

to 26 min., which means that inhibition of RNA synthesis eliminated the delay for about one-half of the X-AMD cells. Furthermore, the remaining X-AMD cells never entered division (even after 30 rads). These observations suggest that radiation has specific effects on RNA synthesis which result in an inhibition in the synthesis or assembly of specific division proteins.

D-13-2 *On the Role of Non-Histone Protein Synthesis in X-Ray Induced G_1 Progression Delays.* EUGENE W. GERNER, RAYMOND E. MEYN, AND RONALD M. HUMPHREY, The Univ. of Texas System Cancer Center, M. D. Anderson Hosp. & Tumor Inst., Houston, Tex. 77025, USA.

Non-histone chromosomal protein (NHCP) synthesis in Chinese hamster ovary cells (CHO) is temporarily coordinated with the initiation of DNA replication. NHCP synthesis rates increase 4 fold as CHO cells traverse G_1 phase to reach their maximum synthesis rate in late G_1 phase. As cells initiate DNA replication and proceed through S phase, NHCP synthesis rates decrease to levels similar to those observed in early G_1. Ionizing radiation causes a dose-independent (150–700 rads) G_1 progression delay of 0.5 hours in CHO cells irradiated in either mitosis or early G_1 phase. In contrast, late G_1 cells are insensitive to ionizing radiation induced G_1 progression anomalies. Radiation induced G_1 progression effects are initially observed as a delay in NHCP synthesis as cells traverse G_1 phase and subsequently as a delay in the initiation of semi-conservative DNA replication. Semi-conservative replication kinetics were monitored by isopycnic centrifugation of density labeled DNA, and then by measuring the amount of DNA that acquired hybrid buoyant density in CsCl gradients. In order to test whether damage to the DNA of mitotic or early G_1 cells might account for the observed G_1 progression delays, 5-bromodeoxyuridine (BUdR) substituted cells were irradiated in mitosis with fluorescent light (FL). Using survival levels similar to those resulting from 300 rads of ionizing radiation (3–4%), it was found that BUdR substituted mitotic cells irradiated with FL undergo a G_1 progression delay of the same magnitude as that induced by ionizing radiation. These data suggest that ionizing radiation damage resulting in G_1 progression delays may be at the level of the DNA. This damage may have to be repaired before transcription can occur leading to factors required for the initiation of S phase.

D-13-3 *The Dependence of Radiation Sensitivity on the Growth Fraction in Continuous Cultures.* JÜRGEN KIEFER AND ELISABETH WAGNER, Strahlenzentrum, Justus Liebig University, Giessen, West Germany.

Continuous cultures of diploid yeast, Saccharomyces cerevisiae, were used as model systems to simulate the conditions in irradiated tissues. The growth fraction as determined by the percentage of budding cells was varied by changing the dilution rate. The kinetics of cell multiplication and the doses leading to the extinction of the whole cell population after gamma ray exposure were determined. It was found that extinction doses decrease with increasing growth fraction in a linear manner. The possible implications for radiotherapy are discussed.

D-13-4 *Changes in CHO Cell-Population-Kinetics Investigated by Colony-Size-Spectrometry.* D. TÖLLNER, H.-ST. STENDER, AND G. HAGEMANN, Medical School Hannover, Dept. of Radiology, Experimental Radiology, 3000 Hannover, Karl-Wiechert-Allee 9, West Germany.

The colony-size-spectrometry may serve as an analytical tool for measuring changes in the population kinetics of culture cells caused by proliferation damages after irradiation. These damages could be the response to either x-irradiation, or to the effectiveness of proliferation poisons, or even to influences of temperature. Supposition for the applicability of this method is the knowledge of its limits. Possible methodical errors which might happen because of the formation of satellite colonies have been investigated. The ambiguity of measured multiplicities will be indicated and in comparison confronted with calculated spectra. Different colony-size-spectra after a freeze-thaw-cycle will demonstrate the sensitivity of this method. The proliferation damages are considered mainly to be caused by reproductive lethal injuries. Therefore, after different radiation doses, several survival curves calculated from the measured colony-size-spectra were derived that way, that small colonies were not taken into account. The changes of the survival curves caused by this way, will be demonstrated.

D-13-5 *Changes in the Cell Proliferation Kinetics Occurring During the Life History of EMT6/M/CC Mouse Tumour Cells Growing in Monolayer Culture.* JAMES V. WATSON, PETER R. TWENTYMAN, AND NORMAN M. BLEEHEN, Middlesex Hospital Medical School, London, Great Britain.

Following subculture of 10^5 EMT6/M/CC mouse tumour cells into 25 cm^2 Falcon flasks the cell number increases exponentially for 3–4 days. The number, in cultures with daily medium change, then remains constant for about 10 days before a decline begins. During exponential growth the ^{3}HTdR pulse labelling index is 55%. The labelling index falls to 25% at day 4 and remains at this level until Day 7, when there is a further rapid fall to less than 5%. Plating efficiency remains in excess of 70% at least until day 10. Between days 4 and 7, cells are shed from the monolayer into the medium at a rate which balances cell production.

Further kinetic analysis by the PLM and continuous labelling techniques will also be presented together with a discussion of the implications.

D-13-6 *Cell-Cycle Analyses of Candidate Lines for RBE Studies with Negative Pions.* D. F. PETERSEN, L. L. DEAVEN, AND M. M. KLIGERMAN, Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico 87544, USA.

Cellular models for RBE studies with densely ionizing radiations (e.g., the Los Alamos Meson Physics Facility negative pion beam) must fulfill several criteria to ensure relevance to eventual clinical application: (1) radiosensitivity approximating radiotherapy experience with some class of normal or neoplastic tissue; (2) plating efficiency and tolerance to culture conditions sufficient to provide acceptable cell accountability and depth-dose distribution; and (3) growth parameters capable of providing sensitive indices of alteration by exposure to doses approximating conventional therapeutic doses (50–400 rads). Murine lymphomas L5178Y and SS L5178Y, mastocytoma P815, Chinese hamster lines CHO, V-79 and Don, human lines HeLa, T-1 and WI-38, as well as several diploid lines with unique karyologic properties, have been examined by flow microfluorometric techniques to determine genome content and cell-cycle distribution. Each of the lines mentioned possesses one or more features supporting inclusion in studies of high LET radiation. For example, P815 cells clone with near 100% efficiency in stabilized medium and share with the lymphoma lines the prominent S phase of hematopoietic RBE models. (Work performed under auspices of the U.S. Atomic Energy Commission and the National Cancer Institute.)

D-13-7 *DNA-Synthesis of Synchronized L-Cells after Irradiation in G1. Incorporation Studies and Impulsecytophotometric Measurements.* KLAUS KÖNIG, WALFRIED A. LINDEN, HEINZ BAISCH, AND MICHAEL VON CANSTEIN, Institut für Biophysik und Strahlenbiologie, Universität Hamburg, West Germany.

Mouse L-929 cells were synchronized by mechanical selection of mitotic cells. 5 hours after synchronisation, i.e., in the middle of G1-phase, the cells were x-irradiated with a dose of 1000 rads. Over a period of about 30 hours we examined 1) the incorporation of 3H-thymidine into DNA by 60 minute pulse labeling, 2) the DNA-content of single cells in the synchronous populations by means of impulsecytophotometry (ICP). Irradiation caused a reduction of the rate of DNA-synthesis to 50% in the following S-phase. The ICP-measurements showed that the beginning of the S-phase was delayed by 2 hours. But the S-phase had a twofold duration compared with the unirradiated controls. Although the rate of DNA-synthesis as well as the progression of cells through the cell cycle and the duration of the different phases were severely altered by the irradiation in G1, a full complement of DNA was synthesized.

D-13-8 *Reproductive Lethal Cell Damage, Colony Survival and Population Kinetics.* GERD HAGEMANN, Medizinische Hochschule, 3 Hannover, West Germany.

A heterogenous line of CHO-fibroblasts with a cycle time of about 13.5 hrs were irradiated in petri-dishes with energy doses up to 800 rads. After vital staining with nigrosine the cultures were fixed in time intervals of 2.5 hrs up to 48 hrs after irradiation. The variations of the counted killed and surviving cells with the time after irradiation give the experimental population kinetics. The reproductive killed cells show a time dependency with maxima and minima in time distances of the cycle time. From these maxima the probability of reproductive cell killing in

every of the three daughter generations have been obtained. The calculation of the fraction r of irradiated cells with reproductive lethal cell damage gives the dose dependency which shows a maximum at a value which is called threshold dose D_s. At higher doses than D_s beside reproductive also interphase death is observed, the dose dependency of which is demonstrated. The comparison of the dose dependency of the reproductive lethally damaged cell fraction r with the colony survival curve shows at doses higher than D_s in the exponential part of the curves similar exponents and differences of about 10% only. For illustration examples of pedigrees of irradiated cells at two doses are given with approximately chosen values for the frequencies of reproductive killed and surviving cells and of the colony forming probability, as derived from the measured population kinetics and colony survival. Parallel investigations of colony size spectra, formed by irradiated cells, which had been cultivated for 77 hrs show, that dose dependent variations of the colony size are mainly influenced by the existence of reproductive death in the cell generations, as illustrated by the pedigree examples.

D-13-9 *Time-Lapse Studies on Generation Time Distributions and Division Probability in Pedigrees of Single Mammalian Cells after Alpha-Irradiation.* KLAUS-RUEDIGER TROTT, Gesellschaft für Strahlen- und Umweltforschung, Institut für Biologie, D-8042 Neuherberg, West Germany.

The proliferation of L-cells growing in Sykes-Moore chambers on Melinex foils was recorded with time-lapse cinephotography. After pro-incubation and recording for 2 generation times the cells were irradiated from below with ^{210}Po alpha-particles and subsequent proliferation was recorded for 4 further generations.

Division delay, generation time distributions in later generations and division rate was taken from the pedigrees of individual cells irradiated with 70 or 140 rad in various age groups and compared to generation time distributions and division rates after comparable doses of x-rays. No marked differences in the effects of both types of radiation on the post-irradiation proliferation pattern could be found.

D-13-10 *Reproductive Lethal Damage of DNA-Synthesis-Outset and Its Influence on Colony Population Kinetics.* J. R. MELLMANN, G. HAGEMANN, AND H. ST. STENDER, Institut für klinische Radiologie, Medizinische Hochschule Hannover, 3 Hannover, West Germany.

CHO-fibroblasts were used to study the colony population kinetics as influenced by the fraction of cells with reproductive lethal damage ("r"-fraction) induced by x-irradiation in the S-phase of the cell cycle (G. Hagemann, H. St. Stender, 1973). The cells were synchronized by the mechanical selection of mitotic cells. The cell cycle time and the duration of the S-phase were determined by the use of a new technique based on 250 nm UV light absorption of cell DNA and contact photomicrograms. The observed cell cycle time is in accordance with the results published by Leeper *et al.* (1973), who used autoradiographical methods. To examine the "r"-fraction CHO-fibroblasts were x-irradiated at hourly intervals from 1 to 12 hours after synchronization. The exposed cells and their controls were incubated for about 50 hours. The grown colonies were stained with nigrosine to detect killed cells (G. Hagemann, 1973) or stained with HE to determine the colony size spectra (D. Töllner, 1973). The time correlation between reproductive killed cells and colony size spectra was measured. The highest amount of reproductive lethal damage was observed at the beginning of the S-phase as the control measurement of the UV absorption showed.

D-13-11 *Influence of Dose-Rate on Cell Cycle Time.* M. COLLYN-D'HOOGHE AND E. P. MALAISE, Institut de Recherches sur le Cancer, B.P. 3567 Lille Cédex 59020, France.

We compared the influence of 2 dose rates classically used in external and interstitial radiotherapy (100 rad/min. and 1.5 rad/min.) on the cell cycle time of EMT6 mouse tumor cells. The study was performed by time lapse cinematography.

Low dose rate irradiation lengthens more the cell cycle time than acute irradiation (1.02 hr/100 rad instead of 0.68 hr). This difference may be explained especially by the influence of low dose rate irradiation on G1 phase (1.06 hr/100 rad) as compared with that one of acute irradiation (0.20 hr/100 rad).

D-13-12 *Significance of Asynchronity of Cell Divisions in Radioresistance of Meristems of Higher Plants.* IGOR N. GUDKOV AND DMITRI M. GRODZINSKY, Institute of Plant Physiology, Ukrainian Academy of Sciences, Kiev 252627, USSR.

For synchronization of cell divisions in the pea root meristems by hydroxyurea a high degree of synchrony is observed during only one mitotic cycle. The cell cycle times in some meristematic zones are shown to be different, but with similar values of the *S*-, G_2- and mitosis stages the differences in the cycle duration are mainly due to the G_1-stage. Since for synchronization by hydroxyurea a block in the cycle is established at the G_1/S, boundary desynchronization is probably a result of differences in duration of G_1-stage and manifests only at the end of the first division cycle. Radioresistance of the meristem cells, being at the different stages of the mitotic cycle, varied by 2–3 times. The most resistant phase is the early G_1-stage and the least resistant—the late G_1-stage, G_2-stage and mitosis. In case of the asynchronous dividing meristem with the cells losing the ability to divide, a part of the cell population with higher radioresistance is capable of dividing. The mentioned fact determines a higher radioresistance of meristems with an asynchronic type of division in comparison with the artifically synchronized ones that is realized only due to cell repopulation which kept the ability to divide.

D-14-1 *The N.S.D. Concept and Radiobiology; New Approaches to Treatment of Radioresistant Tumours.* FRANK ELLIS, Memorial Sloan-Kettering Institute, 1275 York Avenue, New York, NY 10021, USA.

The nominal single dose concept has proved useful in clinical radiotherapy for prescribing unorthodox treatment schedules, comparing techniques on the basis of biological effect and explaining complications. It can be linked to radio-biological data and through this type of approach clinically acceptable variations from the usual radiotherapeutic techniques can be shown likely to be more effective in treating radioresistant tumours.

D-14-2 *Iso-Effect Functions for High-Let Radiations: Dependence of Slope and Shape Upon RBE and OER.* LIONEL COHEN, Michael Reese Medical Center, Chicago, Illinois 60616, USA.

Dose-response functions of cells exposed to heavily ionizing particles are markedly different from those observed with low-LET radiation. Similarly, clinical dose-time factors, including tumor lethal doses and normal tissue tolerance limits for different fractionation schemes, depend on cellular radiosensitivity parameters as well as LET and oxygen tension and the related radiobiological parameters RBE and OER. The two-component model of cellular radiation lethality is well suited for examining these implications theoretically. It is assumed that with high-LET radiations there is a greater probability of direct and irreparable events, so that the single-target radiosensitivity constant is increased, and that under conditions of hypoxia the multi-target component, concerned with chemically-mediated indirect and reversible events, is diminished.

When these two adjustments are entered into the cell population kinetic model, a simple computer program can be used to generate iso-effect curves for both photons and heavily ionizing particles, for oxygenated and anoxic states, and for various tissues and tumors.

D-14-3 *A Trial in Accumulative Effective Dose Computation of Fractionated Radiation Therapy.* ATSUO AKANUMA, University of Tokyo, Tokyo, Japan.

According to Elkind's experiments, the cellular recovery is completed 24 hours after irradiation. When radiation is delivered every day at the same time cellular recovery from the preceeding radiation is completed. If the recovery fraction of each increment dose were constant the total effective dose should be total dose delivered less constant recovery dose times fraction number. This relationship yields a linear curve on a normal graph paper. The total effect from fractionated radiation rather yields a full logarithmic curve. This fact indicates that the recovery dose is not constant and, moreover, the effective dose from each increment increases gradually. Our working model here is to postulate that sensitivity of the irradiated tissue increases gradually with a constant proportion. A few sets of reported data of equivalent total dose in fractionated radiation are applied to obtain the parameter. The results revealed different values of the parameter in normal and malignant tissues which may explain the feasibility of

radiation therapy. The computed theoretical effective dose showed almost full logarithmic relationship between total doses and fractimation numbers.

D-14-4 *The Ellis-Formula. A Study in Applied Radiobiology.* R. W. WIDERÖE, 5415 Nussbaumen/Baden, Switzerland.

1. The Ellis-formula representing quantitative clinical information is used as a bridge, trying to close the gap between radiotherapy and radiobiology.

2. Isoeffect curves calculated by the 2-component theory of radiation using cell parameters from irradiated cell cultures of human kidney cells (T1) are in good agreement with the formula. For tumor sterilization a reduction of tumor cells to between 10^{-7} and 10^{-8} (possibly closer to 10^{-8}) seems necessary. Isoeffect curves for Co-60 gamma rays and high-energy electrons indicate higher exponents, $n = .30$ resp. .36 for fractionation number-curves.

3. The previous results are only valid for euoxic tumor cells. If the tumor contains 10% completely anoxic cells the isoeffect curves change entirely, strongly depending on reoxygenation. For small single doses (250 rad) and 20% conversion the influence of the anoxic cells is negligible. The reoxygenation can be enhanced by suitable treatment programmes; an example is presented.

4. The time factor in the Ellis-formula represents influence of repair and repopulation. Using the repopulation factor e^{T/T_0} proposed by L. Cohen, repopulation values satisfying the Ellis-formula are calculated. The cell sensitivity must be about $\frac{1}{3}$ of that for tumor cells to achieve satisfactory cell survivals. Calculated "Ellis curves" for Co-60 and electrons are presented.

The results are: Only with electron treatment are tolerance doses greater than sterilizing doses. For high-energy neutron irradiation the tolerance doses are only about half of the necessary tumor sterilizing doses.

D-14-5 *On the Human Clonogenic Cancer Cell. II: Predictive Models of Its Response to Fast Neutron Beam Irradiation.* J. ROBERT ANDREWS, Veterans Administration Hospital, Washington D.C. 20422, AND HERBERT W. HETHCOTE, University of Iowa, Iowa City 52242, USA.

An analysis of human cancer radiotherapy experience suggests that the clonogenic cells of carcinomata are a population, or subpopulation, of relatively small size of possibly hypoxic cells (Andrews, 1973. On the Human Clonogenic Cancer Cell. I: Its Number and Its Radiosensitivity. Proceedings of the Annual Hanford Biology Symposium, Richland, Washington). It may be this relatively radioinsensitive population or subpopulation which accounts, in part, for failures of radiotherapy with low-LET photons. An alternative is radiotherapy with high-LET, less oxygen-dependent fast neutron beams. The parameters and their values of the biology and the radiation responses of cancer and normal tissues are sufficiently well known that predictive models may be constructed. Such models are important in decision-making processes where cost-benefit analyses are determinative. The models and their implications for human cancer radiotherapy will be presented.

D-15-1 *Photochemical Isomerization of 1-Hydroxy- to 3-Hydroxyxanthine.* F. L. LAM, JAMES C. PARHAM, AND GEORGE B. BROWN, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA.

Ultraviolet irradiation of 1-hydroxyxanthine (1) causes extensive photoreduction. Concomitantly, there is some photoisomerization to 3-hydroxyxanthine (2) (2–6%), which is also photoreduced. The photoisomerization of 1- to 3-hydroxyxanthine was observed at pH's 0, 3 and 9 indicating that the novel hydroxyl rearrangement can occur from either the neutral species of 1 or its anion. This isomerization is of interest from both chemical and biological respects, since 2 is a potent carcinogen, while 1 is not. An intramolecular mechanism involving consecutive oxazirane intermediates is proposed for the oxygen migration. Two structurally related compounds, 1-hydroxyguanine and 1-hydroxyisoguanine showed no evidence of N-hydroxyl isomerization, but underwent ring opening, apparently from oxazirane intermediates, and photoreduction. (Supported in part by NCI grant 15274 and AEC Contract AT(11-1)-3521.)

D-15-2 *A Kinetic Study on the Reactions Induced into the Gamma Irradiated Solutions of Some Derivates of Phenothiazine.* STEFAN VELEA, VIRGINIA ACHIMESCU, CORNEL GEORGESCU, AND GHEORGHE IONESCU, Institut de Medicină si Farmacie Bucureşti, Bucharest, Romania.

A study carried out on the stability of phenothiazine nucleus derivates also investigated the kinetics of radiolytic reactions of chlorpromazine, promethazine and trifluorperazine under the form of hyper chlorides in aqueous solutions. The quantitative and qualitative study was performed by spectrophotometric and electrophoretic means. Irradiations were from a ^{60}Co source with an integral dose of 0.86 R/h and with various dose outputs from 10^2–10^6 R. The decomposition reaction for the substances investigated is a zero order reaction. The rate constants experimentally determined correspond to the following values: 2.45×10^{-5} for chlorpromazine; 2.65×10^{-5} for promethazine; 4.16×10^{-5} M/s for trifluorperazine. Except for the low decomposition rate it was found out that the substituent of the phenothiazine nucleus as well as the nature of the side chain play no preponderant role in the reaction mechanism.

D-15-3 *Effect of Oxygen and Nitroaromatic Cell Sensitizers on Radiation-Induced Internucleotide Bond Breaking: ApA and dApA.* J. Raleigh and W. Kremers, Medical Biophysics, Whiteshell Nuclear Research Establishment, Atomic Energy of Canada Limited, Pinawa, Manitoba, Canada.

3′-Mononucleotides are 2–3 times more labile than the corresponding 5′-isomers with respect to radiation-induced (^{60}Co-γ) phosphate release in deoxygenated aqueous solution (3′-G(iPO_4) 0.25–0.53, 5′-G(iPO_4) 0.14–0.23). Oxygen and nitroaromatic cell sensitizers (nitrobenzenes, nitrofurans) diminish this 3′-selectivity. Similar effects are now reported for adenylyl-3′,5′-adenosine monophosphate (ApA) and deoxyadenylyl-3′,5′-deoxyadenosine monophosphate (dApA)—compounds which incorporate the basic phosphodiester moiety of nucleic acids. G values for 3′-cleavage (ApA 0.22; dApA 0.19, deoxygenated solution) and 5′-cleavage (ApA 0.10; dApA 0.09, deoxygenated solution) were quantified by liquid chromatography. Oxygen protects against 3′-cleavage (G 0.09 and 0.02 for ApA and dApA, respectively) while nitroaromatic sensitizers mimic this oxygen effect in a way which increases with nitroaromatic electron affinity. The total yield of initial plus alkali-induced 3′-bond breaks (G 0.3, deoxygenated solution) in dApA is the same in the presence or absence of nitroaromatic compounds (alkali labile bonds detected by 30 minute 1 *N* NaOH treatment at 25° after irradiation). In oxygenated solution the total yield of 3′-breaks is ~1.4 times that in deoxygenated solution. The chemical bases for those observations and their possible significance to the radiation chemistry of nucleic acids will be discussed.

D-15-4 *Reaction of Oxygen with Polynucleotides Following Attack by Hydroxyl Radicals.* Howard B. Michaels and John W. Hunt, The Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, Toronto, Ontario, M4X 1K9, Canada.

Although peroxides are known to be produced in DNA irradiated in the presence of oxygen, the actual reaction of O_2 with the radicals formed on the DNA, e.g., due to hydroxyl radical (·OH) attack, has not been observed directly, even though such a reaction has been seen with mononucleotides. Pulse radiolysis experiments with aerated solutions of polynucleotides indicate that the behaviour of single-stranded homopolyribonucleotides is similar to that of their corresponding monomers, and that there are large differences for solutions of purines as compared to pyrimidines. The ·OH adducts of certain single-stranded polymers, including thermally denatured DNA, clearly do react with O_2. However, the absorption spectrum of the ·OH adduct of native double-stranded DNA shows negligible decay from microsecond to millisecond times in the presence of oxygen; repeated radiation pulses cause a gradual unwinding of the DNA, and after ~40 krads, ·OH adducts on these single-stranded regions are observed to react with O_2. In the case of native DNA it is possible that either O_2 does not react with the ·OH adduct species during the time scale studied, or that O_2 does react, but the spectrum of the peroxy adduct is so similar to the original ·OH adduct spectrum that it cannot be detected. It is unlikely that the double-helical structure of DNA provides protection against oxygen at low doses, since ·OH and certain radiation sensitizers are known to react with double-stranded DNA; therefore low dose radiation studies are now being carried out to determine if the DNA peroxides are formed by this process.

D-15-5 *One-Electron Reduction of Ferrihaem.* J. BUTLER AND G. G. JAYSON, Liverpool Polytechnic, Liverpool L33AF, and A. J. SWALLOW, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester M20 9BX, Great Britain.

Haemin in alkaline aqueous solution forms a hydroxy protoferrihaem dimer. The hydrated electron reacts with this with $k = 2 \times 10^{10}$ M^{-1} s^{-1} ($\mu = 0.1$ M) to form a product whose absorption spectrum resembles that of a mixture of the oxidized and reduced haem monomer except that it has a stronger absorption in the Soret band. The product disappears in a first order reaction with $k = 10^3$ s^{-1} at 25°C, activation energy 27 kJ mol^{-1}, entropy of activation −97 J deg^{-1} mol^{-1}, perhaps to form the two monomers. The reduced monomer is a final product but there is evidence of a subsequent process in which the oxidized monomer dimerizes with $k > 10^8$ M^{-1} s^{-1}. The nature of the changes which give rise to these processes is discussed.

D-15-6 *The Effects of Solvent Composition on the Kinetics of the One-Electron Oxidation of the Triplet State of Zinc Uroporphyrin.* P. A. CARAPELLUCCI, Jet Propulsion Laboratory, Pasadena, California 91103, USA.

While investigating the irreversible one-electron oxidation reaction of the triplet state of zinc uroporphyrin by various organic and inorganic electron acceptors, it was observed that the kinetics involved do not follow the accepted dependences on inert salt, solvent, viscosity, and dielectric constant changes. The reaction kinetics for the oxidation reaction appear to be influenced more by the irreversible thermodynamic properties of the reaction medium than the above mentioned factors. Results indicate that these observations are a general phenomenon in kinetics, and not specific for the oxidation of the triplet state of zinc uroporphyrin.

D-15-7 *Spectrophotometry as a General Method for Investigating Radiolysis in Solution.* GEORGE GORIN, NOBUKO OHNO, AND M. QUINTILIANI, Oklahoma State University, Stillwater, Oklahoma 74074, USA, and CNR Laboratorio di Fotochimica, 40126 Bologna, Italy.

Compounds that contain an aromatic nucleus as well as many other substances of biological importance exhibit characteristic absorption spectra in solution, which are altered by irradiation. Spectrophotometry is a powerful means for investigating such systems: fairly accurate measurements can easily be made at several wave lengths (A_λ) and they can then be subjected to rigorous mathematical analysis. In general, radiolysis involves many concurrent and competitive reactions, whereby a solute S may be converted into many products, $P_1 \cdots P_i$. If the weighted sum of the specific absorption coefficients of all products, a_Σ, is less than a_S, A_λ will of course decrease with E_v, the energy absorbed per unit volume. In general, one may write: $A_\lambda = A_\lambda^0 + \alpha_\lambda E_v + \beta_\lambda E_v^2 + \cdots$; α_λ will be negative for the case contemplated above, positive if $a_\Sigma > a_S$. For some compounds in a certain range of wave lengths a_Σ may be negligible relative to a_S, in that case the initial yield for decomposition of S, $G^0(-S)$, will be given by $c_S{}^0\alpha/A^0$ and the identity of the products need not be known. In turn, if $G^0(-S)$ is known, certain deductions can be made about a_Σ at other wave lengths (this is of course also true if $(d[S]/dE_v)$ can be determined by some independent analytical method, as will be necessary if a_Σ is not neglegible at any accessible wave length). If in some range $a_S > a_\Sigma$ and in some other range the opposite is the case, it may be that at some intermediate point $a_\Sigma = a_S$; then A will remain constant over a range of E_v. When this constraint applies, further deductions may be made about the properties of the products. The above approach is applied to the radiolysis of cytosine and adenylic acid.

D-15-8 *Effects of Hematoporphyrin on Photoproducts Formation in UV-Irradiated Thymine.* E. RIKLIS, A. PRAGER, AND E. ELHANANI, Nuclear Research Center, Negev, Beer Sheva, Israel.

Porphyrins tend to localize in certain human and animal tumors. It has been reported that x-ray therapy in conjunction with various injected porphyrins resulted in significant response, and that hematoporphyrins greatly increase the x-radiation sensitivity of paramecia. Bearing in mind that thymine dimers, which are responsible for cell damage following UV-irradiation, are also responsible for tumor production in certain organs, we have decided to study the effects of porphyrins on thymine dimer production. It has been found that the addition of hematoporphyrin sensitizes a frozen solution of thymine. UV irradiation of methyl-^{3}H-thymine frozen

solution and separation of photoproducts by radiochromatography resulted in a 3–5 fold increase in production of thymine dimers as well as in appearance of another yet unidentified photoproduct. This sensitization is pH dependent and concentration dependent with a maximum effect shown by 1×10^{-5} *M* hematoporphyrin with 1×10^{-4} *M* thymine. The effect on DNA and on whole cells is under study, and the significance of these studies will be discussed.

D-16-1 *Effects of Ionizing Radiation on Random Coil-Hairpin Helix Transitions in Poly d(A-T).* JOHN STEPHENS AND CONRAD N. TRUMBORE, University of Delaware, Newark, Delaware 19711, USA.

Exposure of aqueous poly d(A-T) solutions to low doses of γ radiation decreases the first order rate constant for the fastest of the random coil-to double helix conformation changes of poly d(A-T) induced by a pH jump. Small radiation doses also markedly reduce the total extent of the fastest portion of these renaturation processes. With the irradiated polymer, the remaining slow processes take place over a period of hours and ultimately achieve the full hypochromicity change normally observed in milliseconds for the unirradiated polymer. It is postulated that significant, permanent molecular damage to one or more of the constituents of poly d(A-T) occurs at doses less than one kilorad. According to sedimentation studied, these rate alterations should not be caused by strand scission but by some type of base (probably non-chromophoric) or sugar damage. It is suggested that similar, subtle forms of damage should be present in irradiated, natural DNA at very low doses and that this damage may markedly affect rate processes, possibly including replication rates, involving DNA in living systems.

D-16-2 *In Vitro Radiation-Induced Strand Breaks in DNA.* J. F. WARD, I. KUO, AND D. MUHLEMAN, Laboratory of Nuclear Medicine and Radiation Biology, University of California at Los Angeles, 900 Veteran Avenue, Los Angeles, California 90024, USA.

Model systems (Ward and Kuo, Int. J. Radiat. Biol. **23**, 543 (1973)) have indicated that a DNA strand break should be accompanied by release of a damaged nucleoside and that the yield of the release should increase with post-irradiation time. These predictions have been examined in DNA irradiated in aqueous solution. DNA labelled with ^{3}H-thymidine was used. After irradiation the macromolecular material was precipitated with ethanol and damaged thymidine moieties were separated from tritiated water and small oligonucleotides by column chromatography. The yield of thymidine monomers immediately after irradiation is G = 0.06, this yield increases to G = 0.19 after 24 hours. These yields were determined from yield-dose plots at low DNA destruction (less than 1%). In similar experiments G(total damage nucleoside), as measured by ultra violet absorption, increases (at 20°C) from 0.42 immediately after irradiation to 0.67 in 20 mins., 0.83 in 1 hour, 0.98 in 2 hours and 1.26 after 24 hours. This postirradiation time dependence of base release will be discussed in relation to DNA strand breaks. (Supported by the U.S.A.E.C.)

D-16-3 *Mechanisms of Radiation Damage to DNA, and the Action of Radiosensitisers.* D. W. WHILLANS AND G. E. ADAMS, Gray Laboratory, Mt. Vernon Hospital, Northwood, Middlesex, Great Britain.

A study of the modes of radiation damage to DNA in aqueous solution employed intercalating dyes as probes for reactions which occur within the polymer. The reactions of these probes were followed for evidence of intramolecular radical migration in relation to the ordered structure of the complex. The redox reactions of the nucleic acid radicals with an electron-affinic radiosensitiser, p-nitroacetophenone, were characterized by a study of the reactivity of this sensitiser with radicals formed from free bases, nucleosides, nucleotides, and from DNA itself. From these studies it was concluded that with such sensitisers, for which biological activity correlates with electron affinity, electron-transfer oxidation of DNA radicals formed by electron attachment to the bases is most efficient. However a small yield of transfer (<10%) is observed resulting from ·OH and ·H reactions at the sugar residues rather than the bases.

D-16-4 *Effects of Metal Ions on the Formation of Strand Breaks and Radicals, and on Hydrogen Transfer in γ-irradiated DNA in the Solid State.* S. KOMINAMI, V. WEE, AND P. RIESZ, National Cancer Institute, N.I.H., Bethesda, Md. 20014, USA.

The effects of metal ions (1 metal ion per 100 nucleotides) on the formation of strand breaks by γ-radiolysis of calf thymus DNA in the solid state at 298°K in vacuo were investigated. The G-value for strand breaks in DNA is 1.5 and is decreased by a factor of 5 by Cr^{3+} ions, a factor of 3 by Cu^{2+} ions and about 2 by Ni^{2+} and Cd^{2+} ions. Mg^{2+} has no effect. Similar effects of metal ions on the formation of radicals in γ-irradiated DNA at 77°K and 298°K were measured by ESR and were also observed during the transfer of tritium from exchangeable to carbon-bound sites in γ-irradiated DNA at 298°K. Metal ions exert their protective effects at an early stage of γ-radiolysis, prior to stabilization of the radicals at 77°K. The similarity of the effects of the metal ions on these three different processes could be explained by assuming that metal ions decrease the yield of a common precursor species.

D-16-5 *Photoexchange at C(5) of Pyrimidine Nucleosides.* WILLIAM W. HAUSWIRTH AND SHIH YI WANG, Department of Biochemistry, The Johns Hopkins University, Baltimore, Maryland 21205, USA.

Photoexchange at C(5) of cytidine-5-T was studied with the aim of (1) establishing its true light-induced nature by kinetic analysis, (2) determining its quantum efficiency, particularly relative to that for photohydration, and (3) establishing a consistent mechanism of formation and its possible relationship to the process of photohydration. Correcting for a C(5)-tritium isotope effect, photoexchange in both nucleosides is found to be as efficient as photohydration (Cytidine $\phi_{exch} = 0.026$, Uridine $\phi_{exch} = 0.022$) and is, therefore, a major photochemical event in dilute solution. Negative triplet sensitization and quenching results, along with the observation that photoexchange efficiency is independent of the irradiation wavelength, suggest an excited singlet precursor. A mechanism is proposed involving phototautomerization producing a saturated center at C(5) and its possible role in photohydration is outlined. (This research is supported by U.S. Atomic Energy Commission Contract AT(11-1)-3276).

D-16-6 *Serologic Determination of DNA Base Damage.* HAZEL L. LEWIS AND JOHN F. WARD, University of California at Los Angeles, Laboratory of Nuclear Medicine and Radiation Biology, 900 Veteran Avenue, Los Angeles, California 90024, USA.

Serological assays for DNA base damage are being developed. These will permit the identification of low levels of a wide variety of irradiation products. The procedures used remove the problems of chemical detection, i.e., artefact formation. Antibodies have been prepared to a bovine serum albumin conjugate of the oncogenic compound adenosine-N^1 oxide. In complement fixation assays using the adenosine-N^1 oxide ovalbumin conjugate as antigen the specificity was shown to be for the purine base group. Adenosine-N^1 oxide is detected at 0.5 nmole levels by this method. Currently serologic procedures which greatly increase the sensitivity are being applied.

Haptene inhibition tests demonstrated a 100 fold reduced reaction with adenosine and no cross reaction with guanosine, cytidine and thymidine. Specificity may be increased by selective absorption of the antiserum. Application of this method to the assay of base damage in irradiated DNA will be discussed. (Supported by U.S.P.H.S. Grant No. CA13437-02 and by the U.S.A.E.C.)

D-16-7 *Gamma Irradiation of* E. coli *DNA in an Aerated Aqueous Solution. Identification of Radiolysis Products.* R. TEOULE, A. BONICEL, C. BERT, J. CADET, AND M. POLVERELLI, Laboratoire de Radiobiologie, Centre d'Etudes Nucléaires, BP 85 Centre de Tri 38041, Grenoble, France.

DNA selectively labeled with $^{14}C(CH_3)$ in thymine moiety was submitted to γ rays in an aerated aqueous solution. The products resulting from the cleavage of C-N glycosidic bond and the breakage of pyrimidic ring were characterized by comparison with authentic samples in thin layer chromatography and by microreactions. The main substances were: thymine, cis and trans 5,6-dihydroxy-5,6-dihydrothymine, 5-hydroxy-5-methyl barbituric acid, N-formyl-N'-pyruvyl urea and pyruvamide.

Microreactions performed with these compounds are described. Kinetic data from 10 Krads to 1000 Krads are given. Pyruvamide and thymine were the first compounds to be produced at

low doses. This result is in perfect agreement with reaction mechanisms proposed. In the same way, DNA selectively labeled with $^{14}C_2$ in cytosine moiety, was irradiated. The radioproducts were determined as cytosine, cis and trans uracil glycols, isodialuric acid, biuret, hydroxy-5-hydantoin, formylurea, trans 1-carbamylimidazolidone-4,5-diol and 4-amino-1-formyl-5-hydroxy-2-oxo-3,4-didehydroimidazolidine. The degradation of free pyrimidines and that of the heterocyclic bases incorporated in nucleoside or DNA were compared.

D-16-8 *Modification of the Characteristics of Conformational Transitions in X-Irradiated DNA by Metal Ions.* M. I. KOMPANIETS AND V. A. ZHIDKOV, Institute of Plant Physiology, Academy of Sciences of the Ukrainian SSR, Kiev 252127, USSR.

The investigation of the DNA radiosensitivity in different conformational states depending on complexing with metal ions is important for the elucidation of the mechanisms of DNA radiation damage as well as for the interpretation of the peculiarities of metal-DNA interactions. Effect of Mn^{2+}, Zn^{2+}, MoO_4^{2-} ions on thermal- and acid-induced transitions in irradiated DNA was studied in conditions when intermolecular interactions may be neglected. Metals were added to 0.002% DNA solutions, then the irradiation (3 and 10 krads) was carried out after the realization of helium gas saturation.

Mathematical simulation of conformational transitions is based on the computer analysis of absorption data in spectral range of 230–290 nm. Adenine-thymine and guanine-cytosine base pair fractions denaturated in conformational transition range were calculated at several temperature and pH points. Acid denaturation analysis included consideration of spectral changes induced by protonation of nitrogen base aminogroups, and obtaining the protonation course of cytosine.

The specificity of conformational transition changes in irradiated DNA-metal complexes connected with interaction between metal ions and different groups of DNA macromolecule is discussed.

D-17-1 *Yield and Reactivity of Electrons in Viscous Ethanediol-Water Solutions between −90° and −130°C.* IRWIN A. TAUB, JAMES TOCCI, AND PETER A. HURWITZ, US Army Natick Laboratories, Natick, MA 01760, USA.

Pulse irradiation studies on the yield and lifetime of solvated electrons in the presence of solutes in viscous ethanediol-water solutions at low temperatures provide evidence for the existence of an unsolvated electron as a precursor to the solvated electron. Solutes such as HCl, $ClCH_2COOH$, $CuCl_2$, $NaNO_3$, $NaNO_2$, acetone, and acetamide are effective in decreasing the yield of observable solvated electrons, the C_{37} values being on the order of 0.5 *M*. Their efficiencies for scavenging unsolvated electrons in this frozen medium therefore are comparable to the corresponding efficiencies in water at room temperature that have been published based on picosecond time scale results. These solutes also shorten the lifetime of the observed solvated electrons, the bimolecular rate constants correlating approximately with the corresponding constants for hydrated electrons. Although HCl and $NaNO_3$ show similar reactivities towards unsolvated electrons, the former is twelve times more reactive than the latter towards solvated electrons. These results suggest that the precursor to the solvated electron reacts with the solvent in a mechanistically similar process in both frozen and fluid aqueous media, possibly involving pre-existing traps, and that the hydrogen ion is a more active agent than are other solutes in enhancing the decay of solvated electrons in viscous aqueous solutions.

D-17-2 *Electron Decay in the Pulse Radiolysis of Pure Water. Evidence for Spur Overlap.* JAMES E. FANNING, JR. AND C. N. TRUMBORE, University of Delaware, Newark, Delaware 19711, USA.

The rates of decay of the hydrated electron absorbance at 633 nm in pure water have been studied as a function of time and dose per 20 nsec pulse of 15 MeV electrons from the Argonne linear accelerator. Plots of Q, defined as the instantaneous electron fraction decay rate, $[-d(e_{aq}^-)/(e_{aq}^-)]/dt$, vs. time and pulse show that these decay rates diminish with increasing time for a given pulse dose but are essentially independent of pulse dose at a given time following the pulse for low doses (<300 rads/pulse). With increasing pulse dose, the plots of Q vs. time

deviate more and more markedly from the low dose behavior. Plots of Q vs. pulse dose for a given time following the pulse are analyzed in terms of dose independent (intraspur electron decay) and dose dependent (inter- and intraspur electron decay) regions. The transition periods between these regions are analyzed in terms of spur overlap as a function of time and pulse dose.

D-17-3 *Comparison of Reaction Rates of Solvated Electrons in Water and in Ammonia (Pulse Radiolysis Studies).* U. SCHINDEWOLF AND P. WÜNSCHEL, Institut für Physikalische Chemie und Elektrochemie, Universität Karlsruhe, Karlsruhe, West Germany.

Solvated electrons in water are very short lived due to their fast reactions with water or added substrates (reaction rate constants between 10^6 and 10^{11} l/Mol. sec). In pure ammonia on the other hand solvated electrons have a long life time, even the reaction with water is in ammonia extremely slow, due to the small preexponential factor in the rate law. This in turn is due to the negative activation entropy of the reaction or to the large positive entropy of the ammoniated electrons. From this the hypothesis was derived that all reactions of solvated electrons should be slower in ammonia than in water.

Pulse radiolysis experiments prove this hypothesis for a series of substrates like benzene, aniline, nitrate-, nitrite-, chlorate-, bromate-, zinc- and other ions, which in ammonia react with solvated electrons by several powers of ten slower than in water (exception: ammonium ion). For all these reactions the energy of activation in ammonia is about the same as in water. Parallel with the low rates we observe large negative activation volumes.

D-17-4 *A Simple Quantum Mechanical Treatment of Electron-Ion Recombination.* ROBERT SCHILLER, Central Research Institute for Physics, Budapest, Hungary.

For the description of geminate electron-ion recombination the electron is treated as a spherical wave propagating in the potential well of the positive ion. Recombination probability is discussed in terms of electron scattering.

D-17-5 *Nanosecond Pulsed Radiolysis of Liquid Alcohols at Low Temperatures: A UV Study of the Transient Species.* LUCIEN GILLES, BERNARD HICKEL, BERNARD LESIGNE, AND PHILIPPE NACASS, CEN. Saclay, DRA/SRIRMa, 91190, Gif Sur Yvette, France.

Picosecond and Nanosecond Pulse Radiolysis have provided information on the solvation time of the electron.[1,2]

A modified Febetron 707 delivering 8 ns halfwidth pulses of 1.8 MeV electrons is used and doses from 5 to 200 Krad/pulse are obtained.

The monitoring light source (Xe lamp 450 W) being intensified during 1 msec allows one to follow the formation and decay of transient species immediately after the pulse from 260 to 900 nm. In n Propanol and at wavelengths below 350 nm the kinetics of formation and disappearance of the transient species and its behavior towards scavengers show that it is not an electron, but is related to the hydroxyradicals formed in the radiolysis of alcohols.

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[1] Solvation Time of the Electron in Liquid Alcohols and Water at Room Temperature, L. Gilles, J. E. Aldrich, and J. W. Hunt, Nature Physical Science **243**, 70 (1973).

[2] Electrons in Liquid Alcohols at Low Temperatures, J. H. Baxendale and P. Wardman, Journal of the Chem. Soc., Faraday Transactions **69**, 584 (1973).

D-17-6 *Spectral Shifts of the Solvated Electron in Ethanol and Deuterated Ethanol Glasses at 76°K.* NORMAN V. KLASSEN, HUGH A. GILLIS, AND GEORGE G. TEATHER, National Research Council, Ottawa, Canada K1A 0S1 and LARRY KEVAN, Wayne State University, Detroit, Michigan 48202, USA.

A blue shift with time in the optical absorption spectra of solvated electrons in C_2H_5OH, C_2H_5OD and C_2D_5OD glasses has been studied by pulse radiolysis. Values of λ_{max} for C_2H_5OH, C_2H_5OD and C_2D_5OD are 1400, 1300 and 650 nm respectively at 2×10^{-7} sec after a 10^{-8} sec pulse. The ensuing spectral shift seems to be somewhat more rapid, on a sub-microsecond time scale, for C_2D_5OD than for C_2H_5OH or C_2H_5OD. After several msec the spectral shift is largely

complete; e.g., for C_2H_5OH at 40 msec $\lambda_{max} \sim 600$ nm, close to its long term value. These results will be discussed with particular reference to published results of γ-experiments done at 4 K.

D-17-7 *Circular Dichroism of Trapped Electron Absorption Bands in Chiral Media.* ROGER MAY AND DAVID C. WALKER, Chemistry Department, University of British Columbia, Vancouver, Canada.

An investigation, by conventional techniques, of the possible circular dichroic spectra of electrons trapped in chiral alcoholic and aqueous glasses at 77°K shows that their optical absorption bands do not have large asymmetry factors (g). Circularly polarised light pulses from a Q-switched ruby laser have also been used in an attempt to amplify any optical activity at these small g values by subjecting the trapped electrons to multiple excitations and deexcitations. The ramifications of these experiments are discussed in terms of topical concepts of trapped electron absorption bands.

D-18-1 *Reaction Rates of Quasi-Free Electrons in Dielectric Liquids with Various Molecules.* AUGUSTINE O. ALLEN, RICHARD A. HOLROYD, AND THOMAS E. GANGWER, Brookhaven National Laboratory, Upton, New York 11973, USA.

Electrons were generated by x-ray ionization in various non-polar liquids, in which the electron mobilities varied by factors up to 1000. Reactivities of added solutes towards the electron were determined by examining conductivity transients. For some solutes, the rates increased with increasing electron mobility, as might be expected; but for others, the rates were slowest in liquids of highest electron mobility. In many of these cases, the rates actually increased when the temperature was lowered, instead of decreasing as in practically all other chemical reactions. There is some evidence that the electron affinity of these liquids decreases with decreasing temperature, so that the energy of the electron in its mobile state may actually be higher at lower temperatures. The results are discussed in terms of the state of the electron in the disordered solvent, and the known interaction of the various molecules in the gas phase with electrons of varying energies. (Research performed under the auspices of the U.S. Atomic Energy Commission.)

D-18-2 *Electron Scavenging of N_2O in the Radiolysis of Liquid Hydrocarbons.* Y. HATANO, K. ITO, AND S. TAKAO, Laboratory of Physical Chemistry, Tokyo Institute of Technology, Meguro-ku, Tokyo, Japan.

The γ-radiolysis of liquid neopentane and isooctane has been carried out in presence of the lower concentrations of N_2O_4. The addition of N_2O to neopentane at the mole fraction $N_s = 10^{-5}$–10^{-4} gives constant $G(N_2) = 1.1 \pm 0.1$ at 293 ± 1°K. At the further lower concentrations of $N_s = 10^{-5}$–10^{-7}, $G(N_2)$ calculated from the dose dependence on the decomposition of N_2O gives the same value as that at $N_s = 10^{-5}$–10^{-4} which is in good agreement with the free-ion yield in the electric conductivity method. In the case of isooctane, there exists some lower concentration range of N_2O ($N_s = 10^{-6}$–7×10^{-6}) where $G(N_2)$ gives a constant value 0.33 which also agrees well with the free-ion yield. It may be concluded in both cases that, at such low concentrations of N_2O, $G(N_2)$ arises from the complete scavenging of the free electrons and one N_2 molecule is produced per an electron scavenged by N_2O. The results have been compared with that in the case of cyclohexane and *n*-hexane solutions.

D-18-3 *Kinetic Properties of Electrons in Aromatic Liquids.* ALAN J. ROBINSON AND MICHAEL A. J. RODGERS, Chemistry Department, University of Manchester, Manchester M13 9PL, Great Britain.

Electron beam induced ionisation of pure liquid aromatics (benzene and toluene) has been studied by a time-dependent conductivity technique. The reactions of quasi-free electrons in such media with suitable reactants have been studied and the rate constants evaluated. Data relating to electron-ion recombination parameters and the yields of escaped ions have been obtained by the same method. The investigations lead to conclusions about the mobility of quasi-free electrons under such conditions, and the reliability of theoretically derived rate constants.

D-18-4 *Effect of Deuteration on Solvation of Electrons in Ethanol Glasses.* HIROTOMO HASE AND TETSUO WARASHINA, Research Reactor Institute, Kyoto University, Sennan-gun, Osaka, Japan.

In this study we have obtained the spectroscopic evidence that trapped electrons produced in deuterated ethanol glasses at 4°K are mostly solvated, although they are not solvated in non-deuterated ethanol glass at 4°K. Degree of the solvation is in order of: $C_2H_5OD > C_2D_5OH > C_2D_5OD > C_2H_5OH$. This cannot be explained as far as one regards an alcohol molecule as a free point dipole, but imply that the solvation depends strongly on structural factors of solvent molecules and/or on nature of inter-, and intramolecular chemical bonds at a low temperature. A parallel effect of deuteration on the degree of solvation and the strength of intermolecular hydrogen bondings is to be emphasized.

D-19-1 *The Mechanism of Radiation-Induced Ionic Polymerization of Styrenes.* MEISEKI KATAYAMA AND SADASHI SAWAMURA, Hokkaido University, Sapporo 060, Japan.

The radiation-induced polymerization of styrene and α-methyl styrene have been studied by means of pulsed conductivity measurements and also of Hittorf type conductivity measurements. It is well known that for these compounds, the more thoroughly monomers are dry, the larger G(-monomer) becomes. The pulsed conductivity measurements for α-methyl styrene showed that when it is dry, the mobility of anionic species became smaller than that of cationic ones, indicating the relative importance of anionic polymerization at such a condition, although the relationship is reversed for moderately dry samples. Hittorf type conductivity measurements also showed the importance of anionic polymerization at extremely dry condition.

The conclusions from these measurements are as follows: (1) the anionic polymerization is at least as important as the cationic polymerization, (2) the main mechanism of termination for both anionic and cationic polymerizations is the chain transfer to monomer since the number of chain transfers is estimated to be some ten thousands, and (3) the main charge-carrying species are electrons which move very fast in dry liquid styrenes.

D-19-2 *Polymers MW Determination by GPC.* ROBERTO V. A. GABARAIN AND JAIME PAHISSA CAMPÁ, Comisión Nacional de Energía Atómica, Buenos Aires, Argentina.

Radiation and thermal styrene polymerization rate under the influence of different factors was studied. For each condition, the polymer produced was separated. The MW of these polymers (Mn and MW) was determined by using the GPC, a separation method with a column having as the stationary phase a heteroporous solvent-swollen polymer network. The smaller molecules permeate more completely and spend more time, while the larger molecules pass through the column more rapidly. A calibration curve was prepared with well characterized polystyrenes of narrow MW distribution. Some conclusions are obtained. For radiation polymerization, from 20°C till 50°C, the higher the temperature the higher the MW; the higher the catalyst concentration the lower the MW, and the lower the intensity the higher the MW. For thermal polymerization, the higher the temperature the lower the MW and the higher the catalyst concentration the lower the MW. At the same temperature and catalyst concentration, the MW for polymers produced by thermal polymerization are higher than those produced by irradiation. In a general way, it looks possible to produce, at a reasonable rate, a polymer with a pre-determined MW, Mn varying from 16,000 till 770,000, and MW from 29,000 till 1,400,000, by selecting the appropriate polymerization conditions.

D-19-3 *Radiation Induced Solid State Polymerization of n-Butylisocyanate at Low Temperature.* I. SUGAWARA, E. MARCHAL,* H. KADOI, Y. TABATA, AND K. OSHIMA, University of Tokyo, Tokyo, Japan.

n-Butylisocyanate was polymerized at low temperatures (−78° to −196°C) in various (crystalline, glassy, supercooled and liquid) states.

It was found that the polymerization of *n*-butylisocyanate occurs in the supercooled state, but not in the crystalline and liquid states. It was possible to obtain a polymer when the monomer was irradiated in glassy state at −196°C. However, it was confirmed that the polymerization does not take place under irradiation at that temperature, but the post-polymerization

proceeds during the warming. The effect of additives shows that the post-polymerization of *n*-butylisocyanate may proceed via an anionic mechanism.

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* C.N.R.S., Centre de Recherches sur les Macromolecules, Strasbourg, France.

D-19-4 *NMR and ESR Studies of γ Ray-Induced Solid State Polymerization of Methacrylic Acid.* M. Latimier, A. Forchioni, and C. Chachaty, Service de Chimie Physique, CEN de Saclay, B.P. n° 2 91190, Gif sur Yvette, France.

The kinetics of post-polymerization of γ irradiated methacrylic acid at 77°K has been investigated by wide line NMR and ESR spectroscopy. The conversion yield was monitored continuously from 260°K up to 280°K, from the narrowing of the NMR spectrum, due to the progressive amorphization of the matrix. The reaction rate decays exponentially with time, independent of the free radicals recombination. The local concentration of radicals subsisting after prolonged annealing remains unchanged, however, showing that no recombination occurs in the microdomains of polymerization. High resolution ^{13}C NMR spectra of the polymer shows that the contribution of isotactic sequences increases as the post-polymerization temperature is lowered.

D-19-5 *A Note on the Radiation Induced Crosslinking in Solution Grown Polyethylene.* Hisatsugu Kashiwabara, Shigetaka Shimada, and Yasuro Hori, Nagoya Institute of Technology, Showa-ku, Nagoya, Japan.

Crosslinking formation in irradiated polyethylene has been one of the interesting subjects, which has attracted many scientists in the field of radiation chemistry of polymeric systems for more than twenty years. In the present paper, crosslinking formation at the location near the fold end of the crystallite (crystal surface) of solution-grown polyethylene will be discussed based on the data of the grafting reaction and DSC studies. It will be shown that crosslinking formation can be made at the location near the fold end in the early stage of the irradiation and this effect can be removed by the fuming nitric acid treatment which is said to remove the folded region of the crystallites. Correlation with the result of ESR studies of the free radicals in polyethylene will also be mentioned.

D-19-6 *Radiation-Induced Grafting of Hydrogels on Silicone Rubber Surfaces.* G. M. Meaburn, C. M. Cole, and J. L. Hosszu, Armed Forces Radiobiology Research Institute, Bethesda, Md. 20014; C. W. Wade and J. Eaton, U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Md. 21701, USA.

The radiation-induced graft polymerization of 2-hydroxyethyl methacrylate (HEMA) and other acrylic monomers to silicone rubber (Silastic) sheeting has been investigated. Polymerization is initiated by exposure of the elastomer immersed in an aqueous solution of monomer to ^{60}Co γ-radiation or intense beams of high energy electrons. Some of the physical parameters associated with grafting from aqueous solution have been characterized. Factors which influence the depth and morphology of the graft include dose, dose rate, monomer concentration and purity, and the extent of the competing homopolymerization reactions. These laminates of hydrogel and elastomer are being evaluated as synthetic burn wound dressings with the desirable characteristics of biocompatibility and bio-function exhibited by cutaneous allografts.

D-19-7 *Enhanced Crosslinking of Polymers with Acrylic Acid.* A. Charlesby, J. P. Lawler, and P. J. Fydelor, Royal Military College of Science, Shrivenham, Swindon, Wiltshire, Great Britain.

The crosslinking of many saturated polymers is ascribed to the combination of two radicals, at least one of which is produced directly by radiation. This determines the minimum dose required to form a crosslinked gel fraction. In the presence of acrylic acid the dose needed is greatly decreased, indicating some form of chain reaction for crosslinking, which is quite distinct from that involved in grafting. This enhanced crosslinking does not occur with irradiated polyacrylic acid, nor with block or graft copolymers containing polyacrylic chains—acrylic acid must be present as monomer. Evidence for this novel reaction will be presented.

D-19-8 *Track-Effects in the Low-Temperature Radiolysis of Some Polymers.* Bogdan K. Pasalskyi, Valerij A. Vonsyatzkyi, Jaroslav I. Lavrentovich, Andrej M. Kabakchi, L. V. Pisarzhevsky Institute of Physical Chemistry Ukr. SSR Academy of Sciences, Prospect Nauki, 97, Kiev, USSR.

The action of Co^{60} gamma-rays and alpha-particles (22 MeV) on polyisobutylene (PIB) and polycaprolactam (PCL) at 77°K evacuated to 10^{-4}–10^{-5} torr was studied. The kinetics of stabilized radicals formation, the kinetics of their decay at high temperatures and the space distributions of radicals were investigated.

The concentration of radicals in polymers increases directly in proportion to the absorbed dose in the interval 3–200 Mrad. Radiation yield of radicals G_γ is 2.1 and G_α is 0.45 per 100 eV in PIB. In the case of PCL $G_\gamma = G_\alpha = 0.48$ per 100 eV.

The kinetics of radical decay is described by a second order equation. In alpha-irradiated PIB the rate of radical decay is well above that in gamma-irradiated one which is attributed to the different spatial distribution of radicals. The rate of the radical decay in alpha- and gamma-irradiated PCL is the same. In PIB the local concentration of radicals ($C_{loc.}$) in tracks of alpha-particles is 5.1×10^{19} and that in spurs is 7.4×10^{18} cm^{-3}. In irradiated PCL $C_{loc}^{\alpha} = C_{loc}^{\gamma} = C_{av}$. The calculated radius of the alpha-particle tracks in PIB is found to be of the order 15 to 20 Å.

The data obtained show that in PIB the realization of energy of radiation is localized in the tracks and spurs of charged particles. In PCL the effective energy migration in volume on the distance more than 100 Å precedes the radical formation.

D-19-9 *Lightweight Concrete of High Specific Strength.* Jaime Pahissa Campá and Roberto V. A. Gabarain, Comisión Nacional de Energía Atómica, Buenos Aires, Argentina.

In order to obtain high specific strength materials, lightweight concrete was impregnated with monomeric systems, basically methylmethacrylate and the polymerization produced by gamma irradiation.

For the preparation of these lightweight concretes, different proportions of sand were replaced by expanded clay and sawdust. The test specimens were dried to constant weight, evacuated after soaking in the monomeric systems and finally polymerized by the use of Co-60, with dose between 2 and 4 Mrad. Treated specimens were tested for determining the compressive strength and the tensile strength by diametral compression and comparing these with test specimens prepared simultaneously, and without any treatment.

For specimens from expanded clay the best value, averaging three tests, was 3140 Kg/cm^2 by each Kg of material. For specimens prepared with cement and sawdust, without sand, the best result for compressive strength was 2700 Kg/cm^2 by Kg, and for tensile strength, 400 Kg/cm^2 by Kg. For specimens made with cement, sand and sawdust the best value for compressive strength increased to 3040 Kg/cm^2 by Kg.

D-20-1 *The Effect of Gamma-Radiation on Metabolism and Ultrastructure of Neuro-Glial Unit in CNS of Mice.* Z. L. Olkowski, J. R. McLaren, and T. Kumamoto, Emory University Medical School, Atlanta, Georgia 30322, USA.

Previous studies on motor neurons of gamma-irradiated mice have shown a decrease of ^{14}C-Leucine incorporation into the cell cytoplasm and strong increase of incorporation into the nuclei of these cells (Olkowski, Am. J. Anat. **132**, 393, 1971). Significant changes in the activity of ATP-ase, IDP-ase, TPP-ase as well as dehydrogenases from Krebs cycle and pentose shunt in these neurons were also observed (Olkowski, Manocha and Bourne, Strahlentherapie, **143**, 202, 1972).

Since neurons, glia closely apposed and blood vessels in the CNS probably form morphological as well as functional unit, it was interesting to study fine structure of all the components as well as certain aspects of their metabolism.

Experiment was performed on 102 mice. Experimental animals were given 630 rads whole body ^{60}Co irradiation at dose rate 52.4 R/min., and non-irradiated mice served as a control. Part of the irradiated mice were injected with ^{3}H-uridine, and their spinal cord was studied 1, 2, 4,

and 8 hours after irradiation by means of radioautography and part of the animals sacrificed at the same time after irradiation were studied for RNA with use of cytospectrophotometry. Part of the spinal cord was processed for ultrastructural studies. Progressive decrease of ^{3}H-uridine incorporation was found in motor neurons of irradiated mice. Cytospectrophotometry of glia apposed to these neurons has shown decreased RNA content $\frac{1}{2}$ and one hour after irradiation and increasing amount of RNA 2, 4 and 8 hours after irradiation. Changes of fine structure of neuro-glial unit after irradiation were also observed. (Sponsored by Grant 9509 from Teaching and Research Funds, Emory University Medical School.)

D-20-2 *The Long Term Effect of Ionizing Radiation on the Central Nervous System.* TAKERU MINAMISAWA AND TAKEHIKO TSUCHIYA, National Institute of Radiological Sciences, Chiba, 280, Japan.

The long term effect of moderate x-ray doses on the averaged evoked potentials (AEPs) recorded from the visual cortex to photic stimulation was studied in four adult male rabbits with permanently implanted electrodes. Animals were exposed to x-rays directed to the dorsal surface of the head. The head, except for the region of the brain, was shielded with a 4 mm thick lead plate. Two sets of two animals were irradiated with 300 and 100 R, respectively. Two hundred evoked potentials were averaged with a Computer of Average Transients. The AEPs were recorded at biweekly intervals until the animal died. The amplitude of the early three components of the AEPs changed slightly after irradiation of 300 R. The late two components of all four animals gradually decreased in amplitude in the course of time after irradiation, with individual variations as to degree. The decreased value continued over eight months throughout the observation period.

D-20-3 *Late Effects of Irradiation on Different Regions of the Spinal Cord.* A. J. VAN DER KOGEL AND G. W. BARENDSEN, Radiobiological Institute TNO, 151 Lange Kleiweg, Rijswijk (ZH) The Netherlands.

Local irradiation of the rat spinal cord was performed to evaluate the dependence of the development of myelopathy on total dose, dose fractionation regimen, dose rate, and type of radiation used. In order to obtain an adequate comparison with human radiation myelopathy, different regions of the spinal cord were irradiated with 300 kV x-rays or 15 MeV neutrons.

Symptoms of myelopathy develop after a latent period, which decreases with increasing dose, but rapidly reaches a minimum of about 4 months for both x-rays and neutrons. With single irradiations, the largest dose which does not cause observable symptoms of paralysis within one year is 1900 rad of x-rays. Irradiations of the lumbar and the cervical region show about the same tolerance dose and dose-latency relationship, although the development of paralysis has a more acute character when the cervical region is irradiated. The main histological changes consist of demyelinization and necrosis of the nerve roots in the irradiated lumbar region. Irradiation of the cervical region resulted in focal necrosis of the white matter, without damage to the nerve roots.

D-20-4 *Lesions in the Mature CNS of Rats Induced by Proton Beams.* R. F. DE ESTABLE-PUIG, J. F. ESTABLE-PUIG, AND T. A. READER, Département de Pathologie, Faculté de Médicine, Université Laval, Québec G1K 7P4, P.Q., Canada.

The acute effects of proton irradiation on the fine structure of the CNS were examined in 22 adult rats, 15 and 30 minutes, 1, 12, 24, 48 and 72 hours after irradiation. Irradiations consisted of 20,000 or 40,000 rad through the skull from a 100 MeV beam from a synchrocyclotron (Foster Radiation Laboratory, McGill University). A 6.2 brass collimator was utilized in some cases to direct the beam only to a specific region. Irradiated and control animals were sacrificed by intracardiac-perfusion fixation of the CNS.

Similar patterns of responses were observed in different components of the olfactory bulb and cerebellar cortex that parallel those previously described with gamma or x-rays: vulnerability of small neurons, glial reaction, edema, anteroterminal and dendritic degeneration. In

addition lipid inclusions were found in the perikaria and dendrites of mitral cells and interpreted as a neuronal reaction to sublethal injury.

D-20-5 *High-LET Particle Effects upon the DNA in the Non-Dividing Photoreceptor Cells of the Rabbit Retina.* G. E. Powers, Peter Keng, and J. T. Lett, Department of Radiology and Radiation Biology, Colorado State University, Fort Collins, Colorado 80521, USA.

Freshly excised New Zealand White rabbit retinas were irradiated aerobically at 20°C with 17 MeV alphas (Boulder, Colorado, cyclotron) at flux intensities ranging from 10^5 to 10^8 alphas-cm^{-2} or 265 MeV-nucleon^{-1} oxygen-16 ions (Berkeley, California bevatron) at flux intensities of 10^5 to 5×10^6 ions-cm^{-2}.

The retinas were rapidly frozen in buffered saline and stored in liquid nitrogen until DNA analysis. The retinas were thawed in a 40°C water bath, the cells manually separated, and the DNA analyzed by zonal alkaline sucrose gradients.

The definite pattern changes that occurred in the DNA sedimentation profiles indicated damage to most of the DNA structures in the retina following low fluxes. Since this damage was also detected at fluxes at which only one cell in ten was hit by an ionizing particle, the damage to the DNA in the other cells must have occurred by delta-ray contribution or by energy transfer through the electrical or other systems of the organ. At the lowest fluxes an apparent increase in the sedimentation coefficient of DNA to (600S) indicated the destruction of gross cellular structures. At the highest fluxes some of the DNA molecules in the gradient has been reduced to (200S). (This research was sponsored by NASA Grant No. NGR-06-002-128.)

D-20-6 *Late Biochemical Effects in Locally Irradiated Rat Brain.* George B. Gerber, Euratom and Centre Etude Nucléaire, Dép. Radiobiologie, B 2400 Mol, Belgium.

Rats are exposed locally to their head with 2.2 kR of X rays and the animals are sacrificed 1, 15 days or 1, 3, 6, 9, 12, 15 and 18 months later. The following parameters are studied: Uptake of non-metabolizeable α-aminoisobutyrate (AIB), by brain, heart, muscle and liver, content of brain on DNA, proteins collagen, sialic acid, acid phosphatase, β-glucuronidase, cathepsin, serotonin, noradrenalin, dopamin, gamma aminobutyrate. The data available so far indicate that uptake of AIB by heart and somewhat also by brain is depressed. Lysosomal enzymes are temporarily increased, sialic acid is diminished, whereas DNA displays an increase in some animals. Among neurotransmitters, an increase in serotonin is noted after 6 months. The data form part of a cooperative project and are complemented eventually by morphological and physiological studies carried out in other laboratories (Louvain, Rijswijk, Oxford, Ulm). (With the cooperation of the CNS/vascular group of EULEP (European Late Effect Project Group. Publ. N° 878 of the Euratom Biology Division.)

D-20-7 *Postnatal Changes in DNA and Lipid Synthesis in the Brain of Mice after Prenatal X-Irradiation.* G. Konermann, Institut für Biophysik and Strahlenbiologie, Universität Freiburg, D-78 Freiburg i. Br., Albertstrasse 23, West Germany.

After daily fractionated X-irradiations during blastogenesis, neurogenesis and organogenesis the developmental damage in prenatal and postnatal stages remains relatively low, as long as a certain morphogenetic threshold dose is not exceeded. Slightly higher doses induce an unproportional increase of alterations. It is assumed that compensatory growth reactions as well as their onset are a contributory determinant for this kind of dose dependency.

By means of incorporation experiments (^{3}H-Thymidine for DNA synthesis, ^{3}H-Mevalonic acid, ^{3}H-Glucose and ^{32}P-sodium phosphate for lipid synthesis) the occurrence of compensatory reactions at the biochemical level was studied in the brain of mice. At different stages of their embryonic development they were irradiated daily as cited above. The incorporation experiments were done in the period from birth up to three weeks p.p.

Parallel histoautoradiographical experiments were performed in order to correlate the observed changes in DNA and lipid synthesis to the anatomical and cytological subunits of the brain.

It will be shown that DNA and lipid synthesis, although temporarily suppressed, show a pronounced pattern of stimulation, depending on the prenatal irradiation period and the X-ray doses.

D-20-8 *Effects of Postnatal Gamma Irradiation on Behavior, Chemical Composition, Cytoarchitectural Differentiation of the Visual and Somato-Sensory Cortex, and Growth of the Brain, Pituitary and Adrenals in the Rat.* K. R. Brizzee, J. M. Ordy, and B. Kaack, Delta Regional Primate Research Center, Tulane University, Covington, Louisiana, 70433, USA.

Sprague-Dawley 3-day old rats were exposed to 300 or 600 R whole-body gamma irradiation to determine the effects on postnatal development of behavior, the chemical composition of the brain, the cytoarchitectural differentiation of visual and somatosensory regions of the neocortex, and growth of the pituitary and adrenals. Radiated and sham-control rat offspring were compared on maturation of neuromuscular reflexes and open-field exploration on postnatal days 13, 27 and 60. Following testing, they were sacrificed for chemical and morphological evaluations. There was a significant dose-dependent increase in mortality of the irradiated rat offspring. Eye opening, grooming and autonomic reactivity were significantly delayed by irradiation. There was also a dose-dependent decrease in open-field exploration and increase in open-field latency to emerge from the starting compartment. Total brain and regional DNA, RNA, protein, cholesterol, water and electrolytes were significantly altered by radiation. Body, brain pituitary and adrenal weights were significantly reduced by irradiation. Previous studies of the effects of "differential experience" in the neonatal rat on dendritic spine counts and neuronal connectivity have remained inconclusive. In this study, preliminary quantitative ultrastructural observations in lamina I and II of the visual and somatosensory cortex indicated significant decreases in proportion of cortical tissue occupied by synaptic end bulbs following whole-body gamma irradiation. (Supported by AEC Contract AT-(40-1)-3832 and NIH Grant RR 00164-12.)

D-21-1 *Chromosome Aberrations and Cell Death in V79 Chinese Hamster Cells.* J. S. Bedford, H. G. Griggs, J. B. Mitchell, and M. A Bender, Vanderbilt University, Nashville, Tennessee 37232, USA.

Synchronized V79 cells were irradiated with gamma rays in G_1. One hour after irradiation, samples were assayed for colony forming ability. At the first mitosis, cells were again harvested, and individually plated by means of a micropipette into microtest plates. The remainder were allowed to proceed to their next mitosis. A parallel sample, blocked with colcemide at the first mitosis, was scored to determine the frequency of chromosome aberrations. The entire procedure was repeated at the second and third divisions after irradiation. Thus, survival and aberration frequency were always measured in identical populations. A control series of unirradiated cells was carried through the same procedure.

The frequency of all aberration types decreased progressively from the first to the second and third divisions. In contrast, there was no significant difference in colony forming ability for cells assayed immediately after irradiation, at the first, or at the second divisions. Cells reaching the third division showed a significant increase in survival. (Supported by NCI Grant Numbers: CA10410, CA11953 and by USAEC Contract No. AT(11-1)2382.

D-21-2 *The Behavior of Chromosomal Aberrations at Mitosis.* John A. Heddle, Departments of Biology and Natural Science, York University, Downsview, Toronto, Ontario, Canada.

Most of the chromosomal aberrations that can be accurately scored at mitosis are lethal events for the cell. It is not certain, however, just how death occurs, how quickly aberrations kill cells, and just what intermediate stages are gone through. The efficiency of some ways of screening chemicals for mutagenic activity depends upon how aberrations behave. The micronucleus assay, for example, depends upon the frequency with which fragments are left behind at anaphase. What fraction of fragments become micronuclei? Does this fraction depend upon the nature of the aberration (chromatid deletion, isochromatid deletion, asymmetrical exchange, etc.)? Can one fragment result in more than one micronucleus? Similarly, the synchronous anaphase method of screening potential mutagens depends upon the fraction of asymmetrical exchanges that lead to chromosomal bridges. Is this always one-half? Do centromeres co-operate in short dicentrics so that bridges do not result?

In a series of experiments and theoretical calculations, the following conclusions have emerged: (1) about 20% of chromosome fragments from G_1 form micronuclei at the first mitosis, (2) at low doses about one half of the dicentric chromosomes result in bridges, (3) centromere co-operation

is rare or nonexistent, and (4) fragments do not kill many, if any, cells during the first interphase but their loss does kill many cells during the second post-irradiation interphase. Measurements of the frequency with which chromatid aberrations give rise to micronuclei are being made and will be reported.

D-21-3 *The Use of Interaction Experiments to Study the Mechanism of Chromosome Aberration Formation.* J. GRANT BREWEN AND R. JULIAN PRESTON, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

UV doses of 25 or 50 ergs/mm^2 produce very few chromosome aberrations in human peripheral leukocytes. However, when these same doses are given together with X-ray doses of 50 to 200 rad the yield of aberrations was considerably greater than the additive yield for the radiations given separately. This was the case even when the two radiations were given 3 hours apart. The yields from the interaction experiments were independent of the UV dose, but varied with X-ray dose. These results can be interpreted in terms of the known mechanisms and kinetics of repair of UV and X-ray induced DNA lesions. In order to test our interpretations, further experiments were conducted using combined X-ray and N-acetoxy-2-acetylaminofluorene or ICR-171, and UV-X-ray interaction with BUdR incorporation. The results and interpretation of these experiments will be used in an attempt to explain the mechanisms of chromosome aberration formation. (Research sponsored by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.)

D-21-4 *Relationships Between Survival, Chromosome Aberrations, "Mutation" Induction and DNA Repair in Mammalian Cells.* D. SCOTT, MARGARET FOX, AND B. W. FOX, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester, M20 9BX, Great Britain.

The induction of chromosome aberrations, excess thymidine resistant "mutants" and DNA repair replication was investigated in mouse lymphoma cell lines of differential sensitivity to the lethal effects of X-rays. At each dose the most sensitive cells had the greatest amount of chromosome damage and the highest frequency of mutations. The amounts of DNA repair were not correlated with cell killing, chromosome damage or mutation induction.

A pair of Yoshida lymphosarcoma cell lines to identical sensitivity to X-rays showed the same amount of induced chromosome damage and DNA repair. The same pair of cell lines have a pronounced differential sensitivity to killing with bifunctional alkylating agents and, after treatment with sulphur mustard, exhibit a large difference in amount of chromosome damage. DNA repair capacity was, however, equal in the two cell lines.

D-21-5 *Dose-Response Relationship of Lymphocyte Chromosome Aberrations in Locally Irradiated Persons.* SHO MATSUBARA, MASAO S. SASAKI, AND TADASHI ADACHI, Tokyo Medical and Dental University, Tokyo 113, Japan.

The dose-response relationship of chromosome aberrations in blood lymphocytes was studied in the blood samples obtained from patients who were exposed to a single dose of ^{60}Co gamma-rays for their malignant diseases. The frequency of chromosome aberrations was closely related with the radiation dose (D), body weight (W) and irradiated volume (V). The comparison of the frequency of chromosome aberrations in these locally irradiated persons with that obtained by the *in vitro* experiments suggested that the killing of lymphocytes or inactivation of PHA response was another factor influencing the dose-response relationship. The dose-response relationship of chromosome aberrations in the blood lymphocytes of these partially irradiated persons best fitted to an approximation, $Y = a + (v/W)(bD + cD^2) \exp(-D/D_0)$, and the D_0 value of roughly 180 rads was obtained for the survival of lymphocytes as measured 24 hours after irradiation. The frequency of dicentrics and rings among X_1Cu cells was less dependent on these modifying factors and suggested to be reliable biological indicator of absorbed dose in the locally exposed persons.

D-21-6 *Comparative Cytogenetic Damage During the Cell Cycle of Human Lymphocytes Following X and Neutron Irradiation.* ANTHONY V. CARRANO, Biomedical Division, University of California, Lawrence Livermore Laboratory, Livermore, California 94550, USA.

Human lymphocyte cultures were irradiated in the unstimulated state (G_0), 17 hours post-stimulation (G_1), and at 48 hours post-stimulation (late S and G_2) with 250 kVp X rays (100 rads/min) and fission neutrons (0.7 MeV average energy; 6.25 rads/min) from the JANUS reactor at Argonne National Laboratory. Four dose points were used, varying from 50 to 300 rads for X rays and 25 to 150 rads for neutrons. Two four-hour sampling intervals where chosen within a period of 48 to 58 hours post-stimulation. For chromosome deletions the RBE for neutrons is 4.3 in G_0; in G_1 it is less, 4.0, due to the slightly increased effectiveness of X rays in G_1 cells. For two-hit aberrations the RBE decreases with dose in both G_0 and G_1 cells and is about 20 at 25 rads and 3 at 300 rads. After neutron irradiation, the total deletion frequency is 60% higher in G_2 cells compared to S cells and, for doses below 50 rads, exchanges show a similar increase. The cell cycle sensitivity to neutron induced aberrations is as follows: for exchanges, $G_0 = G_1 > G_2 \geq S$ and for deletions, $G_2 > S \geq G_0 = G_1$. (This work was performed under the auspices of the U.S. Atomic Energy Commission.)

D-21-7 *Observed Radiation Intensity Effects on Chromosome Aberration Frequency in Human Lymphocytes—an Analysis of Possible Implications.* A. Michelsen and T. Merz, Johns Hopkins University, Baltimore, Maryland 21205, USA.

Experiments in our laboratory have shown that the frequency of two-hit chromosome aberrations in cultured human lymphocytes is modified when the exposure rate reaches levels on the order of 5×10^{10} R/sec. The data were obtained using a pulsed X-ray beam as a source of radiation. For the same dose, the aberration frequency decreased by approximately 50% with respect to that observed for exposure rates ranging from 0.06 R/sec. to 4×10^6 R/sec. A normal oxygen effect was still recorded at the ultra-high exposure rate. The present paper attempts to explain the implications of these results with respect to the theory of the indirect action of ionizing radiation. The relative reaction rates of the various primary species found in irradiated water are examined in order to seek an explanation of the observed phenomena. Since several assumptions must be made regarding the concentration of the various reactants involved, the sensitivity of the outcome of the analysis to errors in parameter estimation is discussed in some detail.

D-21-8 *Lack of Relationship Between Chromosome Aberrations Induced by and Localized Incorporation of ^{3}H-TdR in Human Leukocytes.* Baldev K. Vig, Department of Biology, University of Nevada, Reno, Nevada 89507, USA.

Leukocyte cultures, made by using human peripheral blood, were treated with 2.5, 5 and 10 μC/ml of ^{3}H-TdR for periods varying from 1–6 hours, and only during the last period of culturing. The cells were harvested at the end of the treatment without removal of ^{3}H-TdR. Autoradiographs were prepared and cells scored for aberrations and presence or absence of grains on chromosomes. A clear lack of relationship between the presence of aberrations and grains was established in cells which were otherwise well labelled. In several instances cells with no grain at all (G_2 cells) also had aberrations of chromatid type. The data indicate that chromosome components being hit by β rays from ^{3}H do not necessarily have to have incorporated ^{3}H in its DNA.

D-22-1 *Somatic Cell Chromosome Changes in Humans Exposed to ^{239}Plutonium.* William F. Brandom, Robert W. Bistline, Arthur D. Bloom, and Philip G. Archer, University of Denver, Denver, Colorado 80210, Dow Chemical Co., Golden, Colorado, University of Michigan Medical School, Ann Arbor, Michigan, and University of Colorado Medical Center, USA.

Preliminary data on 6 unexposed controls, 7 workers exposed to penetrating radiation (<5 rem/yr), and 27 internally exposed Pu-workers (3955 cells) revealed marked differences between the mean prevalence of control level lymphocyte chromosomal changes and those of Pu-worker groups and subgroups. Four of the 27 internally Pu-burdened workers had high proportions of cells containing specific chromosome changes (translocations, deletion, and aneuploid mosaic). We have subsequently analyzed 19 additional unexposed controls (total = 25), 18 additional workers exposed to penetrating radiation (total = 25), 5 workers with recent internal Pu exposure, and 5 workers with internal tritium exposure. The newly exposed Pu and tritium workers do not exceed the externally exposed group in the prevalence of chromosomal breaks and rearrangements. The populational cytogenetic findings and selected individual cases from the 77 workers will be presented. (This work was supported by U.S.A.E.C. Contract AT-(29-1)-1106.)

D-22-2 *The Effect of Acute ^{60}Co or Continuous Intake of HTO on the Liver Chromosomes of Hale-Stoner Brookhaven Mice.* A. L. Brooks, A. L. Carsten, A. Germillion, Diane K. Mead, and Jan C. Retherford, Inhalation Toxicology Research Institute, Lovelace Foundation for Medical Education and Research, Albuquerque, New Mexico 87108, and Brookhaven National Laboratory, Upton, New York 11973, USA.

This experiment was designed to compare the production of chromosome damage in mouse liver cells following continuous ingestion of HTO with that produced by acute exposure to ^{60}Co. Thirty-six Hale-Stoner Brookhaven mice were exposed in groups of six to ^{60}Co irradiation at doses of 0, 50, 100, 200, 400 and 600 roentgens. Ten mice were maintained on HTO at a level of 3 μCi/ml for either 90, 330, 500 or 560 days. Five animals were sacrificed to provide age-matched controls for each of the HTO groups. The aberration frequency following ^{60}Co exposure increased according to the equation $Y = 0.002 + 2.0 \times 10^{-3} D + 3.9 \times 10^{-7} D^2$ where Y = aberrations/cell and D is dose in roentgens. The aberration frequency in the mice drinking HTO seemed to increase as a function of time and dose. The observations of a clone of cells in the controls at 330 days and an unusual distribution of chromosome damage in an animal which had consumed HTO for 560 days will be discussed. Dose response relationships for HTO relative to ^{60}Co will also be considered. (Performed under U.S. Atomic Energy Commission Contract AT(29-2)-1013.)

D-22-3 *Cytogenetic Changes in Blood Lymphocytes of the Rhesus Monkey Following Acute Inhaled Exposure to Polydisperse $^{239}PuO_2$.* R. Joe LaBauve, A. L. Brooks, J. A. Mewhinney, R. O. McClellan, and R. K. Jones, Inhalation Toxicology Research Institute, Lovelace Foundation for Medical Education and Research, Albuquerque, New Mexico 87108, USA.

To determine quantitatively the relationship between blood lymphocyte chromosome aberration rate and dose of internally deposited alpha emitter, twelve Rhesus monkeys inhaled $^{239}PuO_2$ for varying lengths of time to obtain initial body burdens of 7–13, 13–41, 100–550, 590–1150 nCi. This level of activity is expected to result in 8, 20, 170, and 730 rads to the lungs at 120 days, the time of sacrifice. Two controls were sham-exposed. The exposure aerosol was characterized as having an AMAD of 1.6 μ and a σ_g of 1.6. Labeling the $^{239}PuO_2$ with ^{169}Yb made it possible to determine initial body burden and define early retention of the aerosol. By day 7 post-exposure, retention, mostly in the lungs, averaged about 40% of initial body burdens. Daily metabolism collections are being taken and used to reconstruct ^{239}Pu distribution and retention, and determine the usefulness of ^{169}Yb as a tracer. Four additional monkeys were exposed to 375 nCi of $^{239}PuO_2$ and sacrificed at day 0 and 30 to determine internal distribution. Cytogenetic analysis to determine lymphocyte aberration rate and hematologic values will be taken at frequent intervals. At 120 days post-exposure, the animals will be sacrificed, pathological samples taken, and tissues analyzed for plutonium-239. The experiment attempts to relate plutonium lung and body burden to lymphocyte chromosome aberration rate to test feasibility of cytogenetic analysis as a biodosimetric technique. (Research performed under AEC Contract AT(29-2)-1013.)

D-22-4 *Chromosome Aberrations produced in Human Lymphocytes by a 70 MeV Negative Pi Meson Beam.* David C. Lloyd, Roy J. Purrott, and Geoffrey W. Dolphin, National Radiological Protection Board, Harwell, Didcot, Berkshire, 0X11 ORQ, Great Britain.

Human blood samples have been exposed *in vitro* to a beam of negative pi mesons and the damage to lymphocyte chromosomes assessed. The aberrations produced at different depths in a unit density plastic phantom have been compared with the ionisation curve. Dicentric aberrations at the ionisation peak were about 3 times more frequent than in the plateau region, and behind the peak the yield declined rapidly. No dose rate effect could be demonstrated in the peak position and this is consistent with the large proportion of high LET events occurring there. Fractionation of the dose markedly enhanced the peak to plateau ratio so that at inter-fraction times in excess of 6 hours the peak damage was about 6 times that in the plateau. This work emphasises the distribution of biological damage from a negative pion beam which is very attractive for radiotherapy.

D-22-5 *Chromosome Aberration Dosimetry in Partial-Body Irradiation.* Alfred F. McFee, M. Wayne Banner, and Mary N. Sherrill, Comparative Animal Research Laboratory, Oak Ridge, Tennessee 37830, USA.

The use of leukocyte chromosome aberrations as dosimeters is complicated by the non-homogeneity of accidental exposures. Effects are thus influenced not only by uneven dose distribution but also by the movement of cells during exposure. To quantitate damage to peripheral leukocytes from partial-body ^{60}Co irradiation, groups of 4 pigs were given whole-body exposures to 400 R (58 R/min), 400 R to the anterior body only, or 400 R to the posterior body half. Partial-body exposures reduced leukocyte counts almost 50% but recovery was under way at 7 days; counts dropped to 20% of preirradiation values following whole-body irradiation and recovery was not evident until 21 days. Shielding either half reduced both 1-event and 2-event chromosome aberrations to 33% of the level in whole-body irradiated pigs. The rapid loss of aberrations characteristic of swine left only small differences between whole-body and partial-body irradiated animals at 24 hr postexposure. Results indicate that partial-body exposures results in lower level of chromosome aberrations than would be expected on the basis of an average body dose. (Supported by U.S.A.E.C. under contract No. AT-40-1-GEN-242 with University of Tennessee.)

D-22-6 *Chromosomal Estimation of Radiation Damages in Man in Short and Long Lapse of Time after Exposure.* Takaaki Ishihara, Sei-ichi Kohno, and Toshiyuki Kumatori, National Institute of Radiological Sciences, Chiba, Japan.

In 6 persons accidentally exposed to ^{192}Ir gamma rays, repeated observations were made from 10 days after exposure up to 3 years. Significantly elevated levels of chromosome abnormalities were observed. Incidence of chromosome abnormalities at various times after exposure, and the doses estimated on the basis of the aberration yields will be given.

On the other hand, annual follow-up studies were carried out 10–20 years after exposure in 13–18 of the 23 fishermen exposed to fallout radiation at Bikini in 1954. Higher frequencies of chromosome abnormalities than in controls were still observed, from which estimation of absorbed doses was possible.

In either the iridium or Bikini accident, the estimated absorbed doses reasonably correspond to the clinical severity indicated by the hematological or other changes at the critical stages, and also to the physically estimated absorbed doses.

The results indicate that the estimate from chromosome abnormalities may be a reliable measure of radiation damage, which can be used as the basis for the appropriate treatment or for the assessment of risks for the late effects of radiation.

D-22-7 *Chromosome Aberrations in Human Lymphocytes as a Quantitative Indicator of Whole Body and in Vitro Irradiation With ^{60}Co Rays.* Manfred Bauchinger and Ernest Schmid, Gesellschaft für Strahlen- und Umweltforschung, München-Neuherberg, Strahlenbiologisches Institut, Universität, München, West Germany.

15 patients with advanced cancer have been exposed to a therapeutical whole-body 60-Co-γ-irradiation with doses of 10, 19 and 29 rads respectively. The aberration yields in blood samples obtained immediately and 24 hours after irradiation were compared with those after analogous irradiation of the patient's blood *in vitro*. There was no significant difference between the yields of dicentrics and ringchromosomes *in vitro* and *in vivo* and between the 0-hour and 24-hour samples. The dose response relation is well compatible with a linear model. Since the aberration frequency is very low in this dose range questions arises how to apply such findings for a biological dosimetry. Limiting and promising factors will be discussed.

D-22-8 *Microdosimetric Aspects in the Formation of Dicentric Chromosomes in Human Lymphocytes after Exposure to Different Radiation Qualities.* Ernst Schmid and Manfred Bauchinger, Strahlenbiologisches Institut, Universität München, Gesellschaft für Strahlen- und Umweltforschung München-Neuherberg, West Germany.

Comparative results on the induction of dicentric chromosomes by 220 kV X-rays, 3 MeV electrons and 14.1 MeV neutrons in human lymphocytes *in vitro* are given. For the formation of a dicentric chromosome the energy which is actually absorbed within a sensitive region is as essential as the spatial interaction distances of the primary lesions. One possibility to derive the diameter of such a sensitive region is based on the analytical form of the dose effect relation. It

will be demonstrated that one can also get to corresponding results by applying microdosimetric concepts. Relevant quantities are the specific energy z and ζ the 'energy mean' of increments of specific energy z. It is shown that several absorption events must occur on the average for the induction of a dicentric chromosome. From the intercellular distribution of dicentrics (Dispersion) conclusions are drawn for the spatial relation in aberration production.

D-23-1 *Effect of Age on Nuclear and Mitochondrial DNA Syntheses in the Livers of X-Irradiated Mouse.* AKIHIRO SHIMA, Zoological Institute, University of Tokyo, Tokyo 113, Japan.

Male ICR/JCL mice of different ages were irradiated with 400 R of X-rays and nuclear and mitochondrial DNA syntheses in the livers were studied by intraperitoneal injection of tritiated thymidine.

In un-irradiated mouse liver, the nuclear DNA synthesis decreased exponentially with age up to 36 weeks, rising to a higher level at 50 weeks. Age-dependent increase in average nuclear size was observed in histological sections. The mitochondrial DNA synthesis did not show age-related change.

X-irradiation inhibited the nuclear DNA synthesis in the livers of 8- to 36-week-old mice during 24 hours after irradiation. The recovery to about 70% of the control occurred in 50-week-old mice. The mitochondrial DNA syntheses were less radiosensitive than the nuclear ones, although some inhibition was observed during initial period after irradiation. At 24 hours after irradiation, mitochondrial DNA syntheses in 8-, 24-, 36- and 50-week-old mouse liver were 49, 114, 111 and 207% of the control, respectively.

Further studies on older animals are now in progress.

D-23-2 *The NAD Metabolism and DNA Synthesis in Mouse Spleen after Irradiation.* CHRISTIAN STREFFER, Institut für Biophysik und Strahlenbiologie, Albertstrasse 23, D-78 Freiburg, West Germany.

In mammalian tissues the NAD metabolism is highly connected to the cell nucleus. The activity of the NAD pyrophosphorylase and of the NAD glycohydrolase is localized in this cellular compartment. It will be reported that these enzyme activities decrease very rapidly after low radiation doses (whole body X-irradiation) in the mouse spleen, especially the NAD glycohydrolase activity which is bound to the chromatin. These enzymatic changes will be compared with the radiation induced inhibition of the DNA synthesis. We have further observed that the DNA synthesis can be decreased by an alteration of the NAD metabolism. Under these conditions the radiosensitivity of the DNA is also changed in the mouse spleen.

D-23-3 *Liberation of DNA as DNA-Protein Complex from Nucleoprotein in X-Irradiated Rat Spleen.* KUNIHIKO KATOR, Zoological Institute, University of Tokyo, Tokyo 113, Japan.

It is well known that whole body X-irradiation causes a temporary increase in saline-soluble DNA (soluble in 0.14 M NaCl) in rat spleen. This type of DNA had been considered to be free from nuclear proteins and therefore was called "free-DNA". From the present study, however, its complexing with protein was clearly demonstrated.

Rats were exposed to 600 R of X-rays and "free-DNA" was extracted from spleen at 6 hours after irradiation. To the extract was added the appropriate amount of CsCl and the mixture was centrifuged to equilibrium. According to this analysis, "free-DNA" was the material of density approximately 1.7, which banded at its position of neutral buoyancy. Next, the splenic extract containing "free-DNA" was fixed with formaldehyde, and then addition of CsCl and centrifugation were performed. In this case, almost all "free-DNA" floated on the CsCl solution and formed the pellicle. But by means of pronase treatment, formaldehyde-fixed "free-DNA" became free from protein and could restore the density that of before fixation.

D-23-4 *Temporal Order of DNA Replication Following γ-Radiation of* Physarum polycephalum. THOMAS E. EVANS AND HELEN H. EVANS, Case Western Reserve University, Cleveland, Ohio 44106, USA.

Sensitivity to ionizing radiation in *Physarum polycephalum* (as measured by mitotic delay) is most marked at the beginning of the S period; however, DNA replication occurs at almost the

normal rate following irradiation (Nygaard, *et al.*, in Advances in Radiation Research, Duplan and Chapiro, eds., Gordon and Breach, London, in Press). In order to determine if the temporal sequence of DNA replication is altered following radiation, density shift experiments were carried out (*cf.* Braun and Wili, Biochim. Biophys. Acta, **174**, 246, 1969). It was found that the administration of 10 KR either at the beginning of S or in mid-S (5 and 60 min following metaphase, respectively) had no effect on the ordered progression of DNA replication. Thus, neither premature synthesis of late replicating species nor premature re-initiations at the temporal origin were observed. We conclude that the mitotic delay caused by irradiation of *Physarum* during the S period does not result from an alteration in the rate or order of DNA synthesis. (Supported by U.S.AEC Contract W-31-109-ENG-78.)

D-23-5 *Protein Synthesis in Liver Mitochondria of Irradiated Rats.* A. S. Aiyar, K. C. Alexander, and A. Sreenivasan, Biochemistry & Food Technology Division, Bhabha Atomic Research Centre, Bombay 400 085, India.

The exposure of rats to X-rays (800 R) enhances the *in vivo* incorporation of ^{14}C-leucine into liver mitochondrial proteins upto 48 hr post-irradiation. A similar increase in labeling is also observed with liver slices from irradiated rats incubated *in vitro*, but not with isolated liver mitochondria. These differences in the labeling pattern may arise from differential effects of whole-body irradiation on protein synthesis in the mitochondrial and the nuclear-ribosomal systems. Studies on fractionation of mitochondrial proteins, following their *in vivo* labeling, have provided evidence of such a possibility.

The increased labeling of liver mitochondrial protiens following whole-body irradiation, however, does not reflect a true stimulation of the rate of synthesis. There is a considerable contraction in the size of endogenous free leucine pool, as also an increased labeling of the pool by the radioactive precursor, the latter effect presumably arising from altered membrane permeability. Taking into consideration the alterations in the free leucine pool, it would appear that liver mitochondrial protein synthesis is inhibited following wholebody radioexposure of rats. This may not be related to decreased availability of energy, since the radiation-induced impairment of oxidative phosphorylation does not involve an interference with the formation of high energy intermediates.

D-23-6 *The Effect of Ionizing Radiation on the Structure Subunits of Supramolecular Chromatin DNA.* N. B. Strazhevskaya and V. A. Struchkov, Institute of Biological Physics, the Academy of Sciences of the USSR, Moscow Region, USSR.

Based on the fact that in supramolecular DNA native tissue specific acidic proteins (1.5–3.5%), neutral lipids (1.5–3%) and mRNA have been found, the supramolecular DNA is assumed to be an essential part of the DNA-membrane complex. The supramolecular DNA isolated by a gentle phenol method is shown to consist of DNA subunits with molecular weight of $1–2 \times 10^8$ daltons connected by lipoprotein bonds. The functioning mechanism of ionizing radiation on different levels of supramolecular DNA organization in chromatin (DNA subunits, supramolecular DNA) is discussed.

D-23-7 *Effect of Radiation Damage of DNA-Template in Vivo and in Vitro on its Priming Activity for Replicative and Reparative DNA-Polymerases in Rat Thymus.* I. V. Fillippovich, L. N. Belovskaya, and E. F. Romantzev, Institute of Biophysics, Ministry of Public Health, Moscow, USSR.

Partially-purified fraction possessing DNA-dependent DNA-polymerase activity and using both native (reparative polymerase) and denatured (replicative polymerase) primer DNA has been isolated from rat thymus extracts.

The priming activity of DNA irradiated *in vivo* and *in vitro* with 600–1000 r of γ-rays for these two DNA-polymerases was compared.

D-23-8 *The Effect of γ-Irradiation on Phosphorylation of Nuclear Proteins of Rat Liver and Thymus.* S. R. Umansky and R. N. Zotova. Institute of Biological Physics, Academy of Sciences of the USSR, Pushchino, Moscow Region, USSR.

Rat liver and thymus nuclei were irradiated with γ-rays ^{137}Cs at doses 1, 5, 10 and 20 krad and incubated for 10 min in the presence of (γ-^{32}P) ATP. The phosphorylation of thymus nuclear pro-

teins is inhibited at all the doses investigated. The phosphorylation of liver nuclear proteins enhances at 1 and 5 krad (by 27% and 10%) and falls with further dose increase. In the other set of experiments rats were irradiated with a dose of 1 krad. After 15 min, 1 hr and 2 hrs rat liver and thymus nuclei were isolated and the phosphorylation of nuclear proteins was studied. 15 min and 2 hrs after irradiation the phosphorylation of liver nuclear proteins exceeds the control by 37% and 10%, respectively. The phosphorylation of thymus nuclear proteins at the same periods decreases by 10% and 18%. Nuclear proteins were fractionated into proteins of nuclear sap, histones and nonhistone proteins. 15 min after irradiation the phosphorylation of liver nuclear sap proteins does not change whereas that of thymus decreases slightly. The phosphorylation of histones and, to a lesser degree, of nonhistone proteins of rat liver chromatin enhances, that of thymus decreases. Electrophoretic data evidence that phosphorylation of different fractions of chromatin nonhistone proteins in both organs changes to a different extent. A possible role of the revealed effects in the radiation disturbance of the transcription process is discussed.

D-24-1 *UV- and X-Ray Effects on Nucleoside Kinase Production During the Mitotic Cycle of* Physarum polycephalum. W. Sachsenmaier, E. Dworzak, H. Madreiter, and W. Linser, University of Innsbruck, Innsbruck, Austria.

Thymidine (I)—and deoxycytidine (II)—kinase activity in *Physarum polycephalum* undergo cyclic variations related to the synchronous 10 hr mitotic cycle. Both enzymes exhibit a distinct maximum in early S-phase. UV light (λ_{max} 254 nm, 5–10,000 ergs/mm^2) applied during early and middle interphase delays the enzyme maximum parallel to the delay of the next mitosis without affecting the yield of enzyme production. Treatment during late interphase or during mitosis partially inhibits the increase of (I) but stimulates excess production of (II) beyond the time of the control maximum. X-irradiation (25 kV, 1 kr) during a narrow period (pro-metaphase) largely stimulates production of (I) and (II) at the time of the next (delayed) mitosis. Sensitivity to actinomycin of irradiated plasmodia is markedly reduced probably due to repair processes. A model is discussed suggesting that (I) and (II) are controlled by gene activation (A) at the end of G_2-phase followed by a "shut-off" mechanism (B) in early S-phase. UV light appears to affect the (B)-process of (II) more than (A) allowing excess enzyme production. X-rays on the other hand may interfere with the formation of a repressor acting on the translation level. (Supported by the Fund of Austrian Cancer Research Institutes).

D-24-2 *Loss of Regulatory Properties of Rat Liver Uridine Diphosphoglucose Dehydrogenase Exposed to Gamma-Rays.* G. B. Nadkarni and S. M. Kelkar, Biochemistry & Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay 400085, India.

Uridine diphosphoglucose (UDPG) dehydrogenase from rat liver, obtained as a homogeneous preparation, was inhibited sigmoidally by UDP-xylose. The allosteric responses with the inhibitor were observed with the enzyme irradiated as aqueous solution up to 10 KR. However, the preparation exposed to higher doses (upto 25 KR) gave hyperbolic responses to UDP-xylose, pointing to loss of regulatory properties. The structural studies indicated that this tetrameric protein remained in associated state up to 10 KR with partial disaggregation at higher doses, with possible separation of a subunit. The relative sensitivities to radiation of catalytic and allosteric functions respectively, indicated that the latter was more susceptible. The substrate as well as the inhibitor exerted similar protective action indicating the same binding sites for these two ligands. However, the coenzyme (DPN) partially protected the catalytic activity while retaining the regulatory function even at higher doses that could otherwise inactivate the enzyme. The results therefore suggest that the loss of regulatory properties of this enzyme could not be correlated with the binding sites but with the specific conformation of the protein in associated state.

D-24-3 *Superoxide Dismutase; Radiobiology and Biochemistry.* E. Martin Fielden and Peter B. Roberts. Physics Dept., Institute of Cancer Research, Clifton Avenue, Sutton, Surrey, SM2 5PX, Great Britain.

Superoxide dismutase (S.O.D.) is an enzyme found in all oxygen metabolizing cells. Its function is to dismute the O_2^- radical, which it does at a near diffusion controlled rate.

$$O_2^- + O_2^- \xrightarrow{\text{SOD, } 2H+} H_2O_2 + O_2$$

O_2^- is produced by various enzymes or, of course, by radiation. Pulse radiolysis has proved to be the method par excellence for investigating the activity and mechanism of this important enzyme.[1,2] A radiolytic assay has been developed that enables the level of S.O.D. to be determined accurately and rapidly in whole blood or homogenized tissue. A study of blood and tissue levels in sickness and health is being carried out and the effect of insults such as exposure to radiation and hyperbaric oxygen is to be measured and reported.

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D-24-4 *Steroid 11β-Hydroxydehydrogenase Activity in Beagles Bearing ^{241}Am.* C. J. NABORS, JR., J. S. HINCKLEY, AND K. L. FOWKES, Department of Anatomy, Radiobiology Division, University of Utah, Salt Lake City, Utah 84132, USA.

Skin of beagles bearing injected burdens of ^{241}Am (0.3 μCi/kg) and control, vehicle-injected dogs was incubated *in vitro* with an equimolar mixture (0.27 n moles) of ^{14}C-cortisol and ^{3}H-cortisone. Biopsies of 5 ^{241}Am and 6 control animals obtained under pentothal anesthesia were minced and incubated in Krebs phosphate buffer, pH 7.4 for 3 hr at 37°C. The metabolites of these two steroids were isolated by extraction and paper chromatography. The conversion of cortisol to cortisone was higher in skin of ^{241}Am injected animals (8 incubations) than in controls (10 incubations, $p < .05$). The reverse reaction, cortisone conversion to cortisol appeared to be higher in controls although there was no statistical difference. This metabolic picture represents increased destruction of cortisol, the most potent anti-inflammatory, lymphocytolytic glucocorticoid produced in the beagle. Cortisone has no ability to inhibit fibroblast proliferation in a test for anti-inflammatory activity. Preliminary measurements of serum cortisol show that this steroid is decreased in blood of ^{241}Am injected animals. Radioimmunoassay measurements of plasma cortisol and cortisone in these animals will also be presented. (Supported by USAEC Contract AT(11-1)-119).

D-24-5 *Mechanisms of Interphase Death of Thymocytes: X-Ray Modification of the Allosteric Properties of Phosphofructokinase.* TAKESHI YAMADA AND HARUMI OHYAMA, Division of Biology and Division of Radiation Health, National Institute of Radiological Sciences, Chiba, Japan.

Lesions in energy metabolism have been implicated in the mechanisms leading to interphase death which occurs in thymocytes shortly after X-irradiation. In an attempt to obtain better understandings of the energy metabolism in the irradiated cells, the specific facilitation of phosphofructokinase, one of the glycolytic enzymes which catalizes the reaction to form fructose-1,6-diphosphate from ATP and fructose-6-phosphate, was found. ATP depletion after irradiation seemed to be closely related to the above phenomenon, and brought about the decrease in the cell viability.

In order to elucidate the mechanism underlying the enhancement of the phosphofructokinase reaction in irradiated rat thymocytes, the effect of X-rays on the phosphofructokinase preparations extracted and purified from rat thymocytes was examined in the present work. The results of the kinetic experiments show that some alterations in kinetic and allosteric properties of the enzyme, such as sensitivity to ATP inhibition and the binding affinity for a substrate, are induced by X-irradiation and result in a marked stimulation of phosphofructokinase activity under the intracellular conditions of the irradiated rat thymocytes.

D-24-6 *Histochemical Enzyme Changes in Fractionally Irradiated Mouse Tumors.* E. SAMOUHOS AND P. J. MELNICK, From the Radiology Service, and Histochemical Research Section, Veterans Administration Hospital, Martinez, California, USA.

The purpose of this study was to examine the sequence of events in transplantable mouse tumors during the course of irradiation, chiefly with histochemical enzyme and isozyme methods; some representative tumors were also examined with electron microscopy.

Spontaneous mammary tumors in females of a high tumor strain of C3H mice were cut into small fragments that were implanted under sterile conditions into the subcutaneous tissue of the back of 36 males of the same strain, where they grew as transplantable tumors. When the tumors were about 1½ cm. in diameter they were treated with daily fractional radiation, the rest of the body being shielded by a lead shield. 17 tumors were treated with from 150 to 250 r daily of x-ray, of half value layer 0.6 mm. copper. 19 tumors were treated with from 200 to 500 r daily of cobalt radiation. Animals were sacrificed periodically so that a range of total radiation of from 200 to 5000 r could be studied.

As cellular ultrastructure became increasingly disorganized by the radiation, many enzymes of lysosomes, mitochondria, Golgi and endoplasmic reticulum gradually increased markedly in activity, up to a total dosage of about 3800 r, after which activity abruptly diminished or disappeared. Disintegration of nuclear ultrastructure became evident when calcium appeared, due to binding to uncoupled phosphate components of DNA,[1,2] and also the appearance of large numbers of virus particles.[3]

(Acknowledgments are due to S. Abraham, Ph.D. and Mrs. Evelyn Hilbert for contributing animals from a colony of C3H mice. The above research was supported in part by the Veterans Administration Research Service, and by U.S. Public Health Service Research Grants No. CA 05205 and No. CA 07468.)

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D-24-7 *"Nothing Dehydrogenase" Isozymes in Radiation-Induced Thymic Lymphomas of the Mouse.* ROBERT N. FEINSTEIN AND ERMA C. CAMERON, Argonne National Laboratory, Argonne, Illinois 60439, USA. (Withdrawn)

D-24-8 *Expression of Lactate Dehydrogenase-X in Testes of Mice Irradiated In Utero.* NARESH PRASAD, RUPI PRASAD, AND STEWART C. BUSHONG, Baylor College of Medicine and V. A. Hospital, Houston, Texas 77025, USA.

Lactate dehydrogenase-X (LDH-X) is a sperm specific isozyme found exclusively in the testes of mammals that causes infertility in females immunized with this protein. LDH-X may also be implicated in radiation-induced sterility following *in utero* irradiation. To investigate this relationship pregnant female mice were given 100 rads X-radiation on day 10, 14 and 17 following conception. All male offspring were sacrificed 20 days after birth and testes tissues obtained. One testis of each mouse was fixed for histological examination; the other was homogenized and a crude extract prepared for starch gel electrophoresis and zymogram staining for LDH-X.

Results to date show that the LDH-X is not expressed in the testis when fetal irradiation occurred at 10 and 14 days following conception. Faint bands of LDH-X were observed in testes homogenates of mice whose mothers were irradiated 17 days following conception. Pronounced LDH-X bands were present in all control preparations. Correlation of these findings with the histological examinations will be discussed.

D-24-9 *ESR Study of Transfer and Localization of Radiation-Induced Electrons in Enzyme-substrate Complexes.* L. P. KAYUSHIN AND M. K. PULATOVA. Institute of Biological Physics, Acad. Sci. USSR, Pushchino, USSR.

Using ESR photosensitized electron transfer and the nature of electron-acceptor groups have been studied for three systems: 1) enzymes lysozyme and α-chymotrypsin; 2) their substrate-inhibitors; 3) enzyme-substrate complexes. Carboxyl, disulphide and peptide groups were shown to be electron acceptors in enzyme molecules. The reactions involving anion-radicals of different protein groups lead to breaks of disulphide bonds and polypeptide chains. In enzyme-substrate complexes the electron capture occurs on substrate molecules. In lysozyme-inhibitor complexes the N-acetyl group of the inhibitor is the electron acceptor. Localization of the unpaired electrons in mixtures of a modified lysozyme with inhibitors depends on the enzyme molecule state and aminoacid residues in the active centre. The N-acetyl anion-radicals are assumed to play an im-

portant role in breaks of glycoside C_I–O bonds. The sites of localization of unpaired electrons in α-chymotrypsin-substrate complexes depend on the aminoacid content of the latter. Ester and amide groups and the peptide bond formed by aromatic aminoacid residues are strong electron acceptors. Donor-acceptor interactions in enzyme-substrate complexes of the proteins studied are assumed to be responsible for short-lived active anion-radical states of the substrate molecule which participate in the catalytic act of bond break.

D-25-1 *Cyanophage (Blue-green Algal Virus) Survival Curves.* MARJORIE P. KRAUS, Department of Chemistry, University of Delaware, Newark, Delaware 19711, USA.

To explore molecular mechanisms accounting for the high radiation resistance of certain blue-green algae to ionizing radiation, we have isolated a large number of strains of blue-green algae in axenic culture. In certain of these strains, blue-green algal viruses (cyanophages) have been lysogenized, forming, altogether, a long host range upon which differences in properties of cyanophage can be recognized. Survival curves for ^{60}Co gamma-irradiated cyanophage have been compared as plated out on hosts of varying lysogenies. It is found that clear-plaquing mutants unable to lysogenize or free cyanophage give survival curves with uniform steep slopes, whereas irradiated cyanophage plated out on lysogenic hosts gives survival curves which show discontinuities and alterations of slope which correlate with changes in host range and plaque morphology of the irradiated virus. The cyanophage, S3, produces ringed plaques. As discovered by host-range, plaque-morphology assay, the composition of the cyanophage at successive doses of irradiation corresponds with differences in the viral composition of these rings. We conclude that the decreased slope of cyanophage survival curves is dependent on the ability of the superinfecting irradiated virus to recombine at episites of chromosites carried by the lysogenized host upon which the irradiated cyanophage has been plaqued.

D-25-2 *Change in Sulfhydryl-Content and in Radiosensitivity of* Chlorella *during the Cell Cycle.* GÜNTER A. NOWAK, Strahlenzentrum, Universität Giessen, Giessen, West Germany.

Synchronously growing cells of *Chlorella pyrenoidose* show changes in the concentration of acid-extractable sulfhydryls while progressing through the cell cycle. These changes in the content of nonprotein SH resemble the variation in the proportion of cells able to form colonies after X-irradiation. These findings and measurements of cell growth during the cycle led to the hypothesis that the content of acid-extractable SH might be an important factor responsible for the different resistance of cells at different stages in the cell cycle. The basis for discussing the significance of SH-groups for age-response are experiments on cell survival after irradiation under hypoxic conditions.

D-25-3 *Different Kinds of Damage after Gamma Irradiation of Eukaryotic Cells* Chlorella *in G_0 Stage.* R. GILET AND S. SANTIER, Laboratorie de Biologie Végétale, DRF, Centre d'Etudes Nucléaires de Grenoble, France.

Chlorella cells, unicellular green algae, in G_0 stage (quiescent cells) have been irradiated with ^{60}Co gamma and their survival studied by cloning. Four types of damage have been shown: the well-known sublethal damage, by dose fractionation; the potentially lethal damage, by postponing of growth resuming after irradiation; the directly lethal damage, by accurate survival curves at high and low dose-rate; the unrepairable sublethal damage, perhaps bound to the lethal sectoring, to explain the remaining shoulder of the survival curves obtained at low dose-rate.

After an acute irradiation, the desoxyribonucleic acids of *Chlorella* G_0 cells were labelled with ^{32}P and ^{14}C-uracil; these incorporations are generally considered as evidence of the repair of sublethal damage. During a low dose-rate irradiation, the same kind of incorporation is obtained and considered as evidence of the repair of sublethal damage during this low dose-rate irradiation.

D-25-4 *The Effect of Bacterial Strain on the Sensitivity of* Dictyostelium discoideum *to γ-Rays.* N. E. GILLIES, BETHAN M. HUBBARD, AND C. N. ONG, The Middlesex Hospital Medical School, London W1P 6DB, Great Britain.

The survival of spores or amoebae of the cellular slime mould *Dictyostelium discoideum* strain NC-4 was scored by their ability to form plaques on a lawn of bacterial cells. Farnsworth and

James (J. Gen. Microbiol. (1972), **73**, 447) showed that the rate of growth of *D. discoideum* is bacterial strain dependent, being faster with *E. coli* DB11 than with either *E. coli* B/r or *E. coli* B_{s-2}. An exponential survival curve with a D_0 of about 60 Krads was obtained for spores γ-irradiated in the presence of oxygen, grown on *E. coli* DB11. On *E. coli* B/r or *E. coli* B_{s-2}, irradiated spores yielded survival curves with large shoulders and final slopes approximately the same as that for spores grown on *E. coli* DB11. Amoebae irradiated at different times after the beginning of germination became progressively more sensitive to γ-rays. The same relative differences in survival dependent on the bacterial strain used were observed. The results indicate that lower survival may be associated with faster growth rate, suggesting that a relatively slow growth rate may provide more time for repair mechanisms in *D. discoideum* to operate.

D-25-5 *Recovery from Gamma-Radiation-Induced Dissociation of Polysomes in* Tetrahymena pyriformis. Nancy L. Oleinick and Ronald C. Rustad, Division of Radiation Biology, Case Western Reserve University, Cleveland, Ohio 44106, USA.

Sub-lethal irradiation of log-phase *Tetrahymena* causes a rapid, transient dissociation of polysomes, which can be prevented by cycloheximide, and a nearly complete inhibition of amino acid incorporation into protein. Within 2–5 min after 75 kR, amino acid incorporation returns to the control rate and the polysomes begin to recover with a biphasic time course. Radiation delays the recovery of polysomes previously dissociated by puromycin or chilling to 0°C. These results suggest that radiation *may* inhibit the initiation of protein synthesis but *must* interfere with the systematic elongation and termination of nascent peptides. Studies with actinomycin D suggest that synthesis of some new RNA is required for recovery. Polysomes from cells 1 hr post-irradiation contain only ribosomal RNA synthesized prior to exposure, along with both old and newly synthesized mRNA. We conclude that: radiation interferes with the synthesis and/or processing of rRNA; those ribosomes present at the time of irradiation remain functional; and new mRNA may be necessary for polysome recovery. (Supported by USAEC and NIH.)

D-25-6 *The Localization of Structural and Biochemical Changes in Bacteria after Ionizing Radiation.* W. A. Cramp and P. E. Bryant MRC, ERU, Hammersmith Hospital, Ducane Road, London W.12, Great Britain.

In recent years the structure and location of macromolecular constituents of bacteria and indeed of many eukaryotic cells have become more clearly defined. In particular the role of an RNA-DNA-protein complex closely associated with cytoplasmic or nuclear membrane has been specifically associated with the initiation and continuation of DNA synthesis.

The effects of various anti-biotics applied to bacteria irradiated with either electrons or neutrons leads us to suggest that as well as different capacities to repair radiation damage, the structure of RNA-DNA-protein complexes may be different in cells of varying radiation response.

It may be possible that a detailed biochemical and physico-chemical analysis of the structure and location of these complexes may enable us to predict the radiosensitivity of the cell to ionizing radiation.

D-25-7 *Role of Population Recovery by means of Trichomes Fragmeniation in Radioresistance of Blue-green Alga Plectonema borianum.* Juri A. Kutlakchmedov and Dmitri M. Grodzinsky, Institute of Plant Physiology, Ukrainian Academy of Sciences, Kiev 252 627, USSR.

Filamentous blue-green microalga P. b. is an object of an extremely high radioresistance (LD_{90} = 200 Krad). The effect of survival increasing was discovered during the investigation of alga radioresistance modification by visible light when the simultaneous irradiation and light action were taking place (photomodification, PM). DRF of PM is 1.5. The phenomenological features of PM were investigated: 1) kinetics of PM; 2) survival in darkness and on light; 3) influence of light position; 4) action spectrum of PM; 5) temperature dependence; 6) dependence of PM on dose-rate and light intensity; 7) repeated PM. Hypothesis of alga trichomes fragmentation into viable fragments is proposed as the explanation of PM effect; this fragmentation results in increas-

ing survival. Composition of alga population changes as a result of irradiation and PM effect owing to the viable fragments appearance. This hypothesis is confirmed by experiments including trichomes irradiation in solid medium and by experiments with lysozyme as a fragmenting factor. A mathematical model of PM is proposed which gives an opportunity to estimate the effectiveness of the alga trichomes fragmentation process. DRF of the fragmentation process relative to hypothetical population without fragmentation capacity, is 2.2 for irradiation in darkness—and 3.3 in the light.

D-25-8 *Post-Irradiation Sensitization of the Blue-Green Alga,* Anacystis nidulans *With a Rifamycin Derivate (DMB) Which is Active on the RNA-Directed DNA Polymerase.* ALEXANDER P. DMITRIEV AND DMITRI M. GRODZINSKY, Plant Physiology Institute, Kiev, USSR.

The radiosensitizing effect of a rifamycin derivate-dimethylbenzyldesmethylrifampicin (DMB) (20 g/ml) was compared with that of actinomycin D (10 g/ml) in unicellular blue-green alga, *Anacystis nidulans*. Preirradiation treatment of cells with these agents was ineffective. However, they were active when added after irradiation and potentiated the damage produced by gamma-irradiation. DMB which is strong inhibitor of RNA-directed DNA polymerase (reverse transcriptase) much greatly decreased the survival of irradiated *A. nidulans* cells than actinomycin D did. The radiosensitizing action of actinomycin D may be considered as a result of stopping RNA synthesis. Post-irradiation sensitization with DMB might be a result of inhibition of reverse transcriptase. This suggests that there may be additional to dark-repair type mechanism of post-irradiation recovery, ensuring extreme radioresistance in the blue-green algae. We make a supposition that existence of the long-lived mRNA and reverse transcriptase in the blue-green algae give a possibility for synthesis of DNA copies on RNA-template in order to substitute damaged DNA. The details of this supposition and the results are discussed.

D-26-1 *Differential Effects of Total-Body Pre-irradiation on the Growth and Metastasis of Line 1 Alveolar Cell Carcinomas.* JOHN M. YUHAS AND J. O. PROCTOR, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830 USA.

Exposure of BALB/c mice to 500R of X-rays prior to SC transplant of the Line 1 lung cancer decelerates the growth rate of the tumor but accelerates the rate of spontaneous metastasis. Neither of these results is obtained when the pre-irradiation is confined to the injection site and lungs, although the development of artificial metastases following the IV injection of tumor cells is increased. Further the total-body irradiation effects can be obtained with other immunosuppressants and can be reversed by the transplantation of spleen cells from syngeneic mice. Through an analysis of tumor growth and lung colony development in mice receiving SC transplants, IV transplants or both, it has been demonstrated that metastatic spread itself is responsible for the deceleration of the growth of the SC tumor. Mice which received both the SC tumor and the IV transplant (artificial metastases) showed reduced tumor growth, yet they developed as many lung tumor colonies as mice which received only the IV transplant. We interpret these and similar data obtained on spontaneous metastasis as indicating that total-body preirradiation compromises the local control of the tumor and thereby allows metastatic spread. These metastases in turn are available to the recovering immunologic system and induce a cytotoxic reaction, which they themselves escape. The basis of their ability to escape immunologic control would appear to involve a combination of their location (most often the lung) and serum factors which block the cytotoxic reaction. (Supported by the U. S. Atomic Energy Commission).

D-26-2 *Loss of Induced Immunoresistance to Viral Leukemia in Spleen Cells Transplanted into Lethally Irradiated Mice.* J. P. OKUNEWICK, P. M. KUHNERT, E. L. PHILLIPS, AND B. BROZOVICH. Allegheny General Hospital, Pittsburgh, Pennsylvania 15212, USA.

Experiments have been performed to test the feasibility of combining immunotherapy and radiation therapy in the treatment of Rauscher viral leukemia. It was found that through the use of formalin killed leukemic cells it was possible to induce an immunity to Rauscher virus. Serum from these immunized mice was also found to be effective in inactivating splenic colony forming

units from leukemic mice. Such CFU when transplanted into normal irradiated mice after incubation with the immunized mouse serum showed a 50% reduction in the number of colonies formed as compared with controls incubated in normal serum. On the other hand, when the spleen cells from the immunized mice were studied these were found to be ineffective in eliminating the disease. Following their transplantation into lethally x-irradiated (1100 R whole body) mice with active Rauscher leukemia all of the recipient leukemic mice still succumbed to the leukemia. From the data we would conclude that the cellular mechanism responsible for the production of the immune response to Rauscher leukemia exists in a more differentiated cell than the pluripotent stem cell or in a different cell line than that immediately derived from the splenic colony forming unit. (Supported by U.S.A.E.C. and N.I.H.-N.C.I.)

D-26-3 *Radiation-Induced Premature Decay of Immunologic Control of a Harbored Oncogenic Virus.* N. H. Pazmino, R. E. Toya, and J. M. Yuhas. Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

IM injection of murine sarcoma virus (MSV) into adult (6 month) mice induces no tumors at the injection site within 6 months, while similar injection of newborn and senescent (2 to 3 years) mice yields 100% progressively growing tumors (Pazmino and Yuhas, Cancer Res. **33**, 2668–2672, 1973). This age dependency for resistance can be traced to the development and decay of the ability to mount specific immunologic reactions which inhibit the virus. We report here that late in life mice succumb to the oncogenic effects of the MSV they had received earlier and radiation can accelerate the rate at which this resistance decays. No tumors appear in control BALB/c or CD_2F_1 mice within 6 months after MSV injection, i.e., by the age of 1 year. By 1.5 years of age, 50 and 35%, respectively, developed progressively growing tumors at the injection site. Exposure of these MSV injected mice to 500 R one month after injection hastens the appearance of the tumors. The first are detected 2–3 months after exposure. By the age of 1 year the tumor incidences are 60 and 18%, respectively, and by 1.5 years of age both irradiated groups show a 70% tumor incidence. These tumors did not occur in the absence of MSV injection. We conclude that the resistance of adult mice to MSV is only temporary, that the virus itself or a cell it has transformed are merely held in check until age compromises immunologic control below some critical level, and that radiation exposure can shorten the amount of time it takes for the immune control mechanism to decay below the threshold level. We are currently determining whether the tumor appearance late in life results from persistent tumor cells, viruses, or both. (Supported by U.S.A.E.C.)

D-26-4 *Leukemic Transformation of Donor Spleen Cells following their Transplantation into Superlethally Irradiated Rauscher Leukemic Mice.* Paul M. Kuhnert and James P. OKunewick, Allegheny General Hospital, Pittsburgh, Pa. 15212, USA.

Fialkow *et al.* previously reported leukemia induction in donor-type cells after treating patients for acute lymphoblastic leukemia with total-body irradiation and hematopoietic cell transplantation. Utilizing a murine model and paralleling their treatment protocol, we have documented that induction of leukemia can occur in normal donor cells transplanted into Rauscher viral leukemic mice at 0, 1 and 2 days after irradiation. The induction of leukemia in the grafted cells was verified by: 1) the occurrence of splenomegaly; and 2) secondary spleen cell transplants, whereby the secondary donors were transplanted mice still alive at 30 days and the secondary recipients were normal unirradiated mice. The spleen weights of the grafted leukemic mice were found to be significantly greater than those of the controls and all secondary recipients that received spleen cells from the primary grafted leukemic mice also died of leukemia. Verification that the regenerating hematopoietic tissue was from donor (males) and not host source (females) was accomplished by spleen chromosome preparations taken from randomly selected mice during the second week after cell transplantation. In these preparations, the Y chromosome was clearly distinguishable on the basis of size, shape, and differential staining. The data indicate that induction of leukemia after whole-body irradiation and hematopoietic cell transplantation can occur in the donor cells when a viral agent is present and that the incidence of this induction is not affected by a time delay between irradiation and transplant. (Supported by U.S.A.E.C. and N.I.H.-N.C.I.)

D-26-5 *Oncogenesis in Mice After Repeated Whole Body Irradiations and Immunorepression by Antithymocyte Serum.* Ion I. Corneci and Octav H. Costăchel, Oncological Institute, Bucharest 12, Romania.

The effect of chronic administering of rabbit antimouse thymocyte serum on development of x-rays induced or spontaneous leukemia in AKR mice, was studied. Two rabbit antimouse thymus cells serum were used: one obtained by rabbits immunized with normal thymus cells (NRAMTS), the other with thymus cells harvested from leukemic AKR donors (LRAMTS). Whole body irradiations were performed with 4 doses of 150 rads given at weekly intervals. Rabbit antimouse serum therapy (0.5 ml/mouse/week) started concomitantly with the last irradiation when associated and continuated for 6 months in all treated lots. Continuous NRAMTS treatment markedly accelerated the onset (23 weeks in treated NRAMTS versus 27 and 29 weeks in normal rabbit serum (NRS) and untreated animals, respectively), growth and lethality (50% at 27 weeks and 100% at 45 weeks lethality in NRAMTS treated animals as against 50% at 38 and 86% at 52 weeks in NRS treated and 50% at 42 and 84% at 51 weeks in untreated mice). Similarly results were obtained in irradiated animals. More dramatic effects were scored in the groups in which irradiation was followed by NRAMTS chronic treatment. On contrary, the LRAMTS administered in the same conditions prevented or delayed the onset, growth and lethality of lymphoma in normal as well as in irradiated mice.

The conclusion is that immunorepressive effect (alteration of immune surveillance mechanism) is rather responsible for the increased susceptibility to oncogenic influences than other ATS characteristics.

D-26-6 *Immune Recovery and Repair after 60 Co Exposure in Normal and Tumorous Mice.* J. B. Dubois, B. Serrou, H. Pourquier, T. Reme, and B. Delor, Centre Paul Lamarque, Hôpital Saint-Eloi, 34.000, Montpellier, France.

Three groups of 6–8 week old C57 B1/6 × DBA2 normal mice were given total body irradiations. Group I received a single dose of 500 R, group II received two doses of 250 R separated by a 3-day interval, and group III was control. Three other groups were formed using tumorous mice and the same irradiation parameters as the previous 3 groups. The plaque-forming cells (PFC) test (evaluating T and B lymphocyte co-operation) was used to follow the immune status of the mice every two days until the 30th day after irradiation. In every group we observed an immunodepression especially the group receiving single dose irradiation, (50 PFC/10^6 lymphocytes for irradiated mice, 1200 PFC/10^6 lymphocytes for control mice). We noticed, particularly after the immunodepression phase, a return to normal and even amounts of PFC surpassing original (before irradiation) amounts, (150 PFC/10^6 lymphocytes after irradiation and 120,000 PFC/10^6 lymphocytes 15 days after irradiation). This is an immunological overshoot phenomenon (I.O.P.) that followed 8 to 10 days after irradiation and which lasted about 5 days. In addition, mice from the same strain, thymectomized, lethally irradiated (900R), and reconstituted with either a population of thymic lymphocytes or a population of anti-theta serum treated lymphocytes, were irradiated according to the same protocol. Results of the PFC tests clearly indicate that the I.O.P. is largely due to T-lymphocytes. ($P < 0.001$). This seems to emphasize the value of possible radio-immunotherapeutic associations.

D-26-7 *Implications of the Effects of Ionizing Radiation on Lymphocyte Activity during Cancer Growth.* B. Serrou, J. B. Dubois, H. Pourquier, T. Reme, and B. Delor, Department of Clinical and Experimental Immunology, Centre Paul Lamarque, Hôpital St Eloi, 34.000, Montpellier, France.

Six groups of 6–8 week-old male C57 B1/6 × DBA2 mice with Lewis sarcoma transplanted in the right leg were irradiated by a 60 Co unit, (dose rate of 69 R/min, dsp: 60 cm). Group I, received total body irradiation at a single dose of 500 R; group 2, received two doses of 250 R separated by a 3-day interval; group 3, received localized irradiation on the right leg at a single dose of 2100 R; group 4, received two doses of 1200 R with a 3-day interval; groups 5 and 6 were controls. The plaque-forming cells (PFC) test was used to follow the immune status of the mice. In each group we evaluated the tumor surface area and weight, the number and volume of lung metastases, and the survival time of the mice. All these irradiations turned out to be

immunodepressive especially the total and single irradiations (early effect after 48 hours, on T and B lymphocyte co-operation). ($P < 0.001$). The groups with the most favorable tumor evolution were those that received localized and repeated small dose irradiations, particularly in those cases in which an immunological overshoot phenomenon was observed 8 to 10 days after irradiation.

D-26-8 *Radiation-Induced Intestinal Tumors in Rats: Detection of Circulating Antigen Using an Immunoradiometric Procedure.* H. F. (Frank) Cheng, John G. Sharp, and James W. Osborne, Radiation Research Laboratory, University of Iowa, Iowa City, Iowa 52242, USA.

Radiation-induced intestinal tumors (carcinoma) in rats were excised and processed to prepare a saline-soluble tumor extract. An immunosorbent was made to contain soluble tumor protein components. Rabbit antisera against rat intestinal tumors (total homogenate) were adsorbed with normal rat intestine and rat serum, then reacted with the immunosorbent. By dissociation from the immunosorbent, the purified antibody was labeled with I-125. The immunoradiometric assay procedure was based upon the reaction between the immunosorbent (carrying tumor antigen[s]) and the I-125 labeled antibody. When serum samples from tumor-bearing rats were tested, there was competition between the serum from the tumor-bearing rat and the immunosorbent for the labeled antibody, indicating the presence of a substance[s] which could bind the tumor-associated antibody. A relationship existed between the appearance of this substance and the development of the tumor. This infers that the substance is common in different rats bearing tumors. An investigation which is attempting to relate the amount of the circulating antigen to tumor size is in progress.

D-27-1 *Cellular Evidence for the Protective Effect of MEA on Intestine-Irradiated Rats.* James W. Osborne, Victoria L. Y. Tseng, and Jerald W. Bybee, University of Iowa, Iowa City, Iowa 52242, USA.

The temporarily exteriorized ileum and jejunum of 200–250 gram male Holtzman normal or MEA-pretreated (100 mg/kg) rats was irradiated with 806–1614 rads of 250 kVp x-radiation. Four days after irradiation, tritiated thymidine (1 μCi/g) was injected I.V. and the animals killed one hour later. Appropriate radioassays of weighed pieces of intestine and microdissected crypts were made and crypt survival curves constructed. Crypt size and shape were also noted.

MEA markedly shifted the survival curve to the right. With MEA pretreatment, the dose for 50% crypt survival was 1400 rads; without pretreatment, it was 800 rads. Bifurcation of crypts, usually seen with low frequency in controls, was increased in several of the irradiated groups. The results obtained provide cellular evidence for the known radioprotective effect of MEA on the irradiated rat intestine.

D-27-2 *Intestinal Crypt Survival: A Chemical Protection Study.* Curtis P. Sigdestad and Ralph M. Scott, Radiation Center, University of Louisville, Louisville, Kentucky 40201, USA.

The new anti-radiation drugs WR-2721, WR-638 and WR-77913 were compared to MEA and AET for their protective effects against 4 MeV x-rays and fission neutrons in C57/B1 6J male mice. The parameters tested were intestinal crypt survival, intestinal lethality and cellularity in the intestine.

The results demonstrated that, of those tested, WR-2721 was the best for gut lethality, and an RI (rad increase) of 726 rads was obtained for intestinal crypt survival. The protective effect was reduced when fission neutrons were used. (Supported by U.S. Army Medical Research and Development Command Contract No. DADA 17-72-C-2038.)

D-27-3 *Chemical Radiation Protection with 2-Mercaptopropionylglycine and its Related Compounds.* T. Sugahara, T. Tanaka, M. Man-I, H. Nagata,* H. Tamura,* and E. Kano.** Kyoto University, Kyoto, Japan.

No chemical radioprotectors so far reported can be used in man with appreciable effects because of their high toxicity. In the authors' laboratory 2-mercaptopropionylglycine (2-MPG), which is a commercially available synthetic drug under the name Thiola for the treatment of chronic hepatitis, cystinuria and heavy metal poisoning has been shown to be radioprotective

in mammalian cells *in vitro*, in mice and in man for colony forming capacity, 30 day survival, and chromosome aberrations in peripheral lymphocytes, respectively. The study has been extended to various related compounds with sulfhydryls, some of which have been found to be radioprotective as well in doses far below their toxic doses. Their protective property and its relationship with chemical structure will be discussed. They belong to a new group of chemical radioprotectors different from cysteamine in view of chemical structure and toxic properties.

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D-27-4 *Effect of WR2721 on Normal Tissues During Fractionated Irradiation.* JOELLA F. UTLEY M.D., THEODORE L. PHILLIPS M.D., AND LAWRENCE KANE B.S., University of Kentucky, Lexington Kentucky 40506, USA.

The radioprotective effect of S-(2-aminoethyl)phosphorothioate (WR2721) has been studied in two normal tissues, skin and small intestine, comparing the effect of the drug in divided-dose irradiation to that of graded single dose irradiation. With the single dose experiments 2/3 of the toxic LD/50 of WR2721 could be given prior to irradiation. However toxicity increased with repeated administration. Less than 1/2 of the single dose concentration could be administered safely in fractionated experiments. In fractionated skin irradiation WR2721 showed protection to a degree slightly less than seen in single fraction irradiation. The dose modifying factor (DMF) in fractionated doses was 1.3 to 1.5 compared to the DMF in single dose experiments, ranging from 1.5 to 1.7. The DMF decreased with increasing dose of irradiation in both experiments. The protection seen in small intestine was essentially the same in the single dose and fractionated dose experiments: DMF single fraction = 1.64; fractionated = 1.72. This degree of radioprotection, in spite of a greatly reduced drug concentration, indicates again a possibility for clinical use.

D-27-5 *Modification of Radiosensitivity of* Artemia *eggs under Various High-Pressure Gas Conditions.* T. IWASAKI AND Y. KUMAMOTO, National Institute of Radiological Sciences, Chiba, Japan.

We have studied the radiosensitization of dry eggs of *Artemia* to γ-irradiation by various high-pressure gases. In these experiments, the eggs were dried under high vacuum (10^{-5} Torr) and then exposed to nitrogen, oxygen or nitrous oxide at 1, 3 and 5 atm. They were irradiated with 5 to 600 krads of γ-rays, a dose-rate being 9 krads/min. The biological damage was estimated on the basis of hatchability of the eggs.

The shape of the hatchability curves of the eggs irradiated in nitrogen gas was sigmoid, and the slopes of the dose-response curve for all atmospheric pressures of nitrogen gas did not differ significantly. On the other hand, when the eggs were irradiated in oxygen, the shoulder in the dose-response curve disappeared, and the hatchability tended to decrease as the oxygen pressure is increased. The radiosensitivity of the dry eggs irradiated in mixtures of oxygen (1 atm) and nitrogen (4 atm) was similar to that of oxygen (1 atm). The results of sensitizing effect by nitrous oxide will be also reported.

D-27-6 *EPR Studies on the Radioprotective Role of Melanins.* S. LUKIEWICZ AND E. ABLEWICZ, Jagiellonian University, 31-001 Kraków, Poland.

The role of melanins as natural, endogenous radioprotectors is examined. This concept—based on theoretical grounds, radiobiological studies, and model experiments—seems to deserve more attention in the light of new EPR data reported in this paper.

The quantitative EPR determinations of melanin content in living cells of several animal and plant organisms (e.g., amphibians, fungi, hamster melanoma—melanotic and amelanotic), followed by a subsequent test of their radioresistance against x-rays (50 kV), clearly indicate that the latter property is correlated with the inherent amount of pigment.

Moreover, rigorous model experiments on melanins fixed in a solid-state matrix (an organic polymer), and x-rayed at room temperature upon simultaneous registration of the resulting free radical yield, due to radiolytic products, demonstrate in a spectacular way that melanins are able to modify radiation damage and the rate of decay of transient paramagnetic species.

All these facts are in favour of the assumption that melanins may act as radioprotectors in chemical and biological systems, and suggest possible molecular mechanisms of this effect.

D-27-7 *Our Experiences with Biological Radioprotection.* V. Hlavatý, J. Martínek, and Z. Dienstbier, Institute of Biophysics, Charles University, Faculty of General Medicine, Prague.

In the last years we have proved the possibilities of the application of some biological radioprotections for influencing the post-irradiation syndrome after single and after repeated whole-body irradiation by means of gamma rays. The protective effect of Betalactine (Spofa), Yatrein-Casein (Bayer) and the effect of the complete Freund adjuvant (Difco) was investigated. For the protective effect some compounds of antigenous character as human serum albumin and active immunization by phytourease were applied. The mechanism, biological effect and possible utilization of these mechanisms for the radioprotection in human terrain are discussed.

D-27-8 *The Protective Properties of Fungal Melanine Pigment of Some Soil Dematiaceae.* Nelli N. Zdanova and Vitaly D. Pokhodenko, Institute of Microbiology and Virology, Institute of Physical Chemistry of the Ukrainian Academy of Sciences, Kiev 252127, USSR.

The action of γ-radiation and other extreme factors on the vital activity of some Dematiaceae species (Hyphales, Fungi imperfecti) and coloured mutants of *Cladosporium transchelii* was investigated.

By means of the EPR method it was shown the paramagnetic properties of dark coloured fungi.

It was shown that the radioresistance of Dematiaceae species is determined to a great deal by their melanin content. The radioresistance of apigmented mutant is significantly lower; however the postirradiation recovery rate of pigmented and apigmented mutants was the same.

Data are obtained on evident protective action of melanine pigment under unfavorable conditions: hyper and hypoxia, dryness of medium. A conclusion is made as to the biological role of fungal melanine pigment. It consists in ability of melanine to act as the donor or acceptor of electrons in biochemical reactions of fungal organisms under extreme conditions.

In contrast, the UV resistance is connected with the melanine concentration in the cell wall.

Melanine pigment in our opinion takes part in a complex adaptive reaction, that represents the monotonous cell's answer to different irritants.

D-27-9 *Natural Radioprotective Factors.* N. N. Klemparskaya, Institute of Biophysics, Ministry of Public Health, Moscow, USSR.

It is well known that warm-blooded animals and human beings have in their blood certain protein autoantibodies, that can neutralize the harmful action of tissue metabolism products.

We have discovered that these proteins are important natural radioprotective factors. If the level of normal autoantibodies could be increased with the help of different stimulators (for example—microbe vaccines, tissue homogenates, hemostimulation) prior to radiation exposure—the radiation sickness will be lessened and the resultant lethality will decrease.

The most favourable condition for the action of these radioprotective factors is a low-level continuous radiation exposure or a fractionated exposure to high-level irradiation.

The autoantibodies circulate in the blood and get deposited in tissues. After any damaging action (including radiation) their level in the blood is quickly increased, which can be considered as an early protective natural reaction of the organism.

We used in our experiments female mice weighing 20–22 grams that were irradiated with ^{137}Cs or ^{60}Co gamma-rays.

Autoantibodies were detected by us with the help of our modifications of the Hoigne-reaction and Serne method.

D-28-1 *The Effect of Radiation on the Incidence of Pulmonary Tumors in B6CF$_1$ Mice.* R. J. M. FRY, E. J. AINSWORTH, E. STAFFELDT, A. SALLESE, AND K. ALLEN, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439, USA.

The incidence of pulmonary tumors has been determined over the life span of both male and female B6CF$_1$ mice by serial killing. In the female the incidence rose from 2% at 200 days of age to 45% at 1000 days of age, whereas in the male the incidence had reached 62% at 829 days of age. The effect of single and fractionated exposures to ^{60}Co gamma radiation and fission neutrons (fn) on the incidence of these tumors has been examined. In the females, exposed to 240 rads fn in 72 fractions, the cumulative incidence reached 73%, and 60% after exposure to 80 rads fn in 24 fractions. The size of the tumors was greater in the irradiated mice and the labeling index, one hour after ^{3}HTdR, was higher in the tumors in the irradiated mice than in tumors in controls. The question of distinguishing induction from acceleration will be discussed. (Work supported by the U.S. Atomic Energy Commission.)

D-28-2 *Pathological Features of Radiation-Induced Murine Pulmonary Carcinomas and Their Metastases.* C. C. LUSHBAUGH AND J. M. YUHAS, Medical Division, Oak Ridge Associated Universities and Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

The spontaneous and radiation-induced pulmonary neoplasms of the mouse are usually benign, and, if malignant, non-metastasizing. We have shown elsewhere, however, that total-body irradiation with gamma rays delivered at intermediate dose rates (7–28 rads per day) induces, in the BALB/c mouse, a high proportion of malignant pulmonary tumors which metastasize early and widely, and that these characteristics are retained upon *in vivo* and *in vitro* propagation. Pathologic evaluation of these metastases is complicated by the long, but not widely, known fact that they often appear sarcomatous, i.e., unlike the adenocarcinomas which shed them. As an example, after IV transplant, the tumor grows in many cases as a well-differentiated adenocarcinoma in the lung, as a poorly differentiated squamoid neoplasm in the pleural space, and as a spindle-cell sarcoma (rhabdomyosarcoma?) in the myocardium. While it appears that organ environment must contribute to pathologic type, instances where both the primary and metastases show glandular formation are sufficiently common to cast doubt on the proposal that the environment is the sole factor or that viruses shed by the tumor induce new growths in other sites. In our hands, tumor burden relative to host resistance appear to be more important factors. (Supported by the U.S. Atomic Energy Commission.)

D-28-3 *Pulmonary Neoplasia Associated with Inhalation of Insoluble Forms of Beta-Emitting Radionuclides by Beagle Dogs.* FLETCHER F. HAHN, BRUCE B. BOECKER, CHARLES H. HOBBS, ROBERT K. JONES, ROGER O. MCCLELLAN, AND M. BURTON SNIPES, Inhalation Toxicology Research Institute, Lovelace Foundation, 5200 Gibson Blvd. S.E., Albuquerque, NM 87108, USA.

Radioisotopes of yttrium, strontium and cerium are important constituents of a nuclear reactor inventory and in certain accident situations may pose an inhalation hazard. The current status of studies in which 368 dogs have been exposed to aerosols of ^{90}Y, ^{91}Y, ^{144}Ce or ^{90}Sr in fused clay show the relationship between pulmonary radiation dose, dose rate and pulmonary tumor incidence. One hundred forty six dogs have died or been euthanized at 7 to 1318 days post-exposure with the survival time and cumulative doses to lung at death being dependent upon initial dose rate and the effective half-life of the isotope in the lung. Twenty dogs which inhaled ^{144}Ce or ^{90}Sr fused clay died of pulmonary neoplasms, 644 to 1318 days post-exposure and with pulmonary radiation doses of 29,000 to 66,000 rads. Ten dogs with pulmonary radiation doses greater than 29,000 rads are surviving 881 to 1716 days post-exposure, only 3 of which are longer than 1300 days post-exposure. Dogs with similar radiation doses due to inhalation of ^{90}Y or ^{91}Y die at early times with radiation pneumonitis. (Research performed under AEC Contract AT(29-2)-1013.)

D-28-4 *Polonium-210 Alpha Radiation as a Cancer Initiator in Tobacco Smoke.* EDWARD P. RADFORD, Johns Hopkins Univ., Sch. of Hyg. & Public Health, Baltimore, Md. 21205, USA.

Exposure to alpha-emitting radon daughters is now firmly established as increasing the risk of bronchial cancer among a number of underground miners. Naturally occurring polonium-210 and its parent lead-210 are found in tobacco smoke, and increased polonium activity is found in bronchial tissues of smokers versus nonsmokers; we have thus postulated that polonium alpha radiation may be a significant cancer initiator in cigarette-induced bronchial cancer. Quantitative assessment of its importance depends on comparing the cumulative rad dose to the bronchial epithelial cells in smokers to dose-response data in miners populations. Central to this comparison is whether local "hot-spot" doses are comparable to the diffuse bronchial dose received by miners. The rad dose to the bronchial basal cell layer of miners is difficult to define per unit of cumulative radon daughter exposure, and current data probably underestimate the local dose from polonium decay in smokers. Nevertheless with reasonable assumptions it appears that polonium decay as an initiator accounts for a substantial fraction, if not all, of cigarette-induced lung cancers in males. Besides the assumptions used in dose comparisons, some related issues in lung cancer production will be discussed briefly, such as the role of other smoke constituents or viruses, and the observed cell types of bronchial cancer in smokers and miners. Lung cancer offers an example of the difficulty of identifying causative factors in cancer induced by environmental agents, and thus constitutes an important model in this field. (Discussion of the recent U.S. uranium miner data with Dr. Victor Archer of NIOSH is acknowledged with thanks. Work supported in part by grant ES00454 from the NIH.)

D-28-5 *Lung Cancer and Uranium Miners.* MAURICE DELPLA AND SUZANNE VIGNES, Electricité de France, 3, rue de Messine, 75008, Paris, France.

When they study the lung cancer increase risk (i.e. = number of cancers observed/expected) as a function of the dose (as expressed in WLM), Archer, Wagoner and Lundin [Health Physics 1973] find a plateau. From their study we conclude:

1st—It seems that doses under 120 WLM have an effect of cancer inhibition (this result is confirmed by a study in South African gold/uranium miners) this inhibition effect breaks the plateau.

2nd—It seems that the increasing factor of about 5 which extends from 120 to 1800 WLM results from cancerogenic agents other than radon daughters. We do not agree with Archer *et al.* concerning the independence between these cancerogenic agents and cancer risk.

3rd—It seems that a cancerogenesis threshold appears at about 2000 WLM.

So it is enough to keep the working conditions of the miners at such a level and it seems useless, if not inappropriate, to increase beyond reason the mine's ventilation.

D-29-1 *TLD Measurements with Terbium-Activated Magnesium Orthosilicate.* KLAUS BECKER AND J. S. JUN.** Health Physics Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

Terbium-activated magnesium orthosilicate has been prepared by heating finely powdered mixtures of MgO and SiO_2 impregnated with a dilute solution of a Tb^{3+} salt to temperature between 1400 and 1800°C in Pt, SiO_2, or Al_2O_3 crucibles. The UV to gamma radiation response ratio of the phosphor was found to depend on the thermal treatment. Optimized preparation resulted in a material which is about thirty times as sensitive as the widely used LiF:Mg, Ti (TLD-100); exhibits less fading at elevated temperatures; and does not require a complicated, multistep annealing cycle prior to reuse. Some supralinearity of response is observed at higher low-LET radiation doses. The photon energy dependence (oversensitivity by a factor of about 4.5 for ~40 keV x-rays) can be substantially reduced by embedding the finely powdered phosphor into Teflon disks and/or optimized metal compensation filters. (Research sponsored by the U.S. Atomic Energy Commission under contract with Union Carbide Corporation.)

** IAEA Fellow, on leave from Korean Atomic Energy Research Institute, Seoul.

D-29-2 *On Coherent Scattering of Gamma Rays.* S. C. Roy, A. Nath, and A. M. Ghose, Bose Institute, Calcutta-9, India.

Large departures of the measured coherent scattering cross-sections at low momentum transfers for heavy elements from the form factor values as observed by Hauser and Mussgnug (Zeit Phys. **195** (1966)), 252 was checked by systematic measurements of coherent scattering cross-sections for a wide range of elements (C, Al, Fe, Cu, Sn, Pb, Bi and Th) in the angular range 2°–20° using 145 keV photons. The present measurements are in general agreement with form factor values and do not confirm the findings of Hauser *et al.*

As the accurate theoretical estimation of Rayleigh scattering is in general difficult, an analytical formula depending only on photon energy and atomic number of the element is of great importance in the field of radiation research. Such an analytical formula was developed using the recently available accurate cross-section values obtained from the measurements due to Schumacher. (Phys. Rev. **182**, 7, 1969; Nucl. Phys. **A206**, 531, 1973).

D-29-3 *Gamma Ray Detectors with Energy-Independent Efficiency.* A. M. Ghose, Bose Institute, Calcutta-9, India.

Gamma ray detector systems whose efficiency is constant, within $\pm 1\%$ when the energy of paraxially incident photons is varied from low values to about 1.5 MeV, will be described. The systems consist of conventional scintillation counters coupled to "perforated" filters. Modifications necessary for wide angle counting will be presented. Applications to metrology of radionuclides and radiation dosimetry will be discussed.

D-29-4 *Effect of Filters on Clinical Gamma Ray Beams.* Norman K. Sherman and Keith H. Lokan, Physics Division, National Research Council of Canada, Ottawa K1A 0S1, and Ronald M. Hutcheon and Warren Funk, Chalk River Nuclear Laboratories, Ontario, Canada.

To study the production of gamma ray beams best suited to treating deepseated tumors, the N.R.C. $^2H(\gamma, n)$ photon spectrometer consisting of a liquid deuterium target viewed by a photoneutron time-of-flight detector[1] has been used to measure 25 MeV bremsstrahlung energy spectra with high precision. Seven different Ta radiators of thicknesses from 0.012 to 1.0 radiation length were used with and without beam hardeners (27.2 g/cm^2 of Al, or 15.1 g/cm^2 of Pb). For the same nominal attenuation of 10 MeV photons the Al filter produced from each radiator a harder and hence more deeply penetrating spectrum than did the Pb filter. This result has been corroborated[2] by depth-dose measurements.

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D-29-5 *Lyoluminescence Dosimetry.* N. A. Atari, K. V. Ettinger, and J. R. Mallard Department of Medical Physics, University of Aberdeen, Foresterhill, Aberdeen, Great Britain.

Several categories of irradiated solids exhibit lyoluminescence, i.e., emission of light when dissolved in water or other solvents. In the alkali halides color centres (F and V) provide storage of charge which is released during dissolution in the solvent. The stored energy, mediated by the solvated electrons, is emitted as light from the solution. Another category of materials which exhibit lyoluminescence, albeit based on a different mechanism, are the saccharides. In polysaccharides the frozen free radicals formed during the irradiation are on dissolution involved in a series of chemical reactions with dissolved or diffused oxygen leading to the emission of light. This emission is enhanced by the presence, in the solution, of fluorescent chemicals like luminol.

A series of saccharides has been tried as potential materials for lyoluminescent dosimetry, with special attention being paid to the range of doses, response to various types of ionizing radiation, stability of stored radicals under the influence of light, heat and humidity. The most

suitable saccharide was found to be trehalose·$2H_2O$ which gives linear readings from 100 mrad to 150 krad. The summary composition of saccharides is very close to that of human soft tissues which opens the way to true tissue equivalent dosimetry, without the need to consider an average atomic number.

D-29-6 *Differences in Biological and Physical Depth Dose Distribution for Gamma Rays.* DONALD E. CARLSON AND JACQUELYN THORNTON, The University of Texas Health Science Center, Dallas, Texas 75235, USA.

Biologically significant depth dose distribution was compared with that of physical dose using a tissue equivalent phantom permitting assay of cell suspensions at 3 mm depth increments. Maximum energy deposition for ^{60}Co gamma rays occurs 0.5 cm below the surface, followed by exponential attenuation with depth. In contrast, mouse hematopoietic stem cells assayed by spleen colony formation, and V79 hamster fibroblasts assayed *in vitro* indicate that maximum biologically significant dose occurs at approximately 2 cm below the surface. This phenomenon is independent of source distance and field size, suggesting that changes in beam composition are reflected in differential cell survival over the proximal few centimeters of depth.

D-29-7 *Depth-Dose Measurements Using Holographic Interferometry.* ARNE MILLER,* WILLIAM L. MCLAUGHLIN, AND ECKART K. HUSSMANN,** Center for Radiation Research, National Bureau of Standards, Washington, DC 20234, USA.

Holographic interferometry uses radiation-produced temperature gradients causing changes in the index of refraction of liquids in order to measure depth-dose profiles. The liquids are irradiated with broad electron beams at energies from 1–10 MeV. It is also shown that narrow-beam penetration profiles in liquids can be measured in terms of energy deposition by this technique.

The following measurements are included:

1) Depth doses in liquids of different specific gravity.
2) Depth doses in water for different angles of incidence.
3) Depth doses in water layers thinner than the electron range and with different backing materials.

In the case of a liquid having a high temperature dependence of the index of refraction (e.g., hexane), the minimum detectable absorbed dose is ~10 krad. The holographic interferometer has been improved by simplifying the optical components and by superimposing a fixed parallel pattern as part of the holographic procedure.

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* Visiting scientist from Atomic Energy Commission, Res. Establishment Risø, Denmark.

** Visiting scientist Jenaer Glaswerk Schott & Gen., Mainz.

D-29-8 *Applications of the Poly(halo)styrene Dosimeter System in Pulsed and Steady State Environments.* W. P. BISHOP, E. T. SNOW, E. P. ROYER, J. L. BENSON, AND J. D. PLIMPTON, Sandia Laboratories, Albuquerque, New Mexico 87115, USA.

The performance characteristics of the doped poly(halo)styrene dosimeter system are briefly reviewed. Since first described (1970) and since the recent full description of its characteristics (1973), this versatile film dosimeter has been used for many applications under diverse conditions, both in the field and on laboratory sources of photons and electrons.

The material has proven to be an accurate totally-stopping fluence monitor in x-ray beams giving excellent agreement with other measurements and with calculations. Dose vs depth measurements using both stacks of films and solid plugs or wedges have been successful, survival in pulses delivering over 30 megarads has been observed, and dose rate independence to over 10^{15} rads/sec has been confirmed.

Calculations for minor corrections in readout (due to multiple reflection phenomena) and in photon loss by fluorescence (in x-ray applications) are discussed briefly. Finally, preliminary indications of a serious LET effect with 2 MeV protons are noted and other developmental applications are discussed.

D-29-9 *The Dosimetry of Fine-Dispersed Heterogeneous Systems Under Gamma-Ray and Electron Irradiation.* Oleg P. Verkhgradsky and Iosif R. Entinzon, L. V. Pisarzhevsky Institute of Physical Chemistry, Ukr. SSR Academy of Sciences, Prospekt Nauki 97, Kiev 252028, USSR.

All heterogeneous systems may be divided into three types depending on the size of their homogeneous parts: 1) fine-dispersed, 2) crude-dispersed and 3) systems with the wall effect. The dimensions of homogeneous regions in fine-dispersed heterogeneous systems are much smaller compared to the ranges of ionizing particles. These systems may be considered as a statistical plurality of small cavities. The theory of ionization in small cavity may be adopted for every such cavity. It should be noted that among existing methods of dose measuring a procedure with the application of an extrapolating ionizing chamber is the most suitable to the dosimetry of fine-dispersed heterogeneous systems.

A set of systems for dosimetry using chemical dosimeters is proposed. The procedure proposed is based on the same principles as an extrapolating chamber, but it may be used under conditions of small volumes and high intensity of radiation. Experimental data are given for a number of systems involving different metals and organic substances. These data are compared with the computed results.

D-30-1 *Calculations Pertaining to the Use of Fast (<50 MeV) Neutrons in Cancer Radiotherapy.* R. G. Alsmiller, Jr. and J. Barish, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

Calculated results have been obtained for a variety of collimated neutron spectra incident on a tissue-equivalent phantom. The neutron spectra considered are those produced by 35- and 50-MeV deuterons in a thick beryllium target and by 67-MeV protons in a thick lithium target. Collimators producing both cylindrical and square fields at the surface of the phantom have been considered. In all cases, the phantom was taken to be a semi-infinite 30-cm-thick slab. The results include absorbed doses, LET spectra, cell-survival probabilities, OER's, and RBE's as a function of position in the phantom. The cell-inactivation calculations were carried out using the model of Katz *et al.* and parameters for T-1 kidney cells. In the case of incident neutrons from 35-MeV ^2H on Be, the calculated data are compared with experimental measurements of absorbed dose and cell-survival probability. (This work was partially funded by the National Science Foundation, Order NSF/RANN AG-399, under Union Carbide Corporation's contract with the U.S. Atomic Energy Commission.)

D-30-2 *Pretherapeutic Experiments with 30 and 50 MeV Neutron Beams. Dosimetric, Microdosimetric and Radiobiological Studies.* A. Wambersie, Groupe d'Etude pour la Neutron-thérapie à Louvain-la-Neuve, B-1348, Belgium.

Neutron beams produced by bombardment of 30 and 50 MeV deuterons on a thick Be target were studied at the isochronous cyclotron of the University at Louvain-la-Neuve.

A fixed, sandwich-type (steel/lucite) collimator was designed for pretherapeutic experiments. A calibrated tissue-equivalent (TE) parallel plate chamber filled with TE gas was used as reference. Two separate dosimetric methods (Al/Argon gas chamber and GM counter) allow discriminating the γ component.

Depth-dose curves and isodose curves were determined, as well as the build up at the surface. The spectra of the incident neutron beams, the LET spectra in air and at different depths in a TE phantom were evaluated.

RBE values of 2.3 and 1.8 were found respectively for 30 and 50 MeV neutrons both for LD50/5 and LD50/30 on mice. Early recovery for intestinal death was investigated using the usual split-dose technique.

Finally, variation of RBE as a function of dose was studied using chromosome aberrations in onion roots and in human and pig lymphocytes. Correlation between LET spectra and observed RBE values is discussed.

D-30-3 *Neutron Dosimetry at the Beam Port of a TRIGA Core Reactor.* Joseph L. Beach, Radiation Biophysics, University of Kansas, Lawrence, Kansas 66045, and Evan B. Douple, Norris Cotton Cancer Center, Dartmouth College, Hanover, NH, USA.

A system has been developed for determining the dose rate due to high flux mixed neutron and gamma sources and the system has been tested on a 1 Megawatt TRIGA core research reactor. The dosimetry system employs an LET proportional counter to measure the dose rate due to intermediate and high energy neutrons, $CaSO_4$ TLD crystals to determine the gamma contamination, and gold foils to measure the thermal neutron flux. The dose rate for small animals placed in a biological irradiation facility at the end of a modified beam port was found to be: gamma rays, 17 ± 1 rads/hour; thermal neutrons, 5 ± 1 rads/hour, and intermediate and fast neutrons, 128.5 ± 2.0 rads/hour.

D-30-4 *Perturbation of Fast Neutron Dose Distributions by Lung Tissue.* PATTON H. MCGINLEY, Emory University Clinic, Radiation Therapy, Atlanta, Georgia 30322, USA.

Dose distributions for use in neutron radiotherapy were experimentally determined for 14.7 MeV neutrons produced by the $_1{}^3H(d, n)_2{}^4He$ reaction and for neutrons with an average energy of approximately 15 MeV generated by bombarding a beryllium target with 35 MeV deuterons. Muscle-equivalent ionization chambers were used to establish the total dose due to neutrons and gamma rays in homogeneous and heterogeneous phantoms irradiated by small beams of fast neutrons at a target to skin distance of 125 cm. The heterogeneous phantom was equipped with lungs constructed of granulated muscle-equivalent plastic.

An examination of the depth dose data showed that for depths greater than 8 cm along the beam axis the absorbed dose is increased due to the presence of lung tissue. Corrections of the soft tissue dose distribution of the order of 18 to 20% were found for points located behind the lungs.

D-30-5 *Uncertainty Analysis for Dosimetry in a Mixed Neutron and Gamma-Ray Field.* LEON J. GOODMAN, Columbia University, 17 Cornell Avenue, Upton, New York 11973, USA.

A method using two different dosimeters is commonly employed to evaluate the separate neutron and gamma ray absorbed doses in a mixed radiation field. One of the dosimeters is constructed so as to have low sensitivity to a neutron absorbed dose relative to its sensitivity to an equal gamma ray absorbed dose. Uncertainty in this neutron sensitivity produces uncertainties in the computed values of the two dose components. A quantitative analysis is presented of the uncertainties produced in the doses by this neutron sensitivity and its uncertainty, and by the amount of gamma ray absorbed dose relative to the neutron component. Several conclusions are drawn with regard to these parameters, and a recommendation for minimizing uncertainties is made.

D-30-6 *Determination of Total Cosmic-Ray Neutron Flux by the Measurements of 2.22 MeV Capture Gamma-Ray from Hydrogen and Estimation of Neutron Dose.* MASAHARU OKANO, KOICHI IZUMO, TAKEO KATOU, AND TATSUJI HAMADA, Institute of Physical and Chemical Research, Wako-shi, Saitama, Japan.

The intensity of 2.22 MeV gamma-ray emitted by neutron capture of hydrogen has been measured on the ground at Mt. Norikura, covered with snow of about 3 m thick and on the floor of DC-8 aircraft covered with paraffine blocks of 30 cm thick. The measurements were made with a 23 cm^3 Ge(Li) detector and a 3 in. diam. sphere-type NaI(Tl) detector. The values of factor for converting neutron flux to gamma-ray flux has been calculated and found to be 0.3 to 0.4 depending on the moderator geometry. The values of cosmic-ray neutron flux thus determined were 0.015 $cm^{-2}\ s^{-1}$ at 9000 ft and 25°N geomagnetic latitude and $0.1 \sim 0.2\ cm^{-2}\ s^{-1}$ at 33,000 to 35,000 ft and 15°N to 35°N. They are a little less than those reported previously. The cosmic-ray neutron dose has also been estimated from the present value of total flux assuming the neutrons to have the energy spectra given by W. H. Hess *et al.* and T. W. Armstrong *et al.*

D-30-7 *Fast Neutron Dosimetry Intercomparisons of Facilities Engaged in Neutron Radiation Therapy.* ALFRED R. SMITH, JAMES B. SMATHERS, PETER R. ALMOND, AND VICTOR A. OTTE,

M. D. Anderson Hospital, Texas A & M University Fast Neutron Therapy Project, Houston, TX, USA.

Neutron dosimetry intercomparisons visits have been made by physicists from the M. D. Anderson Hospital—TAMVEC Project to the Naval Research Laboratory, University of Washington Hospital, and Hammersmith Hospital. Cyclotron time at TAMVEC has been made available for reciprocal visits. The parameters that are measured during these visits are: tissue kerma in air, tissue dose at depth of dose maximum, depth dose, beam profiles, neutron/gamma ratios in air and in phantom, and photon calibrations of ionization chambers. Other parameters that are compared are values of W for the beam components, stopping power ratios, and calculations and transformations that lead to the statement of tumor dose in a patient. A preliminary report of these intercomparisons will be given including a comparison of the calculation and statement of tumor doses for each institution. (This work was supported by NCI Grant CA-12542.)

D-30-8 *Physical Properties of Shonka A-150 Tissue Equivalent Plastic for an Absorbed Dose Neutron Calorimeter.* J. C. McDonald, T. R. Canada, R. Freeman, and J. S. Laughlin, Biophysics Laboratory, Memorial Sloan-Kettering Cancer Center, New York, New York, USA.

Several authors[1,2,3] have demonstrated that, for both high and low LET radiations, Shonka type A-150 plastic exhibits a thermal defect of approximately 4% at high dose levels. One such measurement (2) indicates an excess production of heat, ~10%, which is modified to ~4% thermal defect after irradiation with 0.1 MRad of 7 MeV x-rays. The relative thermal response per rad as a function of accumulated dose for 300 kVp x-rays has been measured and shows a decrease of 14% ± 1% between 0 and 50 krad, and thereafter it is approximately constant, in general agreement with Bewley *et al.* (2). The internal resistance of the plastic is employed for electrical calibration. The resistivity was measured to be 41.4 ± 0.4 ohm-cm at 0°C and was found to vary linearly between 0 and 50°C with a positive coefficient of 0.227 ± 0.02 ohm-cm/°C. A technique for achieving low constant resistance coatings has been developed and will be described. (Supported in part by AEC(11-1)-3522 and NCI 08748-08B.)

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D-31-1 *Dissociative Ionization of Molecules by Electron Impact.* J. A. D. Stockdale, C. E. Klots, and R. N. Compton, Health Physics Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

The dissociative ionization process $e + AB \rightarrow A^+ + B + e + e$ may often contribute a large fraction of the total ionization produced by electron impact on molecules.[1] Measurements of the angular distributions and kinetic energies of the products shed light on the symmetries[2] and positions of the molecular states involved in the dissociation process. They are also of fundamental importance to radiation chemistry and dosimetry since the nature and distribution of the final products of irradiation is dependent on the nature and kinematic properties of the primary dissociation products. We will summarize the results of recent experimental studies at this laboratory of dissociative ionization of D_2, N_2 and O_2 by impact of electrons in the energy range from 0 to 300 eV. The relevance of these measurements to gas phase radiation chemistry will be discussed. (Research sponsored by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.)

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D-31-2 *Dipole Oscillator Strength Distributions Derived for Several Hydrocarbons from Electron Energy Loss Spectra.* Russell H. Huebner, Argonne National Laboratory, Argonne, Illinois

60439, USA, AND R. J. CELOTTA, S. R. MIELCZAREK, AND C. E. KUYATT, National Bureau of Standards, Washington, D.C. 20234, USA.

An important input for calculating the radiation chemical yields of primary products is the dipole oscillator strength distribution of the molecular target. Such distributions can be derived not only from photoabsorption measurements but also from electron energy loss spectra by a method described in detail elsewhere.[1] We have derived relative oscillator strengths for methane, benzene, and other hydrocarbons from energy loss spectra measured for 100 eV incident electrons scattered in the forward direction. After these data are normalized to an optical value at one energy, the electron impact values are found in generally good agreement with optical values for other energies below 15 eV. This work is being extended to higher incident energies and larger energy losses. (Work supported in part by the U.S. Atomic Energy Commission.)

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D-31-3 *Analysis of Track Structure due to Delta-Rays.* NOBUO ODA,* SATOSHI MOROOKA,** TAKAO NUMAKUNAI,† AND HIROSHI RYUFUKU.†

The experimental double differential cross sections measured by Oda and Nishimura for the ionizing collisions of electrons with some molecules are applied to the analysis of the track structure due to delta-rays. First, several kinds of theoretical models to reproduce the experimental data are discussed. Secondly, these models are applied to get the energy and angular distributions of electrons as a function of the distance from the primary particle track and the event size distributions in small spherical targets traversed by electrons.

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D-31-4 *Angular and Energy Distributions of Secondary Electrons from Molecules by Electron Impact.* NOBUO ODA AND FUMIO NISHIMURA, Tokyo Institute of Technology, Meguro-ku, Tokyo, Japan.

The double differential cross sections, differential in angle and energy of scattered and ejected electrons, produced from some molecules (methane, ethane, etc.) by electron impact are presented, for angular range $5° \sim 130°$ and for impact energy range $100 \sim 2{,}000$ eV. The single differential cross sections, differential only in energy, for the production of secondary electrons are derived from the above data, where the contributions of Auger electrons due to carbon K shell ionization are also estimated.

D-31-5 *Electron Emission Spectra from Proton Ionization of Molecular Gases.* L. H. TOBUREN AND W. E. WILSON, Battelle Pacific Northwest Laboratory, Richland, Washington 99352, USA.

Cross sections for secondary electron production by fast charged particles are of major importance in the calculation of energy degradation and track structure. During the past few years numerous measurements and calculations have been made of the distributions of electrons which are ejected from gas targets by fast protons. Cross sections calculated using binary encounter theory are in much better agreement with measurements than are calculations using the free electron model; however, sizeable discrepancies still exist. Theoretical studies of the angular distributions of ejected electrons exist for only the simplest systems and the measurements of proton ionization of several hydrocarbon molecules indicate that molecular structure effects are evident in the angular distributions. A strong dependence on molecular structure is observed in the shape of the low-energy end of the energy spectra of ejected electrons. These energy and angular distributions will be discussed for ionization of a number of molecular targets by fast protons. (This paper is based on work performed under United States Atomic Energy Commission Contract AT(45-1)-1830.)

D-31-6 *An Elementary Approach to the Systematics of W for Monoatomic Gases.** ROBERTA P. SAXON,† MITIO INOKUTI, AND J. L. DEHMER, Argonne National Laboratory, Argonne, Illinois 60439, USA.

The average energy required to form an ion pair (usually denoted by W) in *monoatomic gases* has been measured only for rare gases and mercury vapor.[1] Contemporary knowledge of dynamical properties of atoms now permits us to predict theoretically the W value for most atoms and to discuss its systematics across the periodic table. We have done so for the atoms He through Ar by use of realistic atomic potentials. In our initial exploration we use the elementary method of Fano[2] which partitions inelastic collisions into two classes, i.e., glancing collisions and hard collisions. The former is characterized chiefly by the spectral distribution of the dipole oscillator strength. The latter is treated in weak-binding approximations. It is hoped that our work will not only stimulate W measurements on monoatomic gases, but also elucidate merits and shortcomings of the Fano method.

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* Work performed under the auspices of the U.S. Atomic Energy Commission.

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[1] W. P. Jesse, J. Chem. Phys. **55**, 3603 (1971).

[2] U. Fano, Phys. Rev. **70**, 44 (1946).

D-32-1 *A Theoretical Approach to the Evaluation of the Molecular Excitation and Ionization Yields by Impact of High Energy Electrons.* P. P. DELSANTO AND R. A. LEE, University of Puerto Rico and Puerto Rico Nuclear Center, Mayaguez, Puerto Rico.

A new method of evaluating inelastic scattering cross sections of electrons against simple molecules is proposed. This method has been recently derived and successfully applied to the calculation of cross sections for photonuclear reactions in the Giant Dipole Resonance energy region in the nuclear 1p-1h Shell Model approximation. The calculations already performed have shown the practicability of the method from the computational point of view. In fact it requires only a small fraction of the computer time needed for solving the same problem with the same accuracy by means of a previous widely used method: the so called Eigenchannel Theory. Being a diagonalization technique, it has the advantage over other methods that it can be easily adapted to more sophisticated models or different problems.

An application of the method to the problem mentioned above is in progress. Once the cross sections are calculated, the yield of any particular process (excitation or ionization) can be obtained by averaging over that part of the degradation spectrum that can induce the process. (R. F. Barrett, P. P. Delsanto, A New Treatment of the One Particle Continuum in Nuclear Reaction Theory. Submitted to Physical Review.)

D-32-2 *Fragmentation Scheme of Methanol, Judging from Its Emission Spectrum Produced by Electron Impact.* KOZO HIROTA, MOTOYOSHI HATADA, AND IWAO FUJITA, Chiba Institute of Technology, Yatsu, Narashino City, Chiba Pref. 275, Osaka Lab., Genshiryoku Kenkyusho, Neyagawa City, Osaka 572, and Osaka Denki-tsushin Daigaku, Neyagawa City, Osaka 572, Japan.

The emission spectra of CH_3OH, CH_3OD and CD_3OH produced by the impact of low energy electron beam give the Balmer lines of H and D of the superexcited state of methanol with electron impact. The ratios of the intensity of H and D obtained at low pressure reached the conclusion that scissions of both OH and CH bond occur primarily. This finding agrees well with the explanation proposed by Hirota *et al.* (Nippon Kagaku Zasshi **78**, 129 (1957)) on the ratio of H_2:HD:D_2 in the produced hydrogen by gamma-ray radiolysis, taking the molecular detachment of hydrogen into consideration (Baxendale and Sidgwick, Trans. Faraday Soc. **57**, 2157 (1961)). It is also found that the scission of the deuterated bond is more difficult by a factor of 0.8 than that of the non-deuterated one.

D-32-3 *Interpretation of Super-Excited States of CH_4 and Other Isoelectronic Molecules.* TSUTOMU WATANABE AND SHIGEAKI NISHIKAWA, Dept. of Applied Physics, The University of Tokyo, Bunkyo-ku, Tokyo, 113, Japan.

The energy spectrum of super-excited states is calculated using ultraviolet photoelectron data for CH_4 and oscillator strength data for the isoelectronic atom, Ne. Recent measurements of ultraviolet photoelectron spectroscopy show that the totally symmetric configuration (T_d symmetry) of CH_4 is unstable in the ionized ground state as a result of the Jahn-Teller effect due to the ejection of an electron from the $1t_2$ orbital. In the case of $1t_2$ electronic excitation, the resultant CH_4^+ ion core suffers a strong distortion and remains in a high vibrational state. This process results in a super-excited state. Furthermore, we can predict that the potential energy surface of a highly excited Rydberg state is similar to that of the CH_4^+ ion. With these considerations, an estimation has been carried out empirically. We have concluded that the super-excited states of CH_4 can be interpreted in terms of the Jahn-Teller distortion of the high Rydberg states with reference to the $^2T_2$ state of CH_4^+. The inconsistency between the UV spectrum data and results of other measurements and analyses is pointed out and discussed. Similar analyses are also attempted for other isoelectronic molecules.

D-32-4 *New Mechanisms for the Jesse Effect.* C. E. KLOTS, M. G. PAYNE, AND G. S. HURST, Health Physics Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

The well-known increase of radiation-induced ionization in, especially, the noble gases in the presence of suitable additives has served to catalyze investigation of energy transfer mechanisms, super-excited states, and autoionization processes. The actual vectors involved are only now succumbing to understanding. Resonance states, metastable atoms and diatomic rare gas molecules have already been implicated as important progenitors. Two additional mechanisms will be discussed. Internal photolysis, i.e., the interception of an imprisoned quantum by an impurity, can be important under certain well-prescribed conditions. Sensitized chemi-ionization, exemplified by the sequence: $Ar(^1P_1) + Xe \rightarrow Xe^* + Ar$; $Xe^* + Xe \rightarrow Xe_2^+ + e$ has also been demonstrated recently. Conditions favoring this mechanism will be delineated. In addition, two-body collisional quenching of helium 2^1P to the 2^1S state, involving a break-down of the Born-Oppenheimer approximation, now appears to be the important pathway leading to the Jesse effect in helium. (Research sponsored by the U.S. Atomic Energy Commission under contract with Union Carbide Corporation.)

D-32-5 *Associative Ionization in Slow Collisions between $He(2^3S)$ and Ar.* HIROKI NAKAMURA, Department of Applied Physics, University of Tokyo, Tokyo, Japan.

The velocity dependence of the branching ratio of associative and penning ionization processes in the system of metastable helium and ground state argon atoms is investigated theoretically within the framework of the adiabatic approximation with use of the local complex potential method.

The complex curve obtained by Olson is used for the initial excited molecular state. Using a screened coulomb repulsive potential and an attractive polarization potential, a potential curve for the final ground state molecular ion is constructed. The previously proposed cross section formula is employed to estimate the total associative ionization cross section. The result is compared with the recent experiment by A. Pesnelle *et al.*

The method of obtaining vibrational energy distribution in the final product molecular ions is also discussed which does not require detailed information on the individual electronic matrix element $V_l(R)$ with an electronic angular momentum l, but requires only the knowledge of the total resonance width (Imaginary part of the complex potential) $\Gamma(R) = 2\pi\sum_l |V_l(R)|^2$.

D-32-6 *Effect of Total Ionization Cross Section of Gases on the Self-Focusing of Pulsed Electron Beam.* HIROSHI HOTTA AND RYUICHI TANAKA, Japan Atomic Energy Research Institute, Takasaki, Gunma, Japan.

The depth-dose curves of high-intensity pulsed electron beam, generated from Febetron 706, in a stack of aluminum absorbers and blue cellophane sheets were measured as a function of pressure of gaseous media. They indicate that the beam is self-focused twice at the low and

high pressures. The total ionization cross section of medium gases by high-energy electrons can be obtained relatively from the rise of the first pinch at the pressure lower than 10 torr. These relative values are in good agreement with those measured at 1 kev by electron impact. The rise of the second pinch above 10 torr is attributed to the recombination of secondary electrons with positive ions and their attachment to neutral molecules. The second pinch is collapsed mainly by the lateral dispacement of the primary beam.

D-32-7 *Intramolecular Energy Transfer in Fluorogenic Derivatives of Thymine and Uracil.* JOHN G. BURR, W. A. SUMMERS, AND YUNG JAI LEE, Department of Chemistry, University of Oklahoma, Norman, Okla. 73069.

Intramolecular energy transfer has been observed in several fluorogenic derivitives of thymine and uracil. We will discuss the efficiency of this energy transfer process, the nature of the donor pyrimidine excited state, and the effect of several solution variables (pH and viscosisty variation) upon the energy transfer process.

The fluorogenic derivatives were obtained by treatment of 1-(3-aminopropyl)thymine (3-APT) or 1-(3-aminopropyl)uracil (3-APU) with NBD or Dansyl chlorides. The derivatives were crystalline and well characterized. 3-APT and 3-APU were prepared in the following manner. Thymine (or uracil) was silated, then alkylated with 1,3-dibrompropane; the 3-bromopropyl thymine was heated with hexamethylenetetramine, followed by acid hydrolysis. The amine hydrochloride so obtained was converted to the free amine either by liquid-liquid extraction from an alkaline solution, or by treating an ethanol solution of the salt with K_2CO_3.

D-33-1 *Detection Methods for the Picosecond Range.* G. BECK, Hahn-Meitner-Institut für Kernforschung Berlin GmbH, Bereich Strahlenchemie, Berlin 39, West Germany.

Design parameters for an optical detection system for the picosecond range are discussed, including the influence of transit time in the photodetector on signal shape. A fixture is described which allows to operate a photodiode at high voltage and with minimum signal distortion. An overall risetime for the system of 60 psec has been measured.

In addition the possibility of detecting conductivity changes with picosecond resolution is discussed and experimental results are given.

D-33-2 *Recording and Processing of a Complete Time-Resolved Transient Absorption Spectrum Produced by a Single Pulse of Radiation.* KLAUS H. SCHMIDT, SHEFFIELD GORDON, AND WILLIAM A. MULAC, Chemistry Division, Argonne National Laboratory, Argonne, Illinois 60439, USA.

This technique, an improved version of a method previously reported by us, represents a combination of the now classical method of kinetic spectrophotometry and pulse spectroscopy. The transient absorption spectrum produced by a conventional pulse radiolysis arrangement is converted, by an image converter camera with streak capability, into a two-dimensional display of light intensity *vs.* wavelength and time. This image is scanned by a TV-camera and stored in a video-disk recorder. From there it is transferred into a Sigma-5 computer, where the raw data, reduced to 12,000 relevant data points, are stored. From the stored data, plots of absorbance *vs.* time and/or wavelength can be obtained on a graphic display terminal. One of the first applications of the new system will be the study of radiation-induced valency changes in actinides. Such an investigation would have been made prohibitive by the frequent changing of samples required with conventional pulse-radiolysis methods. (Work performed under the auspices of the U.S. Atomic Energy Commission.)

D-33-3 *A Computer Controlled Pulse Radiolysis System.* LARRY K. PATTERSON, Radiation Research Laboratories and Center for Special Studies, Mellon Institute of Science, Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213, USA.

A computer controlled pulse radiolysis unit for the gathering and analysis of data related to transient optical absorption has been developed around the PDP-8 minicomputer and Biomation 8100 transient recorder. Because of computer-recorder interaction and constant monitoring of experimental parameters (I_0, dose, Δ transmittance), the conditions of measurement (e.g., recorder sensitivity, photodetector backoff) may be automatically altered by computer. A substantial

number of manual operations are eliminated and provisions are made for storage of kinetic and spectral data on paper or magnetic tape. Further, the analysis of data for kinetic information and construction of time dependent spectra are greatly simplified by computer operation. It is shown that such a system may be constructed using facilities commercially available in the computer system with a minimum of additional circuitry required. Formats for storage and presentation of information are illustrated by application of the apparatus to several chemical systems.

D-33-4 *An AC Conductivity Cell for Pulse Radiolysis of Liquids.* JEAKOOK PARK, W. H. VOELKER, AND E. C. GREGG, Case Western Reserve University, Cleveland, Ohio 44106, USA.

Although most studies of ionic processes in liquids following short pulses of ionizing radiation are made in DC conductivity cells, such measurements can be plagued with space charge effects under certain conditions. These can be avoided through the use of low voltage alternating electric fields which do not produce a continuous drift of ions toward the electrodes and, hence extensive sheaths of charge at the electrodes. Charge collection at electrodes is also minimized. The design of such a device and its associated circuitry will be presented along with data for ionic recombination in hexane. These data, in turn, will be compared with that obtained in a DC cell, demonstrating the magnitude of the space charge and charge collection effects. If time permits, comparison will also be made with theory. (Work supported by U.S.AEC.)

D-33-5 *Polarographic Studies of Pulse Radiolytically Produced Short-Lived Radicals in Aqueous Solutions.* KRISHAN M. BANSAL, Hahn-Meitner-Institut für Kernforschung Berlin GmbH, 1 Berlin 39, West Germany.

The oxidation and reduction behavior of radicals, produced by a 20 ns pulse of high energy electrons in nitrous oxide saturated aqueous solutions of various compounds was investigated at a hanging mercury drop electrode. Polarograms of the radicals were recorded by plotting currents 15 μs after the pulse versus the potential. They generally contain irreversible waves of oxidation and reduction which are characteristic of the various types of radicals. The position and shape of these waves are discussed in the light of the electrochemical theories of electron transfer. In some cases, the radicals undergo rearrangement at certain pH-values and are accompanied by significant change of polarographic behavior. These studies included radicals of biochemical interest. Macroradicals were also investigated and in this case the adsorption at the electrode surface plays an portant role.

D-33-6 *Time-Resolved ESR Studies of Pulse Irradiated Aqueous Solutions.* RICHARD W. FESSENDEN AND NARESH C. VERMA, Radiation Research Laboratories, Center for Special Studies and Department of Chemistry, Mellon Institute of Science, Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213, USA.

The use of time-resolved ESR spectroscopy (1 μsec resolution) to study radical formation and decay kinetics in pulse irradiated aqueous systems is described. This technique brings the power of the ESR method in making precise radical identifications to kinetic experiments carried out under conditions directly comparable with those used in more conventional optical pulse radiolysis experiments. Emphasis has been put upon the rate of formation of radical species which have been identified by ESR in steady-state radiolysis experiments. Reactions have been studied involving both initial attack of the primary water radicals upon the solute (OH abstraction of a hydrogen atom from $CH_3CO_2^-$ and e_{aq}^- addition to $^-O_2CC{=}CCO_2^-$) and further radical transformations (protonation of the e_{aq}^- adduct to $^-O_2CC{=}CCO_2^-$, enol to keto rearrangement of the OH adduct to $^-O_2CC{=}CCO_2^-$, ring opening of the OH adduct to 3,4-furan dicarboxylate, and loss of water by OH adducts to ascorbic acid). The kinetics of these and other reactions will be discussed.

D-33-7 *Excitation of Vacuum Ultra-Violet Lasers by Relativistic Electrons.* STEPHEN C. WALLACE, IBM Thomas J. Watson Research Center, Yorktown Heights, New York, 10598, USA.

Several vacuum ultra-voilet lasers have been recently developed using high-current relativistic electron beams for excitation. Of principle importance are the noble gas molecular dissociation lasers (xenon at 173 nm and krypton at 145 nm), because of their superior coherence properties

intrinsic to the use of an optical cavity. The elucidation of the radiation chemistry of high pressure noble gases ($p > 10$ atm), in particular the kinetics of their excited state diatomic molecules which are the upper laser level, has been essential in the development and understanding of these lasers. Pertinent physicochemical aspects of vacuum ultra-violet lasers and laser excitation by relativistic electrons will be discussed.

D-34-1 *Effects of an Ultraviolet Treated Episome on Growth and Division in* Escherichia coli. R. M. Teather and W. D. Donachie, MRC Molecular Genetics Unit, University of Edinburgh, Edinburgh EH9 3JR, Great Britain.

The introduction of an ultraviolet damaged episome into a repair-deficient strain of *Escherichia coli* results in an immediate and permanent block to cell division. The effect depends on the nature of the episome introduced. The physiology of the cells has been examined and the results suggest that the effect is due to the failure of the episome to complete a round of replication and not to the presence of U.V. photoproducts within the cell.

D-34-2 *Differential Inhibition of RNA-Synthesis after UV-Irradiation in Yeast.* Herbert Koch and Jürgen Kiefer, Strahlenzentrum der J. Liebig-Universität, 63 Giessen, West Germany.

The synthesis of long-lived RNA immediately after irradiation with UV, X-rays and α-particles was measured in stationary diploid yeast *Saccharomyces cerevisiae*.

After UV-exposure of 880 ergs/mm^2 leading to no loss in colony forming ability (CFA), the synthesis of high molecular weight r-RNA is reduced to about 15% of the control level, at twice the dose (CFA 65%) to about 5%, the synthesis of <5S-RNA being reduced to only 60% and 25% respectively. Photoreactivation after UV-exposure increases the amount of RNA synthesized substantially. In contrast to these observations RNA-synthesis after X-irradiation doesn't seem to be similarily affected at doses leading to about 50% CFA. The synthesis of high molecular weight r-RNA is not significantly altered, that of <5S-RNA seems to be slightly decreased.

D-34-3 *Elongation of RNA Chain in Cells Infected with UV-irradiated T2 Phage.* Keiichi Nozu and Takeo Onishi, Osaka University, Osaka, Japan.

Premature termination of RNA chain elongation at UV-lesion of DNA is not likely happen for RNA synthesis in cells infected with UV-irradiated T2 phage, because (1) Size of RNA is not smaller, at least distinctively, than that of RNA synthesized in control cells, (2) No early stoppage of RNA synthesis is detected in UV-phage-infected cells in the presence of rifampicin, although premature chain termination is true in RNA synthesis *in vitro* templated by UV-irradiated T2 DNA.

The RNA synthesized *in vivo*, however, must have some base sequence uncomplementary to T2 DNA in its chain and the occupation must grow with the increase of UV-dose to T2 phage, because the hybrid-forming ability of the RNA decreased with increase of the dose. The size of complementary part of RNA chain got smaller with UV dose. The RNA chain terminated in the midst of transcription *in vitro* was made longer by incubating it with 4XDP and polynucleotide phosphorylase.

We assume that RNA chain elongation does not stop *in vivo* at UV-lesion, but go further beyond it by making polymer uncomplementary to DNA.

D-34-4 *Changes in the UV Sensitivity of* Bacillus subtilis *through its Life Cycle.* Nobuo Munakata, York University, Downsview, Ontario, Canada

A strain of *B. subtilis*, UVSSP-42-1 (*hcr ssp*), produces highly UV-sensitive spores and vegetative cells, while its germinated spores express more than twenty-five times greater UV-resistance than the spore. Kinetics of the shift of sensitivity are characterized as a rapid de-senitization upon germination and a gradual re-senitization during outgrowth. The UV-resistance at the germinated phase is impaired greatly by introducing *recA*, or to a lesser extent, *pol* mutation. Thus, in order to understand the changes in the sensitivity through its life cycle, two basic features should be taken into account: one, the photochemical reactivity of the DNA at each phase, the other, variations in the activities of at least three repair systems: "spore repair," "excision repair,"

and, "*subtilis-recA*-dependent repair." (Supported by research grant A5861 to I. E. Young from the National Research Council of Canada.)

D-34-5 *Generation Cycle Sensitivity to UV and Photoreactivating Light in Synchronized* Chlamydomonas reinhardi. LAWRENCE E. ROCHA AND D. STUART NACHTWEY, Oregon State University, General Science Department, Corvallis, Oregon 97331, USA.

The survival of *Chlamydomonas reinhardi* after 254 nm ultraviolet irradiation with and without photoreactivation (PR) at various stages in the synchronized vegetative cycle was studied. The results show that there is an increase in UV sensitivity as the cells progress through the vegetative cycle, with maximum sensitivity occurring just prior to the first cellular division. During the time when nuclear DNA is reported to be replicated, a biphasic survival pattern is observed, which is attributable to a mixture of cell-cycle-dependent UV-resistant and UV-sensitive subpopulations. PR substantially reduces the lethal effect of the UV irradiation with a dose reduction factor of 0.12 at the LD_{50} level. This survival enhancement by photoreactivation declines with time between UV-irradiation and PR treatment. The cell stage at which survival enhancement by photoreactivation is minimal is also the stage at which the cells are most UV sensitive.

D-34-6 *Tryptophan Photoproduct as a Genetic Probe: Effects on Bacteria.* A. EISENSTARK, F. LANDA, G. YOAKUM, AND L. GLATZER, Division of Biological Sciences, University of Missouri-Columbia, Columbia, Missouri 65201, USA.

Recombinationless (*rec*) mutants of bacteria are sensitive to visible and near-ultraviolet wavelengths of light. In addition, these mutants are sensitive to a tryptophan photoproduct that results from irradiation of this amino acid by 280–365 nm wavelengths. The physical and biological damages are different from those produced by 254 nm UV. Both the longer wavelengths and the tryptophan photoproduct are mutagenic and influence the genetic recombinational process in bacteria. The photoproduct inhibits closure of chromosomal replication gaps. It also uncouples cell division from DNA synthesis. It binds more strongly to single-stranded than to double stranded DNA. Since the natural environment includes an abundance of both free tryptophan and sunlight, the relevance of the effect of tryptophan photoproduct is provocative. (Supported by National Science Foundation (GB 33869), and by Public Health Service, Bureau of Radiological Health USPH 1-R01-RL-00748-02.)

D-34-7 *Mechanism of Near Ultraviolet Light Irradiation Inactivation of* Escherichia coli. JULIUS PETERS AND CRELLIN PAULING, University of California, Riverside, California 92502, USA.

Irradiation of growing bacteria with near ultraviolet light (365 nm) results in the loss of colony forming ability. The primary effect appears to be the loss of the capacity to replicate DNA, as deduced from the following observations: Ribonucleotide diphosphate reductase has an absorption maximum at 360 nm. The sensitivity of bacterial cells to near UV irradiation can be enhanced by the addition of hydroxyurea to the plating medium on which survival is determined; hydroxyurea is a specific inhibitor of RDP reductase. The lethality of near UV irradiation can be spared by the addition of deoxyadenosine to the plating medium. Replication of DNA phages is markedly inhibited by near UV irradiation of the host cells prior to phage infection; RNA phage replication is not affected by such irradiation. Derepression of RDP reductase by growth in medium supplemented with 2 ug/ml thymine lowers sensitivity of near UV irradiation relative to cells grown in medium supplemented with 20 ug/ml thymine. Accordingly, we interpret these results as indicating that a primary target for near UV light in the cell is the enzyme RDP reductase, and that the consequent deficiency of deoxynucleoside derivatives results in the inhibition of DNA synthesis and a consequent loss of viability. Supported by grant AI-07798.

D-34-8 *Biological Consequences of Pyrimidine Dimers and Single-Strand Breaks Induced in Bacterial DNA by 365 nm UV.* ROBERT B. WEBB AND MICKEY S. BROWN, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439, USA.

Near UV at 365 nm induces pyrimidine dimers (R. M. Tyrrell, 1973, *Photochem. Photobiol.* 17, 69–73) and single-strand (SS) breaks (R. M. Tyrrell, R. D. Ley, and R. B. Webb, *Photochem. Photobiol.*, submitted) in bacterial DNA at rates of 6×10^{-5} dimers and 3×10^{-5} SS breaks per

genome per J m^{-2}. Maximum photoreactivation (PR) ratios for 365 nm lethal damage of 1.3 to 1.5 in *E. coli* K12 AB strains 1886 (*uvrA*), 2463 (*recA*), and 1157 (wildtype) suggest that less than half of the lethal damage at 365 nm can be attributed to dimers in these strains. Initial measurements indicate that 365 nm UV induction of SS breaks in bacterial DNA is completely oxygen dependent, whereas, dimer formation is at least as great when oxygen is excluded. The strong oxygen dependence of 365 nm lethality in stationary phase wildtype and *uvrA* strains (oxygen effect ratio of 4 in *E. coli* B/rHcr) suggests that SS breaks may be responsible for a major part of the lethal damage in these strains at this wavelength. In contrast to the strong oxygen dependence for 365 nm lethality, mutation to tryptophan independence in stationary phase *E. coli* B/r Hcr was at least as great in the absence of oxygen as in its presence. In addition, mutational lesions induced in both the presence and absence of oxygen were effectively removed by PR treatment. This result implicates dimers as the primary mutational lesions induced at 365 nm under these conditions. (Work supported by the U.S. Atomic Energy Commission.)

D-34-9 *RNA Synthesis Rate in BrU-Labeled* Escherichia Coli *Irradiated in vivo with Long Wavelength UV. The Influence of Hydrogen Donors on Single-Strand Breakage Rate and Transcription.* Wolfgang Köhnlein, Institute for Radiation Biology, University of Münster, Münster, West Germany.

In BrU-substituted DNA the major photochemical alteration results in a single strand break. The reactions leading to the single strand breaks involve radicals and hydrogen abstraction. Thus hydrogen donors can prevent the formation of single strand breaks and restore the process of transcription.

The RNA synthesis rate after UV-irradiation in the presence and absence of hydrogen donors was measured and the molecular weight distribution of the RNA molecules was determined using radioactive labeling and sucrose gradient sedimentation. This was done for the total RNA and for stable ribosomal RNA. The number of strand breaks in the DNA produced by UV (313 nm) *in vivo* in the presence and absence of hydrogen donors was also determined. A correlation between strand break and transcription inhibiting event will be given. (This work was supported by the Deutsche Forschungsgemeinschaft.)

D-35-1 *Electron Spin Resonance Study of Transient Hydrogen Atoms in Ices under Electron Irradiation.* H. Shiraishi, H. Kadoi, Y. Katsumura, Y. Tabata, and K. Oshima, University of Tokyo, Hongo, Tokyo, Japan.

Behavior of hydrogen atom in ice has been investigated. Steady state concentration of hydrogen atoms were measured at different temperatures and the effect of dose rate on the concentration was examined.

On elevating the temperature, the narrowing of the line width begins at about −130°C, where the signal intensities are extremely low. Above −110°C, there appears anomaly which is characteristic of the CIDEP phenomenon; the low-field line in emission and the high-field line in absorption.

From these experimental results, the formation and the behavior of hydrogen atom in pure and acidic ices are discussed.

D-35-2 *Electron-Electron Double Resonance Study of Trapped Electrons in Irradiated 2-Methyltetrahydrofuran Glass.* D. F. Feng and L. Kevan, Department of Chemistry, Wayne State University, Detroit, Michigan 48202, USA.

Electron-Electron double resonance (ELDOR) of trapped electrons and radicals in -irradiated 2-methyltetrahydrofuran (MTHF) glass at 77K was investigated. Magnetic energy pumped into the radical spin system is transferred to the trapped electron spin system to partially saturate it. The cross relaxation mechanism is consistent with dipolar cross-relaxation in which a radical spin flips down and an electron spin flips up. Analysis of the rate of spin relaxation shows the reciprocal ELDOR reduction is linear with pumping microwave power with an intercept which gives the ratio of the cross relaxation time (T_{pd}) to the electron spin relaxation time (T_{1d}). The experimental data are found to fit this relationship. T_{pd} are generally in the 10^{-14} sec range. (T_{pd}/T_{1d}) as a function of temperature, concentration of the trapped electrons and frequency difference between the pumped hyperfine line of the radical and the electron line have been studied. The analysis of cross-relaxation under these various experimental conditions support the concept that the electron

and radical are spatially correlated and provide an estimate of the distance over which cross-relaxation can occur between the electron and the radical.

D-35-3 *Trapping Site Structure of Trapped Electron in γ-Irradiated Glassy Methyltetrahydrofuran Deduced from Electron Spin Resonance, Electron Nuclear Double Resonance and Electron Spin Echo studies at 77K.* MICHAEL K. BOWMAN AND LARRY KEVAN, Wayne State University, Detroit, Michigan 48202, USA, AND IAN BROWN, McDonnell Douglas Research Labs, St. Louis, Missouri 63166, USA.

Trapped electrons, e_t^-, have been produced in a series of selectively deuterated samples of glassy Methyltetrahydrofuran (MTHF) at 77K with γ-irradiation. A study of the Electron Spin Resonance (ESR) linewidths and Electron Nuclear Double Resonance (ENDOR) linewidths of e_t^- allows the relative orientation of MTHF molecules in the first solvation shell and the spatial extent of the e_t^- wavefunction to be determined. The Electron Spin Echo envelope modulation due to e_t^- has been simulated theoretically for these samples and allows limits to be set on the radius of the trapping site and the number of MTHF molecules in the first solvation shell.

D-35-4 *ESR Studies on an X-Irradiated Single Crystal Complex Containing 1-Methylcytosine and 5-Fluorouracil.* R. FARLEY AND W. BERNHARD, Dept. of Radiation Biology and Biophysics, The University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642, USA.

The structure of a free radical trapped in single crystals of a hydrogen-bonded complex of 1-methylcytosine and 5-fluorouracil irradiated between about 20°K and 300°K has been determined. The radical is formed by hydrogen atom extraction from N(1) of the 5-fluorouracil moiety, the unpaired electron interacting with the N(1) and F nuclei of this molecule. The principal values of the hyperfine and g-tensors are: $[A_{xx}^N = 14.4,\ A_{yy}^N = A_{zz}^N = 0.0]$; $[A_{xx}^F = 156.4,\ A_{yy}^F = 16.4,\ A_{zz}^F = 16.1]$; $[g_{xx} = 2.00124,\ g_{yy} = 2.00564,\ g_{zz} = 2.00620]$. The radical is present below 20°K and decays upon warming to room temperature. No new radicals are detected during this decay. There are at least two additional radicals formed in this crystal, but the extreme spectral overlap resulting from the fluorine hyperfine interaction in the known radical makes an analysis of these radicals very difficult. The assignment of the structure of the known radical and the spectral characteristics of the other will be discussed. (Supported by USAEC Contract AT(11-1)3490.)

D-35-5 *Effect of some Quaternary Nitrogen Compounds on Free Radical Production in Mucopolysaccharide Materials.* RAYMUND A. PECKAUSKAS, IRA PULLMAN, AND MARVIN Z. ROTMAN, New York Medical College, 1249 Fifth Avenue, New York, New York 10029, USA.

The effect of some quaternary nitrogen compounds on the yield of free radicals in x-ray irradiated mucopolysaccharides and in mucin was studied by means of electron spin resonance (ESR). ESR intensity measurements were made at near liquid nitrogen temperatures and at room temperature to determine free radical content after irradiation. Radical yields showed marked changes when the target substance was recovered by lyophilization from aqueous solution with certain of the compounds tested e.g. cetylpyridinium chloride and pralidoxime methiodide. The mechanism of these effects was investigated by noting spectral changes after annealing samples irradiated at low temperatures. Evidence of spin transfer was found. (This work was partly supported by Grant No. In-96A from the American Cancer Society.)

D-35-6 *Free Radicals in 5-Substituted Pyrimidines: An ESR Study.* BRENT BENSON AND PATRICK LORENZ, Lehigh University, Bethlehem, Pa. 18015, AND PETER GUTIERREZ, Tulane University Medical School, New Orleans, La. 70112, USA.

We have shown that free radicals are formed by irradiation of 5,5-disubstituted pyrimidines (barbituric acid derivatives) by the selective abstraction of one of the 5-substituents leaving the unpaired electron on C(5). The preferential ordering for ease of abstraction at room temperature is: $H \gg CH_2CH_3 \gg CH_3$, $OH > CH_2CH_3$, $OH > CH_3$, $C_6H_5 \gg CH_2CH_3$. Additional evidence from low temperature studies indicates that all of the ethyl-abstraction from 5-ethyl-5-methyl-barbituric acid occurs by a mechanism which includes a hydrogen abstraction from the methylene carbon on the ethyl group. Dose response studies comparing dimethyl- and tetramethyl-barbituric acid indicate that methyl abstraction occurs via a hydrogen addition mechanism. We have de-

termined the hyperfine coupling and g-value tensors for all of these radicals from single crystal ESR studies.

5-nitropyrimidines generally form iminoxy radicals by abstraction of an oxygen from the nitro group. The exception to this is 5-nitro-6-methyluracil where the room temperature radical is formed by hydrogen addition to O(4). We have shown in a N^{15} labeling experiment that the low temperature precursor to this radical has its unpaired electron in the nitro-group, and is probably due to a hydrogen addition to a nitro-group oxygen.

D-35-7 *Atomic Hydrogen EPR Spectra in Silica-gel Irradiated in a Nuclear Reactor.* T. V. Ceckhladze, L. Sh. Nadirashvili, B. G. Berulava, and Ts. T. Tarkashvili, Institute of Physics, Academy of Sciences of the Georgian SSR, Tbilissi, USSR.

At irradiation in many solids free hydrogen atoms are formed captured by centres of different nature. Low temperature investigation permits to establish the character of interaction of the formed atomic hydrogen with the environment and its reactivity.

Silicagel specimens were irradiated in the reactor at 90°K. The integral dose of fast neutrons was 10^{15} n/cm^2. At irradiation in the vacuum a hydrogen doublet was observed with hyperfine splitting close to that of free hydrogen. With an increase of superhigh frequency the signal saturation is observed. Irradiation carried out together with H_2, O_2 or C_2H_6 did not change hydrogen concentration, but in specimens with H_2 or C_2H_6 a new component appeared in the doublet.

Introduction of oxygen into the specimen being in the vacuum resulted in a decrease of the time of spin-lattice relaxation.

When the specimen treated in the vacuum was kept in liquid nitrogen, four months later a new component appeared in the spectrum.

Thus hydrogen diffusion in silicagel takes place at 77°K as well and it shows the presence of two different centres of hydrogen capture: one of them is in the surface layer and the other in the depth of the globule and can diffuse to the surface. The observed complex spectrum was decomposed into the components showing the existence of at least two centres of hydrogen capture.

D-35-8 *ESR Method Studies of Primary Processes Occurring in Histones and Nucleohistones under UV Light.* O. A. Azizova, T. Kh. Nikitina, Yu. V. Nikolaev, B. A. Korol', and V. A. Kopylov. Institute of Biological Physics, USSR Ac. Sci., Pushchino, USSR.

Primary free radicals and their reactions occurring in histones and nucleohistones under UV light are studied by the Method of Electron Spin Resonance (ESR). Free radicals (FR) of amino acid residues of tyrosine, lysine and HCO radicals are shown to be formed in UV-irradiated water solutions of total histones of calf-thymus and their fractions at 77°K. Free radicals of tyrosine are formed due to photoionization of tyrosine residues under UV light. Lysine radicals arise as the results of desamination at the expense of adding an electron to NH_3^+ group with the following break of CN-bond. Desamination is observed at pH in the region from 1.5 to 8.5. Radicals of tyrosine amino acid residues and peptide ones arising when adding an electron to the peptide group, are observed in dry histones at 77°K. It is found that the absorption of UV light with $\lambda = 280$ nm by histones in the solution of nucleohistones induces both the formation of histone radicals and thymine ones of DNA.

D-36-1 *Electron Spin Resonance Studies of Radical Trapping in the Radiolysis of Organic Liquids.* Jorge A. Wargon and Francon Williams, Department of Chemistry, The University of Tennessee, Knoxville, Tennessee 37916, USA.

The technique of "spin trapping" has been used to identify the free radicals produced in the radiolysis of aliphatic alcohols, amides, organophosphorus compounds, and organosulfur compounds. Both 2-methyl-2-nitrosopropane and phenyl N-*tert*-butyl nitrone have been employed as radical traps in these systems at low temperatures to produce stable nitroxide radicals. Studies on alcohols have provided proof of alkoxy radical formation; hydroxyalkyl radicals are also trapped but the dependence of the results on the concentration of spin trap suggests that these radicals arise mainly from secondary H-atom abstraction by the alkoxy radicals which are probably produced in a primary ion-molecule reaction. Electron scavenging by methyl bromide in the radiolysis of methanol-d_4 has been demonstrated by the trapping of the $CH_3\cdot$ radical. Alkyl radicals

are trapped in the radiolysis of trialkyl phosphates whereas both alkoxy and phosphorus-centered radicals are produced from dialkyl and trialkyl phosphites. Thiyl (RS·) radicals are the most abundant species in the radiolysis of thiol (RSH) compounds. Nitrogen-centered radicals such as $R_2N\cdot$ are produced from amides. The information from these studies will be used to discuss the mechanisms of radical formation.

D-36-2 *Technique of Quantitative EPR Studies in the X-Ray Field.* S. Łukiewicz and K. Reszka, Jagiellonian University, Kraków, Poland.

Only a few attempts at immediate EPR detection of X-ray induced free radicals during radiation exposure have so far been reported, though kinetic EPR studies on steady-state concentrations of such radicals in solid or liquid samples are highly informative.

Hence, a special method of measuring the EPR absorption upon simultaneous X-irradiation of samples were developed. It has at least two new features:

1—it offers a chance of experimentation with living cells or tissues without disturbing their normal structure and metabolic functions,

2—it involves a system of chemical dosimeters and double internal standards suitable for comparing relative signal intensities of sample, dosimeter, and reference substance which all are simultaneously present in a resonant cavity.

Thus, one can read out from an EPR spectrum not only the changes in the free radical content of an examined material but also the actual level of the absorbed dose at any moment of exposure.

These advantages seem to qualify the new technique for useful application in both radiation chemistry and radiobiology.

D-36-3 *Electron Nuclear Double Resonance (ENDOR) Studies of Radiation Damage Processes.* Harold C. Box, Biophysics Dept., Roswell Park Memorial Institute, Buffalo, New York 14203, USA.

Primary oxidation and reduction products produced by ionizing radiation in organic compounds can be stabilized at liquid helium temperature (4.2°K). These primary products are paramagnetic and can be detected, but often not identified, by ESR spectroscopy. Identification from ESR spectra alone is generally difficult because paramagnetic absorptions arising from different products usually superimpose. Application of the ENDOR technique has made it possible to sort out these superimposed absorptions observed in a variety of organic single crystals x-irradiated at 4.2°K. The primary oxidation and reduction products have been identified in several amino acids (cysteic acid, histidine hydrochloride, serine, β alanine), peptides (acetylglycine, glycylglycine hydrochloride) and carboxylic acid salts (potassium hydrogen malonate, zinc acetate). ENDOR provides a means for measuring accurately the hyperfine coupling tensors of each paramagnetic species. For example, from 6 proton couplings obtained from ENDOR measurements on histidine hydrochloride crystals irradiated at 4.2°K, it could be deduced that: (1) One primary oxidation product results from decarboxylation. (2) Another product results from oxidation of the imidazole ring and (3) the reduction product is formed by adding an electron to the carbonyl oxygen.

D-36-4 *EPR and ENDOR Studies on 5-Thymyl Radicals at 77°K and 4.2°K.* Teymoor Gedayloo* and John D. Zimbrick, Department of Radiation Biophysics, University of Kansas, Lawrence, Kansas 66045, USA.

Radicals formed by hydrogen atom attack on thymine (5-thymyl radicals) in gamma-irradiated acidic glass at low temperature were investigated by EPR and ENDOR spectroscopy. One broad ENDOR line is observed at magnetic field positions corresponding to each of the eight EPR lines at 77°K. At 4.2°K the EPR spectrum of the 5-thymyl radical consists of 16 lines whose external magnetic field positions are such that the average field per set of two adjacent lines corresponds to the field at the center of the analogous single line in the eight-line spectrum at 77°K. Each ENDOR line observed at 77°K and 4.2°K has a narrow region of decreased intensity near its center at a frequency of 42.6 MHz/10 kG. This region is hypothesized to arise from NMR of uncoupled protons located near the 5–6 bond area of the 5-thymyl radicals. (Supported in part by NIH grant no. GM 18927.)

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D-36-5 *Effect of Ionizing Radiation on the Electronic Configuration of Heme proteins.* D. P. DUBEY, Dept. of Biophysics, Panjab University, Chandigarh, India.

Electron Spin Resonance spectroscopy of irradiated heme proteins suggest that the crucial site for dissipation of excitation energy is the prosthetic group. The initial high energy electronic excitations are quickly delocalized by resonance transfer along the polymer chain of the molecule to the heme group. The ground state electronic configuration of heme proteins have been extensively studied using Mossbaur Spectroscopy. We report the result of our investigation on the effect of radiation on the electronic configuration of iron in heme protein using Mossbaur Spectroscopy.

The isomer shift and quadrupole splittings of Cytochrome C were measured after radiation exposure on different times. The isomer shift showed no statistically significant change from normal value (0.40 ± .02 mm/sec.). The quadrupole splitting changed to 1.78 ± .02 mm/sec. immediately after irradiation, however, after 48 hours the quadrupole splitting reduced to normal value (1.54 ± .02 mm/sec.). A large quadrupole splitting immediately after irradiation suggests an increase in the anisotropic covalency of d and p orbitals due to the radiation effect.

D-36-6 *Dosimetry of the Absorbed Dose of Ionizing Radiation Based on the Electron Spin Resonance Spectroscopy of Stable Paramagnetic Centers Induced in Skeletal Tissues.* A. DZIEDZIC-GOCŁAWSKA, AND K. OSTROWSKI, Inst. of Biostructure, Medical School Warsaw; W. Stachowicz and J. Michalik, Inst. of Nuclear Research, Warsaw, Poland.

On the ground of the literature data (Brady *et al.* Health Physics **15**, 43 1968) and our experience (Stachowicz *et al.* Nukleonick **17**, 425 (1972)) a dosimeter of adsorbed dose of ionizing radiation has been elaborated, based on the ESR measurements of the spin concentration of radiation-induced defects in the crystalline lattice of hydroxyapatite. The linear relationship between applied dose and spin concentration of stable paramagnetic centres was found in the range to 1.5 Mrads. The linear relationship was also proved in the low dose range from 50 to 20,000 rads with the use of Co-60 and X-ray (250 kV, 15 mA) irradiations. Bone tissues and tooth enamel were applied as the ESR active detectors of irradiation *in vitro* and *in vivo*. The linear relationship was also found with X-ray radiation although the slope of the curve is different than with gammas. The curves are proposed to be used as nomograms for biological dosimetry with bone powder as dosimeter. The lowest detectable dose of ionizing radiation is: for bone—about 200 rads and for tooth enamel lower than 50 rads.

D-36-7 *Effect of Cysteamine and Cysteine on Gamma-Induced Free Radical States of Plant Meristems.* KOBA A. GIGINEISHVILY. Institute of Plant Physiology, Ukrainian Academy of Sciences, Kiev 252627, USSR.

The gamma-induced free radicals are studied in the horse bean and pea root meristems by ESR method. The tissue samples treated in cysteamine and cysteine solutions of various concentrations were lyophilized and irradiated by gamma-radiation at 77°K. ESR spectra were registered at 77°K and after subsequent heating to room temperature.

A tendency of decreasing radiation yields of primary and stationary free radicals was observed. This tendency of decaying radicals became more obvious when protector concentration in tissue increased. The tendency expressed more clearly when the meristems of horse beans treated by cysteine were irradiated by doses of 100 and 200 krads.

It can be concluded from microwave power saturation curves of ESR spectra that the types of primary and stationary radicals in these objects were different within a wide range of irradiation doses and under different radioprotector concentrations.

It should be noted that the occurence of radioprotectors in tissues in high concentrations influenced to a greater extent the radiation yields of primary radicals than the decaying radicals.

D-36-8 *Adsorbent Surface as a Chemical Reagent in Radiation-Induced Transformations of Adsorbed Substances.* VLADIMIR V. STRELKO, KIRA A. SUPRUNENKO, DMITRY I. SHWETZ, NICKOLAY T. KARTEL, AND VALENTINA N. DOROSHENKO, L. V. Pisarzhevsky Institute of Physical Chemistry, Ukr. SSR Academy of Sciences, Prospect Nauki 97, Kiev-28, USSR.

From the results of investigations of the mechanism of radiation-induced transformation in surface layer of a number of adsorbents a conclusion has been drawn that surfaces of silica, alumina

and silica-alumina gels take part as a chemical reagent in processes of radiolysis of adsorbed substances. It has been shown by ESR studies, deuterium labelling and mass-spectrometry that primary chemical effect of gamma-irradiation is homolytic breakage of O—H (O—D)—bonds of surface ≡SiOH (≡SiOD) groups. Generated "hot" hydrogen (deuterium) atoms radicalize adsorbed hydrocarbons, inorganic gases (NH_3), reduce some oxides (CO_2) and so on.

Macroradicals ≡SiO· produce in their turn radicals from hydrocarbons by addition to aromatic rings, double bonds or abstraction of hydrogen atoms reverting to ≡SiOH groups. Existence of "grafted" compounds ("grafted" radicals) was proved by h.f.s. analysis of ESR spectra and quantum chemical calculations with EH and CNDO/2 theories.

It has been determined that the surface of the dispersed oxides appear to act as a chemical reagent in radiolysis of some polymers containing dispersed inorganic substrates and affected significantly the progress of a number of radiation-chemical synthesis reactions.

E-1-1 *Chemical Carcinogenesis in Man and Experimental Animals.* JAMES A. MILLER, McArdle Lab. for Cancer Res., Univ. of Wisconsin Medical Center, Madison, Wis. 53706, USA.

Epidemiological studies show that large differences exist in the incidence of many important cancers in man between various countries and that migrants tend to assume the cancer incidences characteristic of their new habitats. From these data it has been concluded that a high percentage, perhaps as much as 80%, of human cancer has strong environmental factors in its etiology. Chemical carcinogens, both man-made and of natural occurrence, are under strong suspicion in the etiology of much cancer in man. Solar UV light is the major cause of cancer of the skin of exposed parts. Ionizing radiations are involved in the induction of some cancer in man, but their role has not been quantitated.

Experimental chemical carcinogens comprise a large and structurally diverse group of synthetic and naturally occurring organic and inorganic compounds with various species and tissue specificities. Most of the organic chemical carcinogens require metabolic activation *in vivo*, and the ultimate reactive and carcinogenic forms of most if not all organic chemical carcinogens appear to be strong electron-deficient or electrophilic reactants. These electrophiles combine with numerous electron-rich or nucleophilic centers in cellular informational macromolecules such as nucleic acids and proteins. Carcinogenic ionizing radiations also produce electrophilic species in the radiolysis of water and other cellular molecules. Like ionizing radiation, the carcinogenic electrophiles are mutagenic. However, it is not certain that a causal connection exists between carcinogenesis and mutagenesis by chemicals. Direct or indirect (e.g., via viruses) genetic or epigenetic mechanisms of action appear possible with carcinogenic chemicals and radiations.

E-1-2 *Genetic Aspects of Hazards of Radiation Relative to Other Environmental Agents.* PER OFTEDAL, Institute of General Genetics, University of Oslo, Norway.

The genetic effects of ionizing radiations have been known for almost 50 years; those of some chemicals for about half as long. The complexities of the genetic effects of radiation on man are so great that it is still impossible to base regulations on a strict quantification of undesired effects. The quantification of chemical genetic effects is even more difficult at present. The gap between an increasingly refined understanding of the cellular and molecular events and the highly needed simple, practical and yet acceptable and credible principles on which to base legal and regulatory devices does not appear to be closing. Among the important papers which have appeared during the last couple of years are those of Abrahamson *et al.* (Nature **245**, 1973) on the relationship between radiation mutagenesis and genome DNA content, and that of Lyon *et al.* (Nature **238**, 1972) on the relationship between radiation mutagenesis and dose rate. The first paper underlines the simplicity and uniformity of reaction pattern, the latter the complexity. This contrast will be discussed in the light of other experimental data on radiation and chemical mutagenesis, especially with regard to mutational and cell killing events, and their interaction in the low dose region.

E-2-1 *Energy Loss and Ranges of Charged Particles in Matter.* PETER SIGMUND, Denmark.

E-2-2 *Channeling: A Tool for the Study of the Interactions of Energetic Particles Penetrating Solids.* S. DATZ, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

When energetic particles enter a crystal lattice at relatively low angles with respect to low index crystal axes or planes they undergo a series of correlated small angle collisions with lattice atoms which steer them away from violent atomic collisions. This effect, called "channeling" and the complementary effect called "blocking" have been investigated and applied extensively to problems of crystalline structure in the past ten years. Unlike particles moving in random directions, channeled particles in many cases undergo orderly oscillatory motion. From measurements of the detailed trajectories of the ions interatomic potentials and impact parameter dependent stopping powers in solids are determined. Finally, many channeled ions are constrained to interact only with valence or conduction electrons. A consequence of this is that charge capture and loss are strongly surpressed and additional information is obtained concerning ionic interactions with dense electron gases and the nature of the states of ions penetrating solids. (Research sponsored by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.)

E-2-3 *Transmission of Fast Molecules Through Solids.* J. Remillieux, Institute de Physique Nucleaire, Universite Claude Bernard, Lyon, France.

It has been shown that fast molecular H_2^+ ions, incident upon a thin foil, can emerge in a bound molecular state. For MeV H_2^+ ions the "molecular transmission" probability is typically 10^{-6}–10^{-8} through foils of a few hundred Å thickness.

The transmission of molecular ions through axial and planar channels of thin epitaxially grown gold crystals has been measured. In a channeling configuration the transmission probability is a few orders of magnitude higher than in a random orientation. The planar channeling effect on the molecular transmission has been measured as a function of the ion velocity, the crystal thickness and the planar spacing. The striking effect is that the transmission probability is found to be maximum along planar directions which are not the most open ones.

The experimental results suggest a model for the molecular transmission. The model includes three stages: i) the breakup of the molecular ion at the entrance surface, ii) the movement of the two protons inside the crystal, following more or less independent trajectories determined mainly by the planar potential and the screened Coulomb repulsion between the products of the breakup, iii) the molecular recombination taking place at the back surface of the crystal and involving an electron capture process. This mechanism is also consistent with the measured production of H_2^+ ions emerging from a crystal bombarded by an incident H_3^+ beam. (Address 1973–74: Argonne National Laboratory, Argonne, Illinois 60439, USA.)

E-2-4 *Primary Processes and Track Effects in Irradiated Media.* R. H. Ritchie, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

A review will be given of the early physical stages of energy deposition by charged particles in condensed media. The infratrack, a concept of primary importance in connection with energy deposition by projectiles heavier than electrons, will be defined and the impact parameter dependence of energy deposition will be discussed. Emphasis will be given to non-scaling aspects of radiation interactions in condensed media. This term refers to those aspects of phenomena which cannot be described adequately by merely scaling, according to density, data relevant to the same phenomena in gases. Under this heading we include: collective electron states (plasmons), non-linear interactions among the products of ionization and excitation in a charged particle track, spatial polarization effects and shakeoff effects. (Research sponsored by the U.S. Atomic Energy Commission under contract with Union Carbide Corporation.)

E-3-1 *Chemical Aspects of the Photosensitization Without Oxygen.* G. Rodighiero, Institute of Pharmaceutical Chemistry of the Padua University, 35100 Padova, Italy.

There are two groups of substances which are able to produce photosensitized effects on biological substrates without requiring the presence of oxygen, having own characteristic mechanisms of action:

a) A group of ketones (for instance acetone, acetophenone, benzophenone) absorbed radiation in the long wavelength ultraviolet region and transfer the energy to biological substrates, in particular producing the dimerization of pyrimidine bases in native DNA.

b) A group of furocoumarins (psoralens) under irradiation with long wavelength ultraviolet light give C_4-cyclo-addition reactions with the 5,6-double bond of pyrimidine bases present in the nucleic acids (thymine, cytosine, uracil). As furocoumarins have two reactive sites, they can form two types of covalent monoadducts and also a double adduct. In last case, when occurring in native DNA, an inter-strand cross-linking takes place.

These chemical modifications, occurring both *in vitro* and *in vivo*, which are the bases of the biological effects produced by the two groups of substances under irradiation with long wavelength ultraviolet radiation, are discussed especially in relation to their mechanisms, the excited states which are involved in some interesting physical-chemical aspects.

E-3-2 *Psoralen Cross-Links in DNA: Biological Consequences and Cellular Repair.* RONALD S. COLE AND RICHARD R. SINDEN, Departments of Biochemistry and Microbiology, University of Georgia, Athens, Georgia 30602, USA.

Psoralens are a class of drugs that covalently bind to DNA in the presence of light, forming interstrand cross-links and adducts to single strands. The cross-linking product appears to be primarily responsible for the biological consequences of psoralen-plus-light treated bacteria and phage. Normal cells survive treatments producing many cross-links in their DNA, and recovery is dependent upon the excision-repair and genetic recombination functions and the presence of an intact homologous duplex.

Genetic and biochemical studies on the repair of cross-linked DNA suggest the following mechanism involving sequential excision and recombination. Excision-repair enzymes first make two cuts in one DNA strand at each cross-link. The second arm of the cross-linking residue still attached to the other strand blocks repair polymerization in the excision gap, thus stabilizing the gap. However, strand exchanges with a homologous duplex insert an intact strand complimentary to the single stranded region still containing a partially excised cross-linking residue. When the bihelical structure is restored to the damaged region, excision-repair enzymes can remove the second arm of the cross-link and complete the repair sequence.

Experimental evidence concerning biological consequences of DNA cross-links, cross-link removal, DNA strand cutting, strand exchanges, repair polymerization, and the genetic control of these processes will be described.

E-3-3 *Photosensitized Reactions with Molecular Oxygen that May be Involved in Photodynamic Actions.* KLAUS GOLLNICK, Institut für Organische Chemie der Universität München, Karlstrasse 23, D-8 München 2, West Germany.

In the presence of molecular oxygen, light absorption by photosensitizers in living organisms may cause damage of the cell by oxidizing cell constituents. The two well-investigated mechanisms of Type I (radical) and Type II (singlet oxygen) photosensitized oxygenation reactions will be discussed: in Type I reactions, the electronically excited sensitizer will abstract hydrogen atoms from the substrates RH to give substrate radicals R·that are consequently oxidized by triplet molecular oxygen according to the long-known thermal autoxidation mechanism. In Type II reactions, the electronically excited sensitizers (mainly in their triplet states) react with triplet molecular oxygen to give singlet ground-state sensitizers and a singlet molecular oxygen that reacts with various classes of organic compounds such as 1) cyclic 1,3-dienes (and aromatics and heterocyclics) to give transannlar peroxides, 2) olefins to give allylic hydroperoxides, 1,2-dioxetanes or their decomposition products by fragmentation into carbonyl compounds, 3) sulfides to give sulfoxides, 4) phenols to give 4-hydroperoxy-2,5-cyclohexadienones among other products, and 5) amines to give dealkylation products or amides. The role of the two types of photosensitized oxygenation reactions in Photodynamic Actions on amino acids, peptides, proteins, nucleic acids, and lipids and their involvement in Porphyria and other "photodynamic diseases" will be discussed.

E-3-4 *The Effects of Photodynamic Action Involving Oxygen upon Biological Systems.* AUBREY KNOWLES, MRC Vision Unit, University of Sussex, Falmer, Brighton, BN1 9QG, Sussex, Great Britain.

The rapid growth of photochemistry and the sophistication of experimental techniques has revolutionised the study of photodynamic systems in the past few years. Until recently, the

inactivation of biological materials by dyes in the light was regarded merely as a curiosity but increasing knowledge of the mechanisms and possible regulation of these reactions means that they are becoming a valuable tool in the modification of biological materials. In general, the mechanisms involved in the photodynamic inactivation of intact cellular material are still poorly understood, but great advances in the selective modification of proteins and amino acids by mild and selective photodynamic reactions means that the possibility of 'genetic engineering' is approaching reality.

In the past, it was assumed that all cases of photodynamic action involved oxygen. Fortunately, the majority of investigators now look for proof that oxygen *is* consumed and that the sensitiser *is not* consumed when the system is illuminated. Having established that a particular reaction is sensitised oxidation, it is very much more difficult to identify the reactive intermediate and hence the reaction mechanism; in fact, this has been done in very few cases. Beyond the conventional photodynamic reaction, there is now the possibility that oxygen is involved in non-oxidative sensitised reactions, such as the isomerisation of carotenes.

Another aspect of the action of light on living material now under debate is the *lack* of photodynamic action in organisms that apparently have built-in sensitisers. For example, chlorophyll, carotenes and haemoglobin have all been shown to act as sensitisers in *in vitro* experiments, and yet there are very few instances of cells containing these substances being damages as a result of the absorption of light. There must be protective systems present that inhibit degradative reactions by the quenching of excited states or oxidising-species generated by light.

E-4-1 *Biological Basis of Heavy Particle and Fast Neutron Radiotherapy.* G. W. BARENDSEN, Radiobiological Institute TNO, 151 Lange Kleiweg, Rijswijk (ZH), The Netherlands.

The application of heavy particles and fast neutrons in radiotherapy will provide an advantage if due to specific depth-dose and collimation characteristics or due to differences in dose-response relationships, in comparison with conventional radiations more damage can be induced in the tumor cells without exceeding tolerance doses for normal tissues. With respect to differences in dose-response relationships, the relevant criterium is that in the treatment considered, the RBE for damage to the tumour is larger than the RBE for tolerance of the dose-limiting normal tissue. This depends on the type of tumor irradiated and the fractionation regimen. Several factors have been shown to play an important role with respect to differences among responses of tumours and normal tissue reactions: intrinsic radiosensitivity of cells and their capacity for repair of sublethal damage, the presence of hypoxic cells and kinetics of reoxygenation in fractionated treatments, variation of radio-sensitivity with age in the cell cycle, proliferation of cells in intervals and the presence of nonproliferating cells. Differences in intrinsic radiosensitivity and the presence of anoxic cells are considered to be the main factors which produce higher RBE values for tumour responses as compared with normal tissue tolerance.

E-4-2 *The Use of Proton Beams in Radiation Therapy of Malignant Tumours.* A. I. RUDERMAN, Institute of Experimental & Clinical Oncology, Academy of Medical Sciences of U.S.S.R., Moscow, USSR.

It has been possible to obtain medico-biological beams on synchrocyclotron (680 MeV) of the United Institute of Nuclear Research (in Dubna) and on synchrotron (7 GeV) of the Institute of Theoretical and Experimental Physics (Moscow). Proton irradiation was used to treat 230 patients with malignant tumours: melanoma and skin cancer, metastases in lymph-nodes, cancer of the vulva and cervix, of oesophagus, larynx, osteogenic sarcoma and other tumours as well as to suppress hypophyseal function. Various physico-technical parameters of the accelerators enabled us to try different irradiation variants: fractional, external and intracavitary, multiple field and mobile, with large and very small fields, with Bragg peak or transit irradiation and also for pre-operative single irradiation with very high doses.

It was found that destructive effect in the target tissues can be obtained with practically no, or with complete absence of lesions in the surrounding tissues and general reactions of the body to radiation.

Clinical effects obtained must be related to especially favourable distribution of absorbed radiation doses as the relative efficacy of protons as found in the medicinal beams was within the limits of 1.

Accumulated experience enables us to give more precision to proton beam parameters, to criteria for selection of patients and to other conditions of effective corpuscular therapy and also to define its place along with other radiations in the radiation therapy of malignant tumours.

E-4-3 *The Middle Atlantic Neutron Therapy Association Clinical Trial.* CHARLES ROGERS, USA. (*See page 316.*)

E-4-4 *A Preliminary Report of the M. D. Anderson Hospital-TAMVEC* Neutron Therapy Clinical Trial.* DAVID H. HUSSEY, GILBERT H. FLETCHER, AND JESUS B. CADERAO, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas, USA.

Between October 1972 and February 1974, 122 patients were treated with neutrons produced by bombarding beryllium with 16 and/or 50 MeV deuterons. The patient population included 46 patients with head and neck cancer, 14 with breast carcinoma, 14 with gynecological carcinoma, 11 with superior sulcus bronchogenic carcinoma, 7 with squamous carcinoma of the esophagus, and 28 with a variety of miscellaneous neoplasms. The patients were treated twice weekly with doses calculated to include both the neutrons and gamma components.

Dosage schedules have progressed through three phases: 1) initial schedules based on RBE determinations of the pretherapeutic radiobiology studies; 2) a subsequent dosage increase based on a clinical evaluation of acute oral mucosal reactions; and 3) a subsequent dosage reduction based on a clinical evaluation of late radiation sequelae. The preliminary results are discussed in terms of local control, survival, and complication rates.

* Texas A&M Variable Energy Cyclotron.

E-4-5 *Clinical Experience of Therapy with Cyclotron Neutrons.* R. G. PARKER, University of Washington, Seattle, WA, USA.

E-4-6 *Animal Experiments on RBE and a Radiobiological Interpretation of the Clinical Results Presented.* STANELY B. FIELD, Hammersmith Hospital, Ducane Road, London W12 OHS, Great Britain.

The RBE for high LET radiation is dependent on the size of dose (or dose per fraction), being large for small doses and decreasing as the dose is increased. This is consistent with the shapes of cell survival curves, which tend to be exponential for high LET radiations, but have a "shoulder" followed by an exponential region after X-rays. However the RBE is also dependent on the tissue being irradiated. At Hammersmith, using neutrons produced by 16 MeV deuterons on beryllium, a family of RBE curves has been obtained for damage to a variety of tissues in animals including gut, oesophagus, skin, cartilage, lung and haemopoetic tissue. At any dose level there is a considerable variation from tissue to tissue: e.g., the RBE for damage to oesophagus is about twice that for haemopoetic tissues.

Skin has been investigated in mouse, rat, pig and man, and the data for all four species fall on the same smooth curve. We do not know whether extrapolation from animal data to man is reasonable for other tissues, but it may be possible to provide a link between the experimental and human data by using the information now accumulating from the various radiotherapy centres now using fast neutrons.

Tumours, as well as normal tissues, show a wide, and not yet predictable variation in RBE. The data currently available will be presented.

E-5-1 *The Effects of Radiotherapy on the Elastic Properties of Human Skin.* HARCHARAN S. RANU, TERENCE E. BURLIN, AND WILLIAM C. HUTTON, Polytechnic of Central London, 115 New Cavendish Street, London, W1M 8JS, Great Britain.

The *in vivo* elastic properties of irradiated and non-irradiated human skin are being studied. An apparatus has been constructed. This consists of two arms, one movable, and the other fixed. A displacement transducer between the two arms measures the extension of the skin as the arms are moved apart by a motor driven leadscrew. The force exerted on the skin is measured by strain

gauges attached to the base of the two arms. The ends of the arms are attached to the patient's skin by means of double-sided adhesive tape. The tests were conducted on female patients being treated for carcinoma of the breast. Two areas on each patient were tested, one being irradiated and the other normal skin on the opposite nonirradiated breast.

Results to date indicate that skin stiffens with increasing radiation dose. In addition, two treatment patterns are being studied, i.e., three times per week and five times per week. Preliminary results indicate that radiation given three times per week produces a lower increase in skin stiffness, as compared to five treatments.

E-5-2 *A Quantitative Analysis of in vivo Irradiated Human Connective Tissue.* GEORGE WIERNIK AND DAVID PERRINS, University of Oxford, The Research Institute, Churchill Hospital, Oxford, Great Britain.

Whilst it has been suggested that normal tissue tolerance to irradiation is directly related to the connective tissues rather than to the overlying epithelial cell systems, it has so far proved difficult to quantitate radiation damage in the connective tissues. An organised cell system of fibroblasts has been identified lying immediately under the basement membrane of the intestinal crypts, termed the peri-cryptal fibroblasts, and a method for assessing these cells has been devised by one of the authors (D.P.). This cell system has been analyzed in serial human intestinal biopsies obtained during and after radical courses of therapeutic irradiation. In the same biopsy specimens the cell kinetics of the overlying mucosa have also been established so that the two cell systems can be compared and contrasted. Specimens are available during the phases of acute and late radiation damage from patients irradiated in air and in three atmospheres of oxygen. This work was made possible by a long-term grant to one of the authors (G.W.) from the Medical Research Council.

E-5-3 *Ureteral Stricture in Swine After Intracervical Exposure to Californium-252.* JAMES L. BEAMER, THOMAS D. MAHONY,* AND MAURICE F. SULLIVAN, Biology Department, Battelle, Pacific Northwest Laboratories, Richland, Washington 99352, USA.

The organs found most susceptible to acute injury in our studies are the same tissues that are at risk in treatment for cancer of the human cervix uteri. Of particular concern to the therapist is the late response of these tissues and knowledge of the times and dose levels at which complications are most likely to occur.

Follow-up studies on 21 swine exposed intracervically to ^{252}Cf afterloading tandems have diagnosed 12 animals with ureteral strictures as determined by intravenous pyelography or as confirmed at postmortem examination. These animals received gamma-neutron doses of 2000-2800 rads as measured at 2 cm distance.

Although ureteral stricture is not a major complication in therapy with ionizing radiation, this organ system could become critically important if ^{252}Cf is used for intracavitary tumor treatment. (This paper is based on research performed for the National Cancer Institute under Grant No. 13127.)

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* Consultant for Battelle, Pacific Northwest Laboratories.

E-5-4 *Enhancement of Radiation Tolerance of Liver.* HAROLD M. SWARTZ, JEANNIE J. KINZIE, J. DAVID LEWIS, GEORGE HENSLEY, AND BARBARA REICHLING, Medical College of Wisconsin, Milwaukee, Wisconsin 53233, USA.

Radiation therapy of hepatic metastases is usually limited by the relatively low radiation tolerance of the liver. We are attempting to increase this tolerance by two approaches. In the first, we attempt to interfere directly with the accepted pathophysiological mechanism of radiation hepatitis: thrombosis of hepatic central veins. This is done by the administration of an anticoagulant, heparin. We have found that rats treated with depo-heparin had less hepatic radiation damage than unheparinized rats as measured by histological examination, serum alkaline phosphatase levels, colloid clearance and albumin space determinations. The second approach we are using is to attempt to stimulate the growth of new liver cells after one half of the course of the radiation, with the rationale that these new liver cells (and their supporting tissues) will then receive only the second half of the radiation course. Experimentally we achieve this stimulation by partial

hepatectomy two weeks after the first half of the radiation. In preliminary experiments, we have found that rats with partial hepatectomies performed between radiation doses survived higher levels of hepatic irradiation than controls treated identically except for the omission of the partial hepatectomy. These data suggest that it may eventually be feasible to cure hepatic metastases by radiation therapy.

E-5-5 *Testes DNA as a Biological Dosimeter to Measure RBE of Cyclotron Neutrons.* J. P. Geraci, K. L. Jackson, G. M. Christensen, R. G. Parker, M. M. Fox, and P. D. Thrower, University of Washington, Seattle, Washington 98195, USA.

Decrease in mouse testes DNA 28 days after exposure has been used as a dosimeter to biologically define the University of Washington fast neutron radiotherapy facility. These studies have involved measurement of RBE as a function of depth in an absorber and field size, and at various positions in the primary beam, penumbra, and adjacent shielded area.

The RBE for cyclotron fast neutrons was found to be independent of depth in a tissue equivalent absorber. However, the RBE did increase with decreasing field size and was greater in the penumbra and shielded area as compared to the primary beam. The RBE was slightly higher at the edge of the primary beam relative to the central axis. Interpretation of these data and their significance to therapy will be discussed.

E-5-6 *Use of the Mouse Anagen Hair Coat to Evaluate Neutron Radiation.* Melvin L. Griem, Franca T. Kuchnir, L. A. Buechle, and L. Dangel, Department of Radiology, University of Chicago, and the Frnaklin McLean Memorial Research Institute,* Chicago, Illinois 60637, USA.

We have been interested in evaluating the *in vivo* effects of various kinds of ionizing radiation on a rapidly proliferating cell population which produces hair. A technique has been developed by which the radiation response can be evaluated over several decades.

Using 250 kV x-rays, a D_0 value of 135 rads and a D_q value of 510 rads was found. With fast neutrons from an 8.3 MeV deutron beam on a thick beryllium target, a D_0 value of 45 rads and a D_q value of 185 rads was obtained. The relative biological effectiveness of approximately 3 was determined.

We have evaluated the shoulder of the dose response curve in two dose experiments with a separation of approximately two hours between fractions. There was no sublethal radiation repair observed in these preliminary experiments using neutrons, whereas a similar 2-dose x-ray experiment showed repair between fractions.

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* Operated by The University of Chicago for the United States Atomic Energy Commission.

E-5-7 *The Relative Biological Effectiveness of Cyclotron Fast Neutrons for Spinal Cord Injury.* J. P. Geraci, K. L. Jackson, G. M. Christensen, R. G. Parker, P. D. Thrower, and M. S. Fox, University of Washington, Seattle, Washington 98195, USA.

The time interval between local x-irradiation or neutron-irradiation of the lumbar spinal cord of mice and the onset of hindquarter paralysis was observed to be inversely related to the amount of radiation administered. Over a range of x-radiation doses from 2 krad to 15 krad, this mean latency period decreased exponentially with dose from 39.5 weeks to 1.5 weeks. Using the length of the latent period as an endpoint in animals followed over a period of one year postirradiation, the neutron RBE for spinal cord damage as a function of neutron dose was measured. The RBE increased from 0.9 to 1.2 as the neutron dose was decreased from 4500 rad to 1700 rad and the RBE appeared to further increase to about 1.9 as the neutron dose was reduced to 675 rad. The implications and uncertainty of these data will be discussed.

E-6-1 *Oxygen Depletion in Cells Irradiated at Ultra-High Dose Rates and at Conventional Dose Rates.* H. Weiss, E. R. Epp, C. C. Ling, J. M. Heslin, and A. Santomasso, Laboratory of Physical Biology, Sloan-Kettering Institute for Cancer Research, New York, NY 10021, USA.

Irradiation at conventional dose rates of an oxygen-equilibrated bacterial cell suspension in a sealed vessel produces breaking survival curves similar to those observed for cells irradiated as a thin layer at ultra-high dose rates. A mathematical model previously derived by this labo-

ratory which also yields breaking survival curves is compared to the surviving fraction measured at both dose rates. This comparison yields, at the two extremes, determinations of the rate of radiochemical depletion of dissolved oxygen and of the factor K, the oxygen concentration at which the radiosensitivity is halfway between the anoxic and fully oxygenated values. It is found that there is a dose rate dependence in K. For the sealed vessel, the determination of the rate of depletion is in essential agreement with that measured independently with an oxygen electrode. The agreement supports the assertion that the breaking behavior is due to oxygen depletion. An additional result obtained from the application of the model to the data may be interpreted to suggest a transport mechanism for oxygen into living cells although this remains speculative. (Supported in part by USPHS Grant CA 08748.)

E-6-2 *The Kinetics of Some Fast Processes in Cells Irradiated at Ultra-High Dose Rates.* C. C. LING, H. WEISS, AND E. R. EPP, Laboratory of Physical Biology, Sloan-Kettering Institute for Cancer Research, New York, NY 10021, USA.

Mammalian cells and bacterial cells exposed to single high intensity pulses of ionizing radiation can show survival curves that break sharply from an oxygenated to an anoxic response. This can be attributed to the radiochemical depletion of intracellular oxygen and to the decay of radiation-induced oxygen-dependent damage before extracellular oxygen diffuses to the damaged sites. A model is proposed to deal with the kinetics of oxygen depletion and of the decay of oxygen-dependent damage, and to correlate these processes with observed cellular survival. The model assumes that a radiation-induced agent reacts with and binds oxygen with a certain rate constant in competition with the oxygen-dependent damage which reacts with oxygen with another rate constant. The simultaneous differential equations of these reactions cannot be solved analytically but a computer-generated numerical solution has been reached. Calculations performed for the case of ultra-high dose rate irradiation of cells indicate that breaking survival curves can be produced given certain relationships between the rate constants of the competing reactions. Comparison between experimental data and calculated results will be presented. Speculations on the nature of such oxygen-binding agents will be discussed. (Supported in part by USPHS Grant No. CA 08748.)

E-6-3 *Oxygen Diffusion in Irradiated Bacterial Cells: Lifetime Information on Oxygen-Dependent Damage.* E. R. EPP, C. C. LING, H. WEISS, A. SANTOMASSO, AND J. HESLIN, Laboratory of Physical Biology, Sloan-Kettering Institute for Cancer Research, New York, NY 10021, USA.

Breaking survival curves have been measured for *Serratia marcescens* on Millipore filters equilibrated with known oxygen concentrations and irradiated by single pulses at ultra-high dose rates. This phenomenon is attributed to the radiochemical depletion of intracellular oxygen and provides a basis for double pulse experiments conducted to measure diffusion of oxygen in irradiated cells. A first pulse with sufficient dose to deplete intracellular oxygen precedes a similar second pulse by an accurately known interpulse time, variable from 10^{-6} sec to 30 sec. The amount of oxygen diffusing to critical sites in the cell during the interpulse time is inferred by comparison to cellular response measured under various oxygen concentrations with single high intensity pulses. Oxygen diffusion curves obtained show that a significant amount of oxygen diffuses to these sites by 10^{-4} sec. This can be interpreted as an upper limit to the lifetime of the radiation-induced oxygen-dependent damage. This limit is in agreement with that previously obtained here for *E. coli B/r* (Rad. Res. **54**, 171–180, 1973) using the double pulse technique but is five times shorter than the lifetime found for *Serratia marcescens* by Michael *et al.* (Rad. Res. **54**, 239–251, 1973) using a fastmixing technique. The reason for this discrepancy is not known. Also to be discussed is the observed reduction in spacing between the survival curve obtained under nitrogenated conditions and the anoxic segments of breaking survival curves when *Serratia marcescens* is conditioned by a radiation dose prior to single pulse irradiation at ultra-high dose rates. (Supported in part by USPHS Grant CA 08748.)

E-6-4 *Changes in Radiosensitivity, with Particular Reference to the Oxygen Effect, of Mammalian Cells After Prolonged Storage.* J. L. MOORE AND J. A. V. PRITCHARD, Tenovus Laboratory, Velindre Hospital, Whitchurch, Cardiff, Great Britain.

An estimate of "K" value for mammalian cells after high dose rate (100 rad/min) irradiation was obtained (Int. J. Radiat. Biol. **22**, 149, 1972) and a similar estimate after low dose rate (1 rad/min) initiated.

At 37°C the Chinese hamster cells were unable to survive prolonged anoxia and plating efficiency had dropped to less than 1% after 40 hrs. Any analysis of survival characteristics with a population of dying cells is unlikely to give meaningful results. A reduction in storage temperature to 25–30°C gave plating efficiencies of greater than 20% storage times of 100 hrs. Survival curves with acute irradiation after different times of prestorage showed large changes in radiation sensitivity for anoxicly stored cells. The oxygen enhancement ratio increased from 3 to 5 for cells subjected to storage times in excess of 100 hrs. This observation was not related to changes in synchrony monitored throughout storage by pulse labelling with tritiated thymidine.

These results and the initial experiments to establish "K" value at low dose rate (1 rad/min) will be presented and their significance for radiotherapy of tumors containing hypoxic cells discussed.

E-6-5 *5-Bromouracil and Oxygen Effects in Bacterial Spores.* Mohammad A. S. Al-Shaickly and Alan Tallentire, Pharmacy Department, University of Manchester, Manchester M13 9PL, Great Britain.

Spores of *Bacillus subtilis* 168 thy⁻ try⁻ have been produced in a chemically defined liquid medium containing different concentrations of 5-BUdR as fractions of a total thymidine moiety fixed at a concentration of 5 μg/ml. Sensitivities of these spores to ^{60}Co γ-rays are greater than that obtained with control spores (thymidine only in growth and sporulation medium). Spores showing maximal sensitivity when irradiated wet (grown in presence of 0.25 mole fraction 5-BUdR) have been irradiated in a dried condition (equilibrated to 5×10^{-4} Torr H_2O vapour pressure) and given appropriate gas treatments for measurement of the three basic classes of damage (E. L. Powers, R. B. Webb, and C. F. Ehret (1960), Radiat. Res., Suppl. **2**, 96). Incorporation of 5-BU increases the size of O_2-independent class I damage by about 45% and those of the O_2-dependent classes of damage, III and II by 29 and 32% respectively. These findings indicate that DNA is the site of primary damage in dried spores. Comparisons of the magnitudes of 5-BU effects in dried spores with effects obtained in anoxic and oxic wet spores reveal that a) enhancement by water of O_2-independent damage (Class I) is independent of 5-BU incorporation and b) O_2-dependent damage in wet spores has two components, one affected and the other unaffected by the presence of the analogue in the spore.

E-6-6 *The Oxygen Effect Under the Action of Ultra High Energy Photons on Biological Objects.* Ts. M. Avakian and E. A. Kochinian, Yerevan Physics Institute, USSR State Atomic Energy Commission, 375036, Yerevan, USSR.

Oxygen effect has been shown for irradiation by 50 MeV electrons and 2–4 GeV γ-photons of *Pisum sativum* seeds, *Endomicis vernalis* and *E. coli* microorganisms at rest. A reverse dependence has been discovered between the amount of endogenous thiols and the intensity of chemoluminescence. The experimental data concerning changes in the mitotic index, chromosome aberrations, survival and free radical states indicate the presence of oxygen effect at low pressures and the reverse dependence at high pressures. At high pressure oxygen probably loses its sensitizing action.

E-7-1 *Early Skin Reaction and Late Subcutaneous Fibrosis after Fractionated X-Irradiation in the Pig: the Failure of the NSD System to Predict Iso-Effect Doses.* Roger J. Berry, George Wiernik, Tom J. S. Patterson, and John W. Hopewell, Churchill Hospital Research Institute, Oxford OX3 7LJ, England.

Fields on the flanks of domestic swine were irradiated with 250 kVp x-rays in courses of 1, 6 or 30 fractions. The acute skin response was scored quantitatively using a numerical scheme; late skin tolerance up to one year after exposure was assessed by the presence or absence of skin necrosis in the irradiated fields; subcutaneous fibrosis was assessed quantitatively by the linear contraction of the irradiated fields. The Nominal Standard Dose (NSD) was a poor predictor for early skin response; over 30% higher equivalent dose was required to produce moist desquamation in 6 fractions than in 30. However, equivalent doses calculated on the NSD

system *did* predict an equivalent incidence of late necrosis. The degree of late fibrosis did *not* correlate well with the severity of the early skin reaction, and the NSD was again a poor predictor, in the *opposite* direction to its error in predicting equivalent early skin reactions. The results suggest that the NSD formula is *not* suitable for use when planning new radiotherapeutic schedules in which the number of dose fractions is to be reduced from current clinical practice.

E-7-2 *Time-dose Relationships in Radiation Cataractogenesis.* LARRY L. SCHENKEN AND RONALD F. HAGEMANN, Allegheny General Hospital, Pittsburgh, Pennsylvania 15212, USA.

Fourteen different time-dose schedules were selected to determine the response of the mouse lens to fractionated radiation exposure. Among the groups, exposure per fraction ranged from 100 to 1100 R; number of fractions varied from 1 to 30; overall treatment time varied from 1 to 40 days. Male Ha/ICR mice were irradiated head-only with 270 kVcp x-rays, 70 R/minute in these studies.

The development of lenticular changes was followed, and the onset and number of Stage III cataracts was scored at bi-weekly intervals for the duration of the study. The log-log plots of 50 percent Stage III cataracts *vs.* either overall treatment time or number of fractions yielded straight lines with slopes of .2882 and .3437 and y intercepts of 990 and 900 rets, respectively. The CD_{50} for single exposures was determined to be 825 R.

Log-log plots of other endpoints (20%, 80%, 0% cataract formation, etc.) yielded similar results. The significance of these data for interpretations of model systems exhibiting orderly progression of damage following radiation exposure will be discussed.

E-7-3 *Response of Mouse Skin to Short-Interval, Multi-Fraction Irradiation.* ANTHONY E. HOWES AND J. MARTIN BROWN, Stanford University, Stanford, California 94305, USA.

There is current clinical interest in the use of radiotherapy schedules which give fractionated treatments at the rate of more than one fraction each day. Radiobiological studies indicate that, for both normal and malignant cells, recovery of sublethal radiation damage is essentially complete by 6 hours after a single exposure. However, with multi-fraction treatments, possible effects due to repopulation and radiation-induced cell synchrony are unknown and, consequently, radiotherapists have tended to reduce total treatment dose when decreasing inter-treatment time rather than risk increased complications. This problem has been studied experimentally by assaying the response of C3H mouse foot skin to 250 kV x-rays given as 10 equal fractions, each separated by intervals of 6, 12, or 24 hours. The range of doses was chosen to produce reactions varying from erythema to complete moist desquamation during the acute phase. Preliminary results show small, but significant, differences between the three regimes such that approximately 10% further recovery takes place during the interval of 6 to 24 hours of each day. Data for late reactions are pending.

E-7-4 *Response of Mouse Spermatogenic Stem Cells to Multiple Doses of γ-Rays.* H. RODNEY WITHERS AND NANCY HUNTER, Section of Experimental Radiotherapy, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77025, USA.

It was shown previously in two dose experiments using an *in situ* cloning technique that sparing of testis stem cells by dose-fractionation was greater when the intervals separating doses was short. For example, more stem cells survived a total dose of 1200 rads when the fractionation intervals was 4 hours than when it was 1 day or 4 days. We have measured the multifraction response of mouse testis using up to 10 dose fractions separated by either 4 hours or 4 days. The results support the earlier observations that recovery decreases with protraction of the fractionation interval. We have attributed this to redistribution of first-dose survivors through the division cycle with an appreciable proportion entering into sensitive phases.

In the 4 hour fractionation studies, doses per fraction of 200 to 750 rads were used. Recovery in 4 hours accounts for sparing equivalent to about 50% of the dose per fraction. The iso-effect curve relating total dose for a certain effect to number of dose fractions, plotted on logarithmic coordinates is not linear, being convex upwards. These studies are continuing and the results

will be discussed in terms of radiobiology and radiotherapy. (This work was supported by NIH grant No. CA 11138-05 and CA06294-12.)

E-7-5 *Multifraction Dose Response of Mouse Bowel Mucosa.* H. Rodney Withers, Kathryn A. Mason, and Betty O. Reid, Section of Experimental Radiotherapy, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77025, USA.

The multifraction dose response of the mucosal cells of the stomach, jejunum and colon has been investigated. In all 3 tissues, there is extensive repair of sublethal injury and regeneration. Recovery is measured in terms of the incremental dose required for an iso-effect, e.g., survival of 50 cells per circumference of bowel wall. The relative contributions of repair and regeneration to the total measured recovery have been estimated by varying the fractionation interval. Up to 20 dose fractions have been given at 3-hour intervals, and up to 10 at 24-hour intervals. Most of the recovery observed when 3-hour intervals were employed is attributable to repair of sublethal injury. The additional recovery measured in 24-hour fractionation experiments results from regeneration of surviving cells during the course of irradiation. At low doses, about 60% of the dose is recovered through repair of sublethal injury and this decreases to less than 50% at doses of the order of 800 rads.

Regeneration rate varies and our discussion will center on the large bowel. The rate at which the clonogenic population regenerates has been estimated to accelerate from one doubling every two days to one doubling every 20 hours during a course of 10 doses given at daily intervals. (Work supported by NIH Grants CA 11138-05 and CA06294-12.)

E-7-6 *Cellular Recovery and Proliferation for Skin, Intestine and Lung During Fractionated Irradiation. Implications to Radiotherapy.* J. Dutreix and A. Wambersie, Cancer Institute, Capucienenvoer 35, B-3000, Leuvain, Belgium.

The total dose necessary to reach a given biological effect with a multifraction irradiation has been investigated for human skin and for mouse skin, intestine and lung.

For the same overall-time, increasing the number of fraction needs increasing the total dose. This increase, mainly related to cellular repair, tends to level off for small doses per fraction (<300 rads), in the range of doses usually given in radiotherapy.

It is concluded that, for this range of dose per fraction:

1. the number of fractions is not critical,
2. no significant differential effect related to fraction number is to be expected between the investigated tissues.
3. cell killing is mainly due to direct lethal events.

The increase of the total dose when increasing overall time (with the same fraction number) is significantly different for the investigated tissues: triggering of repopulation occurs more rapidly and repopulation is faster for intestinal mucosa than for other tissues.

Our data are compared with the formulas usually accepted for expressing the iso-effect dose as a function of fraction number and overall-time.

E-7-7 *The Effect of Single Versus Fractionated Doses of X-Radiation on Growing Tissue.* Yoav Horn, Hadassah University Hospital, Jerusalem, Israel.

Strandquist introduced in 1944 the concept of an isoeffect line in radiation therapy. To achieve the same biological effect with divided doses of radiation as with a single dose, he found it necessary to increase the total given dose over a longer period of time.

In our experiments two setups were used in rats: 1. Developing teeth (incisor and molar). 2. DMBA induced mammary carcinoma. The advantage of these experimental groups lies in their continuous growth, enabling study of the radiation effect on the tissue.

One, three and five fractions of x-rays were used. Total doses were calculated according to Strandquist's factors. The parameters examined were histological and roentgenological changes in the teeth formations and measurements of tumor diameter and histological changes in the mammary carcinoma in the rat. These data will be presented.

E-8-1 *Radiation Effects on Host Defense Mechanisms.* PATRICIA C. BRENNAN AND E. JOHN AINSWORTH, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439, USA.

The integrity of defense mechanisms in the $B6CF_1$ mouse is being assessed as part of the JANUS Neutron and Gamma Radiation Toxicity Program. The parameters measured include: (1) the ability to mount a response to challenge with *Mycoplasma pulmonis;* (2) the functional state of the pulmonary antibactericidal mechanism measured by clearance of *Pasteurella pneumotropica;* (3) cellular immune competence measured by susceptibility to transmissible murine leukemia cells and spleen thymus-derived (T cell) content.

Neutron-related injury was qualitatively greater than gamma-related injury following midline tissue doses of 288 neutron or 740 gamma rad. Challenge with *M. pulmonis* 5, 11 and 21 days after irradiation was characterized by a diminished cellular response and extensive invasion. The ability to clear *P. pneumotropica* was similarly impaired. Irradiated survivors challenged with leukemia cells at 250, 300 and 340 days showed a marked increase in susceptibility compared with appropriately aged controls. The mean survival time of neutron-irradiated animals was shorter at all ages. Based on these endpoints, the RBE is greater than 2.6. Spleen T cell content 525–750 days after a single dose of 240 neutron or 855 gamma was ~40% less in neutron- than in gamma-irradiated or control mice. (Work supported by the US. Atomic Energy Commission.)

E-8-2 *Early and Late Ultrastructural Changes in Mouse Vibrissae Following Ionizing Radiation.* FREDERICK D. MALKINSON, ROGER W. PEARSON, AND RUZICA MARIANOVIC, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois 60612, USA.

CF_1 mouse vibrissae were exposed to single doses of 1000 to 1500 rads delivered at 45 kV. Contralateral vibrissae served as controls. At multiple time intervals ranging from 15 minutes to 1½ years post-radiation, single vibrissae were dissected free, fixed in buffered osmium tetroxide, and embedded in epon. Thin sections were examined with a Phillips 300 microscope.

Changes up to 72 hours were characterized by marked autophagic vacuole formation and minor nuclear damage in the rapidly proliferating matrix cells, followed by resultant hair keratinization anomalies.

From 72 hours to 21 days post-radiation there was continuing, though diminishing formation of autophagic vacuoles in matrix cells. Nuclear damage was reflected in significant contour changes. Several months post-radiation there was fibrous replacement of some perifollicular vasculature, persistent nucleolar changes in dividing matrix cells, and degenerative alterations in the hair shaft and hair sheaths. Most long-term changes appear to reflect the severe vascular damage observed, though persistent *cellular* abnormalities may result from other radiation effects.

E-8-3 *Eye Lesions in Beagles After Prenatal or Neonatal Irradiation.* ARTHUR C. LEE AND ROBERT D. PHEMISTER, Collaborative Radiological Health Laboratory, Colorado State University, Fort Collins, Colorado 80521, USA.

The incidence and characteristics of eye lesions produced by single whole-body gamma (^{60}Co) exposures are being studied in a colony of beagles that were irradiated at 8, 28, and 55 days postcoitus (dpc) or at 2 days of age with exposures ranging from 125 to 435 R. The major clinically recognizable lesions observed thus far in dogs up to 4 years of age are cataracts, retinal degeneration and/or dysplasia, retinal detachments, large hyaloid remnants, optic disc hypoplasia, and microphthalmia. Histopathologic changes in the retina have included generalized or multifocal dysplasia, degeneration, and atrophy. Volumetric measurements have also been made on the eyes of dogs killed at 70 days, 2 years or 4 years of age. Both the incidence of eye lesions and a marked reduction in eye volume that appears to be dose dependent indicate that the canine eye is more radiosensitive at 28 and 55 dpc and at 2 days of age that at 8 dpc. (Research supported by Contract FDA 72-302 with the Bureau of Radiological Health, Department of Health, Education and Welfare.)

E-8-4 *Late Effects of Whole Body Gamma-Irradiation of Cattle.* T. R. NOONAN, R. A. REYNOLDS, F. H. CROSS, AND R. L. MURPHREE, UT-AEC Comparative Animal Research Laboratory, Oak Ridge, Tennessee 37830, USA.

Cattle (18 month old Hereford females) were given whole body gamma-irradiation at levels of 200, 300, or 400 R in a single exposure, two 300 R exposures eight weeks apart, or no exposure. Half of the animals entered the study in 1960, half in 1961 and all were kept under conditions approximating a commercial operation until July 1973. Animals were bred annually, examined (including routine hematological evaluation) semiannually, and almost always autopsied at death. Deaths from acute radiation effects occurred in the 300 R, 400 R, and 2 × 300 R exposure groups. All irradiated animals displayed a transient depression of thrombocytes and a prolonged depression of leukocytes, especially lymphocytes. No late hematological changes were detected in any of 214 initial survivors. Irradiated cattle were not reproductively different from controls. Causes of death were varied. Overall cumulative mortality was 31% and no dose dependent radiation effect on tumor incidence or longevity was established. (Research sponsored by the U.S. Atomic Energy Commission under contract No. AT-40-1-GEN-242 with the University of Tennessee.)

E-8-5 *Late Structural and Functional Changes in Rat Liver Following the Local Irradiation.* DJORDJE A. JOVANOVIC, AND PAUL MALDAGUE, Laboratoire de Radiobiologie and Laboratoire de Cytologie et Pathologie Tumorale. Université Catholique de Louvain, Kapucijnenvoer 37, B-3000 Leuven, Belgium.

Late proliferative and functional changes in rat liver induced by administration of radioactive colloids or by external irradiation of hepatic region in rats, were studied up to 24 months after their administration. Special emphasis was laid on the possible correlation between the alteration of the liver blood flow, functional capacity of hepatocytes and tumor inducing properties of above mentioned agents. In irradiated rats a definitive decrease in the liver blood flow was found before proliferative changes occurred in the liver, both after external and internal irradiation. Ability of hepatocytes to remove dye from the blood stream was normal in a majority of cases, except in high dosage groups. Remodelling of liver parenchyma was proportional to the radiation dose. Abundant proliferation of bile duct cells with or without attempts to form new bile ducts and megacaryohepatocytes were more frequently found after internal than after external irradiation. Nodules of small hepatocytes were surrounded with more or less pronounced fibrosis. Modified distribution of elastic tissue and sporadically appearance of "onion-like" arteries could not always be dissociated from aging changes in control rats. Increase in radiation dose resulted in significant shortening of life span of irradiated animals.

E-8-6 *Late Pathophysiologic Effects of Regional X-Irradiation in the Dog.** S. M. MICHAELSON, M. W. KRAMER, S. LU, S. E. PETTIT, AND W. J. QUINLAN, JR., Univ. of Rochester, Rochester, NY 14642, USA.

Correlative pathophysiologic studies have been performed in dogs exposed to 1000 R–2000 R, 100 kVp x-rays to the upper body (UB), neck or head to assess perturbations in the hypothalamic-hypophysial-thyroid (HHT) axis. Dose-time dependent changes in the thyroid over a ten year period, follow a sequence of active destruction, apparent repair, hyperplasia, atrophy, and tumor formation (primarily adenoma and adenocarcinoma). It appears that for the dog, doses of 1000 to 1200 R (50 R/min) UB are the maximal carcinogenic dose. Injury from 1800 R UB or more is too severe to permit survival of the animal over a long enough period of time for carcinogenesis to express itself; in such cases the predominant findings are hypofunction and compensatory hypoplasia. Thyrotropin (TSH) seems to promote the progressive changes and sequence of events leading to permanent hyperplasia or carcinogenesis.

Two to three years after cranial exposure to 1000 R, development of subtle alterations in autonomic control of heart beat, temperature regulation, thyroid function, electroencephalogram and neurologic status are evident. Specific deficiencies of growth hormone (GH) and thyrotropin (TSH) are noted 5–6 years after cranial exposure, at which time there is a greater tendency to obesity and apparent hypothyroidism.

These studies indicate deficiencies in metabolic and hormonal adjustments and compensatory mechanisms, thus permitting the assessment of the influence of ionizing radiation on the integrated endocrine metabolic efficiency of the organism.

* This paper is based on work performed under contract with the U.S. Atomic Energy Commission at the University of Rochester Atomic Energy Project and has been assigned Report No. UR-3490-447.

E-9-1 *The Mechanism and Organization of DNA Replication in Drosophila Chromosomes.* ALAN B. BLUMENTHAL, Laboratory of Radiobiology, University of California, San Francisco, California 94143, USA.

The time needed to replicate chromosomal DNA in fruit fly (*Drosophila melanogaster*) cells varies from 3.5 min in early embryos to 600 min in cultured somatic cells. Examination of the rapidly replicating embryonic DNA by electron microscopy reveals many replicating intermediates, or "eyes," in this DNA. These eyes represent sites of bi-directional replication and have an average eye-to-eye distance of 9.7 thousand bases (kb). Analysis of the distances between neighboring eyes indicates a spacing for replication origins in the DNA of 3.4 n kb, where n is an integer. In somatic cells, the organization of replication eyes was determined by DNA fiber autoradiography. The mean eye-to-eye distance was estimated at ~40 kb and the repeat pattern of origins was ~28 n kb.

Rates of replication fork movement were estimated in both cell types to be ~2.6 kb/min, at 25°C. The approximately 200-fold difference in replication time, then, does not appear to be related to replication rate or mean spacing of replication origins (approximately a 4-fold difference) in the DNA. A model is proposed to explain the spacing of replication origins in terms of the chromomeric pattern of DNA condensation in *Drosophila* chromosomes and to explain the regulation of replication times by heterochromatic condensation of DNA in somatic cell chromosomes. (This work was done in conjunction with H. J. Kriegstein and D. S. Hogness, Department of Biochemistry, Stanford University.)

E-9-2 *Mammalian Chromosome Structure: Ultrastructural Aspects of Specialized Regions and Chromosome Aberrations.* B. R. BRINKLEY, MANLEY MCGILL, AND MYLES L. MACE. Division of Cell Biology, Department of Human Biological Chemistry and Genetics, The University of Texas Medical Branch at Galveston, Galveston, Texas 77550, and the Department of Biology, The University of Texas, M. D. Anderson Hospital and Tumor Institute at Houston, Houston, Texas 77025, USA.

Although the structure of mammalian chromosomes still remains an enigma, newer techniques for chromosome research involving both light and electron microscopy have provided further insight into the architecture of the metaphase chromosomes. When examined by transmission electron microscopy, mitotic and meiotic chromosomes appear to be composed of numerous deoxyribonucleoprotein (DNP) fibrils which range in size from 30 to 350 Å in diameter. Although the DNP fibrils are seemingly folded in a random pattern within the chromosome, the regularity of banding patterns (G-bands) along the metaphase chromosome is indicative of a highly ordered packing arrangement which is characteristic for each chromosome pair of the complement. Chromosomal regions which are rich in constitutive heterochromatin are differentially stained by the Giemsa C-banding technique of Arrighi and Hsu (Cytogenetics **10**, 81, 1971). Further evidence that these regions contained highly repetitious DNA sequences was obtained by differential immunofluorescent labeling with antisera specific for single strand DNA (Mace *et al.*, Exptl. Cell Res. **75**, 521, 1972). Subsequently, ultrastructure studies have now been carried out on these heterochromatin regions. In addition, further electron microscopic studies have been conducted on specialized chromosome regions, such as kinetochore, nucleolus organizer, and telomere.

In addition to studies of normal chromosome ultrastructure, combined light and electron microscopic procedures have been carried out in order to evaluate drug and radiation-induced chromosome aberrations including breaks, gaps, exchanges, attenuations, and "chromosome stickiness."

E-9-3 *Structure and Replication of the Yeast Chromosome.* WALTON L. FANGMAN, University of Washington, Seattle, WA, USA.

The yeast, *Saccharomyces cerevisiae,* is a eukaryotic microorganism with very small chromosomes. The haploid genome consist of about 9×10^9 daltons of DNA distributed among seventeen chromosomes observed by genetic analysis. The average chromosome, therefore, contains only about 5×10^8 daltons of DNA, some 100-fold less than an average mammalian chromosome and only 4-fold larger than the T4 bacteriophage chromosome. Studies of DNA molecules released from the yeast nucleus by sedimentation and by electron microscopy have shown that the average DNA molecule has a mass of 4 to 6×10^8 daltons, the size expected if a single DNA duplex corresponds to a chromosome. Individual molecules range in size from about 10^8 to 10^9 daltons. In spite of this small size, yeast chromosome replication is similar to that in animal cells: it is initiated at multiple sites along a DNA molecule, and initiation sites are spaced along the DNA at short, 20 micron (0.5×10^8 dalton), intervals. Recent experiments indicate that portions of these chromosomes replicate at discrete and programmed times during the DNA synthesis (S) period in the cell cycle.

E-10-1 *The Equilibrium Optical Absorption Spectrum of the Solvated Electron in Polar Liquids and in Binary Solutions.* LEON M. DORFMAN AND JAMES F. GAVLAS, The Ohio State University, Columbus, Ohio 43210, USA.

Optical absorption spectra are now known for a considerable variety of compounds ranging from strongly polar to weakly polar liquids in which λmax may fall in the region 500 nm to 2300 nm. These systems include alcohols, amines and amides (current results), ethers and one hydroxy ether. The results will be discussed with reference to the dependence of spectrum upon the type of compound and upon the dielectric properties of the liquid. Data for binary solutions comprised of weakly polar and strongly polar components will be discussed with reference to evidence for selective interaction. With regard to the question of structure in the optical spectrum of e_{sol}^-, our recent observations do not sustain reports that structure may be resolved. Some correlation of the broad range of experimental data with theoretical and empirical models will be presented. (This work is supported by the United States Atomic Energy Commission.)

E-10-2 *Theory of the Optical Spectra of Solvated Electrons.* NEIL R. KESTNER, Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70803, USA.

During the last few years better theoretical models of solvated electron have been developed. These models allow one to calculate *a priori* the observable properties of the trapped electron. One of the most important and most widely determined properties is the optical spectrum. In this paper we consider the predictions of the theories not only as to the band maximum but line shape and width. In addition we will review how the theories predict these will depend on the solvent, pressure, temperature, and solvent density. In all cases extensive comparisons will be made with experimental work. In addition four new areas will be explored and recent results will be presented. These concern electrons in dense polar gases, the time development of the solvated electron spectrum, solvated electrons in mixed solvents, and photoelectron emission spectra (PEE) as it relates to higher excited states.

This paper will review all recent theoretical calculations and present a critical review of the present status and future developments which are anticipated. The best theories are quite successful in predicting trends, and qualitative agreement concerning band maximum. The theory is still weak in predicting line shape and line width.

E-10-3 *Electron Yields and Reaction Kinetics in Polar Liquids.* JOHN W. HUNT AND K. Y. LAM, The Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada.

Using a stroboscopic pulse radiolysis technique, the electron yield produced by the radiation, the fast electron attachment process to solute molecules, the formation of solvated electrons e_{sol}^-, and the e_{sol}^- reaction rate constants in concentrated solutions have all been studied in the time region from 20 to 350 picoseconds (ps). Some important questions have been posed by these ps studies. For example, in concentrated solutions, the electron attachment is faster

than any normal diffusion process. The two most likely mechanisms are a precursor of the e_{sol}^- which diffuses quickly through the medium, or a long range interaction between the electron and the solute molecules. Recently, we have attacked this problem by studying the absorption spectrum of electrons as they orient the polar alcohol molecules. In the different wavelengths (<1100 nm) we have observed the absorption spectrum of electrons in the infrared, in which a shallow trap in the alcohol (a "damp" electron) relaxed to a deeper trap, the normal e_{sol}^-. The decay kinetics of the damp electrons in the presence of different electron scavengers indicate that the electron attachment process is extremely fast, $<2 \times 10^{-11}$ s. We have also found a close relationship between the fast electron processes, and the e_{sol}^- rate constants in concentrated solutions of solutes, both in water, alcohols, and water-alcohol mixtures. The initial yield of electron adducts in different solvents have been studied, and indicates that the electron yield is $5 \pm .5/100$ eV.

E-10-4 *Reactions Rates of Electrons at Short Times.* GIDON CZAPSKI, Dept. Physical Chemistry, Hebrew University, Jerusalem, AND E. PELED, Dept. of Chemistry, Telaviv University, Tel-Aviv, Israel.

Due to various experiments, where e_{aq} was observed at very short times and generally in very concentrated solutions of scavengers, it was suggested that dry electrons play a chemical role in these systems.

It seems to us, that in most of these studies where the authors interpreted their results as an evidence for dry electrons, several corrections should have been made. If in these concentrated solutions the initial formation of encounter pairs of solutes with e_{aq} are taken into account, this does explain in many of these studies the observed behaviour of the systems without having to attribute a chemical reactivity to dry electrons in reactions with solutes. Also in those systems where this effect does not explain the entire behaviour, this correction should be made.

E-11-1 *A Seven-Year Study of the Pulmonary Retention and Clearance of ^{137}Cs Inhaled in Fused Aluminosilicate Particles by the Beagle Dog.* BRUCE B. BOECKER, RICHARD G. CUDDIHY, FLETCHER F. HAHN, AND ROGER O. MCCLELLAN, Inhalation Toxicology Research Institute, Lovelace Foundation, 5200 Gibson Blvd., S.E., Albuquerque, NM 87108, USA.

When radionuclides are inhaled in relatively insoluble, respirable-sized particles, they may be retained for long periods of time, producing chronic irradiation of the lung and contiguous tissues. Long-term pulmonary retention and clearance were studied using 30 Beagle dogs given single, 15-minute inhalation exposures to ^{137}Cs-labelled fused aluminosilicate particles (AMAD = 1.5 to 2.7 μm, σ_g = 1.6 to 1.8). An average of ~60% of the initial body burden was excreted rapidly, primarily in the feces; the remainder was retained for much longer periods. In 6 dogs observed for 3 years after exposure, the longest component of whole-body retention had an associated biological half-life of ~500 days. Tissue analyses for dogs sacrificed in pairs out to 7 years after exposure showed prolonged retention in the lung, transfer of significant quantities to the tracheobronchial lymph nodes and some soft tissue accumulation of released ^{137}Cs. The results have been incorporated in a kinetic model to study the relative importance of various lung clearance mechanisms. These data on long-term lung retention and translocation to tracheobronchial lymph nodes will be compared with existing data for plutonium dioxide, another aerosol shown to have long retention in the respiratory tract of the dog. (Work performed under AEC Contract AT(29-2)-1013.)

E-11-2 *The Effect of Chemical Form on the Tissue Distribution and Excretion of Plutonium Following its Deposition in The Respiratory System of the Rat.* JOHN W. STATHER AND SUE HOWDEN, National Radiological Protection Board, Harwell, Didcot, Berkshire, Great Britain.

In this study the effects have been investigated of chemical form on the tissue distribution and excretion of plutonium following its intubation into the various regions of the respiratory system of the rat. The injection volume was normally less than 3 μl and the activity administered (2–3 nCi Kg^{-1}) was comparable to a few maximum permissible body burdens of plutonium in man.

The absorption of various chemical forms of plutonium from the pulmonary region was in general agreement with the conclusions of the ICRP Task Group on Lung Dynamics. However the absorption of both plutonium nitrate and citrate from the pulmonary region was approximately 4 times greater than the absorption from either the tracheobronchial or nasopharyngeal regions. About 13% of activity entering the blood during the first week after pulmonary administration of plutonium nitrate, citrate or oxide, was retained in the liver at 7 days implying that plutonium was circulating in the blood in a "monomeric" form. The cumulative excretion of plutonium in the urine in the same period was equivalent to about 4.5% of the total activity deposited in tissues from the blood. The experimental results suggest that this value could be used for calculating the total tissue deposit from urinary excretion measurements.

E-11-3 *Early Fate of Inhaled $^{238}PuO_2$, $^{239}PuO_2$ and $^{244}CmO_x$ in Rodents.* CHARLES L. SANDERS, DOUGLAS K. CRAIG, AND VICTOR H. SMITH, Biology Department, Battelle Pacific Northwest Laboratories, Richland, Washington 99352, USA.

The pulmonary deposition, clearance and translocation of inhaled, freshly prepared $^{238}PuO_2$, $^{239}PuO_2$ and $^{244}CmO_x$ and of $^{238}PuO_2$ aged in water suspension, were studied in rats at 2–3 widely different levels of initial pulmonary deposition, for a period of 30 days after exposure. Initial upper respiratory tract and alveolar depositions were related to the activity median aerodynamic diameter, which ranged from 1–3 μm for PuO_2 and 0.3–0.8 for "aged" $^{238}PuO_2$ and $^{244}CmO_x$. The ultrafilterability of $^{238}PuO_2$ was increased by aging in water, while $^{239}PuO_2$ was not changed by aging in water. The ratio of amounts of transuranic in feces and urine over the 30-day period averaged 37 for $^{239}PuO_2$, 22 for $^{238}PuO_2$, 12 for "aged" $^{238}PuO_2$ and 7.7 for $^{244}CmO_x$. The lung contained about 90% of the 30-day body burden of $^{239}PuO_2$, 80% of $^{238}PuO_2$, 40% of "aged" $^{238}PuO_2$, and 20% of $^{244}CmO_x$. The ratio of 30-day lungs and skeleton burdens averaged 24 for $^{239}PuO_2$, 16 for $^{238}PuO_2$, 1.2 for "aged" $^{238}PuO_2$ and 0.5 for $^{244}CmO_x$, while the ratio of lung and liver burdens were 120, 48, 6.1 and 1.2 respectively. Total body clearance of inhaled transuranic oxide was more influenced by the amount deposited in the lung than by the species of transuranic. *In vivo* solubility was reflected by *in vitro* ultrafilterability, both of which increased with increasing specific activity and age of the transuranic oxide in water suspension prior to aerosolization. (This paper is based on research performed under the United States Atomic Energy Commission Contract AT(45-1)-1830.)

E-11-4 *The Early Toxicity of Inhaled $^{239}PuO_2$ and $^{238}PuO_2$ in Syrian Hamsters.* CHARLES H. HOBBS, JAMES A. MEWHINNEY, DAVID O. SLAUSON, ROGER O. MCCLELLAN, AND JOHN J. MIGLIO, Inhalation Toxicology Research Institute, Lovelace Foundation, 5200 Gibson S.E., Albuquerque, NM 87108 USA.

Syrian hamsters (12 weeks of age) were exposed to aerosols of $^{239}PuO_2$ or $^{238}PuO_2$ to achieve graded initial lung burdens (ILB) ranging from levels equivalent on a body weight basis to the maximum permissible lung burden for man to levels which have caused death at about 100 days post-inhalation from radiation pneumonitis and/or pulmonary fibrosis. The aerosols were generated from plutonium (IV) hydroxide suspensions and degraded to plutonium oxide by passing through heating columns maintained at 325° and 1150°. The aerosols had activity median aerodynamic diameters from 1.9 to 2.4 μm ($\sigma_g \sim 1.5$) for $^{239}PuO_2$ and from 0.7 to 1.9 μm ($\sigma_g \sim 2.0$) for $^{238}PuO_2$. Due to the much higher specific activity of $^{238}PuO_2$ problems were encountered in obtaining the desired aerosol concentrations and thus the ILB achieved for $^{238}PuO_2$ were about 2 to 5 times higher than desired and higher than those achieved with $^{239}PuO_2$. Through 64 days post-inhalation exposure no marked differences in the retention or translocation patterns of ^{238}Pu or ^{239}Pu have been observed. Animals exposed to $^{239}PuO_2$ or $^{238}PuO_2$ with ILB of about 200 nCi or greater have died from radiation pneumonitis and/or pulmonary fibrosis at from 60 to 150 days post-exposure. Although no pulmonary tumors have been observed to date, marked focal epithelial hyperplasia and cytologic atypia have been common findings. (Research performed under AEC Contract AT(29-2)-1013.)

E-11-5 *The Effects of Repeated Inhalation Exposure of Mice to $^{144}CeO_2$.* ROGER O. MCCLELLAN, DAVID L. LUNDGREN, AMBROSE SANCHEZ, GEORGE J. NEWTON, AND FLETCHER F. HAHN.

Inhalation Toxicology Research Institute, Lovelace Foundation, Albuquerque, NM 87108, USA.

The effects of repeated inhalation exposures to radioactive aerosols in mice are being compared with those in mice given single acute inhalation exposures. Mice were repeatedly exposed to $^{144}CeO_2$ to maintain lung burdens (LB) near 0.2, 1.0 and 4.5 μCi ^{144}Ce. All mice are being held for lifespan observation. Results are being compared with those from mice in three different age groups having approximately similar initial LBs of ^{144}Ce after single inhalation exposures. During the first seven days after repeated exposures, clearance of inhaled ^{144}Ce was lower and retention after this period was higher than in mice given single inhalation exposures. These effects were most evident in the mice with the highest LBs. All mice in the highest μCi level of the repeatedly exposed group died by day 224 after the initial exposure, whereas 83% of the mice at the highest μCi level after a single exposure at the same initial age have died by that time. Mortality among the mice in the two lower μCi levels was similar for both single and repeated exposures and did not differ significantly from mortality among control mice through day 224. Older mice cleared inhaled ^{144}Ce at the same rate as younger mice after a single inhalation exposure. (Performed under U.S. Atomic Energy Commission Contract AT(29-2)-1013.)

E-11-6 *The Effect of Influenza Virus Infection on the Retention and Distribution of ^{144}Ce and Mortality in Mice and Syrian Hamsters After Inhalation Exposure to $^{144}CeO_2$.* David L. Lundgren, Ambrose Sanchez, Fletcher F. Hahn, and Roger O. McClellan. Inhalation Toxicology Research Institute, Lovelace Foundation, Albuquerque, NM 87108, USA.

Impaired pulmonary clearance of particulate matter in mice infected with influenza virus before and at the time of particle administration has been reported by other investigators. This report presents the effects of influenza virus infection on the retention and distribution of inhaled ^{144}Ce in $^{144}CeO_2$ particles in mice and Syrian hamsters after inhalation exposure. Two groups of mice with average estimated initial lung burdens (ILB) of 1.0 and 4.8 μCi and one group of Syrian hamsters with an estimated ILB of 12 μCi were inoculated with mouse adapted influenza virus strain PR-8 at 3 and 6 months after inhalation exposure to $^{144}CeO_2$. Control animals were either inoculated with a normal mouse lung suspension in diluent or left untreated. In mice with ILBs of 1.0 μCi the effective retention of ^{144}Ce was slightly higher than in controls. This was in contrast to the effective retention of ^{144}Ce in mice with ILBS of 4.8 μCi and the Syrian hamsters which did not change after influenza virus infection. Influenza virus infection did not significantly alter the distribution of inhaled ^{144}Ce in the tissues of mice and Syrian hamsters. The mortality rate among the mice with ILBs of 4.8 μCi and Syrian hamsters after infection was significantly higher than that among the mice with ILBs of 1.0 μCi and control animals. (Performed under U.S. Atomic Energy Commission Contract AT(29-2)-1013.)

E-11-7 *Lung Tumors in Rats after Thorium Dioxide Administration.* Giuseppe Grampa, Department of Pathology, State University Medical School, Milano, Italy.

Epithelial and connective tissue tumors (adenomas, adenocarcinoma, fibroma and fibrosarcoma) were observed in Sprague-Dowley's rats injected intravenously with thorium dioxide and sacrificed from 10 to 22 months after treatment. Histological examinations and radiochemical analysis by alpha-spectrometry showed a dose effect response.

The findings are compared with lung tumors observed in man after exposure to internal alpha-emitters. (Work done in part under contract n. 70.01529/23 of the Italian National Research Council (C.N.R.))

E-11-8 *Skin Decontamination for Plutonium Lung Counting.* Bob Robinson, Ralph Brown, and F. Keith Tomlinson, Monsanto Research Corporation, Mound Laboratory, Miamisburg, Ohio 45342, USA.

Practical techniques have been developed for removing Plutonium 238 contamination from the skin prior to lung counting subjects who must be checked as a result of minor incidents. Various cleaning solutions are used for gross decontamination and this is followed by using a portable "steam" cabinet for approximately 30 minutes. Preliminary indications show that

the "steam" cabinet helps remove small quantities of contamination from skin pores and wrinkles that are not normally detected with an alpha survey meter. It is necessary to remove all surface contamination prior to lung counting because as little as 60 dis/min of Plutonium 238 surface contamination could result in a false lung deposition measurement near a maximum permissable lung burden. Special lung counting techniques that are used for counting subjects following minor incidents is also included.

E-11-9 *Turnover Time of Insoluable Plutonium Out of The Lungs for Two Occupational Cases of Acute Uptake.* F. KEITH TOMLINSON AND BOB ROBINSON, Monsanto Research Corporation, Mound Laboratory, Miamisburg, Ohio 45342, USA.

Lung deposition measurements by whole body counting have been made on two persons who recieved acute occupational exposures to Plutonium 238 oxide during the past five years. Significant details of their exposures and the data collected are given, including the half-time of Plutonium 238 out of the lung.

In vivo measurements of Plutonium in lungs of humans have not been available until recently. Experimental data obtained is in agreement with that of others and less than the theoretical 500 day half-time for long term elimination from the lungs given by the ICRP Task Group lung model.

E-12-1 *Photosensitized Oxidation of Tyrosine Derivatives by Dyes Bound to Alginate.* G. R. SEELY AND R. L. HART, C. F. Kettering Research Lab., Yellow Springs, Ohio 45387, USA.

Many dyes that sensitize photodynamic action also bind to their substrate, often producing metachromatic effects from change in environment or aggregation. The present work examines the relation between the state of aggregation of dye, the charge on the polyelectrolyte to which it is bound, and the rate of photosensitized oxidation. The system consists of the oxidation of tyrosine and tyramine by thiazine dyes bound to the anionic polyelectrolyte, Na alginate. Certain dyes, including methylene blue and thionine, tend strongly to bind to this polymer as dimers whose absorption bands lie about 100 nm to the blue of the monomer bands. Oxidation of tyrosine is fastest at pH >9 but is measurable at pH 6. The reaction is characterized spectrally by loss of phenyl peaks *ca.* 275 nm and appearance of a series of broad bands extending from *ca.* 480 nm into the UV. At the level of 10^{-3} *M* tyrosine or tyramine-HCl, the rate of oxidation is much less in monochromatic light absorbed by dimeric bound dye than in light absorbed by bound monomer. However, the rate and extent of oxidation of 10^{-4} *M* tyramine-HCl with thionine in unbuffered solution are greater when alginate is present, even though the dye is mostly in dimeric form, probably because of attraction of tyramine to the polymer under conditions where the lifetime of $'\Delta\ O_2$ is rate limiting.

E-12-2 *Formation of Alkali Labile Bonds Upon Triplet Sensitization of PM2 DNA.* RONALD O. RAHN, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830, USA.

Triplet sensitizers such as acetone, benzophenone and acetophenone when irradiated at 313 nm in the presence of DNA, transfer their triplet energy to DNA causing thymine dimerization. Sensitization is accompanied by single-strand chain breakage as determined by alkaline sucrose sedimentation.[1] To determine the extent to which the number of chain breaks observed in alkali represent alkali labile bonds, the DNA from PM2 phage was employed. This DNA is a covalently closed circle which undergoes a change in sedimentation velocity when a single nick is present. By comparing the number of chain breaks measured under neutral and alkaline conditions, an estimate of the number of alkali labile bonds was obtained. The number of chain breaks as determined in alkali was in general agreement with the values obtained previously with linear DNA.[1] Fewer chain breaks were observed with neutral sucrose sedimentation implying that some of the breaks observed in alkali represented alkali labile bonds. The percentage of such bonds was estimated to be 27–39% for irradiation in the absence of oxygen and 30–60% for irradiation in the presence of oxygen. The efficiency of making chain breaks as well as the extent of alkali labile bond formation was greatest for benzophenone and least for acetophenone. Since the percent of alkali labile bonds is approximately the same as that obtained with ionizing

radiation, it is concluded that common mechanisms may be operative such as hydroxyl radical attack. (Research sponsored by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.)

[1] Rahn, Landry and Carrier, Photochem. Photobiol. 19, 75 (1974).

E-12-3 *The Mechanism of the Photodynamic Action of DNA and the Binding Between the Sensitizer and the Substrate.* F. A. GOLLMICK, Akademie der Wissenschaften der DDR, Forschungszentrum für Molekularbiologie und Medizin, Zentralinstitut für Mikrobiologie und experimentelle Therapie Jena, Abteilung Biophysikochemie, East Germany.

In most of the mechanisms proposed for photodynamic action the triplet state of the sensitizer is involved. For the systems DNA—thiopyronine or methylene blue—the oxidized radical of these dyes acts as the primary oxidizing agent. This could be shown by measuring the kinetics of the transients in the system sensitizer-guanosine after flash photolysis. Because of the binding sensitizer-DNA the influence of this binding to the transients needs to be taken into consideration. For these further investigations thiopyronine as model sensitizer was used.

If the dye molecules are externally bound to the DNA the triplet lifetime increases by suppressing of second order reactions. On the other hand the intercalation between base pairs leads to a further increase of the lifetime. The reactions of other transients are nearly not affected in the presence of DNA.

From these results one can conclude:

1. Even at relatively high DNA concentrations the free triplet plays an important role as a precursor of oxidized radical.
2. The longer lifetime of the triplet in the externally bound state will favour the Photodynamic action.
3. The efficiency of oxidized radical as oxidizing agent for guanine is not affected by a binding to DNA.

E-12-4 *The Effect of* uvrB *Deletion on Base-Pair Substitution and Frameshift Mutagenesis by Photodynamic Treatment.* F. PAULA IMRAY AND DONALD G. MACPHEE. Genetics Department, La Trobe University, Bundoora, Victoria, 3083, Australia.

Photodynamic treatment sensitized by acridine orange or methylene blue can induce *HIS*$^+$ revertants of the base-pair substitution mutation *hisG*46 and of the frameshift mutation *hisD*3052. In strains with a deletion over the *uvrB* gene the frequency of photodynamically induced reversion of *hisG*46 is increased while that of *hisD*3052 is decreased. The mechanism by which photodynamic treatment produces basepair substitutions may as with UV mutagenesis depend on recombination repair, so that if damaged bases are not excised from DNA more of these are passed on to an errorprone recombinational repair system and more mutations are induced. In contrast to this fewer frameshift mutations are induced following photodynamic treatment if the bacteria are excision-deficient, suggesting that one of the later steps in the excision repair pathway may be responsible for introduction of at least some of the frameshift mutations into the DNA. This step could possibly be resynthesis by DNA polymerase I. (This work was supported by the Australian Research Grants Committee.)

E-12-5 *Viral Disinfection of Lysogenic* Staphylococcus albus *by Photodynamic Action with Maintenance of Homo-Immunity.* L. R. CALDAS, S. MENEZES, AND R. ALCANTARA GOMES, Instituto de Biofisica-UFRJ and CNEN, Rio de Janeiro, Brazil.

In cultures of the UV inducible $P(\omega)$ strain of *Staphylococcus albus* at the end of the log phase of growth in tryptone broth medium, circa 10% of the cells are spontaneously induced yielding ω mature phages. If such cultures are submitted to photodynamic treatment in the presence of 2 μg/ml of methylene blue (the cells are centrifuged, washed and incubated with methylene blue in phosphate buffer M/15 pH 7.0 for 1 hour at 37°C prior to the illumination which is followed by immediate plating) with increasing doses of light (from a GE PAR 38 lamp) the number of infectious centers progressively decrease and finally no more viruses are detected. The surviving cells (expressed as colonies) while maintaining their immunity against the phage

ω lose their ability to yield mature phages even when submitted to UV induction. (This work has been supported by CNEN, CNPq, CPEG-UFRJ and BNDE (Contracts FUNTEC 74 and 143).)

Reference: "Estudo radiobiológico do sistema lisogênico do *Staphylococcus albus*" L. R. Caldas, 1963, Thesis, Instituto de Biofísica-UFRJ.

E-12-6 *A Possible Common Pathway for Repair and Mutagenesis Induced by 5-bromouracil (BU) and UV-Light.* IRENA PIETRZYKOWSKA, Institute of Biochemistry & Biophysics, Polish Academy of Sciences, Warsaw, Poland.

It has been shown that for efficient BU-induced mutagenesis of λ phage the *red* or rec_A as well as *lex* gene functions, known to be involved in UV-induced mutagenesis, are required (I. Pietrzykowska, Mut. Res. 19, 1, 1973). It was also observed that deficiency in excision repair of the host (mutation uvr_A) increases BU-induced mutagenesis of λ phage several-fold. This suggested that BU incorporated into DNA introduces damages which may be repaired by dark repair processes. This is further supported by the following observations:

(a) The double mutant E. coli $rec_A uvr_A$ shows growth inhibition in the presence of BU, while the single mutants rec_A or uvr_A, or the wild type, grow normally.
(b) *E. coli* CR-34 strain grown in the presence of BU is subject to a lethal effect which may be reversed if bacteria are submitted to the liquid holding recovery (LHR) conditions.

The repair of BU-containing DNA during LHR was investigated by centrifugation in alkaline sucrose gradients and by incorporation of ^3H thymidine (repair synthesis). It was observed also that LHR results in a significant drop in the number of mutants induced by BU.

E-13-1 *Induction and Repair of Single-Strand Breaks in Mammalian DNA Studied by Means of Rate of Alkaline Strand Separation and Hydroxyl Apatite Chromatography.* G. AHNSTRÖM, Wallenberg Laboratory, University of Stockholm, Stockholm, Sweden.

Velocity sedimentation in alkaline sucrose gradients according to McGrath and Williams has been the major technique for studying the induction and repair of single strand breaks in bacterial and mammalian DNA. The technique is however less suitable for low dose investigations due to the anomalous sedimentation behavior of very high molecular weight DNA. We have recently developed a simple sensitive and reproductive technique for determination of single strand breaks based on the principle that single strand interruptions serve as untwisting points during strand separation in alkali, increasing the rate of the transformation of double stranded DNA to single strands. In this technique, cells are lysed in alkali for a fixed period of time, brought to neutral pH with NaH_2PO_4 and immediately sonicated. The amount of DNA released in single stranded form is proportional to the number of strand interruptions—radiation dose—and can easily be determined by means of hydroxylapatite chromatography. Data concerning the induction and repair of radiation induced strand breaks will be presented.

E-13-2 *DNA Repair in Cells After Gamma and Neutron Irradiation.* HELGA TUSCHL, WOLFGANG KLEIN, FRITZ KOCSIS, KAROLY TURANITZ, AND HANS ALTMANN, Institute of Biology, Research Center Seibersdorf, A-2444 Seibersdorf, Austria.

Unscheduled DNA synthesis was estimated in mouse spleen cells after gamma and fast neutron irradiation by autoradiography. Likewise DNA repair of these cells was investigated by gradient centrifugation in neutral and alkaline sucrose. The number of strand breaks was calculated after gamma or fast neutron irradiation, respectively. DNA repair of *E. coli* was also measured after gamma and neutron irradiation by sucrose gradient centrifugation and survival curves were established.

E-13-3 *Cyclic Repair of DNA and Induced Cyclic Radiosensitivity in Mammalian Cells.* JERRY R. WILLIAMS AND JOHN B. LITTLE, Laboratory of Radiobiology, Harvard University, The School of Public Health, Boston, Mass., USA.

Immediately after exposure to ionizing radiation, alternate incision and rejoining of cellular DNA can be observed in mammalian cells using a modification of the technique of McGrath and Williams which allows precise incubation intervals and sudden lysis. The period of this

cycle is approximately one to one and a half minutes. This process has been observed after doses as low as 1000 rads. Further, cells induced by small radiation doses exhibit a cyclic radiosensitivity in survival to a second dose of radiation also with a period of one to one and a half minutes. We have observed these cyclic phenomena in diploid human cells, diploid rodent cells, aneuploid rodent cells and aneuploid human cells. We, therefore, believe this to be a general phenomenon in the radiation response of mammalian cells which has not heretofore been reported. The implications which these observations may have in understanding the molecular basis for radiation response, and in devising more rational fractionation schemes for radiation therapy will be discussed.

E-13-4 *^{125}I Induced Damage in Late Replicating DNA Is Most Efficient in Causing Reproductive Death in Cultured Chinese Hamster Cells.* H. JOHN BURKI, Division of Medical Physics and Donner Laboratory, University of California, Berkeley, California 94720, USA.

Chinese hamster cells strain V79 were synchronized by mitotic shake off, labeled in the first S period with ^{125}IUdR, cooled to 4°C in the G2 stage and then stored up to 4 days to accumulate damage due to ^{125}I disintegrations in cell DNA. There was a large difference in the efficiency of induction of reproductive death when damage accumulated in the DNA which replicated in the second half of the DNA synthesis period was compared to damage accumulated in the DNA which replicated in the first half of the DNA synthesis period. Damage accumulated in the *late* replicated DNA appears to be the most critical. This result suggests that the mammalian cell nucleus is not homogeneous with respect to the damaging events leading to reproductive death and may stress the importance for cell survival of the integrity of the late-replicating, heterochromatic DNA near the nucleus membrane.

E-13-5 *Induction and Repair of Single-Strand Breaks after Incorporation of ^{125}I-iododeoxyuridine into Mammalian DNA.* B. R. YOUNG, H. J. BURKI, AND R. B. PAINTER, Lab of Radiobiology, University of California, San Francisco and Donner Lab, University of California, Berkeley, USA.

Chinese hamster V79 cells were labeled with ^{14}CTdR and then for one generation with ^{125}I-iododeoxyridine and frozen in liquid N_2. At various intervals, cells were thawed, and either layered immediately on top of alkaline sucrose gradients, or allowed to incubate at 37°C for 3 hrs. To measure the repair capacity of the cells to external radiation under these conditions, frozen cells labeled with only ^{14}CTdR were irradiated with x-rays, thawed and incubated at 37°C. Measurements of single-strand breaks showed a continuing decrease in molecular weight with increasing numbers of ^{125}I decays, so that breaks accumulated at a rate of 1.8 breaks/decay. Upon incubation, one third or more of these breaks were not rejoined. Since x-ray induced strand breaks were rejoined more completely within this time, the damage caused by ^{125}I in DNA appears to be different than that caused by x-rays. These data suggest that the irraperable component of this damage is responsible for the unique toxicity of ^{125}IUdR after its incorporation into mammalian DNA (Burki *et al.*, 1973, Int. J. Rad. Biol. **24**, 363).

E-13-6 *Characterization Studies of the Rejoining of α-Particle and γ-Ray Induced Double-Strand Scissions in Mammalian DNA.* W. GRANT COOPER AND ARTHUR COLE, Univ. of Texas System Cancer Center, M. D. Anderson Hosp. & Tumor Inst., Houston, TX 77025, USA.

Cellular rejoining of radiation induced double strand (DNA) breakage has been investigated, using γ-ray and α-particle irradiations. The induced double strand scissions are measured in terms of number average molecular weights on neutral sucrose gradients. Chinese hamster cells irradiated with Cs-137 γ-rays at 25 kR yield DNA profiles with the peak position in the 75–80 S region, whereas the peak position is located in the 105–110 S region when the irradiated cells are allowed a post radiation incubation at 37°C for 90 minutes. The rejoining of double strand scissions obeys first order kinetics and the average rate of 3 to 6 rejoining events per cell per second is not altered by 5 to 10 mM of the DNA synthesis inhibitor hydroxyurea. Rejoining is completely inhibited by the identical concentrations of EDTA or incubation at 2°C. At other temperatures in the range ($0 \leq t \leq 41$°C), the process exhibits an enzyme-like temperature dependence. Preliminary α-particle irradiations imply that the cell can also rejoin these lesions.

E-13-7 *The Kinetics of Repair of Double-Strand Breaks in the DNA of Mammalian Cells and the Organization of the DNA in their Chromosomes.* CHRISTOPHER S. LANGE, DANIEL F. LIBERMAN,* PAMELA MITCHELL,* AND LORRAINE E. SHECK,* The University of Rochester School of Medicine & Dentistry, Rochester, New York 14642, USA.

Native mammalian DNA has been accurately sized by the use of a semi-automated speed-dependence-free sucrose gradient system. A monodisperse distribution of eighth-of-a-chromatid pieces (1.67×10^{10} D) was found. Ionizing radiation rapidly breaks each of these pieces into 48 subunits and with increasing dose the subunits themselves are randomly broken down into even smaller pieces. Post-irradiation incubation permits the cells to repair both DNA double-strand breaks in the subunit and the intersubunit linkages at the same, dose independent, rate (T_{37}) of about 55 min; the same rate as that found in *M. radiodurans*. The repair data are compatible with a first order kinetics repair system, analogous to the post-UV excision repair system, which becomes saturated at high doses (60 krad). Specially constructed "enzyme" gradients show that the linkages are/contain a covalently bound protein. Correlation of cell survival and DNA break kinetics yield two possible models. These are that the 50% of lethal events which are due to improperly or unrepaired double-strand breaks result from either (a) a misrepair frequency of 6.5×10^{-4} or (b) the induction of a double-strand break in a single subunit which cannot be repaired, possibly because that subunit contains the double-strand break repair system gene(s). (This paper is based on work performed under contract with the US. Atomic Energy Commission, in the Department of Experimental Radiology (Contract No. AT(30-1)-4282), and at the University of Rochester Atomic Energy Project and has been assigned Report No. UR-3490-448. C.S.L. would like to acknowledge the support of an N.I.H. Research Career Development Award.)

E-13-8 *Radiosensitivity of DNA Single-Strand Break Rejoining Mechanisms.* K. T. WHEELER AND J. D. LINN, Univ. of Calif., San Francisco, Ca. 94143, USA.

It has been hypothesized that the repair of radiation-induced single strand breaks in mammalian DNA involves two rejoining mechanisms. The first mechanism reforms the Lett subunit while the second mechanism restores the chromosomal DNA structure found in unirradiated cells. When rat 9L sarcoma cells are heavily X-irradiated these rejoining mechanisms can be resolved in time so the sensitivity of these cells to a second dose of X-rays can be measured as a function of their position in the rejoining process. The data from split dose experiments indicate that a second X-ray dose delivered at the time the Lett subunit is reforming leads to a greater accumulation of DNA damage than is found after a single dose equivalent to the sum of the 2 split doses. If the time reference were telescoped to that expected for biologically significant doses, and if the DNA damage measured is significant for cell survival, these results predict that small pulsed doses separated by very short intervals would produce a decrease in cell survival with its subsequent ramifications for radiotherapy. (This work supported in part by MSC #24 Breon Fund, NIH Cancer Center Grant CA-13525, UC Cancer Research Fund, Spinco Division of Beckman Instruments, and the USAEC).

E-14-1 *Induction of Radiation Chimaera by Allogeneic Bone Marrow Transplantation. I. Inducing Conditions.* ITSURO TAMANOI,* TOSHIO TANAKA,** SADAHITO USUI,*** AND TAKEHIKO TSUCHIYA.***

GVHR and HVGR are known to be caused by immunological reactions after allogeneic bone marrow transplantation. The induction of a radiation chimaera is assumed to be affected by the following conditions: 1) breakdown of immune mechanism by radiation, 2) high doses of allogeneic transplants (antigen), and 3) time of transplantation after irradiation. Accordingly, the survival rate of the irradiated mice was studied with respect to the relation of radiation (900 R, 605 R ($LD_{90/30}$), 400 R) and various cell doses ($25 - 1 \times 10^6$), using C57BL/6j (H-2^b) as donors and CF#1/Nrs (H-$2^{k?}$) as hosts. Also the ratios of donor and host type cells in bone marrow and peripheral blood were analyzed in long-survived animals by indirect membrane immuno-fluorescence. The 900 R-irradiated group with a great number of transplanted cells (25×10^6) survived

longer than those treated otherwise. The donor type cells and host type cells in bone marrow and peripheral blood of 900 R-irradiated animals were about equal in number.

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E-14-2 *Induction of Radiation Chimaera by Allogeneic Bone Marrow Transplantation: II. Kinetics of Thymic Cells in Radiation Chimaera Mice and their Influence on Spleen.* T. TANAKA,* I. TAMANOI,** AND T. TSUCHIYA.***

Our hypothesis is that the transplantation of allogeneic bone marrow cells after X-irradiation will establish the chimaera in case the thymus of recipient is largely occupied by the transplanted donor cells which have been and are proliferating. This proved true experimentally under the limited conditions of the X-ray doses (900 R) and cell number (25×10^6); the donor cells in thymus were evident on the second day after transplantation, and proliferated markedly and continuously on the fifth day and later days, while in 605 R no demonstrable proliferation was noted in spite of the presence of the donor cells on the third day. Number of cells, ratio of the donor type cells to host cells, and changes of differential cell population were followed daily in the thymus, and immunological influence of these cells upon the spleen will be discussed, as regards on the reactivity to the anti-θ antiserum.

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E-14-3 *Kinetics of the Development of Anti-Host and Anti-Donor Reactive Immunocompetent Cells in Spleens of Allogeneic Mouse Radiation Chimeras Maintained Under SPF Conditions.* TOSHIHIKO SADO AND HITOKO KAMISAKU, National Institute of Radiological Sciences, Chiba, Japan.

Lethally irradiated mice protected with allogeneic bone marrow can escape from fatal 'secondary disease' if they are reared under the specific pathogen free conditions. Experiments were carried out, therefore, to examine the immunological status of such chimeras. The following donor-host combinations were used: CBA/H-T6 (H-2^k) – C57BL (H-2^b), C3H (H-2^k) – C57BL (H-2^b) and C57BL – C3H. The results indicated that at all time intervals studied, practically 100% of the proliferating hematopoietic cells of such chimeras were donor-derived. On the other hand, it was shown that depending on the time after induction of chimeras their spleens contained immunocompetent cells which were reactive to the host-type as well as to the donor-type alloantigens, as judged by the mortality of lethally irradiated secondary recipients of spleen cells derived from such chimeras. In spite of this fact, immunological reactivity of these 'potentially harmful' cells were suppressed *in situ* by as yet unknown mechanism. Significance of these findings to the mechanism of the maintenance of immunological homeostasis in these chimeras will be discussed.

E-14-4 *Decline of Immunological Reactivity in Irradiated Old Mouse Spleen.* JUN-ICHIRO HAYAKAWA AND TAKEHIKO TSUCHIYA, National Institute of Radiological Sciences, Chiba, 280, Japan.

In order to clarify the genesis of the decline of immunological reactivity in later life of irradiated animals, the GVH reactivity and the number of CFU of spleen were compared with continuously and single irradiated C57BL mice of 15-month old. The GVH reactivity and the number of CFU were assessed by the spleen weight method of Simonsen and the method of Till and McCulloch, respectively. Despite of large accumulated doses the continuous irradiation was affected the decline of the reactivity to a lesser extent as compared with the single irradiation. Furthermore, the reconstitution of hematopoietic tissues by syngeneic bone marrow cells immediately after single irradiation did not prevent the decline of the reactivity of spleen cells 12 months later. These results together with the results of CFU assay support the view that the decline of immunological reactivity in irradiated old spleen is probably due to alterations in nature of environment factors which are necessary to sustain growth and differentiation of the reactive cells.

E-14-5 *Effect of Microbial Restriction on Long-Term Survival of the Radiation Chimera with a Strongly Histoincompatible Combination.* TAKEO YAMAGUCHI, National Institute of Radiological Sciences, Chiba 280, Japan.

In the conventional (CV) C3H/He mice spared from lethal irradiation by transplantation with $5 - 15 \times 10^6$ bone marrow (BM) cells from C57BL/6J, no recipients survived 90 days postirradiation. Under a specific pathogen free (SPF) condition, on the contrary, all the recipients survived 270 days postirradiation. When the SPF recipients had been immunized with the spleen cells of donor strain, all the irradiated recipients died during the first month after the allogeneic BM transplantation. If the irradiated SPF mice were given 5×10^8 spleen cells from the allogeneic donor, a severe GVH reaction also killed the recipients.

When the SPF C3H mice given C57BL BM were transferred to CV condition at various times postirradiation, the long-term survival rate thereafter decreased depending on the time of transfer. Bacterial species found in the heart blood from dead animals were identified. Continuous administration of aureomycin alleviated this decreased survival rate after the transfer from CV to SPF condition.

E-14-6 *Immunogenetic Response of F_1 Hybrid Rats to Bone Marrow Transplantation.* GHISLAIN F. LINCHET, Laboratoire de Radiobiologie U.C.L., Institut du Cancer, Kapucijnenvoer 37, B-3000 Leuven, Belgium.

Hybrid F_1 rats conditioned with lethal doses of gamma rays or Myleran are treated with an increasing number of bone marrow cells from parental J013 or R strains. The quantitative evaluation of the proliferative capacity of the graft, depending on the origin, is calculated by probit analysis of the 30 days survival rates. In Myleran conditioned animals, the number of transplanted bone marrow cells required for obtaining a 50% survival reflects the antigenic differences between the host and the donor. In the same experimental system when the bone marrow graft is composed of equal parts of each parent, we find that after irradiation a greater number of cells is required than in syngeneic transplantation. Paradoxically, if Myleran is used as the host-conditioning agent, a mixed graft is more efficient than syngeneic or parental transplantation. Late mortality is analysed in relation to the number of transplanted lymphoid cells, in order to assess the importance of the donor cells in the GVH reaction. (This work is performed under the contract (G.29) Belgian Armed Forces).

E-14-7 *Mitigation of Secondary Disease in Lethally Irradiated and Allogeneic Bone Marrow Grafted Mice by Selective Elimination of Immunocompetent Cells.* OCTAV H. COSTĂCHEL, ION I. CORNECI, AND TRAIAN ANDRIAN, Oncological Institute, Bucharest 12, Romania.

The secondary disease evolution and recovery were studied in lethally irradiated (900 R) AKR mice infused with allogeneic bone marrow obtained from C57BL/6 donors after partial and selective elimination of immunocompetent cells by one of the following methods: (a) donor pretreatment by antithymocyte serum (ATS); (b) *in vitro* exposure of bone marrow cells after harvesting to the ATS action; (c) separation of immunocompetent cells from bone marrow inoculum by filtration on glass bead columns; (d) by combination of methods from points (a) and (c); (e) treatment of irradiated receivers by ATS beginning with the 5th day after grafting, and (f) by combination of methods from points (a), (c) and (e).

The most favourable results expressed by delaying of secondary disease onset, medium life-span and the greatest percentage of surviving animals at 100th day after irradiation and grafting were obtained in decreasing succession in the groups (f), (d) and (a), where survivals of 75, 70 and 60%, respectively, were recorded. Conclusion: the most important contribution to elimination and therefore to secondary disease mitigation in all cases was represented by donor treatment with ATS before bone marrow harvesting, because when treatments were separately applied the best survival was registered in the animals from these lots. Contribution of the other methods represented an increase of 100th day survival by only 10–15%.

E-15-1 *Iodine-131 Induced Dominant Lethal Mutations in Mice.* O. S. REDDI, P. P. REDDY, AND M. KRISHNA, Department of Genetics, Osmania University, Hyderabad, Andhra Pradesh, India.

^{131}Iodine as NaI (carrier free) in normal saline injected intraperitoneally in 8–10 week old random bred C_3H/He mice induced dominant lethal mutations in both males and females.

In males mutations could be recovered in all the spermatogenic stages, the spermatid stage being most sensitive.

Mating of the treated females with normal males (1–28 days) likewise produced intrauterine deaths showing that the oocyte stages are also sensitive to the mutagenic action of Iodine-131.

F_1 progeny of treated males and femals showed no signs of semisterility indicating the absence of translocation heterozygotes in the F_1 generation.

E-15-2 *Genetic Effects of Phosphorous-32 in Female Mice.* M. KRISHNA AND O. S. REDDI, Department of Genetics, Osmania University, Hyderabad, Andhra Pradesh, India.

A significant increase in the incidence of intrauterine deaths occurred following the administration of phosphorous-32 (as sodium orthophosphate) in doses of 5, 10 and 25 uCi in 9–10 week old random bred C_3H/He female mice—The loss observed for 0.5–28.5 day period exhibited the dose effect since the % dominant lethals calculated by the ratio of live embryos to corporalutea increased from 8.09% with 5 uCi to 23.75% with 25 uCi—The period-wise analysis of the data in all treated series indicates that the dominant lethals decreased from first to fourth period with the highest incidence in the first period suggesting high sensitivity to mutation of oocytes sampled in the first period than those sampled in subsequent periods—The mean number of corporalutea in treated series did not show an increase in their number when compared to control indicating the absence of superovulation—Similarly the frequency of matings in all treated groups did not differ significantly from the control—There was however a reduction in the ratio of fertile matings in 10 and 25 uCi dose groups.

E-15-3 *New Ways for Determining the Magnitude and Nature of Genetic Risks from Radiation.* LIANE B. RUSSELL AND W. L. RUSSELL, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn. 37830, USA.

Use of the specific-locus method for estimating mutation frequency in mice has also furnished new genetic tools and facilitated experiments that provide information on total mutation frequency, on the nature of genetic changes induced by different conditions of treatment, and on the effects of these genetic changes on fitness of the organism. Complementation analysis using mutants at 3 loci (*d, se, c*) has identified at least 30 complementation groups and at least 13 new functional units. It has also led to an estimate of about 20 functional units per crossover unit; and this has made possible, for the first time, a direct estimation of the number of loci in the entire genome, one of the essential figures in the calculation of total genetic risk.—Using complementation methods for characterizing genetic changes, we have shown that mutations induced in spermatogonia by X or γ rays (excluding 24-hour fractionation) closely resemble spontaneous mutations in that only a small proportion are known deficiencies. Although the proportion is greater with other conditions of irradiation, most deficiencies are short, and probably result from single-track events.—Several mutations that have been characterized as to the functional units they span are being studied for their effects in heterozygous condition, a highly important parameter for risk evaluation.—A method has been developed, using deficiencies characterized by our complementation studies, that detects mutations induced within a delimited chromosomal segment, thus yielding another approach to the estimation to total risk. (Research sponsored by U.S. Atomic Energy Commission under contract with Union Carbide Corporation.)

E-15-4 *Genetic Effects of Internal Emitters.* HAKON FRÖLÉN, Research Institute of the Swedish National Defence, Box 416, S-172 04 Sundbyberg 4, Sweden.

Radionuclides of plutonium, americium and strontium have been tested for genetic effects in mice. The main task has been to look for effects in various stages of spermatogenesis. Different doses of the nuclides were applicated to male mice of the CBA strain intraperitoneally as well as intravenously. In the tests with strontium earlier results were partly confirmed. The total rate of intrauterine death was significantly higher than in the control series, but genetic effects of ^{90}Sr in offspring to F_1– and F_2 could not be confirmed. It has been possible to demonstrate a significantly increased frequency of dead fetuses even at as low dose as 0.04 μCi americium per male. Males given 0.5 μCi and 0.1 μCi plutonium gave an exceedingly high frequency of dominant lethals during the whole mating period of 22 weeks. Some weeks the intrauterine death exceeded the control series with more than 100%. All the males in the high dose series were sterile 22 weeks after injection.

E-15-5 *Failure (?) of X-Rays to Induce Spermatogonial Histocompatibility Mutations.* HENRY I. KOHN AND ROGER W. MELVOLD, Harvard Medical School, Boston, Massachusetts 02115, USA.

The H-test, employing dermal grafts, screens at least 30 and perhaps more than 100 H-loci in the (BALB/c × C57BL/6) hybrid mouse. To test 250 kVcp X-rays, BALB/c males were irradiated to the pelvis and beginning 3 months later were mated to C57BL/6 females for periods of 1.5–2.0 years. The dosage-groups included single doses of 0, 350, 500, 650 and 800 rads; split doses, 1 day apart, of 500, 650 and 800 rads; and split doses, about 1.5 months apart, of 650 and 800 rads. Suspected mutations were proven by demonstrating their transmission to backcross progeny. No evidence for the X-ray induction of mutations has been obtained thus far (16 proven mutants, 8,700 progeny, January 1, 1974), a result that confirms a previous report for a single dose of 522 rads (Bailey and Kohn, Genet. Res. 6:330, 1965). A positive result, however, was obtained with triethylenemalamine (Kohn, Mutation Res. 20:235, 1973). Additional data from the X-ray trial will be reported.

E-15-6 *Relative Biological Effectiveness of 14.1 MeV Neutrons to X-rays for Mutation Induction in Relation to Stage Sensitivity in* Drosophila melanogaster. T. SHIOMI, I. YOSHIKAWA, AND T. AYAKI, Department of Genetics, Nagasaki University, 852 Nagasaki, Japan.

RBE values were obtained from induced frequencies of sex-linked recessive lethals and of 2–3 translocations in *Drosophila melanogaster* after irradiation of mature spermatozoa and late spermatids with 14.1 MeV neutrons and 180–200 kV X-rays. Employed dose range was 500–2380 rad. 14.1 MeV neutrons showed about the same effectiveness as X-rays for the induction of sex-linked recessive lethals when spermatozoa or spermatids were irradiated. RBEs for lethals tended to be higher with spermatid than with spermatozal irradiation, though the values are not far from unity. For the induction of 2–3 translocations the X-ray yield increased exponentially with the dose, while the neutrons showed the linear response with the dose. Thus the shape of the dose-response curves indicates that RBEs for translocations will tend to increase with decreasing dose. This tendency is more remarkable at the stage of spermatids than the spermatozoa.

E-15-7 *The Whole and Partial Y Chromosome Losses Induced by X-Rays and Neutrons in Drosophila Spermatozoa.* NORI NAKAMURA AND YOSHIO NAKAO, Zool. Inst., Hiroshima Univ., Hiroshima, Japan.

The marked *Y* chromosome is very useful for the detections of chromosome losses because any *Y* hypoploidy does not affect the viability of the flies, but fertility in the male carrying it. D. melanogaster males of 6 ± 1 day old which have the genotype $y\ sc^{8I}\ In\ 49\ sc^{8}/y^{+}\cdot Y\cdot B^{s}$ were irradiated with X-rays (1.5, 3, 4.5 and 6 kR) and with neutrons (1296, 2916 and 5183 rads). Then, they were mated with 3 females of the genotype $yf\colon = /y^{+}\cdot Y\cdot B^{s}$ individually for 24 hrs in order to sample mature spermatozoa only. The exceptional F_1 females crossed with their brothers and F_2 males were examined for their fertility. In the case of single marker loss (Partial loss) induced by X-rays, the fertile Y chromosomes increased linearly with dose, whereas sterile Y chromosomes increased with $\frac{3}{2}$ power of the dose. In the case of double marker losses, the yield increased linearly with the doses of both X-rays and neutrons. In this case, nearly all (97%) of X-ray-induced and all of neutron-induced altered Y chromosomes were sterile. From these results, we could assume that (1) the fertile Y chromosome which show single marker loss originated from a small deletion, (2) double marker lossed possibly produced by three mechanisms, (i) ring formations resulted from inter-arm unequal exchanges, (ii) interstitial deleted-X and (iii) complete loss.

E-15-8 *Attempts to Induce Non-Disjunction by Means of Irradiation and Chemical Treatment in the Silkworm.* YATARO TAZIMA, National Institute of Genetics, Misima, Japan.

Since the induction of aneuploidy by radiation attracted our great concern from the view point of genetic hazard to man, experiments have been carried out in our laboratory with silkworm. The results obtained hitherto indicate that non-disjunction can hardly be induced by direct means, although innumerable trisomic strains have been known in this organism. The mutagenic agents used so far are ionizing radiations, high temperature, CO_2 gas, organic mercury compounds and MMC. Both sex-chromosome and autosome systems have been employed. The latter system, in which egg color genes were used as markers, permitted to deal with large number of experimental individuals with relative ease.

Females heterozygous for *pe*+/+*re* were subjected to mutagenic treatment and crossed to non-treated pe re males. Individuals due to non-disjunction, if occurred, could be recovered as a wild type among F_1 eggs which comprise 1 *pe*: 1 *re*. The incidence of wild type was fairly high both in the control and treated groups: for instance, 13/59120 in the control, 25/99765 in X-irradiated groups and 41/123413 in methyl mercuric chloride groups. Wild type individuals analysed so far were 70 females and 1 male. 70 females comprised 1 *4n*, 63 *3n* and 6 *2n* and 2*n* females were confirmed as the products of recombination. One male was 2*n* but its genotype was not determined. Thus, even a case of non-disjunction of marked chromosomes has not been recovered yet, contrasting sharply to the findings in Drosophila. Almost all trisomies thus far obtained in the silkworm occurred as a consequence of chromosomal unbalance due to deficiencies, inversions and translocations. Why non-disjunction can hardly be induced by direct means may be ascribed to the holokinetic nature of the chromosome. Further analyses are in progress.

E-16-1 *Quantitative Studies on the Vascular System in the Living Tumor Tissue after Irradiation.* H. YAMAURA AND T. MATSUZAWA, Radiology and Nuclear Medicine, The Research Institute for Turberculosis, Leprosy and Cancer, Tohoku Univerity, 4–12 Hirosemachi, Sendai 980, Japan.

To observe quantitatively the tumor blood vessels in the living tissues, our newly devised transparent-chamber technique was applied to the transplanted rat tumor (AH109A) through the experiments. The details of this technique have been described elsewhere (H. Yamaura, et al 1969). The process of the tumor vessel development was divided into four stages, according to the changes of the vascular three parameters (volume, suface area and length). From the first to second stage the vascular formation in the developing tumor tissue was 40–50 times as rapid as in that of the non-tumor granular tissue. In third and fourth stage, these blood vessels decreased rapidly and reached to zero. The reproductive capacity of the blood vessels in the normal and tumor tissues after irradiation was estimated by our technique. The mean lethal dose (Do) and the extrapolation number (n) of the capillaries will be reported in the present paper.

E-16-2 *Oxygenation in the Radiation Response of Three Hamster Sarcomas.* HUBERT A. EDDY AND GEORGE W. CASARETT, University of Rochester School of Medicine and Dentistry, Rochester, N.Y. 14642, USA.

The radiation responses of three hamster sarcomas (Reticulum Cell Lymphosarcoma, Malignant Neurilemmoma and Fibrosarcoma) were compared following single brief X-ray exposures delivered during tumor euoxia or anoxia. In the nonirradiated tumors, quantitative determination of vascular density gave values of 15.5, 9.6 and 6.7 vessels per 150,000 u^2 of tumor area respectively. Under euoxic radiation exposure conditions, 50% tumor control values showed a direct relationship between vacular density and dose required for tumor control. Lifetime TCD_{50} values for the three sarcomas were 6,300, 6,050 and 4,060 R respectively. Anoxia, imposed for 30 minutes before, during, and for 30 minutes after irradiation of each tumor, increased the TCD_{50} for the Reticulum Cell Lymphosarcoma and the Malignant Neurilemmoma by factors presently calculated to be 2.27 and 2.03 respectively. Anoxic conditions did not significantly alter the TCD_{50} of the Fibrosarcoma. These and observations following radiation exposure during hypoxic conditions will be discussed.

E-16-3 *^{133}Xenon Clearance Test in Investigations of the Vascular System in Human Tumors.* G. ARCANGELI, V. AURELIO, M. BENASSI, C. NERVI, AND R. PAOLUZI, Regina Elena Institute for Cancer Research, Roma, Italy.

The functional aspect of tumor vascularity has been studied by means of interstitial and intra-arterial ^{133}Xe Clearance Tests in primary and metastatic squamous cell carcinoma of the head and neck in man. Local Clearance Test was not reproducible because the probable inhomogeneity of the tumor vascular system does not allow one to extract a significant index of vascularization. A biexponential-like ^{133}Xe disappearance curve has been obtained in our cases. The analysis by means of a mathematical model suggests that this behavior of the clearance curve is related to inhomogeneity of the blood flow even within a volume as small as .1 ml. Even when injecting the tracer intra-arterially a biexponential-like disappearence curve was obtained, but in these cases a satisfactory reproducibility was reached, so that a good evaluation of the blood flow distribution could

be obtained. The effect of chemotherapy and/or radiotherapy on vascularity will be referred and discussed.

E-16-4 *Hemodynamics During Tumor Growth in Irradiated and Unirradiated Sites.* RANDY JIRTLE AND KELLY H. CLIFTON, University of Wisconsin Medical School, Madison, Wisconsin 53706, USA.

The relative vascular space remains constant as the "grossly viable" mass of adenocarcinoma strains 328, MTG-B, and CA755 increase (*Cancer Research* 33: 764, 1973). However, the host hematocrits decrease from 50% to 30% during tumor growth. With such an apparent decrease in the oxygen-carrying capacity per unit volume of blood, it was of interest to determine the mechanisms which allow the same vascular space to maintain the same grossly viable tumor tissue mass in small and large tumors. The total red cell volume (TRCV) in BDF_1 female mice remained constant when 328 gastric adenocarcinomas were grown in either unirradiated or irradiated host tissue and when CA755 tumors were grown in pre-irradiated sites. However, the TRCV decreased significantly after 10 days of CA755 tumor growth in unirradiated host tissue, due in part to an elevation in the erythrocyte loss rate. The heart rates of resting CA755 tumor-bearing mice increased 100 beats per minute at the time the TRCV decreased. Thus, the reduction in hematocrit in tumor-bearing mice resulted from hemodilution alone, or hemodilution plus erythrocyte loss, depending upon the tumor type and the radiation history of the transplantation site. In the latter case, an increase in heart rate, and presumably cardiac output, accompanied the decrease in TRCV. (Supported by ACS Grant DT-35-0 and NIH Center Grant CA-06295.)

E-16-5 *Quantitative Evaluation of Tumor Vascularity Following X-Irradiation.* DUANE E. HILMAS AND EDWARD L. GILLETTE, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland 21701, and Colorado State University, Fort Collins, Colorado 80521, USA.

Morphometric methods were used to evaluate changes in the microvasculature of 8-mm diameter C3H/Bi mouse mammary carcinomas following exposure to split or fractionated doses of x-irradiation. The contrast agent, colloidal carbon prepared for biological studies, was injected intravenously. Estimates were made of percent vascular volume, vascular surface area, vessel length, average vessel diameter and percent necrotic volume. Changes in tumor volume were also measured.

Following six 500-R daily fractions, tumor volume did not change significantly. However, mean vessel length and surface area reached maximum values 72 hours following the sixth fraction. Similarly, estimates of vessel diameter and vascular volume reached minimum values at this time. In another study, designed to evaluate the early postirradiation period, a single exposure of 1500 R or two 750-R fractions separated by 48 hours, resulted in similar changes in the vascular estimates at 12 hours postirradiation. Changes in these vascular estimates are attributed to possible improvement in colloidal carbon filling of previously existing unfilled, nonfunctional vessels. If anatomically derived values of vascular dimensions are related to a metabolically useful blood supply, then an improved capability for exchange of essential nutrients occurred following radiation treatment.

E-16-6 *The Effect of Temperature and Radiation on Tumor Blood Flow.* R. J. R. JOHNSON, Gray Laboratory of the Cancer Research Campaign, Mount Vernon Hospital, Northwood, Middlesex, Great Britain.

In vivo blood flow and cell loss rates have been measured in 4 tumour lines implanted in the rear limbs of WHT and CBA mice.

The effect of a single dose of 1200–2000 rads and alterations in cell loss rate achieved by maintaining animals at 5°C have been observed.

Blood flow at 22°C and 40°C was assessed with a thermodynamic system which measures the thermal flux induced by an insulated constant temperature heat sink positioned over the tumour.

Cell loss was estimated by an *in vivo* IUdR method which corrects for the skin count factor.

Results show that tumour blood flow is decreased by 50% to 75% at 22°C due to hypothermic vasoconstriction. The vasoconstriction can be reduced by a single dose of 1500 rads, which increases blood flow both at 40°C and at 22°C. The time period at which the increased blood flow occurs is associated with an increased rate of loss of IUdR from the tumour.

No gross change in blood flow occurred following radiation when the cell loss was decreased by keeping the animals at 5°C.

The reduction of the hypothermic vasoconstriction response following radiation suggests the use of hypothermia prior to and during a second dose of radiation since the accompanying decreased O_2 consumption without a radical decrease in blood flow should improve tumour oxygenation.

E-16-7 *Radioprotective Effect of Methylene Blue on Serum Enzymes of Rats.* Sang Yul Nam, Department of Biology, College of Liberal Arts & Sciences, Kyung Hee University, Seoul 131, Korea.

Male Sprague-Dawley rats were given 360 rads of single whole-body gamma-irradiation 28 to 32 minutes following an intraperitoneal injection of physiological saline or methylene blue (40 mg/lg). Serum glutamic oxalacetic transaminase (SGOT), serum glutamic transaminase (SGPT), serum alkaline phosphatase (SALP), and serum acid phosphatase (SACP) activities were determined at various time intervals after exposure. The changes in these serum enzyme activities in the control and the methylene blue-treated rats were followed up to 460 hours after irradiation. Methylene blue delayed singificantly the SGOT rise at 25, 130, and 460 hours, and the SGPT rise at 25, 85, 130, 190, and 320 hours, respectively. Also, methylene blue delayed significantly the SALP rise at 6, 25, and 190 hours, and the SACP rise at 6 and 130 hours, respectively. Therefore methylene blue may protect SGOT, SGPT, SALP, and SACP-liberating tissues. Protective action of methylene blue against gamma-irradiation in rats is discussed.

E-17-1 *Mathematical Analysis of the Destruction of the Base Moieties of Thymidine 5'-Monophosphate and Polydeoxythymidylate in Solution by X Irradiation.* Donald E. Hoard, F. Newton Hayes, and Walter B. Goad, Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico 87544, USA.

Solutions of thymidine 5'-monophosphate (*d-TMP*) also containing additional free-radical scavenging solutes were subjected to x irradiation. Mathematical analysis applied to the changes produced in the ultraviolet absorption spectra indicates that hydroxyl radicals, hydrogen atoms, and hydrated electrons destroy the thymine base moiety. The theoretical treatment which leads to this conclusion is described. Application of the analysis to similar experiments in which solutions of polydeoxythymidylate (poly *d-T*) are irradiated is also discussed. (This work was performed under the auspices of the US. Atomic Energy Commission.)

E-17-2 *Comparative Studies on Carbon Free-Radical Distributions Among Individual Amino Acid Residues of Native and Fully Reduced-Carboxymethylated (RCM) Lysozymes after Gamma-Irradiation and Exposure to Electrical Discharge.* F. H. White, Jr. and A. G. Wright, Jr., N.I.H., Bethesda, Maryland 20014, USA.

The reaction of carbon free-radicals in irradiated proteins with tritiated H_2S, followed by degradative and analytical procedures to determine the distributions of tritium among the amino acid residues, has already been employed as a means for studying free-radical distributions. In continuation of an earlier project (White, Radiation Res. **36**, 470 (1968)) designed to study the radical distribution among individual residues in gamma-irradiated native lysozyme, the native and RCM forms of this protein were exposed to electrical discharge (White, *et al.*, Radiation Res. **45**, 8 (1971)), as an alternative means of radical production, and then tritiated. As had been found for gamma-irradiated lysozyme, many residues associated with the "hydrophobic box" were heavily labelled, and thus a relationship between conformation and radical distribution was confirmed. Further evidence of radical distribution as a function of conformation was seen when native lysozyme, exposed to discharge and then tritiated, was compared with its RCM derivative, identically treated, since many differences appeared in relative specific activity (RSA). Although the RSA value of every residue in a tritiated randomly coiled chain should equal unity, with conformation assumed as the only contributing factor to differences in radical distribution between native and denatured states, many higher activities appeared in RCM lysozyme, particularly from residue 54 to 77. The available evidence is compatible with the hypothesis that the free-radical distribution in RCM lysozyme was influenced by a small amount of remaining conformation.

E-17-3 *Radiolysis of Ribonuclease in the Presence of Ethanol.* HELGA SCHUESSLER, Institut für Physikalische und Medizinische Strahlenkunde der Universität Erlangen-Nürnberg, Erlangen, West Germany.

X-ray inactivation of ribonuclease in the presence of ethanol shows a different extent of the protection effect for the two functions of the enzyme, which may be explained by the prevention of aggregation under these conditions. Comparing the dependence of x-ray inactivation on the enzyme concentration under different conditions yields in the presumption that the radiation protection for both functions by ethanol is caused by the scavenging of OH-radicals. Further investigations, however, indicated an interaction of ethanol radicals with ribonuclease which is very specific as shown by amino acid analysis. This interaction became also obvious by gelfiltration studies. To exclude additionally to OH·radicals the solvated electrons from reaction with ribonuclease the same measurements were repeated after irradiation under N_2O. No increase in radiation protection was observed, and the obtained results confirmed the interaction of ethanol radicals with ribonuclease which must contribute to the inactivation of the enzyme.

E-17-4 *Chemistry and Biology of Irradiated Solutions of Monosaccharides.* JACK SCHUBERT, University of Pittsburgh, Graduate School of Public Health, Pittsburgh, Pennsylvania 15261, USA.

Upon gamma irradiation of oxygen-free aqueous solutions ($\sim$0.06-0.3M) of monosaccharides, each carbon atom is attacked. However, chain scission reactions are minor ($G < 0.1$). The OH radicals are largely responsible for the radiolytic attack as demonstrated by scavenger studies. The primary radiation act is hydrogen abstraction following which the sugar radical undergoes different reactions including loss of H_2O, and disproportionation, leading to the formation of the observed products. Thus, from irradiated glucose we find the principal products include gluconic acid ($G = 0.19$), 2-deoxy-*D*-gluconic acid ($G = 0.62$), and total carbonyls ($G = 1.0$). The yields increase in N_2O, as, for example, carbonyls ($G = 1.7$). The spectrophotometric and cytotoxic properties are due to α,β-unsaturated carbonyl sugars derived from radiolytically produced dicarbonyl sugars and deoxy dicarbonyl sugars which undergo dehydration and enolization. The cytotoxic properties include inhibition of bacterial growth and chromosome breakage *in vitro*. However, no significant biological effects are observed when irradiated sugars are ingested by mammals because the very high chemical reactivity of the α,β-unsaturated carbonyl sugars prevents significant concentrations from reaching target cells (Work supported in part by the U.S. Atomic Energy Commission, Division of Biomedical and Environmental Research, Contract AT(30-11-3641)).

E-17-5 *Effect of Salt Concentration on the Reactions of the Radical Anions $(CNS)_2^-$ and Br_2^- with Several Enzymes.* J. L. REDPATH, L. I. GROSSWEINER, AND J. OVADIA, Department of Medical Physics, Michael Reese Medical Center, Chicago, Illinois 60616, USA.

The radical anions $(CNS)_2^-$ and Br_2^- have been used as selective probes in the study of the mechanisms of inactivation by ionizing radiation of several enzymes in dilute solution.[1] Reactivities of these radicals with the various enzymes have been measured and transient spectra observed and identified. These measurements were generally made at a single salt concentration although preliminary data indicated that the reactivities and transient spectra may be dependent on salt concentration. This paper presents a more detailed investigation of these effects.

Data will be presented on the effect of salt concentration on the following parameters:

1. Reactivity of enzyme with radical anion
2. Nature of transient product spectra
3. U. V. Spectra of the enzymes
4. Activity of the enzymes

(This work was supported by the National Cancer Inst. Grant # CA-13307-01).

[1] G. E. Adams, J. L. Redpath, R. H. Bisby and R. B. Cundall, Israel J. Chem., **10**, 1079–1093, (1973).

E-17-6 *Radiolysis Products of Sugars in Aqueous System and Their Antibacterial Eflect.* MITSUO NAMIKI, YUKIO KITO, AND SHUNRO KAWAKISHI, Dept. of Food Science & Technology, Nagoya University, Chikusa-ku Nagoya, Japan.

To elucidate the antibacterial factors of irradiated sugar solution,[1] glucose and fructose in redistilled water were γ-irradiated (2 Mrad) in air or in air free, and the products were isolated and identified by using GLC and MASS on the reduction products of irradiated solutions with $NaBH_4$ or $NaBD_4$.

Glucose irradiated in air gave *D*-ribo-hexos-3-ulose, *D*-gluconolactone, *D*-arabino hexosulose as main products and other hexosuloses and C-4, C-3, C-2 degradation products as minor ones, while in air free it provided mainly deoxy-dicarbonyl-hexoses as 2-deoxy-*D*-gluconolactone.

Fructose in air gave especially 5-ketofructose as main product and in air free furnished also various deoxysugars as 1-deoxy-*D*-threo-2,5-hexodiulose.

The antibacterial effect was tested by E. coli in the growth and recombination ability. The fractions involving the deoxysugars showed some activity.

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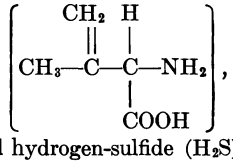
[1] M. Namiki et al, Agr. Biol. Chem., **37**, 989 (1973).

E-17-7 *The γ-Radiolysis of D-Penicillamine in Dilute Aqueous Solutions.* G. C. GOYAL AND D. A. ARMSTRONG, Department of Chemistry, The University of Calgary, Calgary, Alberta, T2N 1N4, Canada.

The Co^{60} γ-radiolysis of *D*-Penicillamine in dilute aqueous solutions has been studied under conditions such that products of the reactions of e_{aq}^-, OH, Br_2^-, $\cdot CH_2OH$ and other radicals can be determined. Satisfactory radical and material balances were achieved, and the main products were: Penicillamine disulfide (PenSSPen), Penicillamine trisulfide (PenSSSPen), valine (PenH), 2-amino-3-methylbut-3-enoic acid



ammonia (NH_3), hydrogen (H_2) and hydrogen-sulfide (H_2S). The mechanism for the formation of these products will be discussed.

E-17-8 *The Molecular Mechanism of Action of WR 2721.* WOLFGANG LOHMANN, A. HAHN, AND M. HILLERBRAND, Abteilung für Biophysikalische Chemie, GSF, D-8042 Neuherberg, West Germany.

The radioprotective effect of WR 2721 on catalase and the type and loci of its interaction with the enzyme have been investigated by means of spectrophotometric and electron spin resonance (ESR) methods. The radiation damage, indicated by a change in enzymatic activity and in the Soret absorption band, has been the less the larger the WR 2721 concentration. In the case of ESR investigations, addition of WR 2721 has resulted in a reduction of the spin concentration of Cu^{2+}. Since cysteamine has exhibited similar results, however, to a lesser extent, it can be assumed that the RS^- ions are responsible for the protective effect. From the results obtained it can be concluded that (the dephosphorilized WR 2721 forms a complex with the enzyme and acts as an electron donor. (This work was supported in part by Fraunhofer-Gesellschaft).

E-18-1 *The CO^{60} γ-Ray Inactivation of Papain—the Nature of the Repairable Damage.* G. M. GAUCHER, W. S. LIN, AND D. A. ARMSTRONG, University of Calgary, Calgary, Alberta, Canada.

Proteins are the cell's most functionally versatile macromolecules. They are essential to cellular structure, metabolism, and regulation and to the repair of damaged DNA. The radiation inactivation of a variety of structurally well characterized enzymes has been examined. However despite an increasing understanding of amino acid residue susceptibilities, the exact nature of the damage responsible for the inactivation of most enzymes is not known. In a continuing study of the γ-ray inactivation of papain in dilute aqueous solution, the molecular identity of a major inactive form

of papain was established. The non-irradiation inactivation of papain solutions by H_2O_2 was shown to be 92% cysteine repairable, 73% of this repairable damage being due to the formation of papain sulfenic acid (PSOH). The irradiation of N_2O and Air saturated papain solutions in the presence of a peroxide scavenger almost totally eliminates any repairable damage. This and other data to be presented indicate that PSOH is the major form of repairable damage. In N_2 saturated solutions, e_{aq}^- appears to compete with or lower the concentration of H_2O_2 to yield less PSOH. These conclusions concerning the nature of repairable papain damage, are supported by the fact that PSOH prepared by H_2O_2 inactivation of papain, as well as non-repairable and repairable components of irradiated papain have been separated from native papain and each other by affinity chromatography.

E-18-2 *Release from Substrate Inhibition in Gamma-Irradiated Lactic Dehydrogenase.* MASAHIRO SAITO AND HIROTOSHI MAKI, Research Reactor Institute, Kumatori-cho, Sennangun, Osaka, Japan.

Dilute solutions of pig heart lactic dehydrogenase and chicken heart lactic dehydrogenase were irradiated by Co^{60} gamma rays. The dose effect curves for the both LDH isozymes varied in shape depending on the pyruvate concentration as substrate. At a lower pyruvate concentration the dose effect curves exhibited exponential loss. However, at a excess concentration of pyruvate, the remaining enzymatic activity initially increased with increasing radiation dose. The *S-V* plot of the irradiated enzymes showed that the pyruvate inhibition at excess pyruvate concentration is considerably depressed by gamma irradiation. Along with this observation, it was also found that the formation of an abortive ternary complex NAD-pyruvate-enzyme is depressed by gamma irradiation. These results show that the regulatory site for substrate inhibition was destroyed without loss of the LDH activity. The acetylation of the LDH isozymes by *N*-acetyl imidazole revealed that the acetylation of tyrosine residues results in a loss of active site along with a decreased substrate inhibition. Tyrosine residues are suggested to be involved in the mechanism of the decreased substrate inhibition of irradiated LDH isozymes.

E-18-3 *Radical Induced Scission of the Glycosidic Bond of Cellobiose in Aqueous Solution.* CLEMENS VON SONNTAG, MIRAL DIZDAROGLU, AND DIETRICH SCHULTE-FROHLINDE, Institut für Strahlenchemie im Max-Planck-Institut für Kohlenforschung, Mülheim (Ruhr), West Germany.

In the γ-radiolysis of deoxygenated N_2O saturated aqueous solutions OH-radicals and H atoms abstract carbon bound hydrogen from cellobiose (10^{-2} *M*). Cellobiose is attacked at all positions although some selectivity still persists. Among the products there is a series of sugars with $C \leq 6$ *G*-values: glucose (2.0), gluconic acid (0.7), 5-keto-glucose (0.1), 4-deoxy-glucose (0.27), 5-deoxy-gluconic acid (0.18), 2-deoxy-gluconic acid (0.13), 3-deoxy-4-keto-glucose (0.23), 2-deoxy-5-keto-glucose (0.34), 4-deoxy-5-keto-glucose (0.14), arabinose (0.07), 2-deoxyribose (0.17).

These products allow conclusions on the mechanism of the radical induced scission of the glycosidic linkage to be drawn. Scission follows only after attack at C-4 and C-1′ either by hydrolysis or by a rearrangement under the formation of a C=O double bond. In a similar way the radical at C-5′ splits by a rearrangement in which the radical position migrates to C-1′. Hydrogen abstraction from other positions does not lead to a scission of the glycosidic bond. In the product formation water elimination and decarbonylation processes often preceed the disproportionation reactions of the radicals. From the G-values of the products it follows that 1/3 of the attacking radicals abstract hydrogen from the positions C-4, C-1′, and C-5′.

E-18-4 *Specific Inactivation of Bovine Carbonic Anhydrase as the Consequence of X-Ray Resonance Absorption in the Constituent Zinc Atom.* B. DIEHN, Department of Chemistry, The University of Toledo, Toledo, Ohio 43606, USA, A. HALPERN AND G. STOCKLIN, Institut fur Nuklearchemie der Kernforschungsanlage Julich, 517 Julich, West Germany.

The inactivation of bovine carbonic anhydrase irradiated in the solid phase with monoenergetic low-energy X-rays has been investigated. Upon irradiation of the metalloenzyme, efficient inhibition of its esterase activity was observed. The inactivation normalized to an equal dose absorbed was shown to depend on the X-ray energy. For photon energies slightly above the Zn *K*-edge the inactivation significantly exceeds that expected on the basis of energy absorption. The loss of Zn atoms from molecules upon irradiation was also studied by means of ion-exchange chromatography.

The amplifier effect is interpreted in terms of Auger charging resulting from inner shell vacancies in Zn atoms. Complete and spontaneous recovery of enzyme activity occurs within an hour after dissolution.

E-18-5 *Radical Formation, Strand Breakage, and Inactivation of DNA Exposed to Excited Inert Gases.* HERMANN DERTINGER, CHRISTINE LÜCKE, AND KARL-FRIEDRICH WEIBEZAHN, Institut für Strahlenbiologie, Kernforschungszentrum Karlsruhe, West Germany.

Inert gases excited to metastable states in a glow discharge provide a useful system for clarifying certain problems in the field of early radiation effects. After exposure of dry DNA and its constituents to excited N_2, Ar and He, radicals resulting from dissociation of CH-bonds of the bases as well as addition products of the atomic hydrogen liberated thereby were identified by EPR-spectroscopy. In addition, DNA of phage øX 174 was tested for strand breaks and the loss of infectivity. Approx. 76% of the DNA molecules were found to be inactivated by strand breaks, this figure as well as radical formation being independent of the energy of the metastables. These results which are in contrast to those obtained after ionizing irradiation are discussed in terms of different mechanisms of energy transfer.

E-18-6 *On the Activity of Glucose-6-Phosphatase and Fructose 1,6-Diphosphatase in the Red Blood Cells of Rat After X-Irradiation.* M. MOISIU AND L. ABABEI, The Institute of Medicine and Pharmacy, The Institute of Public Health and Medical Research, Iassy, Romania.

Glucose-6-Phosphatase (G-6-Pase) and Fructose-1,6-Diphosphatase (F-1,6-DPhase) activity is demonstrated in human, rat, rabbit erythrocytes as in the rabbit reticulocytes. By increasing the irradiation rate, the activity of these two enzymes decrease. The decrease is fully evident in the early 24 hours after irradiation and it seems to be dependent upon the irradiation dose rather than upon the time of irradiation. Some 24 hours after total irradiation with 600 R, the G-6-Pase activity in rat erythrocytes fell from 3.56 to 1.90 μmoles/ml cells/h. Some 24 hours after total irradiation with 600 R, the F-1,6-DPase activity fell from 2.95 to 1.30 μmoles/ml cells/h. Reduced glutathione and cysteine restore the activity of the enzymes.

E-18-7 *Aldolase Inactivation Following Addition of Radiation-Induced Free Radicals.* W. D. FELIX AND D. R. KALKWARF, Battelle Pacific Northwest Laboratory, Richland, Washington 99352, USA.

Studies were made of the role of specific free radicals on the inactivation of the enzyme, aldolase. The technique used allows the study of specific radical-substrate reactions without interference of competitive radical species. Radicals were prepared by exposure of crystalline biochemicals to gamma radiation. Concentration and general structure of the radicals were determined by electron spin resonance measurements. Known quantities of radicals were added to aqueous buffered solutions of aldolase; radical-enzyme reactivity was determined as an inverse function of the enzyme activity. Whereas addition of the irradiated crystals caused inactivation, normal or only slightly decreased activity was noted when aldolase was added to solutions of predissolved irradiated crystals. (This paper is based on work performed under United States Atomic Energy Commission Contract AT(45-1)-1830.)

E-19-1 *Effect of Thymine Hydroperoxide on Nucleic Acid Bases.* SHIH YI WANG AND LINDA PARKHILL, Department of Biochemistry, The Johns Hopkins University, Baltimore, Maryland 21205, USA.

cis-5-Hydroxy-6-Hydroperoxy-5,6-dihydrothymine (TOOH) is the major product resulting from γ-radiation of aerated aqueous solutions of Thy and has been implicated as a major product in γ-irradiated DNA. The studies presented here were undertaken to evaluate the effect of TOOH, if formed in DNA, on its neighboring bases or vice versa. When Ade, Gua, Cyt, Thy, or Ura was treated with an excess of TOOH, Ade and Ura were found to be resistant to TOOH while Gua, Cyt, and Thy gave rise to many products. There are two interesting findings: 1) Cyt is converted to N(3)-oxide in the presence of TOOH which, in turn, reacted to give 85% 5-methyl-5-hydroxyhydantoin and 11% of Thy glycol, and 2) Thy in the presence of TOOH yields Thy glycol as the product for both compounds. This demonstrates dramatically the effect of neighboring bases on the eventual fate of radiation products in nucleic acids and their possible biological consequences.

(This research is supported by US. Atomic Energy Commission Contract AT(11-1)-3286 and is identified as No. COO-3286-8.)

E-19-2 *Irradiation Effects on Deoxyribonucleoprotein in the Presence of Ca Ion.* GIICHI YOSHII, MIKINORI KUWABARA, AND MASANOBU HAYASHI, Radiobiology, Vet. Med., Hokkaido University, Sapporo, Japan.

Larger part of radiation energy is absorbed in the protein moiety of the DNP. ESR studies have shown that the spins induced by irradiation in DNP solution transfer preferentially on the DNA moiety before labilizing the nucleicacidprotein linkage. In the presence of Ca ion, the complex formation between Ca and two neighouring phosphates of the DNA moiety prevents the spin transfer from the protein to the DNA moiety.

The biological activity tests have shown that there is an initial increase in the priming ability of the DNP for RNA polymerase after irradiation, but with greater doses the priming ability decreased. In the presence of Ca, the priming ability of the DNH less increased with smaller doses, but decreased more rapidly with greater doses.

The physico-chemical properties and the biological activities in the irradiated DNP solution without or with Ca ion are discussed.

E-19-3 *Effects of Ultraviolet Light and X-Rays on Poly-L-Glutamic Acid and Poly-L-Lysine in Aqueous Solution.* MITSUO ISHIKAWA, AND KAORU TAKAKURA, International Christian University, Osawa, Mitaka, Tokyo, Japan.

The effects of ultraviolet light and X-rays on poly-*L*-glutamic Acid (PLGA) and poly-*L*-lysine (PLL) in aqueous solution were studied in different secondary structures, helix, β, and random coil forms.

X-rays and ultraviolet light of wavelength below about 2300 Å caused degradation of the polypeptides by indirect action, while light of wavelength above 2300 Å brought about degradation by direct action. It was found that the $[\eta]$-*PH* curve for PLGA passed through a minimum in the transition region.

The decrease in helix content caused by X-rays and ultraviolet light of wavelength above 2300 Å was almost the same, and it was attributed to the increase of small molecules of PLGA which cannot exist in helix form.

Irradiation of monochromatic ultraviolet light showed that light of wavelength near 2000 Å could bring about considerable decrease in helix content, which could not be ascribed only to degradation.

E-19-4 *The Effect of Ionizing Radiation on Protein Fractions of Ribosomes and Their Electrophoretic Mobility.* G. T. LANDRES, E. V. BOUDNITSKAYA, V. N. STOLETOV, A. N. Bach, Biochemistry Institute, USSR Academy, Moscow, M. V. Lomonosov State University, Moscow, USSR.

The composition of ribosomal proteins has been studied by disk-electrophoresis in polyacrylamide gel. Ribosomal proteins were isolated from embryoes of wheat air-dried seeds, and 24, 72 hours after their germination. The seeds were subjected to preliminary radiation at GUPOS-CS-131 unit with a power of 610 r/min. Radiation doses were 0.5; 5 and 40 kr. It has been found that the effect of gamma-radiation resulted in quantitative changes in the protein fractions studied, appearance of minor components, and a change in their electrophoretic mobility. The data obtained indicate that gamma-radiation causes essential changes in ribosomal protein fractions depending on a dose of radiation and functional state of ribosomes.

E-20-1 *Photoconductivity of γ-Irradiated Organic Glasses.* G. C. DISMUKES AND J. E. WILLARD, Department of Chemistry, University of Wisconsin, Madison, Wis. 53706, USA.

This paper will discuss the electrical currents induced in γ-irradiated low temperature organic glasses exposed to narrow bandpass visible and infrared light from a tunable laser. Evidence to date shows that 1) photocurrent thresholds for 3-methylpentane (3MP) and 3-methylhexane (3MHx) at 77°K are near 2500 nm and for 2-methyltetrahydrofuran (MTHF) near 1100 nm; 2) the photocurrents produced in 3MP and 3MHx by 1700 nm light and in MTHF by 659 nm light show first order dependence on the light intensity, with no indication of biphotonic processes; 3) exposure of γ-irradiated 3MP and 3MHx glasses to light at wavelengths shorter than the maximum

of the absorption spectrum of trapped electrons shifts electrons to weaker traps with resultant enhancement of the photocurrent produced on subsequent exposure to infrared light; 4) the enhanced currents decrease more rapidly during infrared exposure than the same current levels prior to enhancement. These results, in conjunction with results on the shifting to trapped electron spectra as observed by optical spectrometry, imply that electrons in hydrocarbon glasses are trapped with a wide spectrum of energies. Further implications about the trapping and detrapping processes in the three matrices will be discussed in terms of the data.

E-20-2 *Electric Field Effect on Free Ion Yields: Influence of Multipair Spurs.* JEAN-POL DODELET AND GORDON R. FREEMAN, University of Alberta, Edmonton, Alberta, T6G 2G2, Canada.

The field effect has been studied theoretically for several liquids that have different free ion yields at zero field. The theoretical model includes a distribution of spur sizes with populations ranging from one to seven ion-electron pairs per spur. The positive charged core of the spur was regarded as a point charge and electron-electron repulsion was neglected. Different distributions of electron ranges were tested. The only distribution function that provided agreement between calculated and experimental yields in all the liquids over a range of temperature was a simple exponential $(b_E^{-1} \exp [(y_0 - y)/b_E])$ to which a longer (y^{-3}) tail had been added for the outermost 3% of the electrons. By contrast, the best distribution function for use in a single-pair-spur model of the field effect was a Gaussian $([y^2/b^3] \exp [-y^2/b^2])$ to which a longer (y^{-3}) tail had been added for the outermost 5% of the electrons. For smaller and smaller free ion yields the multipair-spur model degenerates into the single-pair-spur model, independent of the shape of the inner portion of the electron range distribution function. Introduction of multipair spurs into the model caused the estimated total ionization yield to increase and skewed the electron range distribution more towards the lower ranges.

E-20-3 *The Dose-Rate Dependence of the Yield of Localized Excess Electrons in Crystalline Ice.* GÖSTA NILSSON AND PALLE PAGSBERG, AB Atomenergi, Studsvik, Fack, S-611 01 Nyöping and Danish AEC Research Establ, Risö, DK-4000 Roskilde, Denmark.

The pulse radiolysis technique was used to study the yield of localized excess electrons in single crystals of ice as a function of the dose rate using the Febetron 705 electron accelerator at Risö as the radiation source. The dose was measured by means of a chemical and a calorimetric dosimeter and the maximum dose was about 120 krad. When the temperature was varied between -10 and -55°C it was found that the yield, besides decreasing with decreasing temperature, also approached a constant value at each temperature when the dose rate was increased at constant pulse length. A theoretical interpretation of the results will be presented.

E-20-4 *Electron Mobility in Xenon in the Critical Region.* TOYOAKI KIMURA AND GORDON R. FREEMAN, University of Alberta, Edmonton, Alberta, T6G 2G2, Canada.

Electron mobilities have been measured in liquid xenon at temperatures from 167.5K (triple point 161.3K) through the critical point, 287.7K, up to 315K. A pulse radiolysis technique was used. There is a maximum in the mobility at 220K, larger than the one that occurred in argon near its critical temperature. The minimum in mobility predicted by Lekner to occur in the heavy inert fluids near their critical regions does not exist in xenon.

E-20-5 *Temperature Inversion Effect on the Radiolytic Yield of Free Ions in the Presence of an External Field in Liquid Hydrocarbons.* A. MOZUMDER, Radiation Laboratory, University of Notre Dame, Notre Dame, Indiana 46556, USA.

A theoretical analysis originating with the Onsager formula reveals that, under suitable conditions, the probability that an ion-pair will escape mutual neutralization in the pesence of an external electric field can actually decrease with temperature. This phenomenon, referred to as the *temperature inversion effect,* depends on the form of the initial distribution function, variation of dielectric constant with temperature and on the temperature range itself. Necessary conditions for actual experimental observation are delineated together with suggested experimental liquids and ranges of temperature and field strengths. An explanation of the effect in terms of dipolar orientation is offered.

E-20-6 *Effect of Biologically Important Solutes on Charge-Carrier Decay Processes in n-Hexane.* E. C. Gregg and R. D. McCreary, Dept. of Radiology, Case Western Reserve University, Cleveland, Ohio, and George Bakale, Hahn-Meitner Institut für Kernforschung, Berlin GmbH, Bereich Strahlenchemie, 1 Berlin 39, West Germany.

The kinetics of electron attachment and ion recombination processes were studied by monitoring the electron-ion current decay in a pulse-irradiated parallel plate ion chamber. Solutes of biological importance were dissolved in *n*-hexane and the electron attachment rate constants and the mobilities of the anions resulting from the solute attachment were determined. The electron attachment rate constant to *para*-benzoquinone, a typical radiation-sensitizing agent, is 1.3×10^{12} l.-mole^{-1} sec^{-1} at 298°C which contrasts sharply with attachment to l-cystine, a radiation-protective additive, with which no reaction with the quasifree electron was detectable. The activation energy of the para-benzoquinone attachment reaction is ~7 kcal/mole which is ~2 kcal/mole greater than the activation energy of the mobility of the electron in *n*-hexane. Electron attachment to other solutes will be discussed along with the effect of the product ions' mobility upon the ion recombination rate. (Work supported by U.S.A.E.C.)

E-21-1 *Effects of 1000 Rads ^{60}Co on Baroreceptor Reflex Responses to Phenylephrine and Carotid Occlusion in Monkeys.* A. Bruner, A. W. Neely, E. A. Henderson, and G. K. Weiss, Lovelace Foundation, 5200 Gibson Blvd., S.E., Albuquerque, New Mexico 87108, USA.

Failure of the baroreceptor reflex mechanisms has been proposed as a basis for the early hypotension seen in monkeys following high-dose, whole-body irradiation. The present work involved the testing of baroreflex sensitivity by carotid occlusion and phenylephrine injection before and after 1000 rads ^{60}Co in nine unanesthetized, restrained monkeys. During the early postradiation minutes, at the time of deepest hypotension, both of the baroreflex tests revealed depressed baroreceptor sensitivity (diminished blood pressure and heart rate responses). After 8 to 15 minutes postirradiation, the phenylephrine, but not the occlusion test, demonstrated a reversal to significant baroreceptor hypersensitivity which persisted 24 hours or more. Early shifts in the set level relation between blood pressure and heart rate also occurred following irradiation. No failure of the baroreflex mechanisms was evident, in contradiction to a previous finding.

E-21-2 *The Neurosecretory System of Rats Subjected to Local Head X-Irradiation.* Z. Srebro and Krystyna Dubis, Department of Biology, Medical Academy, Kraków, Poland.

Adult inbred rats were locally x-irradiated on the head with doses from 1000 R to 4000 R. The hypothalamo-neurohypophysial neurosecretory system was studied at postirradiation periods ranging from 7 to 21 days. Following the irradiation cell nuclei of neurosecretory ganglion cells of the supraoptic and paraventricular nuclei showed various degrees of shrinkage depending on the dose of the radiation and length of the postirradiation period. Smallest nuclei were found 7 days post irradiation after 4000 R and 3000 R. High mortality was observed in these groups. Smaller doses caused less pronounced changes which were reversible. Concomitantly with nuclear volume changes a disappearance of the neurosecretory material from the neurosecretory centers, the neurosecretory tract, and the neural lobe was observed. The decrease of nuclear volume was specific for the neurosecretory centers, other brain areas showing much less pronounced changes. The irradiated animals had considerably more Gomori-positive glial cells in their brains than the non-irradiated controls.

E-21-3 *Homing Ability and Head Irradiation in Pigeons.* Sidney Mittler and A. O. Schjeide, Northern Illinois University, DeKalb, Illinois 60115, USA.

Racing pigeons that have been trained to home to the loft from a west to east direction were exposed to 600 R of 225 Kv x-rays. Only the heads received the radiation and the birds were released from points 20 to 50 miles away. Two years later these birds had flown over 500 miles in competition with non-irradiated control hatch mates. They homed as well or better than controls and there was no change in their behavior Another group of pigeons received 1500 R to their heads only; 2 of these birds had also been exposed to 600 R in an earlier test. This group has successfully homed from 30 to 50 miles away for over 300 miles, plus numerous training flights up to 30 miles each. Those birds which had received a total of 2100 R showed signs of

senility after 1½ years, and those birds which received 1500 R could easily be recognized in the flock by moulting on the head which was followed by growth of white feathers and paling of color in the iris. X-ray doses of 10,000 R to a 2 mm diameter area in the middle of the forebrain and extending down, through and between the lobes of forebrain was given to one group and another group received 10,000 R to a 10 mm diameter region in the same part of the brain. These treatments did not interfere with homing ability two months after irradiation.

E-21-4 *Ionising Radiation Effect on Preparations of Mitochondrial DNA of Rat Brain as Revealed by Electron Microscope.* R. Paul Choudhury, P. Sadhukhan, and A. Narayanaswami, Biophysics Laboratory, Saha Institute of Nuclear Physics, Calcutta-37, India.

Adult rats were subjected to whole body irradiation with 1000 rads in a single exposure to a cesium 137 teletherapy unit for 12 min. The animals were sacrificed at the end of 4 and 6 days after irradiation, the cerebral cortex separated and homogenized. The mitochondrial fraction was isolated from the homogenate by differential centrifugation and the DNA released by three different procedures and prepared for electron microscopy. Mitochondrial DNA from the cerebral tissue of control rats displayed super-twisted, half-open and open circular molecules of 5.6 μ average size. While the electron microscopic picture of the DNA molecules obtained from the cerebral mitochondria of irradiated animals was not similar; some open molecules of DNA with free ends, but of the same length (5.8 μ) could be observed. The significance of this finding has been discussed in relation to the process of polynucleotide chain rupture caused by irradiation.

Acknowledgments—We deeply thank Prof. N. N. Saha (Director, Biophysics Division of Saha Institute of Nuclear Physics), to Dr. A. Narayanaswami (Deputy Director, Indian Institute of Experimental Medicine, Calcutta) for their guidance and to Mr. S. Mandal (I.I.E.M.) and B. Bhowmick, (S.I.N.P.) for their technical assistance.

E-21-5 *Effects of X-Radiation on Monoamine Metabolism in the Developing Rat Brain.* Doherty Hudson, Theony Valcana, and Paola S. Timiras, Department of Physiology, University of California, Berkeley, California 94720, USA.

Disturbances in neurotransmission may represent a key biochemical correlate of the morphological and electrophysiological anomalies observed in the brain of neonatally x-irradiated rats. The present study examines the effects of 500 R whole-body radiation at 2 days of age on the metabolism of neurotransmitter monoamines in various brain regions during development. Levels of norepinephrine (NE), dopamine (DA) and serotonin (5-HT) increase consequent to x-radiation, particularly in the cerebral hemispheres (CH) and cerebellum (Ce). The most marked effect was observed at 9 days of age and persisted throughout the experimental period (64 days of age). Turnover studies at 9 days of age employing Pargyline show that there is a significant increase in the turnover of DA, NE and 5-HT in the CH of x-radiated animals; however, no conclusive statement can be made with respect to turnover of these amines in the Ce inasmuch as Pargyline had no effect on turnover in this region. The observed alterations may be of significance to our understanding of the neurological and behavioral abnormalities induced by x-radiation and indicate that neurotransmitter processes are, indeed, very sensitive during maturation of the brain.

E-21-6 *The Effects of X-Irradiation and Surgical Trauma on the Cell Kinetics of the Subependyma Plate of Rats.* Andrew Riches, Peter Willis, and Martin Berry, Department of Anatomy The Medical School, Birmingham, Great Britain.

The subependymal plate of male Wistar rats aged 40 days consists of approximately equal proportions of light and dark cells as classified by their nuclear morphology in coronal sections stained with haematoxylin and eosin. After placing a knife wound stereotaxically in the corpus callosum of the left hemisphere, the number of dark cells and the size of the plate increased markedly by day 5 on the injured side and continued to hypertrophy. The dark cells in the subependymal plate thus respond to injury of the corpus callosum and appear to produce cells which contribute to the glial scar about the wound.

Exposure to 650 rads of x-irradiation damaged the dark cells but not the light cells in head irradiated and whole body irradiated rats. Irradiation to the body alone had no effect on these cells. Recovery of the dark cells was rapid in animals with head irradiation only, reaching control

levels by day 10 but did not occur within this time period in whole body irradiated rats. These results suggest that exogenous factors maybe influencing this recovery.

E-21-7 *Behavioral Suppression By 383 MHz Radiation.* R. J. CUNITZ, W. D. GALLOWAY, AND C. M. BERMAN, Bureau of Radiological Health, Division of Biological Effects, Rockville, Maryland 20852, USA.

Two rhesus monkeys were irradiated in a 383 MHz resonant cavity for one hour immediately before and during performance of a four-choice, forced-choice serial reaction task. Behavioral training sessions lasted a maximum of two hours. Integral dose rates of 0.001 through 17.5 W were delivered to the head. No effects were observed below a critical dose level ($\simeq$23 W/kg) derived from integral dose rate and body mass. Above this level, behavioral suppression occurred, i.e., correct response rate was profoundly altered. The effect was completely reversible and repeatable in one of the subjects—the other subject did not recover completely and was sacrificed for histological examination which revealed no gross or microscopic damage. The nature of the effect suggests a neurochemical rather than a direct electrical or mechanical basis for the results.

E-21-8 *Epileptiform Syndrome as a Cause of X-Irradiation in Utero and in Early Postnatal Ontogeny.* MICHAEL MYSLOBODSKY, Ramat Aviv, Tel Aviv, Israel.

Adult rabbits x-irradiated in utero in the middle of embriogeny (400 r, 20 r/min, 190 kv–15 mA) and rats exposed to 300 r on the 1–3 day after the birth were studied. Both rabbits and rats exhibited similar features of abnormal behavior: freezing during spontaneous movements, low level of performance in instrumental situation, highly ustable conditioned reflexes. These were attributed to a certain derangement of higher nervous functions. Though EEG studies in freely moving animals showed prolonged afterdischarges in the visual cortex, spontaneous bursts of waves and wave-spike discharges 2.5–3.5 per second in rabbits and 5–6 per second spike-wave spindles in rat. Hypersynchronous activity of the cortex was facilitated in stressful situation and during consumatory behavior and was in correlation with the cessation of movements and failures in conditioned behavior.

Evoked potentials studies and single cell recordings in the visual cortex showed that hypersynchronous activity in irradiated animals is due to abnormal facilitation of cortical authorhythmicity. The latter might be the consequence of the derangement of the reticular activating system. It is believed that alterations of behavior in x-irradiated animals is due to epileptiform syndrome of "petit mal type."

E-21-9 *Effects of 14 MeV Neutrons on Development of Neural Explant.* A. MAMOON, B. GAHWILER, R. V. GRIFFITH, AND C. A. TOBIAS, Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720 USA.

Fresh cerebellar explants from newborn rats were exposed to 14 MeV neutrons and then cultured by roller tube technique. After 3 weeks of incubation, control cultures exhibited abundant myelination, apparently normal Purkinje and Golgi type cells and smaller cells (glia and microneurons), spread in essentially a monolayer. Spontaneous bioelectric activity extracellularly recorded from Purkinje cells usually displayed irregular firing patterns. Irradiated cultures were examined for evidence of sublethal and lethal damage to neural cells as revealed by morphological, cytochemical and electrophysiological observations. At 2.0 krad, myelination was inhibited in all cultures and small cell density was reduced to about 10% of normal. At 4.0 krad, 90% of the large nerve cells showed glycogen deposits while about 80% were chromatolytic. Five krad eliminated almost all neural cells with few large nerve cells remaining, exhibiting abnormal morphology and apparently altered bioelectric activity patterns when present. Comparison of these results with similar data from x-irradiated cultures seems to suggest that 14 MeV neutrons at the range of doses used have about twice the effectiveness of x-rays in producing direct damage to developing neural cells.

E-22-1 *Chemical Consequences of Inner Shell Ionization Followed by the Auger Process in Halodeoxyuridines.* A. HALPERN AND G. STÖCKLIN, Institut für Nuklearchemie, Kernforschungsanlage Jülich GmbH, West Germany.

The chemical consequences of inner shell electron vacancies in Br atoms of solid 5-Br-2-deoxyuridine induced by monoenergetic x-rays were studied by means of ESR technique. The radical concentration per unit dose absorbed was determined as a function of photon energy. It exhibits a resonance behaviour near the *K*-absorption edge of Br. This radiation resonance effect can clearly be attributed to the Auger charging rather than to electron self-radiolysis.

Data are presented on radiation damage in ^{125}I- and ^{131}I-labelled 5-I-2-deoxyuridine, which also indicate the importance of the Auger effect in biomolecules.

E-22-2 *Radiation Chemical Effects in Neutron Capture Therapy: Transient Species in Boron Containing Compounds.* BRUCE J. BROWN, JOHN G. HAWKE (School of Chemistry, Macquarie University, North Ryde, NSW 2113 Australia), AND DAVID F. SANGSTER (AAEC Research Establishment, Sutherland, NSW 2232 Australia).

The potential of B^{10} boron hydrides and phenylboric acids for neutron capture therapy of malignant neoplasms has been established. The extent to which the $B^{10}(n, \alpha)Li^7$ transition will initiate chemical transformations which result in the formation of transient and free radical species is being studied. The effect of ionizing radiation on boron hydride ($B_{12}H_{12}^{2-}$, $B_{10}H_{12}^{2-}$, $B_{10}H_{14}$) and phenylboric acid $C_6H_5B(OH)_2$ derivatives has been investigated. Transient species have been identified by pulse-radiolysis and low temperature trapping techniques. Spectrophotometric and electron spin resonance data for selected solvent systems is reported. For the boron hydrides the transient and stable product yields may be interpreted mechanistically in terms of the breakdown of the parent compound structure. For the phenylboric acids, the transients have been rationalised to result from primary product interactions with the aromatic centre and with the boron functional group. The significance of aerobic and anaerobic conditions is considered on the basis of analogous electron scavenging reactions by O_2 and N_2O. This work is undertaken with the support of the Australian Institute of Nuclear Science and Engineering.

E-22-3 *Radiation Chemistry in the Plateau and Bragg Peak Regions of 3.9 GeV N^{7+} Ions.* A. APPLEBY AND E. A. CHRISTMAN, Rutgers University, New Brunswick, New Jersey 08903, USA.

3.9 GeV N^{7+} ions from the Princeton Particle Accelerator penetrate several centimeters of water, and show pronounced plateau and Bragg peak regions. Relative radiation-chemical yields were determined in these regions for aerated and deaerated ferrous sulfate. In the former system, solute concentration effects were much more pronounced in the plateau region than in the peak. In both systems the data are compared with literature data for lighter ions of similar LET, and shown to be consistent with track-core expansion at the high velocities of the N^{7+} ions. (Work supported by the Fannie E. Rippel Foundation.)

The encouragement and assistance of Dr. W. Schimmerling and Dr. K. Vosburgh as well as the entire staff of the Princeton Particle Accelerator are gratefully acknowledged.

E-22-4 *Fission Fragment Radiolysis of 0.4 M Sulfuric Acid Solutions With Dissolved ^{252}Cf.* NED E. BIBLER, E. I. du Pont de Nemours and Co., Savannah River Laboratory, Aiken, S.C. 29801, USA.

Radiolysis of 0.4M sulfuric acid solutions with fission fragments from dissolved ^{252}Cf was studied at very high LET values (~400 eV/Å). This isotope irradiates the solution with both fission fragments and alpha particles. Visible and UV absorption spectroscopy were used to determine $G(Fe^{3+})$ in 10^{-3} *M* Fe^{2+}, $G(Ce^{3+})$ in 10^{-4} *M* $Ce^{4+}/10^{-3}$ *M* Ce^{3+}, and $G(Ce^{3+})$ in 10^{-4} *M* $Ce^{4+}/10^{-3}$ *M* Ce^{3+} containing 10^{-3} or 10^{-2} *M* Tl^+. Values for $G(H_2)$ were determined manometrically. Experiments with alpha particles (LET $\cong$ 9 eV/Å) from dissolved ^{244}Cm verified the technique and established 100 eV yields (G values) for pure α-radiolysis. Experiments with mixtures of ^{244}Cm and ^{252}Cf established that the effects of fission fragments and alpha particles are linearly independent. The following results were obtained for $G(Fe^{3+})$, $G(Ce^{3+})$, and $G(Ce^{3+})$ in 0.001M Tl^+, and $G(H_2)$, respectively: for ^{244}Cm—5.1, 2.9, 3.3, 1.3; for ^{252}Cf—4.4, 2.7, 2.9, 1.8; for fission fragments—3.3, 2.4, 2.4, 2.0. Interpretation of the fission fragment yields leads to the following estimates: $G(H_2) = 2.0$, $G(H_2O_2) = 1.0$, $G(HO_2) = 0.5$, $G(OH) = 0$, and $G(H + e_{aq}^-) = 0$. (Work done under USAEC Contract No. AT(07-2)-1.)

E-22-5 *Radiation Effects on Organic Solids as Studied by Positron Annihilation Technique.* YASUO ITO AND YONEHO TABATA, Research Center for Nuclear Science, Nuclear Engineering Research Laboratory, University of Tokyo, Tokyo, Japan.

We have measured positron lifetimes in various organic solid materials (n-$C_{20}H_{42}$, polyethylene, acrylamide, dimethylitaconate, etc.) as a function of the dose of γ-ray irradiation and the time of heat treatment. Both formation probability and lifetime of ortho-positronium have been shown to be affected by the irradiation and these variations can be explained in terms of two fundamental processes; selective annihilation of positronium in disordered regions (or defects in crystals) and chemical interaction of positronium with active species formed. But it has become clear that in some cases the chemical interaction of positronium is strongly inhibited due to crystalline structure.

E-22-6 *On the Interactions of Positrons and Positronium Atoms with Organic Molecules.* HANS J. ACHE, ALAN L. NICHOLS, AND WILLIAM J. MADIA, Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, USA.

The factors which determine the interactions of positrons and positronium atoms with organic molecules were assessed by measuring the rate constants for *Ps* interactions with a series of diamagnetic organic compounds in benzene solutions. The results observed with substituted nitrobenzenes show a strong dependence of the reactivity on the nature and position of the substituents in the aromatic ring, which emphasizes the importance of inductive and mesomeric effects. The relative rate constants seem to follow the Hammett relationship. Possible mechanisms for e^+ and *Ps* attachment to the molecules are being discussed and compared with the e^- attachment.

E-22-7 *Pulsed NMR Studies of Radiation Damage in Metal Tritides.* ROBERT C. BOWMAN, JR. AND ALBERT ATTALLA, Mound Laboratory,* Miamisburg, Ohio 45342, USA.

The effects of triton decay in LiT and UT_3, which are regarded as representative ionic and actinide metal tritides, have been investigated by pulsed NMR techniques. Lithium metal, molecular tritium, and ^3He are observed in LiT while ^3He is the only species detected in UT_3. These radiation damage products have been characterized with respect to their concentrations, nuclear spin-lattice relaxation times, and spin-spin relaxation times. The influences of temperature and irradiation dose on the formation and distribution of these species have also been studied. The general behavior of metal tritides with regard to possible CTR applications will be briefly discussed.

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* Operated by Monsanto Research Corporation for the U.S. Atomic Energy Commission.

E-22-8 *On the Radiation Stability of Some Liquid Crystals.* M. RODER, K. PINTER, K. RITVAY, AND R. SCHILLER, Central Research Institute for Physics, Budapest, Hungary.

Systematic investigations on the relationship between radiation stability and structure of several liquid crystals were carried out. Irradiation was found to diminish the width of the temperature range for the mesomorphic phase while radiation stability seemed to be commensurate for the real liquid and the liquid crystaline states.

E-23-1 *Ultrasonically-Induced Biological Perturbations in* Vicia faba *Roots.* MORTON W. MILLER, EDWIN L. CARSTENSEN, WINBORN D. GREGORY, FREDERICK L. CATALDO, AND GARY E. KAUFMAN, Department of Radiation Biology and Biophysics, The University of Rochester, Rochester, New York 14642, USA.

2MHz ultrasound exposure at intensities from 2–20 W/cm^2 of *Vicia faba* roots causes an immediate decreasing root growth rate, sharply reduces meristematic mitotic activity, ruptures elongation zone cell walls, reduces subsequent lateral root emergence, and induces chromosomal anomalies in the form of bridges and agglomerations. The effects are non-thermal; the evidence for a cavitational effect will be discussed. (This research was partially supported by Grant USPHS GM 0933 and by the U.S.A.E.C. at The University of Rochester Atomic Energy Project and has been assigned Report No. UR-3490-408.)

E-23-2 *Post-Irradiation Survival, Behaviour and Life Span of Human Peripheral Blood Lymphocytes.* KUMUD C. BORA, Human Development Division, Environmental Health Centre, Tunney's Pasture, Ottawa, Ontario, Canada.

Three patients, two receiving single whole-body gamma-ray doses of 220 and 226 rads, and the third, 308 rads followed by partial doses, were investigated. Following irradiation, peripheral blood samples were obtained at intervals from 0 hrs to 1139 days. Fifty-two hour cultures were analysed for chromosome aberrations with a view to (1) relating the aberration frequency to blood sampling time, (2) studying the survival rate of cells with different aberration types, and (3) providing an estimate of life span of lymphocytes. After an initial increase during the first few days following irradiation, the frequency of aberrant cells exponentially declined with time. There was no clear relationship between the type of aberration a cell contained and its rate of elimination from the lymphocyte population. The selection against chromosome aberrations was random. The mean life span of cells was estimated using the relationship $P_t = P_c + P_0\, e^{-bt}$, where P_t is the percentage of cells with aberrations at sampling time t, P_c the control percentage, P_0 the percentage at $t = 0$ and $1/b$ is the mean life span of aberrant cells. It is estimated that the mean life span of these cells is approximately 1500 days.

E-23-3 *Chromosomal Aberrations by Neutron-Induced Slow Particles.* RONNY BERGMAN, Dept. of Physical Biology, The Gustaf Werner Institute, University of Uppsala, Sweden, AND SÖREN STURELID, Dept. of Genetics and Plant Breeding, Royal Agricultural College of Sweden, Sweden.

Fast charged particles dissipate energy in matter mainly through ionization and excitation. At sufficient low velocities, however, charge exchange and atomic collisions become important in regard of energy transfer. The charged particles are called slow in this velocity interval.

The range of slow charged particles is less than 0.2 μm, i.e., much smaller than the linear dimensions of a typical cell. To study biological effects on nuclei of eukaryotic cells, direct exposure to slow charged particles therefore is impossible.

Intermediate energy neutrons, 0.1–30 keV, induce slow charged particle recoils at considerable depth in tissue. We have developed a technique that allows irradiation with fields of intermediate neutrons of mean energy 8.5 keV at 10–20 rad/h. Cultures of Chinese hamster fibroblasts were exposed to such radiation, or alternatively, to gamma rays from a ^{60}Co source.

The relative biological efficiency (RBE) of intermediate neutrons was evaluated for induction of chromosomal aberrations. The frequencies of translocations and chromatid breaks were analyzed after exposure at comparable dose rates.

E-23-4 *Optical Image Analysis of Radiation-Induced Chromosome Aberrations.* AGNES N. STROUD AND SAYURI HARAMI, Jet Propulsion Laboratory, Pasadena, California 91103, USA.

The introduction of chromosome analysis through the new banding techniques made possible the characterization of each chromosome so that individual chromosomes could reliably be identified with its homologue, except the male sex chromosomes. By using the chromosome banding techniques and the optical image analysis techniques developed at JPL, we have been able to identify and characterize irradiation-induced chromosome aberrations.

Peripheral blood leukocytes were exposed to Cesium^{137} irradiation to induce chromosome aberrations for these analyses. Whole blood cell cultures were incubated in serum-media with phytohemagglutinin at 37°C following irradiation. Fifty to 72 hours after irradiation, mitosis was blocked with colcemid for 1 hour and chromosome spreads were made. The metaphase spreads were treated with 0.05% trypsin for less than a minute to produce banding and stained with Giemsa to enhance banding. The stained metaphase spreads were scanned with an Automatic Light Microscope System (ALMS). The system scans the chromosomes with an optical scanner and the information is digitized and processed by computers. Algorithms have been developed which automatically gives lengths, areas, centromeric indices and profile waveforms of individual chromosomes. The profile waveforms represent the optical density of the bands along the chromosome. From this information, we can quantitatively identify chromosome aberrations, such as, translocations, insertions, etc. This sytem offers a rapid and quantitative approach to the analysis of radiation-induced chromosome aberrations. (This work was supported by the Jet Propulsion Laboratory, California, Institute of Technology sponsored by NIH under Grant RR-00443 by agreement with NASA.)

E-23-5 *Chromosome Banding Analysis of* In Vitro *Cell Populations.* L. L. DEAVEN, P. C. SANDERS, M. M. KLIGERMAN, AND D. F. PETERSEN, Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico 87544, USA.

In preparation for preclinical studies at the Los Alamos Meson Physics Facility, we have characterized euploid and aneuploid cells from humans, mice, Chinese hamsters, and Indian muntjac with respect to chromosome constitution. Each chromosome can be divided into regions and bands so that deletions or translocations involving segments too small to be detected by arm-length measurements are readily visible. In many cell lines used extensively for radiobiological studies, the cell-to-cell variability in chromosome number makes cytogenetic assessment of radiationinduced chromosome damage difficult. Our results indicate that there are definite trends in evolution of chromosome changes in these cell populations. One haploid set of chromosomes remains largely unaltered, and markers are stable rearrangements of normal chromosomes. Variability of chromosome number is restricted to the smaller groups of chromosomes, and total chromatin per cell is nearly constant. These studies suggest that radiation-induced chromosome aberrations or loss can be detected accurately in most heteroploid lines. (This work was performed under the auspices of the US. Atomic Energy Commission and the National Cancer Institute.)

E-23-6 *Assay of Radiomimetic Activity of Thymine Hydroperoxide on Normal Human Lymphocytes.* J. E. T. KELLEY, B. S. HAHN, S. Y. WANG, AND T. MERZ, The Johns Hopkins University, Baltimore, Maryland 21205, USA.

cis-5-hydroxy-6-hydroperoxy-5,6-dihydrothymine (TOOH) is the major product resulting from γ-irradiation of aerated, aqueous solutions of thymine. Furthermore, TOOH reacts with thymine and cytosine producing base alteration. The studies reported here were undertaken to evaluate the possible role of TOOH as a significant radiochemical lesion in irradiated biological systems. Treatment of lymphocyte cultures with TOOH induces achromatic gaps and a marked mitotic inhibition. These effects will be discussed with particular attention to cellstage sensitivity. The effect of the thymidine analog of TOOH is also being studied in lymphocyte cultures as well as in other cell systems. Preliminary results of these studies will be given. (Unpublished data, S. Y. Wang and L. Parkhill.)

E-23-7 *Deoxyribonucleic Acid Stability and the Frequency of Radiation Induced Chromosome Aberrations in* Hordeum vulgare. E. G. SIDERIS, Nuclear Research Center Democritos, Aghia Paraskevi, Attiki, Greece.

The frequency of radiation induced chromosome aberrations in the embryos of the plant *Hordeum vulgare* var. Himalaya was decreased when these embryos were grown in deuterated water. The reduced frequency of the chromosome aberrations was coupled with a delay of the early stages and an inhibition of the late stages of mitotic division and an increase of the melting temperature (Tm) of the deoxyribonucleic acid by 7°C. The lower frequency of radiation induced chromosome aberrations in material grown in deuterated water is being attributed to the immobilization of the broken ends of the chromosomes due to the delay of the mitotic procedures and the increased stability of the deoxyribonucleic acid molecule, both of them favoring the reconstitution of the broken ends.

E-24-1 *Morphological Study of Lesions Produced by the Decay of* ^{32}P *in* E. Coli. S. APELGOT AND J. P. THIERY. Foundation Curie-Institut du Radium. 75231 Paris Cedex 05, France.

It is known that the initial perturbations produced by the decay of ^{32}P incorporated in bacteria are probably not lethal lesions, but that these perturbations evolve to such lesions with a probability depending on experimental conditions (liquid growing medium with ^{32}P, solid plating medium, storage temperature, incubating temperature, etc.). It is likely that the bacteria bearing these perturbations an still able to carry out syntheses and to grow till the lethal lesion occurs. To test this hypothesis, we examined with a phase contrast microscope, the development (on agar) of bacteria which had accumulated ^{32}P decay during storage at -196°C.

We observed very long snake-form cells (growing without division), the percentage of which increased regularly with decay accumulation; then bacteria appeared which had a more and

more pathological aspect, with bulges. We also observed some bacteria put end to end, corresponding to cells the multiplication of which had stopped after 2 or 3 divisions.

E-24-2 *Separation of Bacteria into Differentially Damaged Sub-Populations.* MILTON B. YATVIN, INGE PORTEN-SEIGNE, AND TIKVAH ALPER. Radiobiology Res. Lab., University of Wisconsin Medical School, Wisconsin 53706, USA, and MRC-ERU Hammersmith Hospital, London, Great Britain.

When gamma-irradiated *E. coli B/r* cells are reincubated after exposure they separate into different populations during centrifugation on linear renografin gradients. The number of cells appearing in the farthest sedimenting band increases with increasing radiation dose. At the same dose, greater numbers of cells migrate to this zone when the irradiation is performed in the presence, as opposed to the absence, of oxygen. The band that sediments the greatest distance in the renografin gradient contains approximately 10% as many viable cells as the upper band. After exposure to neutrons, electrons or UV radiation, *E. coli B/r* behavior on renografin gradients was similar to that after gamma-irradiation. Heating the cells for 20 minutes or longer at 52° resulted in differential banding on renografin gradients; however, when the cells were reincubated at 37° there was a decrease in the number of cells which entered the farthest sedimenting band. In summary, we have developed a renografin gradient centrifugation technique which allows bacteria that have been exposed to various cytotoxic procedures to be separated into two major populations which differ in survival. This technique offers an opportunity to increase the precision of studies concerned with radiation damage and "repair." (Supported by NIH Center Grant CA-06295.)

E-24-3 *Dosimetry Considerations in the Radiation Biology of the Bacterial Spore.* BRUCE F. KIMLER* AND E. L. POWERS, Laboratory of Radiation Biology, Department of Zoology, The University of Texas, Austin, Texas 78712, USA.

When using low-energy x-rays to study the inactivation of microorganisms, one encounters several problems in dosimetry. We irradiated spores of *Bacillus megaterium* with 50 kVp x-rays; the spores being either in suspension or on the surface of Millipore filters. The Fricke ferrous sulfate dosimetry technique can be used satisfactorily for the suspension system; but not for the filter system since a homogeneous build-up of secondary electrons is lacking. This becomes increasingly important as the air in the beam path above the spores is replaced with various inert gases (with different attenuation characteristics).

This problem was overcome by using a variety of dosimetric techniques, including the bacterial spore itself. The results provide an accurate determination of the absorbed dose and thus allow a correlation between studies of spore inactivation using the aqueous suspension system and the dry filter system. (This work was supported by NIH Grant number PHS GM 13557-06, AEC Contract AEC AT-(40-1)-3408, and NIH Biophysics and Physiology Training Grant number 5 T01 GM-00836.)

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E-24-4 *Delayed Plating and Interaction Experiments with Ionizing Radiation and UV-Light Using Yeast Cells of Different Sensitivity.* ECKART SCHNEIDER AND JÜRGEN KIEFER, Strahlenzentrum der Universität, 63 Giessen, West Germany.

Delayed plating after ionizing radiation is known to be dose-modifying. Under our experimental conditions this could only be verified in a certain dose range. These experiments were combined with interaction experiments with different kinds of radiation (x-rays, alpha-particles and *UV*-light). The results are discussed in relation to repair processes. Three yeast strains of different sensitivity against ionizing radiation and *UV*-light were used.

E-24-5 *A Defined Growth Medium which Produces an Enhanced Radiation-Resistance in Rec$^+$* Escherichia coli. CYNTHIA RUIZ, BENJAMIN S. FRIESEN, AND JEREMY E. BAPTIST, Radiation Biophysics, University of Kansas, Lawrence, Kansas 66045, USA.

We have previously reported that growth to the stationary phase of Rec$^+$ *Escherichia coli* in glucose-enriched nutrient broth (as compared with growth in nutrient broth) induces an

enhanced resistance to both gamma-rays and methyl methanesulfonate, and that such cells repair a greater number of single-strand breaks in DNA. Because cells grown in commonly used defined media, such as *M*-9, which contain glucose as a carbon source do not exhibit this enhanced resistance, we have looked for the constituents which are necessary for the development of this resistance. It was found that 20% of cells grown in a weakly-buffered salts-glucose medium containing a 0.2% (*w/v*) concentration of both aspartic and glutamic acid exhibited the enhanced radiation-resistance. Because these are the two amino acids that feed directly into the citric acid cycle and because a low *pH* is required for the development of the enhanced resistance, it is suggested that the respiratory chain may be involved in this effect. (Supported in part by the Biomedical Sciences Support Grant PR-07037.)

E-24-6 *Mechanism of Lethal Actions of Thermal Neutrons on Amoebae.* Mitsuhiko Akaboshi, Kenichi Kawai, Toshio Maeda, and Akira Shimizu, Research Reactor Institute, Kyoto University, Kumatori, Osaka, Japan and College of General Education,* Osaka University, Toyonaka, Osaka, Japan.

Lethal effects of thermal neutrons were compared with those of ^{60}Co-γ-rays on the two types of Amoeba proteus. D_{37} doses against γ-rays were $2{,}5 \times 10^5$ (*M*-type) and $7{,}2 \times 10^5$ rads (*P*-type), and those for thermal neutrons were 9,1 and 11,7 $\times 10^{13}$ n. cm^{-2} (total flux) respectively. Additions of AET, D_2O or boron failed to increase or decrease the lethal effects of thermal neutrons. Among the several specific inhibitors such as bleomycin, actinomycin *D*, puromycin, 8-azaguanine and BUdR, only bleomycin (DNA strand breaker) had a strong synergistic action to thermal neutron-irradiation. When amoebae were irradiated by thermal neutrons on pre-γ-irradiated status, the lethal actions were markedly enhanced (γ-*n* effect). Thus the lethal actions by thermal neutrons must arise by the nuclear reaction, namely, $^{31}P(n, \gamma)^{32}P$ on DNA chains, and not by the after effects of the nuclear reactions such as the radiative effects due to γ-rays emitted spontaneously or heavy ionized particles per se.

E-25-1 *Inactivation of Giant Bacteriophage Particles by Gamma Rays.* John D. Childs, Biology and Health Physics Division, Atomic Energy of Canada Limited, Chalk River, Ontario K0J 1J0, Canada.

Giant particles of bacteriophage T4 have heads which are 1.5 to at least 10 times the length of normal particles and contain a linear DNA molecule of correspondingly increased size compared to normal. They are able to inject into bacterial cells multiple genomes, as shown by their greatly increased resistance to ultraviolet irradiation compared to normal size particles. The sensitivity of giant particles to γ rays, however, was found to be similar to that of normal size particles, ($\pm$30%, dependent on the medium in which the irradiation was carried out). Giant genomes also had a similar sensitivity to γ rays as normal size genomes when both were irradiated after infection. Thus the similar sensitivity of giant and normal size particles to γ rays cannot be explained by a failure of irradiated particles to inject all of their DNA. It can be calculated that some of the γ-irradiated giants contain stretches of DNA, without lethal damage, longer than the normal T4 genome and yet they are inviable.

E-25-2 *Influence of Oxygen on the Yield of DNA Double-Strand Breaks in X-Irradiated* E. coli *K-12.* Thomas Bonura, Christopher D. Town, Kendric C. Smith, and Henry S. Kaplan, Department of Radiology, Stanford University School of Medicine, Stanford, California 94305, USA.

We have developed a simple procedure by which radiation-induced DNA doublestrand breaks may be measured in x-irradiated *E. coli* K-12. The neutral sucrose gradients employ SDS and chloroform to aid in the isolation of large pieces ($Mn > 2 \times 10^8$ daltons) of the *E. coli* genome. Sedimentation at low speed (8000 rpm, Beckman SW50.1 rotor) eliminates the speed dependent complications which have been a significant problem in earlier experiments. Using this technique we have observed a 4-fold oxygen enhancement ratio for double-strand breaks. The kinetics of double-strand break formation deviate from linearity at superlethal doses (above 60 krads), however the efficiency of double-strand breakage in the most linear region of the dose response curve is approximately 1450 *ev*/break in air and 5800 *ev*/break in nitrogen. The aerobic breakage efficiency is increased in cells carrying the *polA1* mutation.

E-25-3 *Degradation of DNA in* Haemophilus influenzae *Following X-Ray Exposure.* M. L. RANDOLPH AND JANE K. SETLOW, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

Haemophilus influenzae cells, in exponential or stationary phase and labeled with ^{3}H-dThd, were irradiated with x-rays at 4°C, incubated in growth medium at 37°C for various times and the amount of DNA present measured by counting acid insoluble radioactivity. Initially the amount of DNA is independent of exposure. Following exposure (typically 30 kR) at first the amount of DNA decreases rapidly with time ("half-life" less than 15 minutes) and then settles to a plateau of about 12% of the initial DNA. This value decreases with exposure but has a different dose response curve and is much greater than either colony forming ability or the fraction of unbroken DNA single strands, as measured by alkaline sucrose gradient techniques. Hence we have fit our results to kinetic models of DNA degradation initiated at a single strand break and continuing until interrupted on a random basis which might be the occurrence of an unique sequence in the DNA chain or the result of an enzyme which blocks further degradation. In either case this could be an early step in DNA repair. The model which best fits our data involves unilateral degradation at an initial rate of roughly 4×10^3 nucleotides/minute for exponential phase and about 7×10^3 for stationary phase and the probability of degradation being interrupted is about 3×10^{-4} per second for both cases. (Research sponsored by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.)

E-25-4 *Studies on* in vivo *DNA Base Damage in Gamma-Irradiated* E. coli. R. C. RICHMOND AND J. D. ZIMBRICK, Radiation Biophysics, University of Kansas, Lawrence, Kansas, 66045, USA.

E. coli C 321 thy$^-$ cells grown into log phase in the presence of 10 micro-Ci/ml $^3H_{C6}$-thymine were gamma-irradiated with 283 krads in ozygenated phosphate buffer at 0°C. Cells and RNA were digested with protease and RNase with intervening dialyses. DNA was then hydrolyzed enzymatically to nucleosides. Thin layer radiochromatography (TLC) scans were made on all cell fractions. Complete enzymatic DNA hydrolysis is usually obtained from both control and irradiated cells. Spontaneous release of ca. 1% of incorporated label is observed in control cells after 5 hours in buffer at 0°C. This material is oligonucleotides between 2–5 nucleotides in length. For irradiated cells, this release represents ca. 6% of the incorporated label, of which a significant fraction is oligonucleotides of chain length greater than five. From irradiated cells, a damage product ($G \sim 0.02$) has been isolated from the hydrolyzed DNA fraction. This product, which has not been identified conclusively at this time, appears different from the radiolysis products of the same DNA irradiated *in vitro.* (Supported by NIH grant No. GM 18927.)

E-25-5 *DNA Repair Mechanisms in Yeast.* R. H. HAYNES, M. BRENDEL,* J. G. LITTLE, AND D. W. BRYANT, York University, Toronto, M3J 1P3, Canada.

The genome of the yeast *Saccharomyces cerevisiae* is smaller than that of all other eukaryotes and each of its 17 (or 18) chromosomes is actually about 5 times smaller than the *E. coli* chromosome. We feel that the analysis of DNA repair and the genetic control of radiosensitivity in such a simple eukaryote is a wise, and possibly essential, step toward an understanding of DNA repair in more complex cells. In yeast several phenomena may reasonably be associated with repair: Photoreactivation, liquid-holding recovery, radiosensitive and radioresistant mutants, 'diploid repair,' 'repair-induced UV-resistance,' repair of mitochondrial DNA, mitotic and meiotic recombination and gene conversion. The relation of these phenomena to known macromolecular modes of repair has not yet been worked out in detail; apart from photoreactivation, only excision repair has been demonstrated at the macromolecular level. Almost 40 distinct genetic loci are known to affect sensitivity to x-rays, UV and various chemical mutagens. By studying the interactions among certain of these genes in specially constructed multiple mutants we have been able to 'map' at least 3 interrelated pathways for repair; a similar pattern of pathways has also been inferred by Hunnable and Cox from studies of UV-induced recombination. Recently we have isolated a yeast endonuclease that may be involved in repair. Macromolecular studies of DNA repair (and normal replication) are now more practical because we have recently isolated yeast mutants whose DNA can be specifically labeled with exogenously

supplied thymidine monophosphate. (Work supported by the National Research Council of Canada.)

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E-25-6 *Biochemical Events Involved in the Radioprotective Action of Thiols.* LARS EHRENBERG, IMRE FEDORCSÁK, AND MARIA NÄSLUND, Wallenberg Laboratory, Stockholm University, S-104 05 Stockholm, Sweden.

The radioprotective action of thiols (drf about 3) in *E. coli* at log phase was found to coincide with a suppression of RNA synthesis. When substances like ascorbate were added the RNA synthesis started again and simultaneously the radioprotective action of the thiol was decreased to drf about 1. The addition of ascorbate did not decrease the concentration of thiol, i.e., both high rate of RNA synthesis and low radioprotective action had occurred at that concentrations of thiol which, without ascorbate, resulted in high radioprotection and suppressed RNA synthesis. Ascorbate alone gave no radioprotection and no change in RNA synthesis. On the other hand, suppression of RNA synthesis is certainly not the primary event in radioprotection because inhibition of RNA synthesis by rifampicin did not give protection.

It is concluded that under the present conditions biochemical events are more important than radiation chemical events (radical scavenging etc.) in the radioprotective action of thiols. The biochemical processes involved can be diagnosed by the suppression of RNA synthesis.

E-25-7 *Radioresistance in Yeast* (S. cerevisiae) *is Associated With DNA Synthesis, but not Budding.* RICHARD P. BIRD AND T. R. MANNEY, Kansas State University, Manhattan, Ks. 66506, USA.

Budding yeast cells are highly radioresistant, and account for the resistant tail on x-ray survival curves. Furthermore, budding cells are known to be engaged in DNA synthesis. Studies with cell cycle mutants and with hydroxyurea have established that budding and DNA synthesis are separable events in the cell cycle. Hydroxyurea reversibly inhibits DNA synthesis, but does not inhibit budding. We have used these observations to demonstrate that the radioresistance is associated with DNA synthesis, but separable from budding.—Synchronized diploid cultures, with cells near the time of cell-separation, were obtained from an isopycnic colloidal silica (Ludox) density gradient. Haploid cultures of mating type *a* were synchronized with α factor, a diffusible agent produced by cells of mating type α, which arrests cells at a point just before DNA synthesis and bud initiation. When synchronized cultures are incubated in normal growth medium, the initiation of budding is accompanied by increased radioresistance. However, when synchronized cultures are incubated in medium containing 0.75 *M* hydroxyurea, bud initiation occurs on schedule, but without increased radioresistance. But when the hydroxyurea is removed, allowing DNA synthesis to occur, the radioresistance increases rapidly. (Supported by USPHS Grant GM19175.)

E-25-8 *Survival Kinetics of Diploid Yeast Cells Homozygous for Various Combinations of rad Genes and Mating Type.* JOHN C. GAME AND ROBERT K. MORTIMER, Donner Laboratory, University of California, Berkeley, California 94270, USA.

A minimum of 12 genes have been shown to affect the sensitivity of yeast cells to x-rays. The typical expression of these mutants is the elimination of the radioresistant fraction associated with budding or *S*-phase haploid cells and the partial or complete elimination of the shoulder on the survival curves of diploids homozygous for the mutations. Many of the mutants also have profound effects on meiosis. We have concentrated on a series of diploids homozygous for various combinations of certain of these *rad* genes and in addition either homozygous or heterozygous for mating type in an attempt to determine whether additive or epistatic interactions occur. From such information, a pathway-type model for the role of these genes in the repair of ionizing radiation damage has been developed. Mutants with a temperature-conditional response to x-rays also have been found and their properties will be described.

E-25-9 *A Cyclic Component in Induced Repair.* JOHN CALKINS, University of Kentucky, Lexington, Kentucky 40506, USA.

Caffeine post-treatment of irradiated Tetrahymena produced dose-response relations of a complex nature. Survival of caffeine treated animals is a non monotonic function of dose; relatively low doses of ionizing radiation or ultraviolet light can be highly lethal while at higher levels of dose, survival may equal the survival observed without caffeine treatment. The peaks and valleys of survival relate to position in the growth cycle; however, the pattern of response has proven more complex than was originally assumed. The current observations tend to substantiate the concept that an irradiated organism assess radiation injury and, if the magnitude of the injury is sufficient, a potent (and caffeine resistant) repair system (the *T* system) is triggered. It appears that this process can occur more than once following a single injury and that the paradoxical aspects of response are generated from the complications inherent in an induced repair system in comparison to the relatively direct action of constitutive repair systems. (This research was supported, in part, by the University of Kentucky Tobacco and Health Research Institute.)

E-25-10 *Protection of Transforming DNA Against X-Rays by Histidine, Glycerol, AET and the UVR⁻ Genotype.* M. J. Peak and J. G. Peak, Division of Biological and Medical Research, Argonne National Laboratory, 9700 S. Cass Ave., Argonne, Illinois 60439, USA.

Histidine, glycerol and 2-aminoethylisothiouronium bromide hydrobromide (AET) all protect DNA strongly from loss of genetic activity when irradiated with x-rays in the presence of the agent. Histidine and glycerol protection is of similar magnitude, whereas AET is more than twice as effective. AET and glycerol both seem to protect by a similar mechanism since addition of both AET and histidine to the DNA does not show additive protection, only a small augmentation.

Exposing the x-irradiated DNA to *uvr⁻* cells, compared with *uvr⁺*, has a minor effect. This shows that *uvr⁻* can repair only a small proportion of x-ray induced lesions in DNA.

E-25-11 *Sensitization to Irradiation by Inhibition of Repair of DNA-Single-Strand Breaks.* Ferenc J. Hernádi, Péter Kovács, and József Csongor, Department of Pharmacology, University Medical School, Debrecen 4012, Hungary.

A class of radiation sensitizers will be shown which inhibit the repair of radiation-induced cellular damage. These agents are active when added after irradiation and can potentiate radiation induced death in *rec⁺* strains of *E. coli* K12, while they have no effect on irradiated *rec⁻* strains, which are deficient in the ability to repair the damage. The parameters of radiation induced damage were the loss of colony forming ability and the incidence of breaks in cellular DNA. The effect of acriflavine, 5-bromouracil, Ara-C and hydroxyurea, supposing properties associated with this class of post-irradiation sensitizers, has been investigated. The results and discussion, in connection with our previous results will be given in detail.

E-26-1 *Composition of High-Energy Heavy-Ion Beams.* Howard D. Maccabee, University of Miami Medical School, Ph.D. → M.D. Program, Miami, Florida 33152, USA.

Accelerated high-energy heavy-ions are of increasing interest as a possible therapeutic modality because of desirable depth-dose and high-LET properties. Measurements of various energy-deposition spectra of 240-MeV/nucleon oxygen ion beams were performed at the LBL Bevatron, using silicon and germanium detector systems, and varying thicknesses of water absorber to simulate beam penetration in tissue. Interpretation of data yields values for beam contamination ($\simeq$1%), cross-sections for fragmentation into different secondaries, dose contribution from primaries and secondaries, effects on the Bragg peak, and the Dome distribution. The mean free path of fast oxygen ions in water is 19.4 cm, in agreement with theoretical estimates. (These results appear favorable for continued research on biomedical applications.)

E-26-2 *Dose Distributions in LET through the 'Spread-out Bragg Peaks' of Heavy-Ion Beams.* Stanley B. Curtis and John T. Lyman, Lawrence Berkeley Laboratory, Berkeley, California 94720, USA.

Calculations are presented of LET distributions for oxygen and neon beams in the region of their "spread-out" Bragg peaks in water. In practice, in order to cover a given irradiation

volume, the sharp peak in dose created by the unmodified heavy-ion beam is placed subsequently at many different depths to "spread out" the depth-dose curve, by means of a variable-thickness absorber ("ridge filter"). A comparison is made for two filters for beams with initial energies suitable for possible irradiation of deep-seated tumors. Production of secondary heavy particles is included in the calculations. Results from other beams of "high-LET" radiation are compared with these calculations. The approximations made in the calculations are discussed, and an assessment is made of the relative importance of high- *vs.* low-LET radiation components in the heavy-ion beams chosen. (This work is supported by the U.S. Atomic Energy Commission.)

E-26-3 *Apparatus and Dosimetry for Heavy-Ion Beam Irradiations.* WALTER SCHIMMERLING, LBL, Berkeley, CA 94720, KIRBY G. VOSBURGH, G. E. Research & Development, Schenectady, NY 12345, PAUL W. TODD, Penn State Univ., University Park, PA 16802, JOHN KIDD AND JOHN WEFEL, Naval Research Laboratory, Washington, D.C. 20390, AND ALAN APPLEBY, Rutgers Univ., New Brunswick, NJ 08903, USA.

The instrumentation used for monitoring, dosimetry and characterization of high-energy nitrogen and neon beams at the Princeton Particle Accelerator is discussed. In order to cover the range of several orders of magnitude in beam flux, energy and spot size required, several systems were used. The construction, calibration, electronic readout, and performance of dual argon-filled ionization chambers is described. A precision of $\pm 5\%$ and an accuracy of $\pm 10\%$ were obtained for beam fluxes above 5×10^4 cm^{-2} sec^{-1}. Calibration of the chambers using C-11 activation and scintillation counter telescopes yielded a value of $\bar{W}$(argon) $= 25.6 \pm 2.6$ ev/ion pair for N^{7+} beams. The ionization chambers were also compared with a Fricke dosimeter and the relative variation of G-value with dE/dx measured. Average energies of the beams at the Bragg peak were 20–30 MeV/u, a factor of 2 higher than previous calculations. The possible reasons for this discrepancy are attributed to the effect of nuclear interactions. A scintillation counter particle identifier was constructed. The nonlinear response of scintillator as a function of dE/dx was measured. Preliminary results for the angular distribution of fragments from nitrogen ions interacting with hydrogen are presented. It is seen that the assumption of $\Delta v = 0$, often made, is not strictly valid. (Work supported by the Fannie E. Rippel Foundation, Princeton University, and Naval Research Laboratory. The encouragement of Professor M. G. White and the assistance of Messrs. J. Fennimore, and E. Christman are gratefully acknowledged.)

E-26-4 *Calculations on Tissue Equivalence for Stopping π^--Mesons.* KAY C. CHANDLER AND TONY W. ARMSTRONG, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

Calculations have been made of the energy deposition in tissue-equivalent (TE) plastic (Shonka type A-150) and tissue when both are placed in the stopping region of a negatively charged pion beam. The configuration considered is that of relatively small pieces of TE plastic (thicknesses of 50 and 3000 microns) placed in a large π^--capture region. The multiplicities and energies of the π^--capture products were determined using an intranuclear-cascade-evaporation model, and the energy deposition was obtained using computed stopping powers and ranges.

The results for the particular configuration chosen show that for plastic thicknesses which are small compared to the ranges of most of the particles (50 μ case), the energy deposition (per unit mass) is the same (within $\approx 1\%$, which is the statistical precision of the calculations). For a thickness of 3000 μ, the total energy deposition is about $3 \pm 1\%$ higher in TE plastic, but the energy deposition by individual particle types differs by as much as 30%. (This work was funded by the National Science Foundation, Order NSF/RANN AG-399, under Union Carbide Corporation's contract with the U.S. Atomic Energy Commission.)

E-26-5 *The Effects of Bone in the Use of Negatively Charged Pions in Cancer Radiotherapy.* R. T. SANTORO, R. G. ALSMILLER, JR., AND K. C. CHANDLER, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

The effects of bone in the use of negatively charged pions in cancer treatment planning have been estimated using Monte Carlo methods. The influence of bone along the pion path and of a bone-tissue interface in the beam has been considered. Contours of constant absorbed dose and of cell-survival probability, and calculated OER's and RBE's as a function of spatial coordinates in a 30-g-cm^{-2}-thick tissue phantom with bone both included and excluded suggest that the presence of bone in a negatively charged pion beam can be accounted for in an approximate manner if the absorbed dose and biological damage results are plotted as a function of depth in g cm^{-2}. For bone-tissue interfaces, scaling methods can be applied to absorbed-dose and cell-survival parameters obtained in tissue to estimate approximately the influence of bone at the interface. All biological data are for T-1 human kidney cells. (This work was funded by the National Science Foundation, Order NSF/RANN AG-399, under Union Carbide Corporation's contract with the U.S. Atomic Energy Commission.)

E-26-6 *Effects of π^- Beam Parameters on Calculated Dose Distributions in Tissue.* H. A. Wright, R. N. Hamm, and J. E. Turner, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

The computer code PION-1 (an improved version of the code reported in Rad. Res. **52**, 22, 1972) has been used in a systematic study of the effects on dose distributions in tissue of varying the mean momentum, momentum spread, diameter, density profile, and convergence of negative pion beams. PION-1 uses Monte Carlo methods and includes all of the physical processes which are thought to be significant, e.g., multiple scattering, range straggling, inflight elastic and inelastic collisions, nuclear capture, secondary particle transport (including neutrons), etc. Although it is possible to achieve a much larger dose in a tumor region than in surrounding tissue, a number of the above physical processes prevent the attainment of very sharply defined boundaries of the high dose region. These effects are very important in planning cancer therapy using π^- beams. The results of these calculations are presented in the form of depth dose curves and isodose contours in a homogeneous soft tissue phantom. These studies show the extent to which therapeutically desirable dose distributions can be realized in practice. (Research sponsored in part by the U.S. Atomic Energy Commission under contract with Union Carbide Corporation and in part by the National Science Foundation under order NSF/RANN AG-399.)

E-26-7 *Effects of Inhomogeneities on Dose Distributions from Pion Beams.* R. N. Hamm, H. A. Wright, and J. E. Turner, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

Inhomogeneities such as air cavities or bone are particularly important in planning for tumor radiotherapy using negative pions because the range of the pions must be carefully selected so that the pions will stop in the tumor region. Furthermore, edge effects which are caused by multiple scattering and range straggling when only a portion of the beam penetrates a region of different density must be taken into account. Calculations have been made of the spatial distribution of dose in a homogeneous soft tissue phantom as well as in a soft tissue phantom containing regions of high and low densities to simulate the presence of air cavities or bone located both near the surface and near the stopping region. The Monte Carlo computer code PION-1 (an improved version of the code reported in Rad. Res. **52**, 22, 1972) which has been used for these calculations includes all of the physical processes such as multiple scattering, range straggling, nuclear collisions, secondary particle transport, etc., that are thought to be significant. The results are presented in the form of isodose contours for several configurations of the inhomogeneities. (Research sponsored in part by the U.S. Atomic Energy Commission under contract with Union Carbide Corporation and in part by the National Science Foundation under order NSF/RANN AG-399.)

E-26-8 *Caclulations Related to the Application of Silicon Detectors in Pion Radiobiology.* Tony W. Armstrong and Kay C. Chandler, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

Calculations have been carried out to determine the response of silicon detectors placed in the vicinity of a stopped negatively charged pion beam. A one-dimensional approximation has been used with detectors of thicknesses from 10 to 3000 microns placed at the midpoint of a region of tissue 5 cm in width containing a uniform density of π^- captures. The π^--capture products from the silicon and tissue were determined using an intranuclear-cascade-evaporation model, and the energy-deposition spectrum (the analogue of a measured pulse-height spectrum) was obtained using computed ranges and stopping powers.

The calculations show that the shape of the pulse-height spectrum depends strongly on detector thickness, and for some thicknesses the spectrum can be analyzed to obtain the proportion of the total dose due to individual types of particles [pions, protons, and heavy ($Z > 1$) particles]. Calculations have also been made to compare the dose that would be measured by the detector to the actual dose in the tissue at the same location. (This work was funded by the National Science Foundation, Order NSF/RANN AG-399, under Union Carbide Corporation's contract with the U.S. Atomic Energy Commission.)

E-26-9 *Heavy Ions Tracks by Polymer Grafting.* MICHEL M. MONNIN. Université de Clermont. 63170 Aubière, France.

Trajectories of heavy particles are revealed in polymers by a graft and dye process. Along their path in a polymer sheet, heavy charged particles create active sites. A monomer is made to diffuse into the polymer sheet. Copolymerization is initiated at the active sites. The monomer, namely propenoïc acid, is such that the copolymer formed along the particle trajectory is able to fix a suitable dye. After the dyeing process, the particle trajectory can be observed as a track under an optical microscope. Such tracks have been obtained in several plastics including cellulose triacetate and PTFE, with various heavy particles. This "graft and dye" technique is entirely described as well as some characteristics of this new type of heavy ions detectors.

E-26-10 *The Simple Analytical Expression for Distribution of Absorbed Energy in LET.* EVGENIA I. NIŽNIK, AND JAROSLAV I. LAVRENTOVICH, L. V. Pisarzhevsky Institute of Physical Chemistry Ukr. SSR Academy of Sciences, Prospect Nauki, 97, Kiev, USSR.

All reported LET spectra are based on numerical calculation of the energy spectrum of δ-electrons. We have obtained a simple formula for the calculation of the number of electrons resulting from slowing down of high energy electrons in the infinite medium analysing the method of Spencer-Fano-Attix:

$$\mathfrak{N}(E_0, E) = (1 - \epsilon)\left(\frac{E_0}{2E}\right)^{-\epsilon} + \epsilon \cdot \left(\frac{E_0}{2E}\right)^{1+\epsilon/2}$$

where $\mathfrak{N}(E_0, E)$ is the multiplication coefficient of electrons, the function which characterizes the energy spectrum; E_0 is the initial energy of electrons traversing a material; E is the energy of emerging δ-electrons and slowing down primary electrons; $\epsilon = 1/\ln E_0/\dot{\vartheta}$; $\dot{\vartheta}$ is mean excitation energy of the atoms of a material.

Using this formula we have obtained simple expression for distribution of absorbed energy in LET in the infinite medium at irradiation by high-energy electrons and also at irradiation of thin polymer films by heavy charge particles (under condition of electron equilibrium).

The LET spectra, calculated on the base of obtained formulas are in good agreement with LET spectra calculated numerically.

E-27-1 *Increased Proliferation of Hematopoietic Stem Cells Within Diffusion Chambers Implanted into Irradiated Mice Pretreated with Cyclophosphamide.* T. J. MACVITTIE AND K. F. MCCARTHY, Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20014, USA.

The *in vivo* diffusion chamber (DC) method of bone marrow culture was used to determine if the pretreatment of lethally whole-body irradiated mice (WBI) with cyclophosphamide (CTX) released a long range humoral factor specific for proliferation of the hematopoietic stem cell (CFU).

Host mice were pretreated with CTX (200 mg/kg) (control-saline) i.v. 1, 3, 4 and 7 days prior to 900 rads ^{60}Co gamma radiation and implantation of DC's containing normal or irradiated (400 rad) bone marrow cells. DC's were removed at daily intervals and their content

of nucleated cells, granulocytic progenitor cells (*in vitro*, CFC) and transplantable spleen colony forming cells (CFU) were determined. In all experiments using normal and irradiated marrow, the DC's of animals pretreated with CTX 1–4 days prior to lethal WBI contained significantly higher numbers of both CFU's and CFC's as well as total granulocyte cellularity than did the DC's of saline pretreated host mice. The greatest difference in CFU and CFC growth occurred within the 3 day CTX pretreated groups, both normal and irradiated marrow, while the least response was noted in the 7 day CTX pretreated group. The increased proliferation due to CTX was noted within the first 24 hours of culture. Thereafter, the CFU growth rates were approximately the same. Irradiated marrow implanted within 4 hours of exposure, consistently showed a greater response to pretreatment with CTX than did normal marrow. These data support the contention (Gregory *et al.*, Blood, 37: 196, 1971) that a long range humoral factor(s) responsible for stem cell proliferation is released in CTX pretreated mice.

E-27-2 *Influence of Cysteamine (MEA) on Kinetics of Bone Marrow Colony-forming Units /CFUs/ in Sublethally Irradiated Mice.* STANISŁAW BITNY-SZLACHTO AND STANISŁAW KWIEK, JR., Institute of Hygiene and Epidemiology, Warsaw 86, Poland.

Bone marrow CFUs were determined by the transplantation technique in male CFW mice after x-ray whole body irradiation with 200 R. Before the exposure the CFU count amounted to 41.5 $\pm$ 3.0 per 10^5 bone marrow nucleated cells, as estimated on the ground of formed haemopoietic macroscopic colonies in spleens of recipients irradiated with 800 R. In mice given MEA (150 mg/kg i.p.) 10 min prior to irradiation, the CFU count dropped to 5.52 $\pm$ 1.26 when assayed 30 minutes after the exposure, and it fell subsequently to 0.04 and 0.10 after 24 and 48 hours. In controls given i.p. 0.9% NaCl, the respective CFU counts were 5.04 $\pm$ 1.19, 1.15 $\pm$ 0.26 and 0.77 $\pm$ 0 21. Thus, improvement by MEA of bone marrow recovery after sublethal irradiation has turned out to reflect in enhancement of the post-irradiation loss of CFUs.

E-27-3 *The Effect of Prostaglandin on the Regeneration of Stem Cells after Sublethal Irradiation.* JULIA GIDÁLI AND I. FEHÉR. "Frédéric Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene, P.O.B. 101, 1775-Budapest, Hungary.

Proliferation of haemopoietic stem cells (CFU) has been induced by prostaglandins of the *E* group (PGE_1 and E_2), in BALB/c $\times$ CBA/F_1 hybrid mice. The effective doses which induced a short-term cycling of steady-state CFU were between 10^{-4} to 1 μg/g body weight, i.e. in the range of PGE level proved to be present in human serum, too. This fact has suggested that prostaglandins may play a role in the physiological proliferation induction of CFU. Therefore, the action of a single effective dose of PGE_2 was studied on the regeneration of CFU in 350 R gamma-irradiated mice. 0.1 μg/g PGE_2 was injected one day after irradiation. No difference between the kinetics of CFU regeneration was found in the prostaglandin-treated or non-treated groups, not even in a group where the irradiated mice were treated with PGE_2 repeatedly.

E-27-4 *Ultrasonic Irradiation of Human Thoracic Duct Lymphocytes.* JAMES A. ROSEBORO, AMOS NORMAN, AND RICHARD STERN, UCLA Schools of Medicine and Engineering, Los Angeles, California 90024, USA.

We are exploring the feasibility of destroying thoracic duct lymphocytes for the control of autoimmune diseases. For this purpose we are flowing human lymphocyte suspensions through a hollow cylindrical ultrasonic transducer and observing the survival of the lymphocytes as a function of flow-rate, power, pulse duration, and time after irradiation. The destruction of the lymphocytes immediately after irradiation is found to be an exponential function of the ultrasonic energy to which the cells are exposed during the flow through the transducer. The sensitivity of the cells to destruction is similar to that found for red cells. However, when the lymphocytes are cultured for three and six days after irradiation additional cell destruction becomes apparent; the long term cell sensitivity is from three to seven times greater than the sensitivity measured immediately after irradiation. Ultrasonic radiation thus resembles both ionizing radiation and ultraviolet light in producing cell death at times extending over days following irradiation. (This work was supported in part by the Crump Institute for Medical Engineering.)

E-27-5 *The Liver Irradiation Modifies the Bone Marrow DNA.* MASAMI KIGA, TAKASHI KITAHARA, HIDEO SHIMURA, Department of Radiology, School of Medicine, Showa University, Tokyo, Japan.

Purine-requiring nature of the bone marrow cells was responsible for decrease in DNA synthesis by liver irradiation in rabbit. Contents of purines synthesized *de nova* measured by glycine-2-^{14}C incorporation, were also decreased in bone marrow. Administration of purine after liver irradiation improved the DNA synthetic rate in bone marrow. These results support the role of indirect effect and hepatic factor in radiation leukopenia. It is not impossible that indirect and slight damage to the liver may cancel the purine supply to the bone marrow.

E-27-6 *Estimate of Radiation Dose in Whole-Body Exposed Mammals by Means of Bone Marrow Biopsy.* CAMILLO SEGRETO, ANTONIO MATERA, AND FREDERIC C. LUDWIG. Laboratorio de Radiobiologia, Escola Paulista de Medicina, São Paulo, Brazil.

In the bone marrow of whole body irradiated rodents the loss of nucleated cells is paralleled by an increase of number of erythrocytes. Both responses are dose-dependent. Since the ratio erythrocytes/nucleated cells can be determined in marrow samples of unknown or unpredictable size, the radiation dose received by larger mammals be estimated by smears from marrow biopsies. This was confirmed by experiments with twelve whole-body irradiated monkeys (Cebus Apela, from Fundação Parque Zoologico de São Paulo). Three groups of four animals were irradiated with 150, 300 and 1000 rad, respectively. The source was a 4 Mev Linear Accelerator Clinac 4 (Instituto de Radioterapia Osvaldo Cruz, São Paulo). In each monkey, marrow samples were obtained (by removing 1 cm of the right or the left 13th rib) prior to and 60 and 72 hours after exposure. The ratios erythrocytes/nucleated cells ($\times$10 S.D.) were: controls, 4.8 $\pm$ 0.44; 150 rads 60 hrs., 9.0 $\pm$ 1.3; 72 hrs., 18.1 $\pm$ 2.3; 300 rads 60 hrs., 24.3 $\pm$ 5.2, 72 hrs., 35.0 $\pm$ 4.2; 1000 rads 60 hrs., 41.7 $\pm$ 3.2, 72 hrs., 48.3 $\pm$ 5.1. The technical prerequisites, practical significance as well as the limitations of this "biological dosimetry" are discussed.

E-28-1 *Measured Mobilities of Electrons in Liquids.* GORDON R. FREEMAN, University of Alberta, Edmonton, Alberta, T6G 2G2, Canada.

Electron mobilities have now been measured in about fifty organic and inorganic liquids. To avoid serious overlap with Christophorou's paper in this Symposium, my discussion is restricted to temperatures near and below the normal boiling points of the liquids. Mobilities in the diverse compounds range continuously from 10^{-3} to 10^{3} cm^2/Vs. In polar liquids, such as ammonia, ethers, alcohols and water, the mobilities are low, 10^{-2}–10^{-3} cm^2/Vs. The mobility is also 10^{-2} cm^2/Vs in liquid helium. Electron migration in the polar liquids is restrained by attractive electron-dipole interactions. In helium the restraint is due to strong electron-atom repulsions which cause a bubble to form around the electron; there is viscous resistance to migration of the bubble. In argon, krypton and xenon the mobilities are high, about 10^{3} cm^2/Vs. Electrons apparently migrate in conductance bands in these liquids, and the mobilities are much higher than one would expect by extrapolation from the gas phase values. Mobilities in hydrocarbons range from 10^{3} to 10^{-2} cm^2/Vs and are dependent on molecular structure. In saturated hydrocarbons the mobility increases with increasing sphericity of the molecules. The mobility in olefins depends greatly on molecular structure in a manner that is not understood.

E-28-2 *Conduction State Energies of Excess Electrons in Non-Polar Liquids.* RICHARD A. HOLROYD, Chemistry Department, Brookhaven National Laboratory, Upton, New York 11973, USA.

The properties of excess electrons in non-polar liquids depend on the relative energies of the trapped and conducting states. We have measured the energies of the conducting states, denoted V_0, for about twenty non-polar liquids. Two methods were used: In one the work functions of metals immersed in the liquid were measured. In the other, solutes (TMPD) were photoionized in the liquid and V_0 calculated from the wavelength at which ionization onsets occur. A wide variation in conduction state energies is observed from a high of +0.21 eV for tetradecane to a low of -0.60 eV for tetramethylsilane. In general V_0 shifts to more negative values with increasing molecular symmetry, and correlates well with electron mobility. The

photoionization results indicate that V_0 decreases with increasing temperature. In mixtures V_0 is linearly dependent on mole fraction. It was found empirically for *n*-hexane-neopentane mixtures that $\mu = 0.34 \exp [-15.2(V_0)]$. This equation relating V_0 to the electron mobility also applies approximately to pure hydrocarbons. Thus the role of the conduction state energy in influencing electron mobilities and photoionization onsets is established and recent evidence indicates V_0 also influences the rates of electron reactions in these liquids. (Research performed under the auspices of the U.S. Atomic Energy Commission.)

E-28-3 *Theory of Electron Mobility in Non-Polar Liquids.* ROBERT SCHILLER, Central Research Institute for Physics, H-1525 Budapest, P.O.B. 49, Hungary.

Excess electron mobilities, $/\mu$, and the energies of the quasi-free electrons (or the energy of the conducting states), V_0, in non-polar liquids exhibit an apparent interdependence. The understanding of this can be attempted in terms of the general theory of energy fluctuations. Electrons are thought to be either in quasi-free or in localized state these two differing both in energy and mobility. The dynamical equilibrium of the states is controlled by energy fluctuations in the medium. Hence the probability of finding an electron in localized state, P, is given by

$$P = (2\pi\sigma^2)^{-1/2} \int_{-\infty}^{V_0} \exp[-(E - E_t)^2/2\sigma^2] dE$$

where E_t is the energy of the localized state and σ can be calculated from statistical mechanics. Mobility is expressed as $/\mu = /\mu_F(1 - P)$, i.e. by assuming that only quasi-free electrons contribute to observed mobilities. Similar, though not identical, expressions can be established by an alternative model which suggests that the transparency of the liquid toward electrons is governed by fluctuations. The theory is evaluated for pure liquids and also for mixtures. Calculated mobilities and their energy of activation agree reasonably with experiment taking about the same E_t and $/\mu_F$ values for all the saturated open-chain hydrocarbons.

E-28-4 *Electron Mobilities in Gases and Liquids.* LOUCAS G. CHRISTOPHOROU, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

The mobility, μ, of thermal electrons in molecular gases decreases with increasing magnitude of the permanent molecular electric dipole moment D. For small values of $D(\rightarrow 0)$, μ decreases with increasing static polarizability. In addition to these macroscopic parameters distinct microscopic characteristics of the molecular structure affect μ. Thus μ was found to decrease with increasing number of doubly-occupied π-electron orbitals for a number of linear, cyclic and aromatic hydrocarbons. For linear hydrocarbons with two doubly-occupied π-electron orbitals, μ increases with increasing separation of the two π-orbitals. Thermal-electron mobilities in gases will be discussed in relation to molecular structure and will be compared with those in the corresponding liquids. For a number of relatively spherical hydrocarbons, as well as for the heavier rare-gas atoms, thermal electrons seem to be more mobile in the liquid than in the gaseous phase. Mobilities of thermal electrons in high-pressure gases will be discussed also in an effort to relate gaseous to liquid-state behavior. (Research sponsored by the U.S. Atomic Energy Commission under contract with Union Carbide Corporation.)

E-29-1 *RBE and OER after Neutron Irradiation.* ROBERT KATZ AND S. C. SHARMA, University of Nebraska, Lincoln, Nebraska 68508, USA.

Calculations of RBE, OER, and cellular survival, of hamster and human kidney cells, after sequential irradiation with x-rays and 14 MeV neutrons, agree well with the experimental data of Railton *et al.*[1] for these mixed radiation environments, providing a direct experimental confirmation of the theory of RBE for mixed radiation fields. Calculated values of the RBE *vs.* neutron dose, for 14 MeV, 1 MeV, and MRC (Hammersmith Hospital) neutrons, for HeLa, kidney, hamster, leukemia, and mouse bone marrow cells, yield log-log plots which show a monotonic decline of RBE with increasing neutron dose, the RBE being greatest for neutrons of lowest energy. The calculated curves agree quantitatively with experimental values of the RBE

found for murine cataracts (Bateman *et al.*),[2] and with mouse, rat, pig, and human skin (Field),[3] at values of the dose/fraction less than 500 rads. Above this dose there is a systematic disagreement between the cellular survival calculations, and the clinical observations on skin. (Supported by the USAEC and the NSF(RANN).)

[1] R. Railton, D. Porter, R. C. Lawson, and W. J. Hannan, Int. J. Radiat. Biol., in press.

[2] J. L. Bateman, H. H. Rossi, A. M. Kellerer, C. V. Robinson, and V. P. Bond, Radiat. Res. **51**, 381 (1972).

[3] S. B. Field, Radiology **93**, 915 (1969), private communication.

E-29-2 *The 2-Component Model in the Theory of RBE.* S. C. Sharma and Robert Katz, University of Nebraska, Lincoln, Nebraska 68508, USA.

Many investigators have made use of a mathematical model of the form of a product of an exponential function by a multi-target single hit function to represent cellular survival after x-ray irradiation, as $N/N_0 = e^{-D/D_1}\{1 - (1 - e^{-D/D_2})^m\}$, where $D_1/D_2 \simeq 2$, in many cases. When this formula is incorporated into the theory of cellular survival we find that a plot of the "ion-kill cross-section," σ, *vs.* z^2/β^2 of the incident ion has slope less than 1 (on a log-log plot), when $D_1/D_2 < 10$. Since the nominal value of the RBE in the ion-kill mode is RBE $= D\gamma^{37}\sigma/L$, and $L(\mathrm{LET}_\infty) \propto z^2/\beta^2$, these results imply that the RBE of cells, whose survival after x-rays is given by the two-component model (has an observable initial negative slope), is less than 1, for track segment bombardment with heavy ions, and therefore with neutrons. Since there exist many observations of cells whose survival curve has an observed initial negative slope after x-ray irradiation, and whose RBE exceeds 1 for neutron irradiation, calculations have been made of the RBE which would be observed for a mixed population of cells. We find that for mixed populations it is possible to display both an initial slope after x-ray irradiation, and an RBE greater than 1 after heavy ion or neutron irradiation. (Supported by the USAEC and the NSF(RANN).)

E-29-3 *Examples of Cell Survival Models Incorporating the Fixation Time Concept.* W. R. Garrett and M. G. Payne, Health Physics Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

On the basis of a rather general mathematical formulation of low LET cell survival models,[1] we consider survival models specific to (i) several types of liquid holding recovery experiments on diploid yeast[2] and (ii) survival of stationary phase Tetrahymena Pyriformis when subjected to post-irradiation treatment with caffeine.[3] Results agree well with experimental data and allow a clear distinction to be made between certain choices of proposed mechanisms. (Research sponsored by the U.S. Atomic Commission under contract with the Union Carbide Corporation.)

[1] M. G. Payne and W. R. Garrett (submitted for publication).

[2] V. I. Korogodin, Yu. G. Kapul'tsevich, M. N. Myasnik, A. F. Mosin, and V. V. Gridnev, Adv. Biol. Med. Phys. **12**, 253 (1968).

[3] J. Calkins, Radiation Res. **45**, 50 (1971).

E-29-4 *A Family of Cell Survival Models for Low LET Radiation.* M. G. Payne and W. R. Garrett, Health Physics Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

A rather general statistical problem concerning the effect of radiation on a cell is solved in analytic form, and the solution is used to generate a large variety of cell survival models. These models can include in an approximate way all of the following: (i) the effects of statistical fluctuations associated with both the direct and indirect effects of radiation for an arbitrary time history of the radiation field, (ii) the chemical kinetics associated with the indirect effect and/or with recovery and repair, (iii) possible effects of radiation on the repair mechanism, (iv) the effect of chemicals that are believed to either stop repair or "fix" reparable cell damage, (v) the effect of a "fixation time" or other cell cycle effects, (vi) the accumulation of reparable

damage to give a lethal lesion, and (vii) the possible requirement of multihits in accounting for cell death. (Research sponsored by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.)

E-29-5 *Production and Repair of Sublethal Damage by Different LET of Ionizing Particles in Diploid Yeast.* H. Liesem and W. Pohlit, Gesellschaft für Strahlen- und Umweltforschung, D-6 Frankfurt (M), Paul-Ehrlich-Str. 20, West Germany.

A cybernetic model for radiation reactions in eukariotic cells has been used to analyse repairable and irrepairable radiation reactions at the target molecules. Irrepairable reactions can be observed at extremely low absorbed dose rate, since all repairable damage is repaired during irradiation time. At this extremely low absorbed dose rate also all those reactions are omitted by repair, which otherwise would be unrepairable lethal due to the combination of two repairable lesions at the same locus in the target molecule. Such analysis has been made with different ionizing particles, and the dependance of both components (repairable and irrepairable damage) on the different linear energy transfer is discussed in detail. The main result is that repairable damage is mainly produced by indirect effects of radicals and unrepairable damage is produced mainly by direct effects with a strong dependance on LET of the particles.

E-29-6 *Approaches to the Theoretical Simulation of Radiation Injury in the Organism and its Systems.* I. G. Akoev and V. G. Tyazhelova, Institute of Biophysics, Acad. Sci., USSR, Pushchino, Moscow Region, USSR.

The kinetics of the formation of two groups of radiation effects both structural-morphological and functional ones induced in the organism and its systems by irradiation has been considered. Regularities of transition of a "closed" injury into the visible pathology and the effect of distant connections on these processes have been determined. It has been shown that under certain conditions proportionality between the rate and intensity of the recovery response and the rate and value of the injury should be taken into account. The significance of the potentiality of the recovery mechanisms the degree of their activation, the possibility of their occurance, duration of their action in the organism have been estimated. Time has been shown to play the main role in the adaptation reactions which take place in the organism on different biological levels.

E-29-7 *Multiple Simultaneous Mutation Model for Radiation Carcinogenesis.* J. W. Baum, Health Physics and Safety Division, Brookhaven National Laboratory, Upton, New York 11973, USA.

A mathematical model is proposed which postulates that cancer induction is a multievent process, that these events occur naturally, usually one at a time in any cell, and that radiation frequently causes two of these events to occur simultaneously. Microdosimetric considerations dictate that for high LET radiations the simultaneous events are associated with a single particle or track.

The model predicts: (a) linear dose effect relations for early times after irradiation and low doses, (b) approximate power functions of dose (i.e. D^x) having exponent less than one for small doses and moderately long times after irradiation, and (c) saturation of effect at either large doses or for long times after irradiation.

Data of Vogel, for neutron induced mammary tumors in rats, are used to illustrate the validity of the formulation. This model provides a quantitative framework to explain several unexpected results obtained by Vogel. It also provides a logical framework to explain the dose-effect relations observed in the Japanese survivors of the atomic bombs.

E-30-1 *Markov Chain Model for Chromosome Aberrations and Cell Lethality.* G. A. Sacher, M. Gabriel, and S. A. Tyler, Argonne National Laboratory, Argonne, Illinois 60439, USA.

We present an ad hoc model of the kinetics of chromosome breakage and rejoining, and examine the explicit hypothesis that radiation lethality in cells is due to lethal rearrangements. The "state" of a cell is given by the number of chromosome fragments present. A chromosome

break moves the cell to the next higher state, and rejoining of 2 fragments moves it to the next lower state. Nonviable aberrations (dicentrics or acentrics) lead to an irreversible "lethal" state. The transition probabilities between states are derived, and the set of differential equations for the state probabilities is integrated numerically. The first case assumes that there is at most one break in a chromosome. This case is adequate to explain some features of the dose and dose rate dependence for lethal effects of x and gamma rays in cells and animals. A second case adds assumptions of delayed breaks and intrastrand repair, which are needed to account for the shapes of cell killing curves. Some LET effects are explicable by the model. (Work supported by the U.S. Atomic Energy Commission.)

E-30-2 *Radiation Sensitivity During the Cell Cycle.* WILLIAM C. ROESCH, Battelle, Pacific Northwest Laboratory, Richland, Washington 99352, USA.

The change in the survival curves of synchronized mammalian cells as they move through their mitotic cycle is being studied with the following model: The constituents of a cell move through a large, complex web of biochemical-reaction pathways. The radiation sensitivities and populations of the stages just before rate-limiting reactions determine the survival curves obtained at different stages of the cell cycle. Chemical-kinetic equations give the populations at these stages as a function of time.

Survival data for Chinese hamster cells were modeled this way. The first half of the cycle has three rate-limiting steps with mean lives of about 20 min, 3 hr, and 1 hr. Radiation inactivates by single-event mechanisms in the stages before the first two and by both single-event and two-event mechanisms before the last. The same model may also apply to other phenomena; for example, the radio-"sensitizing" effects of some drugs (hydroxyurea and hydroxyurea plus *n*-ethylmaleimide) may be due to population shifts rather than to sensitization, and the blocking action of cyclohexamine and puromycin in non-radiation experiments may involve the same rate-limiting steps.

E-30-3 *Applications of a High LET Track Model.* A. CHATTERJEE AND C. A. TOBIAS, Donner Laboratory/Lawrence Berkeley Laboratory, Berkeley, Ca. 94720, USA.

The physical processes of the interaction of ionizing particles with matter (living and non-living) are over in the extremely short time of 10^{-17} to 10^{-15} seacond. At this instant of time the track structure of a heavy charged particle can be characterized quantitatively in terms of radial-energy-density function. The dependence of this function on the flow of time has been considered to account for the chain of events which follows the initial physical processes including interactions of free radicals. A model resulting from such considerations will be presented. The model has been applied to predict the relative yield of Ferric ions in the Fricke dosimeter system and also to predictions of survival curves and oxygen effect in mammalian cell radiation biology experiments with high energy heavy ions. A discussion on the distinct possibility of generation of shock waves due to passage of heavy charged particles will be presented also.

E-30-4 *A New Model Describing the Energy Deposition Profile Along the Path of Energetic Heavy Ions.* LUIS MUGA, Chemistry Department, The University of Florida, Gainesville, Florida, 32601, USA.

A new model is presented which describes the energy deposition radially outward from the trajectory axis of an energetic heavy ion. Applied to luminescence production in thin plastic scintillators, the model accurately describes the specific luminescence as a function of heavy ion nuclear charge over a wide range of ion velocity or energy. In brief, the number of electrons scattered into a thin disk of scintillator material (perpendicular to the ion trajectory) is taken, in the absence of saturation effects, as proportional to the luminescence response from that disk. Saturation effects are included by deriving an explicit relation for the number of electrons scattered per unit volume of disk at a distance r from the ion path. Above a critical number density no additional luminescence response is generated from that volume element. From these number density profiles, dose deposition profiles are obtained using known electron range-energy relations. Using the same concept of saturation to define "overkill" or "surekill" of biological cells, the radiation damage profile may be similarly calculated and used to describe the survival

rate of biological cells along and about the path of an energetic heavy ion. Results of calculations to this end are presented. (Supported in part by the U.S. Atomic Energy Commission.)

E-30-5 *Relative Size of Nuclear Components.* ALAN D. CONGER, Temple U. Sch. of Med., Philadelphia, Pa. 19140, USA.

When the relation of nuclear volume (NV) to DNA per cell is tested within a group of related species (flowering plants; amphibians; the genus Rumex), the data best fit the regression $Y(\text{NV}, \mu 3) = a + bX(\text{DNA/cell}, \mu^3)$—but NV begins to saturate toward high D's. Say that the nucleus (N) consists of sap (S), plus chromosomes of protein (P) and DNA (D), or, N = S + (P + D). Then, the a intercept is the group's "residual" sap, and is surprisingly large (32–87 μ^3). The b constant, the increase in NV for each 1 μ^3 increase in D is also large (17–24 μ^3). In these three groups, most of the NV is sap (96–89%), a fairly constant fraction at larger D's, but increasing sharply at low.

Physiologically, "residual" sap volume may be the biochemical "maintenance" volume for a nucleus of the group. The b constant could be the "synthetic" sap volume (17–24 μ^3) required for each μ^3 of active DNA; and the saturation of NV implies that, at large D's in a group, not all the DNA is being synthesized from.

E-30-6 *The Interaction of DNA with Model Membranes upon Exposure to X-Rays.* DENNIS PIETRONIGRO AND HARRY B. DEMOPOULOS, New York University Medical Center, Department of Pathology, New York, New York 10016, USA.

DNA has been shown to be intimately associated with membranes in prokaryotic as well as euckaryotic systems. In bacteria such as *B. subtilis* and *E. coli,* the inner cell membrane is the site of the origin and replication point for DNA synthesis. In eukaryotes, although the literature is somewhat ambiguous, one would have to conclude that the nuclear membrane has many associations with DNA (chromatin), some of which may be non-specific and others more specific such as initiation sites for DNA synthesis.

X-irradiation produces many effects upon cells including mutation, inhibition of DNA synthesis, membrane damage and cell death. We have studied the interaction of DNA and model membranes upon exposure to x-rays. Phosphatidylcholine liposomes and *B. subtilis* DNA were mixed together in suspension and x-irradiated in the presence of various catalysts of peroxidative membrane damage. DNA damage was measured by a decrease in transforming ability, while membrane damage was assayed by gas-liquid chromatography and the thiobarituric acid test. Modification of the radiation-induced DNA damage through interaction of the DNA with the liposomal membrane will be discussed.

E-30-7 *Statistical Aspects of Dose-Effect Relationships for Somatic Mutation Induction in* Tradescantia occidentalis. J. A. DENNIS, National Radiological Protection Board, Harwell, Berks, Great Britain.

Polynomial expansions and other theoretical expressions have been fitted by minimization of the weighted squares of deviations to experimental data for the dependence upon absorbed dose of somatic mutation induction in the staminal hairs of *Tradescantia occidentalis* by x-rays, gamma-rays and monoenergetic neutrons. Although over limited dose ranges the x-ray and gamma-ray data seem to be superficially well fitted by second order polynomials and the neutron data by first order polynomials, a closer study suggests that this is not the case. It is thought that the higher order polynomials which are required to provide statistically "good fits" reflect variations in sensitivity throughout the cell cycle for somatic mutation induction.

E-30-8 *The Biological Toxicity of X-Ray Induced Auger Effect in Nuclides of Medium Mass, Incorporated into Cellular DNA.* L. E. FEINENDEGEN,* G. TISLJAR-LENTULIS,* AND V. P. BOND.**

The decay of I-125 by K-capture and internal conversion results in the emission of Auger electrons. When I-125 was bound to DNA of mammalian cells, the killing efficiency per decay was approximately 0.025 irrespective of radiosensitivity of the cells.

Photoelectric effects of x-rays causing vacancies in inner electronic shells of nuclides also trigger Auger cascades and are expected to be detrimental to biomolecules.

Monoenergetic x-rays corresponding to the K-edge of particular atoms produce a high yield of photoelectric events in comparison to Compton scattering. For producing photoelectric events in nuclides of biomolecules the x-rays energies required are too low for effective dose distribution on organic tissue. Incorporation of heavier nuclides allows higher energies and consequently a more favourable dose distribution. The various factors for optimal dose depth distribution are expressed in a figure of merit for nuclides 23 to 61. Bromine incorporated into DNA of various cell lines served as target for 14 KeV x-rays. Viability studies indicated the biological toxicity of the induced Auger effect and may offer therapeutic advantages over conventional radiotherapy.

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E-30-9 *Optical Properties of Chloroplasts and Red Blood Cells in the Vacuum UV.* M. W. Williams, E. T. Arakawa, R. D. Birkhoff, R. N. Hamm, H. C. Schweinler, and R. A. MacRae.* Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

Absorption spectra have been obtained for chloroplasts from 2 to 22 eV and for packed red blood cells from 2 to 10.6 eV. The spectra are very similar except for the well-known chlorophyll absorption at 1.75 eV. In the ultraviolet, above 4 eV, an analysis is given in terms of the spectra of the main constituents; proteins and lipids in the chloroplasts and protein (globin) and water in the red blood cells. In chloroplasts there is evidence for the existence of an electronic collective oscillation which could provide an important energy transfer mechanism associated with the interaction of radiation with green plants. (Research sponsored by the U.S. Atomic Energy Commission under contract with Union Carbide Corporation.)

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E-31-1 *A 6 MeV Photon Facility.* Geoffrey B. Bishop, Liverpool University, Liverpool L69 3BX, Great Britain.

The nitrogen 16 radiation facility at the Universities Research Reactor Centre has been designed to provide 3 R/h of 6.13 MeV gamma for penetration and dosimetry studies. It utilises the nitrogen 16 produced by the fast neutron reaction $^{16}O(n, p)^{16}N$ in the reactor cooling water as it passes through the fuel element assemblies. On leaving the reactor core, the active cooling water is pumped directly to a disc radiator positioned within a shielded cell. The radiator is a specially designed disc with varying section to produce a well defined uniform source suitable for benchmark experiments. Within the shielded cell is a rotating 1.6 ton lead collimator mounted on a traversing gear capable of remotely controlled movement in three dimensions to an indicated position with an accuracy of microns.

The current research programme will provide benchmark data, with assigned accuracy, for the scalar and angular flux penetration of 6 MeV γ through lead, iron, aluminum, graphite and laminated assemblies. Radiation dosimetry studies will be carried out to determine the effect of 6 MeV γ on thermoluminescent materials and the response of cavity ionisation chambers at this energy.

E-31-2 *KARIN—A Sealed High-Power Generator of 14 MeV Neutrons for Radiotherapy and Activation Analysis.* K. A. Schmidt, Institut für Angewandte Kernphysik, Kernforschungszentrum, Karlsruhe, West Germany.

A 14 MeV neutron generator tube based upon the d-t fusion reaction from a 150 mA mixed deuterium-tritium ion beam (50% atomic) accelerated onto an internal 200 kV titanium-tritium-deuterium target has been developed. The tube is constructed as a compact, closed, ceramic-metal sealed UHV system. It contains about 300 Ci of Tritium and has a life expectancy of several hundred hours. The source strength is 5×10^{12} neutrons per second.

The average flux inside the central hollow target cylinder of 5×10^{10} N/cm^2 sec is nearly isotropic and very homogeneous in a volume of 30 cm^3. The irradiation site is accessible by a 1″ diameter rabbit system.

Clinical radiotherapy with fast neutrons at a kerma dose rate of 20 rad/min. will be performed at a distance of 90 cm from the target center, 10 cm outside a 70 cm long collimator. Optimum collimation is achieved by using a target shaped into a truncated cone with its apex on the collimator axis in the direction of the neutron beam, the apex angle being chosen according to the maximum size of the irradiation field to be used. A movable radiator head shield with exchangeable collimator inserts is in construction.

Operational experience with the prototype of the tube KARIN is presented.

E-31-3 *Activation Control at LAMPF.* J. R. PARKER, University of California, Los Alamos Scientific Laboratory, Los Alamos, New Mexico 87544, USA.

Activation control at a large proton linear accelerator has been attained without unduly limiting accelerator operation and adjustment. Beam spill is sensed by detectors located near the most probable spill regions. Their output is employed to inhibit the beam whenever the average beam spill exceeds a limit determined by maintenance access requirements.

The half-lives of the activities generated and the energy dependence of the activations change the allowed beam spill by over sixty times from the 100 MeV region of the machine to the output at 800 MeV. However, it is shown that the energy dependence of detector sensitivities has to change only by 2X from 100 MeV to 800 MeV.

Calibration of the system *in situ* is done by employing the radiation incidental to the use of wire-scanner beam-profile monitors. Computer codes have been developed which enable calibration checks of over fifty detectors as frequently as the operators wish. These codes also evaluate the system performance and determine whether radiation damage or other failure modes have occurred.

A summary of data on radiation levels along the beam channel is presented.

E-31-4 *Experimental Apparatus at the Bologna Linac.* A. HUTTON, G. ROFFI, AND A. MARTELLI, Laboratorio di Fotochimica e Radiazioni d'Alta Energia, Via dei Castagnoli 1, 40126 Bologna, Italy.

A description of the experimental set up for the 12 MeV Vickers Linac is presented. The system is characterized by the very low level of electrical interference, achieved by complete shielding of the accelerator and avoidance of earth loops during construction of the laboratory. Auxiliary equipment including the optical system, apparatus for rapid mixing and conductivity are described in detail. Results obtained with this equipment are given to indicate the capabilities of the set up. The designs for the electron beam transport system which is to be installed for the study of γ, n reactions are outlined, with particular reference to the solution adopted to enable fast change over between experiments.

E-31-5 *Spectroscopy of Therapeutic Electron Beams.* JOHN C. F. MACDONALD, GARY W. DOUGLAS, AND JERRY J. BATTISTA, Ontario Cancer Foundation, London, Ontario, N6A 4G5 Canada.

The effective ranges of electron beams used in radiation therapy are determined by their energy spectrum. A portable magnetic spectrometer has been built which permits the experimental determination of the spectrum of electrons generated in machines operating at nominal energies up to 35 MeV. After calibration using heavy ions of known energy, it has been used to determine the electron energy spectrum of a therapeutic betatron and of an experimental racetrack microtron over an energy range of 6 to 35 MeV. The results of these measurements will be presented and their significance discussed.

E-31-6 *An Efficient, Low-Cost Shield for a Whole-Body Counter.* PAUL T. SNOWDEN, ROBERT L. LONG, AND HUGO G. PENA, Veterans Administration Hospital and University of New Mexico, Albuquerque, New Mexico 87108, USA.

The typical shield for a whole-body counter is constructed of thick, pre-atomic age steel, usually obtained from old battleships or railroad track. This steel is both expensive and dif-

ficult to obtain. This paper presents a proposal for a less expensive shield, capable of being fabricated on site, and as effective a shield as its steel counterpart. The proposed shield is designed to optimize Compton scattering and the photoelectric effect, two main gamma attenuation mechanisms. It would be of layered construction—the first, or outer, layer composed of gypsum (an inexpensive material chosen to enhance Compton scattering), a second layer composed of dense ferrophosphorous concrete (to enhance the photoelectric effect), and a third layer of thin sections of lead, cadmium and copper. A theoretical calculation shows that if a ^{40}K source with an activity of 1 R/hr were placed on the outside of the shield, the activity inside the shield would be 0.02 micro R/hr. The layered shield would cost about one-third that of an equivalent steel shield.

E-31-7 *Microwave Cavity Irradiation Dosimetry.* WILLIAM P. EDWARDS AND HENRY S. HO, Bureau of Radiological Health, Division of Biological Effects, Rockville, Maryland 20852, USA.

A right circular cylindrical 383 MHz resonant cavity was developed to irradiate a monkey head, with little or no microwave exposure to other tissues. The system is used in studies to determine the behavioral effects of the absorption of radiant power by the head exposed to microwave radiation. Dose rate measurements were made by replacing the head with an electrically equivalent calorimetric load, consisting of a saline-filled plastic cylinder whose geometry and position in the cavity reproduced parameters measured with a test animal. Since integral dose rate P_m (total power absorbed) is proportional to the net power transmitted to the cavity P_t, the constant of proportionality $K_m = P_m/P_t$ must account for the absorption of field energy by the tissue. K_m was determined by comparing the temperature rise produced in a fixed time period by the dissipation of DC power to the temperature rise produced by microwave radiation in the same time. It was found that, at an ambient temperature of 25 $\pm$ 2°C and a relative humidity of 55 $\pm$ 5%, K_m is 0.62.

E-31-8 *An Inexpensive, Continuous, Ultrasensitive Air Sampler for Differentially Sampling Tritium Oxide and Gas.* RALPH BROWN AND BOB ROBINSON, Monsanto Research Corporation, Mound Laboratory, Miamisburg, Ohio 45342, USA.

The instrument described is a small, inexpensive, practical, ultrasensitive, portable air sampler for the simultaneous, differential sampling of air for tritium oxide and elemental tritium. The sampling system occupies a relatively small space (12″ × 12″ × 8″), is light weight (Approx. 12 lbs.) and is simple to operate. The detection sensitivity approaches that of background levels in air. The two tritium fractions are collected in traps of silica gel and a bubbler preceeded by a Palladium catalyst. Details of operation and sampling data are described.

E-31-9 *Solarization Characteristics of Cellulose Triacetate Film Exposed to Ultraviolet Light (270–400 nm).* ROBERT C. WORREST, DONALD J. KIMELDORF, AND D. STUART NACHTWEY, Oregon State University, Corvallis, Oregon 97331, USA.

For studies of biological effects of a simulated increase of the global solar flux in the 290–320 nm region, a combination of fluorescent sunlamps and spectrum-limiting filters is being used. One type of filter is cellulose triacetate film (Kodacel-TA401) which strongly absorbs wavelengths shorter than 290 nm. The transmission characteristics of Kodacel-TA401 were determined over the spectral range of 210–370 nm, using a Shimadzu Spectrophotometer: MPS-50L. Percent transmittances at 310 nm for nonsolarized three mil (0.08 mm) Kodacel is 80%, relative to air. At 290 nm, the respective value is 3%. During continuous exposure to radiation emitted by four Westinghouse-FS40 sunlamps (SSD, 33 cm), at an exposure rate equivalent to three Sunburn Units per hour (Robertson Sunburning Ultraviolet Meter), three mil thicknesses of Kodacel solarized for 24 and 48 hours yielded filter transmittancies of 0.73 and 0.68, respectively, in the 300–320 nm range, relative to nonsolarized Kodacel. A comparable solarization pattern was found with five mil (0.13 mm) Kodacel. Transmission values greater than 80% of the values found at 48 hours were obtained after solarization of the filters for ten days. It is concluded that the filters can be considered relatively stable after the initial 48-hour solarization effect.

E-31-10 *Theory of Interaction Between Nuclear Radiation and Liquid Scintillator and its Practical Results.* HUBERT PROCHÁZKA, JOSEF KATZER, AND RUDOLF JÍLEK, Veterinary Research Institute—Czechoslovak Academy of Agriculture, 621 31 Brno, Czechoslovakia.

The presented interaction-theory is deduced from both the theory of liquid scintillators and the general theory of interaction between nuclear radiation and matter. It allows both theoretical formulation of optimal measurement conditions with the help of calculated balance-point position and interpretation of the measured response of liquid scintillator detector. The balance-point positions are proportional to the average resulting energy values of measured radiations the simplified calculations of which are described. Experimentally gained results are in good agreement with the presented theory. Conventional liquid-scintillation spectrometers can thus be used for direct measurement of low-activity samples of fission-products and other radioactive elements especially in waste-waters of nuclear power stations or uranium industry.

E-32-1 *The Temperature Dependence of Fluorescence from Liquid Alkanes and the Quenching of that Fluorescence.* W. P. HELMAN, Radiation Laboratory, University of Notre Dame, Notre Dame, Indiana 46556, USA.

The decay time of fluorescence from several alkane liquids has been measured from near the melting point to above room temperature. Excitation is provided by a pulsed x-ray source operated at 35 kV. Fluorescence was selected by a 205 nm interference filter and measured by a single photon timing technique. The effect of added CCl_4 as a quenching agent has also been measured. These results confirm previous findings that quenching is faster than expected for diffusion controlled reactions.

E-32-2 *Energy Transfer by Excitons in γ-Irradiated n-Alkane Single Crystals.* TOMAS GILLBRO AND ANDERS LUND, The Swedish Research Councils Laboratory, Studsvik, 2-611 01 Nyköping, Sweden.

Single crystals of *n*-decane-d_{22}($C_{10}D_{22}$) doped with $C_{10}H_{22}$ were γ-irradiated at 77°K. Identification of the radiation-induced alkyl radicals by electron spin resonance showed that mainly two species were present. One radical is of the type—$CD_2\dot{C}D\ CD_2$–, $C_{10}D_{21}\cdot$ (II), and the other radical is of the type $CH_3\dot{C}HCH_2$–, $C_{10}H_{21}\cdot$ (I). For $C_{10}D_{22}$ crystals with a small content of $C_{10}H_{22}$ the relative amount of $C_{10}H_{21}\cdot$ (I) radical was much higher than expected from simple stoichiometric considerations. For example, in a crystal containing 0.25 mol% $C_{10}H_{22}\cdot$, 14% of the alkyl radicals were in the form of $C_{10}H_{21}\cdot$ (I), as estimated by comparison with simulated ESR spectra with the two components added in different relative amounts. Several mechanisms for the energy transfer in *n*-alkanes are discussed including ionic processes, hydrogen abstraction by hot hydrogen atoms and exciton transfer. The exciton model, however, offers by far the best explanation of the experimental results and it was possible to calculate the critical concentration of $C_{10}H_{22}$ for the most effective trapping process. The critical concentration was 0.2 mol%, which corresponds to a critical trapping distance of ca. 30 Å.

E-32-3 *On the Stimulated Neutralization Luminescence in γ-Irradiated Ices and the Triplet State of Water.* A. BERNAS AND T. B. TRUONG, E.R. CNRS 98, Université Paris VI, Avenue Jean Perrin, 91 405-Orsay, France.

The luminescence of pure crystalline ice had been studied so far either during x-irradiation or low energy electron bombardment, or after the irradiation through thermoluminescence.

For γ-irradiated light or heavy crystalline ice, we have now recorded the neutralization luminescence associated with an optical release of the trapped electrons. The luminescence intensity is found higher for heavy than for light ice.

The emission and luminescence excitation spectra will be presented and discussed.

The kinetic energy of the released electrons is two low ($\simeq$1 eV) to lead either to an electron attachment on water or to a direct electronic excitation of OH or OH^-. The lower triplet of water formed upon H_3O^+ neutralization thus remains the most plausible emitting species.

E-32-4 *Chemiluminescent Reactions Induced by Ionizing Radiation in Aqueous Solutions of Aromatic Molecules.* WALTER A. PRÜTZ, Institut für Biophysik und Strahlenbiologie der Uni-

versität Freiburg, West Germany and E. J. LAND, Christie Hospital and Holt Radium Institute, Manchester 20 9BX, Great Britain.

Various aromatic molecules which exhibit fluorescence in aqueous solution can also be excited to the fluorescent state by high energy irradiation in a chemiluminescent process involving oxidation of the fluorescer by OH-radicals and subsequent reduction by hydrated electrons. This general chemiluminescent process is discussed on the basis of pulse radiolysis experiments. Absolute quantum efficiencies of the chemiluminescent reaction were determined for several systems.

E-32-5 *Role of Molecular Excitons in Radiolytic Formation of Molecular Hydrogen from Solid Aromatics.* JAROSLAV BEDNÁŘ, Institute of Nuclear Research, 250 68 Řež, Czechoslovakia.

It is suggested that a part of molecular hydrogen formed in radiolysis of solid biphenyl, naphthalene and similar aromatics originates in monomolecular decay of low-energy excited states of the aromatic π-electron system. These excited states are in the form of optically-allowed molecular excitons.

E-32-6 *Mechanisms of Scintillation in Organic Crystals Bombarded by α-Particles: Tetracene.* NICHOLAS E. GEACINTOV, MICHAEL BINDER, MARTIN POPE, AND CHARLES E. SWENBERG, Radiation and Solid State Laboratory, New York University, New York, New York 10003, USA.

The utility of external magnetic fields ($H \approx 4000$ gauss) as a probe of the scintillation mechanisms in organic crystals is illustrated by a study of the effect of H on the fluorescence (F) and scintillation (S) of single crystals of tetracene at room temperature. Prior to exposure of the crystal to the α-particles the effect of H on the UV excited fluorescence is $F(H)/F(O) \approx 1.30$, but decreases gradually with increasing time of exposure. After a 14 hour irradiation period $F(H)/F(O)$ is 1.23, but if the source is removed for 12 hours, $F(H)/F(O)$ recovers to 1.28. As the irradiation time is prolonged, however, the ability of the crystal to recover is gradually destroyed and $F(H)/F(O)$ appears to reach a limiting value of 1.10–1.15 after 50 hours of irradiation. The decrease in $F(H)/F(O)$ with increasing irradiation time is attributed to the generation of quenching centers such as free radicals. At room temperature, the lifetime of some of these quenchers is of the order of hours, which is indicated by the recovery of $F(H)/F(O)$ after moderate irradiation times. The magnetic field effect on the UV-excited fluorescence is decreased because the quenchers produced by α-particle bombardment can compete with the fission of the fluorescence emitting singlets into two triplet excitons, which is the dominant mode of decay of singlet excitons at room temperature in the case of UV excitation.

The effect of H on the scintillation yield is $S(H)/S(O) \approx 1.02$. This indicates that in the α-particle track there is a high density of transient quenchers ($\sim 10^{18}$ cm^{-3}), which are most likely triplet excitons. Using the values of $S(H)/S(O)$ and the fission rate constant, the singlet exciton lifetime in the α-particle track is estimated to be less than 2×10^{-11} sec. (This work was supported by the U.S. Atomic Energy Commission.)

E-32-7 *A Monte Carlo Study of the Spatial Distribution of H_2O Molecules Excited by Delta-Rays.* J. H. MILLER, G. J. KUTCHER, AND A. E. S. GREEN, University of Florida, Gainesville, Florida 32611, USA.

The distribution around the track of a heavy ion of H_2O molecules excited by collisions with low energy, secondary electrons (delta-rays) was investigated by Monte Carlo techniques. The degradation of electrons ejected from a point on the heavy ion track was simulated using differential cross sections for elastic scattering, vibrational excitation of the H_2O ground state, electronic excitation and ionization. The molecular excitation resulting from each inelastic collision was scored and profiles for the excitation of a given state were calculated. Profiles associated with different ejection energies and angles are folded with the differential, heavy ion impact ionization cross section to obtain the distribution of excited states about the heavy ion beam. The Monte Carlo results are compared with earlier calculations based on a continuous slowing down approximation. (Work supported by United States Atomic Energy Commission Contract AT-(40-1-3798).)

E-33-1 *Biphasic Response of Lymphoid Tissue Under Continuous Irradiation.* BESSIE R. FOSTER, BILLY TAYLOR, PATRICIA LEFEAR, AND MATTHEW WARE, Grambling College, Grambling, Louisiana 71245, USA.

Cell population kinetics of thymic tissue has been examined in mice irradiated at a dose rate of 10 roentgens per day for 105 days. Techniques of electronic cell counting, labeling with tritiated thymidine, and autoradiography, indicate that two different "steady state" phenomena of cellular proliferation obtain during the irradiation period. On the basis of tissue weights, cell counts, distribution of PAS-positive cells and thymidine labeling, the data suggest that the first steady state phenomenon is reached by about day 24 through day 84. At this time, there is a significant decrease in tissue weight, cell counts, etc., for about 2 weeks which is interpreted as a "breakdown" in the system. By day 98 through the remainder of the irradiation period, another steady state of cellular proliferation obtains. Factors which may contribute to this biphasic response of the thymus under continuous irradiation are discussed.

Acknowledgments.—Research jointly funded by NSF Grant GB29136 and NASA Grant NGR-19-011-008, and initiated at The Argonne National Laboratory under the sponsorship of the US AEC's Division of Nuclear Education and Training.

E-33-2 *Proliferative Patterns of Mouse Jejunal Epithelium Following Fractionated X-Irradiation.* JOHN W. COOPER, S. LESHER, AND RONALD F. HAGEMANN, Allegheny General Hospital, Pittsburgh, Pennsylvania 15212, USA.

Male C57 B1/J mice received a series of 1 to 9 (300 R) x-ray exposures (abdomen only). Labeled nuclei and mitotic figures/crypt were determined from 1 to 96 hours after the terminal exposure. PLM curves were prepared 12, 24 and 48 hours after the terminal exposure. As the number of fractions increased, duration of G_2 delay decreased from 3 hours (single exposure) to 2 hours (2 exposures) to essentially zero (3–9 exposures). As the number of fractions increased, the height of overshoot in LN/crypt and MF/crypt decreased. The interval between exposures governed the degree of damage and compensatory response; the shorter the interval, the greater the reaction. By 24 hours post-exposure, PLM curves were similar in shape to controls, but significantly shorter. All stages were reduced, but shortening was due primarily to reduction in G_1. The responses from the third exposure on appeared to be similar. Peak response (height of curve) versus integrated response (area under curve) will be discussed.

E-33-3 *Changes in Intestinal Cell Proliferation Accompanying Lactation.* J. D. HARDING AND A. B. CAIRNIE, Department of Biology, Queen's University, Kingston, Ontario, Canada.

Since some have held that all proliferative cells in the crypt are stem cells, a physiological condition in which intestinal hyperplasia occurs would permit a test of the correlation between stem and proliferating cells. To this end, lactation in mice was investigated.

The total number of crypts and villi did not change during the intestinal hyperplasia associated with lactation. However, the number of cells per crypt more than doubled (virgins: 254 $\pm$ SE 10; lactating mice 585 $\pm$ 22) as did the number of cells in the S period (virgins: 82 cells/crypt $\pm$ SE 5; lactating mice: 168 $\pm$ 7). Cell cycle times of the proliferating cells, analyzed by the percent labelled mitoses method, did not greatly differ (virgins: 11.6 hours; lactating mice: 10.2 hours). It was calculated that the pool of proliferating cells in the crypt almost doubled during lactation.

Dose survival curves of intestinal stem cells of virgin and lactating mice have been undertaken to determine whether the number of stem cells, assayed by the method of Hagemann, Sigdestad and Lesher, has increased in relationship with the increase in proliferative cells in the crypts. (Supported by DRB., Ottawa.)

E-33-4 *Search for the Clonal Origin of Regenerating Islands in Swine Skin.* JOSEPH J. ABATA AND JOHN O. ARCHAMBEAU, Nassau County Medical Center, East Meadow, New York 11554, USA.

The presence of dose-independent giant islands of regeneration in previously irradiated swine skin gives rise to the possibility of the existence of radioresistant clones of epidermal basal cells. To identify these young Yorkshire swine were injected I.V. with tritiated thymidine at 8-hour

intervals for 10 consecutive days. Daily biopsies were taken, and at the end of the injection period the biopsy-free side of the swine were irradiated with 250 KV x-rays. The three 10-cm. fields were the shoulder (2300 r), the side (1700 r) and the ham (1700 r). Biopsies were taken from each field and from an unirradiated area twice weekly for five weeks. Measurements made on the autoradiograms obtained include labeling and mitotic indices, growth fraction, FLM, and the distribution frequency of labeled cells. These results are compared with previously determined cell cycle parameters of normal and irradiated swine skin, and the role of G_0 clones and mitotic delay in the genesis of giant islands of regeneration is considered.

E-33-5 *Inhibitory Effect of X-Irradiation on the Maturation of the Testes of the Fish,* Oryzias latipes, *in Sexually Inactive Seasons.* YASUKO HYODO-TAGUCHI, AND NOBUO EGAMI, National Institute of Radiological Sciences, Chiba 280, and University of Tokyo, Tokyo 113, Japan.

The atrophic winter testes of the teleost, *Oryzias latipes,* become mature within few weeks if the fish are kept at 22° to 26°C (Egami, 1954). The changes in gonad-somatic index, and cytokinetics during the maturation process in the testes of x-irradiated *Oryzias* under warm temperature condition were studied. The gonadsomatic index of the fish incubated at 23°C increased from 0.59 to 0.90% on day 10. After 100, 250, 500, and 1000 rad of x-ray-irradiation, the indices were 0.70, 0.59, 0.29, and 0.33%, respectively, on day 10. Number of first type of primary spermatogonia (Ia)/section remained unaffected until day 3 after 1000 rad irradiation, then rose from 90 to 405 on day 15. The pyknoses of second type of primary spermatogonia (Ib) were observed within a few days and number of the primary spermatogonia (Ib)/section decreased from 396 to 196 on day 3 and 21 on day 15 in 1000 rad-irradiated fish. Mitosis of the spermatogonia (Ib) was markedly inhibited on day 3 (30% of control). The differentiation of secondary spermatogonia (II) to spermatocyte was also markedly inhibited by 1000 rad of x-rays.

E-34-1 *Possible Applications of Flow Microfluorometry Instrumentation in Radiation Biology Related to Radiotherapy.* M. R. RAJU, P. K. HORAN, A. ROMERO, T. T. TRUJILLO, J. C. MARTIN, AND C. J. STERNHAGEN, Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico 87544, USA.

There is increasing interest in the use of flow microfluorometry (FMF) instrumentation for rapid and quantitative measurement of DNA distributions of cell populations. Changes in cell-cycle distribution in KHT sarcoma tumor cells after radiation treatment have been reported. Our preliminary results indicate that, during the course of radiotherapy treatment, changes in normal and tumor cell populations of patients with carcinoma of the cervix can be detected with this instrumentation. Experimental results of changes in cell-cycle distributions in experimental animal tumor systems and changes in normal and tumor cell populations from patients undergoing radiotherapy treatment will be reported. Possible use of such measurements to guide the course of radiotherapy with high LET radiations will be discussed. (This work is being performed under the auspices of the US. Atomic Energy Commission and the National Cancer Institute.)

E-34-2 *Radiation Sensitivity of Various Cell Populations Separated from a Fibrosarcoma by Density Gradient Centrifugation.* DAVID J. GRDINA, LUKA MILAS, KATHRYN A. MASON, AND H. RODNEY WITHERS, Section of Experimental Radiotherapy, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas, 77025, USA.

A characterization of the radiation response of malignant cells in solid tumors is complicated by the heterogenity of these tumors (i.e., presence of both dividing and "resting" tumor cells, as well as non-tumor cells and dead or dying cells). Homogenous populations of viable cells can be separated from disaggregated solid tumors by means of centrifugation in continuous and linear density gradients. After centrifugation, four "bands" of cells, obtained from fibrosarcoma tumors, were observed at densities of 1.064, 1.097, 1.132 and 1.170 g/cm^3. In addition to density, the size and/or morphology of cells differed for each population. Clonogenecity of tumor cells was determined using a lung colony assay. After 14 days, a proportion of i.v. injected cells proliferated in the lungs of recipient test mice and appeared as macroscopic tumor nodules. While no significant difference in clonogenecity was observed for the cells banding

at the three light densities, a significant decrease in tumor-forming ability was apparent for cells collected from the most dense fraction. Data relating to the radiation sensitivity of each of these separated populations, as well as that of the unseparated control population, will be presented. (Work supported by NIH Research Grants CA-6294 and CA-11138.)

E-34-3 *Studies on Population Kinetics of the Walker Carcinoma* in vivo *by Autoradiography and Impulsecytophotometry.* Wolf Erbe, Suram B. Reddy, Walfried A. Linden, and Friedrich Zywietz, University Clinics Hamburg, Hamburg, West Germany.

The experiments were carried out on Walker carcinoma transplanted to the thigh of male inbred rats. The doubling time of the tumor as determined by volume measurements was 35 hrs. The cell cycle time t_c was 20 hrs calculated by the method of labeled mitoses after pulse labeling with ^{3}HTdR. The growth fraction was measured by multiple injections of ^{3}HTdR at various ages of the tumor. From a computer analysis of DNA distribution patterns obtained by impulse-cytophotometry the percentages of cells in the different phases of the cell cycle were determined. The experimental data are discussed in terms of a tumor model.

E-34-4 *Proliferation Behaviour of Cycling Cells in a Rat Rhabdomyosarcoma as Determined by Impulse Cytophotometry.* H. B. Kal, Radiobiological Institute TNO, 151 Lange Kleiweg, Rijswijk (ZH), The Netherlands.

DNA distributions of a large number of R-1 cells from a rat rhabdomyosarcoma which grow either *in vivo* or *in vitro* have been obtained at different time intervals after irradiation with 2000 rad of ^{137}Cs gamma rays. DNA distributions were obtained by quantitative measurements of fluorescent light emission from cells stained with ethidium bromide with an impulse cytophotometer. The R-1 tumour cell population can quite easily be distinguished from the population of normal cells present in the cell suspension derived from the tumour due to differences in the DNA content/cell. From the changes observed in the DNA distributions the following conclusions can be drawn: 1) changes in the fractions of cells in G_1, S and $G_2 + M$ phases are similar for R-1 cells cultured *in vitro* and proliferating R-1 cells irradiated in the tumour; 2) non-cycling cells have a DNA content/cell equal to that of G_1 phase cells; 3) up to 20 hours after irradiation no recruitment of noncycling cells to the compartment of cycling cells could be observed. It can be concluded that comparison of DNA histograms can provide a useful tool for investigating the influence of various types of agents on the proliferative behaviour of cells in tumours and in culture.

E-34-5 *An* in Vivo *Analysis of Cytostatic Effects through Radioactive Prelabeling Technique.* William T. Chu and James M. Slater, Loma Linda University School of Medicine, Loma Linda, California 92354, USA.

Radioactive prelabeling with IUDR-I-125 of L1210 murine leukemia cells grown *in vivo* allows one to study the cytocidal effects of radiation and/or the other external challenges by monitoring residual radioactivity. On the other hand, the dilution rate of the radioactivity in individual cell serves as an index of the doubling time, and its stress-related deviation from the norm can be attributed to the cytostatic effects of the external agents. The dilution rate is, however, not conveniently accessible, and the retention of the radioactivity in cells is measured only at the beginning and the end of an experiment. As the mitotic rate is strongly dependent on the total cell population in the animal, the cytostatic effect is derived through an analysis of a parametric equation relating the population and doubling time. The *in vivo* kinetics of prelabeled L1210 cells under radiation and/or chemotherapeutic agents is analyzed and compared with the results of a computer simulation.

E-34-6 *Cell Kinetics of Ehrlich Ascites Carcinoma in Mice Made Resistant.* Karen L. Brandt, Titus C. Evans, and H. F. (Frank) Cheng, Radiation Research Laboratory, University of Iowa, Iowa City, Iowa 52242, USA.

Only forty cells were required to produce tumors in untreated CF-1 female mice. Pretreatment with subcutaneous injections of lethally irradiated cells made the animals capable of resisting an intraperitoneal injection of as many as a million viable tumor cells. Although the inoculum

disappeared within a few days, it was possible to do some studies of cell kinetics as altered by host resistance mechanisms.

Strong resistance to tumor inoculation was produced by a series of subcutaneous injections of lethally (5000 R) irradiated cells. In the cell cycle studies, both resistant and control mice were injected intraperitoneally with four million viable Ehrlich ascites tumor cells. Twenty-four hours later the mice were injected with 25 μCi of ^{3}HTdR. Smears of ascitic fluid were then prepared at one or two hour intervals for the next twenty-four hours. Autoradiograph preparations were scored for labeled mitoses to determine the cell cycle.

The percentage of labeled cells in the resistant mice was lower than in the controls and the duration of the period of labeled mitoses was also shortened. It appears that changes in ability to move through the generation cycle may precede the morphological evidence of injury and host resistance. (Supported in part by NDEA IV and American Cancer Society Grant ET-37L.)

E-34-7 In vivo *Measurements of Radiosensitivity of Different Cell Populations Within Sarcoma-180 in Mice.* W. Porschen, L. E. Feinendegen, H. Mühlensiepen, W. Piepenbring, and P. Bosiljanoff, Institute of Medicine, Nuclear Research Center, Jülich, West Germany.

The solid Sarcoma-180 in living mice was examined for radiation induced turnover of cells localized within and outside the growth fraction.

After 2 intravenous injections, 50 hours apart, of differently labeled 5-Iodo-2′-deoxyuridine, a special tumour counting device permitted external registration of renewal rates of differently labeled cells over a period of 2–4 days. The results were confirmed by biochemical analysis.

Following x-irradiation (2000 and 4000 rad) 2 accelerated renewal rates were observed at each dose and differed by a factor of about 2.5.

After irradiation with 14 MeV neutrons (300 and 400 rad) both accelerated turnover rates per dose differed by a factor of about 1.5. In tumours irradiated with alpha-particles [B-10 (n; alpha) Li-7] the acceleration of turnover yielded a single component.

The ratios of turnover rates obtained from the different LET radiations closely agree with OER's known from *in vitro* studies.

E-34-8 *The Effect of Local Thoracic Irradiation on the Metastatic Type Growth of a Mouse Mammary Tumour in the Lung.* Susan C. Thompson, Department of Radiobiology, Medical College of St. Bartholomew's Hospital, Charterhouse Square, London, EC1M 6BQ, Great Britain.

Male C3H mice were irradiated with 14 MeV electrons either to the whole thorax or to a hemithorax. Suspensions of C3H mammary adenocarcinoma synegeic cells were then injected intravenously 3 hours, 48 hours, 3½ months or 9½ months after irradiation.

The "metastatic" or growth yield in the irradiated lung was increased at the earliest when cells were injected 48 hours, and also 3½ months after doses of 2000 rad. Not only were there more tumour colonies but the colonies were also larger than those in unirradiated areas of the lung. 9½ months after 2000 rad the lungs were atrophic and quantitation of tumour growth was not possible.

A possible increase was observable when cells were injected at 9½ months, but not sooner after doses of 250 rad.

It is concluded that local thoracic irradiation possibly increases the number and stimulates the growth of tumour cells arrested in the lung. Moreover these effects are apparently increasing or maintained for several months after irradiation.

E-35-1 *Changes in the Lipid Fraction of Eel Gills After X-Irradiation* in Vivo *and a Shift from Fresh to Sea Water.* Heinz J. M. Hansen, Danish Atomic Energy Commission Research Establishment Risø, 4000 Roskilde, Denmark.

16 eels were adapted to tap water for a week. Then 8 of them were irradiated with 1000 R γ-irradiation and 4 of the irradiated group and 4 of the non-irradiated control group were transferred to sea water. The rest remained in tap water. 3 days later all were incubated with ^{14}C-labelled acetate and ^{32}P-labelled phosphate added to the water in the aquariums. Lipids from

the gills were separated by TLC and the individual fatty acids were assayed by paper chromatography.

Results show an enhanced incorporation of ^{14}C-activity into sterol esters in sea water. This was further intensified by x-irradiation. Similarly, x-irradiation intensified an enhanced incorporation in sea water of ^{32}P-activity into lecithin, and there was an increased synthesis of certain ^{14}C-labelled unsaturated fatty acids (mainly $C_{16:1}$) in the gills of animals that had both been irradiated and transferred to sea water.

By measuring lipid biosynthesis in gill tissue it was thus possible to show an interaction between the effect of x-irradiation and the salinity of the environment, in agreement with previous biological findings.

E-35-2 *Radiation-Induced Atheromatosis.* A. W. T. Konings, C. Th. Smit Sibinga, M. W. Aarnoudse, and H. B. Lamberts, Laboratories of Radiopathology and Coagulation of the Medical School, University of Groningen, The Netherlands.

Although rabbits are known to be susceptible to atheroma induction by cholesterolrich diets the carotid artery of this animal appears to be very resistant. Irradiation of these arteries with x-rays or fast neutrons (14 MeV) at a relatively low dose (500 rads) already induces serious atheromatous lesions in hypercholesteraemic rabbits. Feeding rabbits a 0,5% cholesterol diet results in a ten-fold increase of plasma lipids within a period of four to five weeks. The cholesteryl ester fraction appears to be the main lipid component, its concentration exceeding that of free cholesterol by a factor of three to four. The concentration of triglycerides in the plasma has hardly changed and is a minor fraction in the hypercholesteraemic plasma. Lipid analysis of the carotid artery of the cholesterol-fed rabbits, which had been irradiated (2000 rads, x-rays), shows an increased amount of triglycerides. This fraction makes up about 60% of the total neutral lipids. These preliminary results show that the mechanism of radiation induced atheromatosis, is not simply an accumulation of plasma lipids and might depend on metabolic activity of the artery.

E-35-3 *Changes of Adenine Nucleotides Content of Erythrocytes, Lymphocytes and Blood Platelets Following Gamma Irradiation.* Wanda Leyko, Zofia Jóźwiak, Grzegorz Bartosz, and Wanda Retelewska, Department of Biophysics, University of Łódź, Poland.

Nucleotides were estimated in erythrocytic, lymphocytic and blood platelets mass from pig blood. Lymphocytes were obtained: (1) using gelatine solution, (2) using dextran and nylon columns. Blood platelets were obtained by successive centrifugations. Irradiation was carried out in the range 0.050–10 krad. Nucleotide content was estimated using Dowex 1, ammonium formate system. The following compounds were determined: NAD, CMP-uric acid, GSSG, AMP, NADP, UMP, GMP + IMP, ADP, UDP, CTP, ATP, GTP + UTP. Changes following irradiation were expressed in % values (control = 100%). For lymphocytes (gelatine method) control values amounted to: AMP 0.26 ± 0.01, ADP 1.04 ± 0.25, ATP 4.73 ± 0.87 $\mu M/10^{10}$ cells. For blood platelets: AMP 0.23 ± 0.04, ADP 2.38 ± 0.61, ATP 6.16 ± 1.43 $\mu M/10^{11}$ cells. Irradiation did not affect ATP level in erythrocytes. In the case of lymphocytes and blood platelets a decrease of ATP level to about 90% for small doses and an increase to about 110% in the range of 1–3 krad was observed. For doses over 7 krad a decrease of ATP below the control value was noted. It is suggested that changes of ATP may indicate differences in radiosensitivity of the investigated cells.

E-35-4 *Serum Thymidine Levels after Irradiation.* Mary Christine, Ding-Jen Lee, and Walter L. Hughes. Tufts University School of Medicine, Boston, Massachusetts 02111, USA.

Serum thymidine concentration in this study was measured by radioimmunoassay, recently developed by Hughes *et al.*[1] Total body irradiation of CDF_1 mice with 100, 500 and 5000 rads produces an elevation of serum thymidine concentration, increasing with the dose of radiation and maximal between 3 and 12 hours post-irradiation. Similar elevations in plasma deoxycytidine have been observed in rats following irradiation.[2] Irradiation of tumor bearing CDF_1 mice results in a greater increase in serum thymidine. Innoculation of more than 10^7 lethally irradiated or heat-killed tumor cells into normal mice also produces an elevation of serum thymidine, which

increases with the number of cells innoculated. The serum thymidine concentration of patients undergoing radiotherapy is frequently elevated.

The data will be interpreted in terms of the effects of cell death and altered metabolic rates on thymidine pools. The possible importance of these findings in designing chemotherapeutic regimens for the patient will be discussed. (Supported in part by N.I.H. Grant No. CA 10735-06.)

[1] Hughes, W. L., Christine, M., and Stollar, B. D., Anal. Biochem., **55**, 468 (1973).

[2] Guri, C. D., Swingle, K. F., and Cole, L. J., Proc. Soc. Exp. Biol. Med., **129**, 31 (1968).

E-35-5 *On the Mechanism of X-Irradiation Induced Intestinal Folate Malabsorption.* J. M. Noronha, and V. Kesavan, Biochemistry and Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay-400 085, India.

The normal rise in serum folate levels following ingestion of conjugated folates is not observed in x-irradiated rats suggesting a complete block of the intestinal absorption mechanism. The enzymatic activity responsible for breaking down conjugated folates into simpler forms suitable for absorption is not adversely affected by irradiation. However simpler folates accumulate at the jejunal tissue site, suggestive of a radiation-induced lesion in the transolation of absorbed folates.

A partially purified protein which could bind folate has been isolated from intestinal tissue and serum. Its folate binding efficiency is not altered in the irradiated group. However in *in vitro* transport studies across everted intestinal segments of irradiated rats, the folate bound to this carrier protein is found to build up in the tissue. Inclusion of ATP in the reaction mixture allows release and transport of tissue-bound folate. The experiments suggest that x-irradiation damages the energy-generating mechanisms required to sustain the overall active intestinal folate absorption process.

E-35-6 *Biochemical Lesions in the Rat Testis Following Gamma Irradiation.* G. S. Gupta and S. R. Bawa, Department of Biophysics, Panjab University, Chandigarh-14, India.

Response of the rat testis to x and gamma radiation is generally associated with the destruction of germ cells followed by repopulation of the cells of interstitium. After 20 hours of local body gamma irradiation at 720 and 2000 R, we observe that inorganic pyrophosphatase declines continuously for 74 days. The declining levels of pyrophosphatase accompany the accumulation of pyrophosphate. During third week of radiation succinate, isocitrate, malate and glucose-6-phosphate dehydrogenases and nucleotidase activity for ATP and AMP increased along with the proliferation of interstitial tissue. The apparent recovery of dehydrogenases and nucleotidases was matched with a slow recovery in the content and the synthesis of nucleic acids without any evidence of recovery of spermatogenic cells. It is concluded that enhanced activity of glucose-6-phosphate dehydrogenase is associated with hyperplasia of testis interstitium and accumulation of pyrophosphate and loss of inorganic pyrophosphatase are the earliest metabolic changes in radiation injury of germ cells.

E-35-7 *The Effects of Pinealectomy and X-Irradiation on Pituitary Luteinizing Hormone and Ovarian Function in Albino Rats.* Maria Elena Ortiz and Eugene W. Hupp, California Polytechnic State University, San Luis Obispo, CA 93401 and Texas Woman's University, Denton, Texas 76204, USA.

The five treatment groups studied included (1) irradiated-pinealectomized (I-P), (2) irradiated-sham-pinealectomized (I-SP), (3) sham-irradiated-pinealectomized (SI-P), (4) sham-irradiated-sham-pinealectomized (SI-SP), and (5) spayed Sprague Dawley rats. Irradiation at 7 days of age with 150 R of x-rays did not affect pineal weight; uterine and ovarian weights of irradiated groups were decreased while thymus and adrenal weights were increased, approaching those of spayed rats. Pituitary weights of irradiated rats were as great as those of spayed rats. Observation of irregular estrous cycles and continuous estrus in conjunction with organ weight changes indicated reduced ovarian steroid production in irradiated rats. Ovarian ascorbic acid depletion bioassays showed greatest pituitary LH activity in I-SP and spayed rats; that of I-P rats was almost as great. Greater LH synthesis in irradiated groups in response to decreased ovarian steroids was indicated from the experimental results.

Evaluation of pinealectomy indicated a very limited influence of the pineal on the reproductive system as measured by the parameters used in this study. Instances in which a response due to pinealectomy was observed in conjunction with radiation responses.

The effects of pinealectomy in modifying radiation responses were not clearcut. I-P rats had more instances of irregular estrous cycles and a greater percent of time in estrus, indicating pinealectomy enhanced the radiation effect. However, the low pituitary weights and pituitary LH activity of I-P rats than I-SP rats indicated that pinealectomy decreased the response to irradiation.

E-36-1 In Utero *Exposure to Low Dose X-Radiation: Long Term Effects in the Offspring.* V. NAIR, S. GREENBERG, AND A. CHEDID, Depts. Pharmacology and Pathol., Chicago Medical School, Chicago, Ill. and Dept. Pathol., Univ. Cincinnati Schl. Med., Cincinnati, Ohio, USA.

Earlier studies from our laboratories showed that x-radiation of pregnant rats on 14th gestation day (g.d.) suppressed development of a) acetylcholinesterase and carbonic anhydrase within central nervous system (Brain Res. **16**, 383, 1969) and b) microsomal enzymes (mixed function oxygenases) in liver (Radiation Res. **36**, 493, 1968). Enough experimental evidence exists indicating that variations in these oxygenases—developmental, diurnal, or drug-induced—correlate with corresponding changes in endoplasmic reticulum (ER). The present investigation was undertaken: 1) to know whether the radiation induced suppression of enzyme development would be reflected in disturbances of ER and 2) to identify other functional changes. Pregnant rats received 50 R to the pelvic region on g.d. 13. Animals were sacrificed on g.d. 15, 20; on day of birth and at later periods. Fetal and infant livers were processed for electromicroscopy as described in our earlier publications. In control rats, rough ER (RER) appears for the first time on 13th g.d. and smooth ER (SER) on 20th g.d. Prenatal radiation while producing no changes in RER, suppressed the normal development of SER. Furthermore, male irradiated offspring were infertile. Microscopic examination of testes revealed deformed and necrotic sperm. (Supported in part by Easter Seal Res. Foundation.)

E-36-2 *Process of Radiation-Induced Lethal Damage Expression.* YUTAKA OKUMURA, Aichi Cancer Center, Chikusa-ku, Nagoya 464, Japan.

When cultured mouse carcinoma cells, FM3A, were irradiated with 5 krad of x-rays, cells did not die for 40 hours after irradiation, then cells began to die. During these 40 hours, cell volume and protein content in cells increased as much as 5 times of control. But the rate of protein synthesis decreased to 50% of control at 20 hours after irradiation. When the protein biosynthesis was inhibited immediately after irradiation, dead cells began to appear at 80 hours after irradiation instead of 40 hours. It was suggested the cause of reproductive death is the accumulation of nonsense protein in cells.

When cells were cultured at 15°C for 2 days before irradiation with 5 krad, dead cells appeared at 6 hours after irradiation. This type of cell death is different from the cell death described above, it might be inter-phase death. The mechanism of this cell death will be discussed.

E-36-3 *Effects of Irradiated Histidine on Rat Thymocytes* in Vitro. AKIKO M. UENO AND YASUKAZU AKITA, National Institute of Radiological Sciences, Chiba 280 and Ibaragi University, Mito 310, Japan.

We have already reported that incubation of unirradiated thymocytes with the histidine irradiated with γ-rays in Krebs-Ringer's solution caused an increase in interphase death in the cells, as measured by erythrosin-B staining.

In order to clarify the mechanism of cytotoxic action of irradiated histidine, binding of ^{14}C-histidine to chromatin was studied. When the cells were incubated with irradiated histidine at 0°C, the radioactivity increased in chromatin isolated from the cells as compared with unirradiated histidine. Amount of histidine bound was much less in chromatin DNA than in chromatin protein. About two-third of bound histidine were released from chromatin after incubation at 37°C. On the other hand, we have found the formation of some single-strand breaks in the DNA on thymocytes after incubation with irradiated histidine by using sedimentation through alkaline-sucrose gradients.

Based on these observations, possible roles of binding and single-strand breaks in the DNA in the cytotoxic action of irradiated histidine will be discussed.

E-36-4 *The Influence of Ultrasound on Ionizing Radiation Effects.* SHOZO FUJITA AND SADAYUKI SAKUMA, Nagoya City University Medical School, Mizuho-ku, Nagoya 467, Japan.

The conchae of *Oryctolagus cuniculus* were irradiated with ultrasound before, during or after ^{60}Co gamma-rays irradiation. The experiments were performed as follows: (1) gamma-rays only 2780 R (2) ultrasound (1 MHz, 3 Wcm^{-2}) only 60 minutes (3) gamma-rays 460 R and ultrasound 10 minutes (4) gamma-rays 690 R after ultrasound 15 minutes (5) ultrasound 15 minutes after gamma-rays 690 R. The results were as follows: (1) and (5) no damages (2) congestion only (3) remarkably destroyed (4) showed similar tendency in the case of (3).

The aqueous solution of KI starch system added chloral hydrate was irradiated with gamma-rays and ultrasound. The solution changes colorless to purple by gamma-rays over 250 R or a little ultrasonic irradiation. The solution formed with agar was colored by gamma-rays, but not changed by ultrasound.

Mouse carcinoma cells (FM3A) were examined in the similar way as the conchae. The effect of ultrasound will be discussed.

E-36-5 *Alterations of Serum Sialic Acid Levels in Miniature Swine After Split-Dose Exposure to ^{60}Co-Radiation.* P. Z. SOBOCINSKI, J. F. TAYLOR, W. J. CANTERBURY, AND N. S. MATHEWSON, Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20014, USA.

The prognostic significance of serum levels of various protein-bound carbohydrates has been previously reported from this laboratory. The purpose of the present investigation was to determine whether the alterations in serum levels of one of these carbohydrate residues, sialic acid (*N*-acetylneuraminic acid), would provide information useful in assessing the clinical status of the irradiated animal. Thirteen miniature swine received an initial 150 rads of ^{60}Co-radiation followed 28 days later by an exposure to 550 rads. Sera, obtained at weekly intervals, were assayed for neuraminidase-labile sialic acid and protein-bound neutral hexose levels. Elevated sialic acid levels were observed in survivors and diers. However, these elevations were transient in the six animals which survived for 30 days after the second exposure. Alterations in neutral hexose levels were similar to those observed for sialic acid levels. Results indicate that the serum sialic acid level, which is easily and rapidly determined by an automated method, may provide some information useful in the initiation of therapy in radiation injury.

E-36-6 *Metabolism of DNA in the Body During Radiation Disease.* T. A. FEDOROVA, O. YA. TERESHCHENKO, V. K. MAZURIK, V. F. MIKHAILOV, M. P. TARAKANOVA, AND P. G. RUBATCHEV. Institute of Biophysics, Ministry of Public Health of the USSR, Moscow, USSR.

The level of the DNA metabolites in biological fluids provides important information on their metabolism in the body.

It has been established that the three waves observed by us in the level of pyrimidine deoxyribosides excretion by rats, mice, hamsters, monkeys and human beings during the radiation disease constitute a general biological response. The phenomenon is due to the postirradiation disturbances in the DNA metabolism. The increase in the level of deoxyriboside excretion occurs on the 1st–3d, 6–10th and 18–25th days after exposure, the first and the third peaks being the highest.

Biochemical methods combined with isotope labeling technique in experiments on monkeys have made it possible for us to decipher the causes of all the three peaks in the three-peak excretion curve. It has been established that the mechanisms of the hyperexcretion in different periods of the radiation disease are not the same and result, on the one hand, from increased DNA catabolism and on the other hand, from increased biosynthesis of DNA precursors.

The significance of the observed alterations in the pathogenesis of the radiation disease is discussed.

E-36-7 *The Effect of Dose Rate on Size of Radiation Disturbances of Pyrimidine Nucleotide Biosynthesis in the Rat Tissues.* E. F. ROMANTZEV, N. N. KOSHCHEENKO, AND A. IU. ATABEKOV, Biophysics Institute, USSR Ministry of Health, Moscow D-182, USSR.

We studied the effect of total-body lethal brief and prolonged irradiation of rats on the enzymatic conversion of 2-^{14}C-orotic acid and 2-^{14}C-uracil into pyrimidine ribonucleotides in the extracts of some tissues. It was established, the *de novo* synthesis of the precursors in extracts of thymus and spleen is decreased by 30–40% after 3–5 hours and by 70–85 and 20–30% respectively by the end of first day after short-time irradiation with 1000 r (dose rate—340–360 r/min). Inhibition of this process is more pronounced after prolonged irradiation of rats with 1100 r (dose rate—0,8 r/min). In liver extracts the prolonged irradiation does not induce a disturbance of pyrimidine nucleotide biosynthesis. 15 min–2 hours and 1 day after short-time irradiation of rats this process is somewhat increased in this tissue. Enzymatic conversion of uracil into uridine nucleotides and in turn into cytidine nucleotides are less radiosensitive mechanisms.

F-1 *Summary Plenary Session. Chairman* GORDON F. WHITMORE, Canada.

For this session, four speakers have been selected to review what they consider to be some of the salient findings which have been reported during the course of the Congress and some of the questions arising therefrom.

F-1-1 *Physics.* H. E. JOHNS, Ontario Cancer Institute, Toronto, Canada.

F-1-2 *Chemistry.* J. H. BAXENDALE, University of Manchester, Manchester, Great Britain.

F-1-3 *Cellular and Subcellular Biology.* M. M. ELKIND, Argonne National Laboratory, Argonne, Ill., USA.

F-1-4 *Tissue and Animal Biology.* GIOVANNI SILINI, Centro di Studi Nucleari della Casaccia, Rome, Italy.

L-1-1 *Late Somatic Effects of Ionizing Radiation in Mice as a Function of Dose, Dose Rate, and Radiation Quality.* J. B. STORER, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, USA.

A series of experiments have been performed to evaluate the late somatic effects of ionizing radiation as a function of dose, dose-rate, and radiation quality. Gamma rays from ^{137}Cs sources were delivered at rates of 40 rads/minute or 10 rads/day. Fission energy neutrons were delivered at 40 rads/min from a reactor or at 1 rad-day from a ^{252}Cf source. A total of about 40,000 mice, principally of the RFM strain, have been exposed. After exposure, the mice were allowed to live out their life spans and detailed pathologic examinations were conducted at the time of death. The effects of gamma ray doses as low as 10 rads and neutron doses as low as 5 rads have been studied. A preliminary evaluation of the results of these studies, with especial emphasis on tumor incidence and longevity, will be presented.

L-1-2 *An Examination of the Time Parameter that Governs the Effect of Fractionation on Life Shortening.* GEORGE A. SACHER, Argonne National Laboratory, Argonne, Illinois 60439, USA.

Various data indicate that the effectiveness of low-LET radiations for life shortening is a function that approaches an upper limit value at high doses and dose rates, and a lower limit value at low doses. The factors governing the magnitudes of the limiting values and the transition between them are not yet fully understood. The data on life shortening and radiation-specific mortality in mammals are reviewed and analysed in terms of a mathematical model containing an effectiveness term that depends on the square of the dose and on a phenomenological time parameter. This parameter is shown to have a value of 5 to 10 days for mice, and is primarily responsible for species differences in sensitivity to chronic exposure. The mechanistic basis of this parameter is still obscure, so some of the alternative hypotheses are examined to stimulate discussion of an unaccountably neglected problem. (This work supported by US. Atomic Energy Commission.)

L-1-3 *Thyroid Nodules and Leukemia in a Marshallese Population Exposed to Fallout 20 Years Ago.* ROBERT A. CONARD, Brookhaven National Laboratory, Upton, N.Y. 11973, USA.

Twenty-five cases of thyroid abnormalities have developed among 82 Marshallese people on Rongelap due to radioiodines absorbed at the time of accidental exposure to fallout during nuclear tests in the Pacific in 1954. Estimated thyroid doses range from 130–335 rads in adults to 1400 rads in young children. The higher dose in children was related to the smaller size of their thyroid glands. Beginning at 10 years post-exposure 25 thyroid lesions have been seen so far among 82 exposed people, mainly benign adenomatous nodules, but 3 with cancer. Of 19 children exposed at less than 10 years of age 17 (90%) have developed lesions. Some children developed hypothyroidism with growth retardation which improved on thyroid hormone treatment. Thyroid surgery has been performed on 23 cases with no recurrence of the disease. Comparison is made between development of thyroid neoplasia from beta rays of radioiodine exposure and x- or gamma radiation.

A fatal case of acute myelogenous leukemia developed in a Marshallese boy believed to be due to radiation exposure. He showed greater granulocyte depression than did other exposed boys over the 19 years prior to development of the disease. The case is discussed with reference to other cases of radiationinduced leukemia, particularly in the Japanese.

L-1-4 *Late Effects of Spinal Cord Irradiation with 300 kV X-Rays and 15 MeV Neutrons.* A. J. VAN DER KOGEL,* AND G. W. BARENDSEN, Radiobiological Institute TNO, 151 Lange Kleiweg, Rijswijk (ZH), The Netherlands.

Dose-effect relationships were determined for late damage caused by local irradiations of the spinal cord of rats with single and fractionated doses of 300 kV x-rays and 15 MeV neutrons.

After latent periods of 4 to 12 months, symptoms of myelopathy develop. The latent period decreases with increasing dose, but rapidly reaches a minimum of 4 months with both x-rays and neutrons. The relative biological effectiveness of 15 MeV neutrons is 1.1 for single irradiation and 1.7 for irradiation in 5 fractions. For x-rays, the total dose required increases with the number of daily fractions.

The main histological changes consist of degeneration of the nerve roots if the lumbar region was irradiated. Irradiation of the cervical region resulted in focal necrosis of the white matter. An analysis of the data with respect to an hypothesis about the mechanism through which damage to the spinal cord may develop, is given.

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* Fellow of the "Koningin Wilhelmina Fonds," Amsterdam, The Netherlands.

L-1-5 *The Dose-Rate Effect in Murine Radiation Carcinogenesis.* JOHN M. YUHAS, Biology Division, ORNL, Oak Ridge, Tennessee 37830, USA.

It is generally accepted that the slower the rate at which an exposure is accumulated, the smaller will be the incidence of radiation induced cancer. What is lacking is a rigorous definition of the relationship between dose rate and effect(s), and, even more importantly, an understanding of the mechanisms involved. Toward this end a series of experiments are being conducted whose twofold objective is to define the relationship between dose rate (1 rad per day through 1 rad per sec) and effect(s), and to determine the role of potentially critical variables (target cell kinetics, immunosuppression, viral activation, etc.) in the observed patterns. Results to date have demonstrated that the dose rate effect varies as a function of the tumor in question. In certain systems, tumor induction is a rigorous function of dose and dose rate, while in others intermediate dose rates are more effective than higher or lower ones. We have been able to trace certain of these tumor differences to their underlying mechanisms, but it is clear that without a more complete understanding of the mechanisms involved and their relative importance in other species, extrapolation of any single dose rate factor is unwarranted. (Supported by the U.S. Atomic Energy Commission under contract with Union Carbide.)

L-1-6 *Late Effect Studies Using Some Poikilothermal Animals.* NOBUO EGAMI, Zoological Institute Faculty of Science, University of Tokyo, Tokyo 113, Japan.

In order to analyze effects of temperature on the developmental rate of late effects of radiation, experiments using poikilothermal animals have been started.

Irradiated fish, *Oryzias latipes* were kept under different temperature conditions, and the mortality rate and pathological changes were examined.

Life span of the small fresh-water snail *Lymnaea pervia* was recorded by Dr. Takeo Mori. Radiation effects on the snail were observed at different temperatures. Life span of the ascidian, *Ciona intestinalis* in different seasons was recorded and radiation effects were analyzed by Dr. Takashi Nomaguchi.

The nematode, *Rhabditis ikedai,* was used for late effect studies as a model of organisms in which postmitotic cells play a critical role in the determination of life span. On the other hand, *Pelmatohydra robusta* has been used by Dr. Koichi Noda for various experiments, including late effect studies, as a model of cell renewal systems.

The results of these preliminary experiments suggest that temperature effects on the rate of development of radiation induced acute damages are very clear, but those on late effects are not always simple.

L-1-7 *The Syngeneic Mouse Radiation Chimera as a Model for Late Radiation Effects.* PIETRO METALLI AND GIOVANNI SILINI, Laboratory of Animal Radiation Biology, CNEN, CSN, S. Maria di Galeria, Casaccia, Rome, Italy.

Observations on long term survival and pathological data at death were collected from lethally irradiated mice repopulated with either normal or irradiated isogeneic bone marrow cells. The age specific rate of reticular cell sarcoma, thymic lymphoma, myeloid leukemia, and of tumors of other tissues will be described and correlated with radiation treatment to mice and/or cells.

L-1-8 *Life Shortening Effects of Fractionated Doses of Fission Neutron or Gamma Radiation.* E. JOHN AINSWORTH AND R. J. MICHAEL FRY, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439, USA.

Life shortening and age-specific rates of neoplastic and non-neoplastic diseases are being evaluated in $B6CF_1$ (C57BL/6Anl[Anl 70] × BALB/cAnl[Anl 70]) mice given either single doses or terminated-fractionated doses of neutron or gamma radiation over a period of six months. Under conditions of terminated-fractionated exposure, the RBE for life shortening is ~10. Life shortening produced by gamma irradiation is reduced when the dose is administered in 6, 24, or 72 fractions rather than as a single fraction, and the protraction factor is estimated at ~3. In contrast, no sparing effect results from neutron dose fractionation, and fractionation produces higher death and tumor rates, over at least a portion of the lifespan, than does the same single dose. (Work supported by the US. Atomic Energy Commission.)

L-1-9 *The Ultrastructure of the Lung of Mice Exposed to Increasing Doses of Ionizing Radiation on the Thorax.* J. R. MAISIN, Department of Radiobiology C.E.N./S.C.K., B-2400-Mol, Belgium.

Cyto and histopathological lesions in the lung of mice after an exposure to a single high dose of radiation develop in three phases: The early phase (a few hours to 2 months after exposure) is characterized by lesions limited to certain foci. All types of lung cells show changes in ultrastructure and the interstitial tissue is dissociated by oedema. The intermediate phase (from 2 to 7 months after exposure) represents the period during which most animals irradiated over the entire thorax died. The ultrastructure of the lung was characterized by a marked accumulation of myelin-like figures, cell debris and fibrine-like material in the lumen of the alveoli. There was a great increase in size and number of granular pneumocytes and an increase in the number of macrophages. The lung of mice surviving the intermediate phase displayed an extensive fibrosis and hyalinization of the alveolar septa. The fibrosis of the alveolar septa may be a consequence of direct as well as indirect action of radiation.

ABSTRACTS RECEIVED LATE

A-1-1 *Energy Needs, Nuclear Energy, and the Environment.* ALVIN M. WEINBERG, Office of Energy Research and Development, Federal Energy Office, Washington, D.C. 20500, USA.

Every projection of the world's energy needs suggests that nuclear fission will generate an increasing fraction of our total energy, both in the short term and in the long term. In the short run, the role of nuclear energy may be limited by the availability of uranium and by the public acceptability of this energy source. In the very long run, nuclear energy will have to compete with, or be supplemented by, solar, geothermal, possibly fusion. If, as now seems likely, the fission breeder eventually occupies a central role in supplying the world with energy, man will have to solve permanently the delicate balance between risks and benefits implied in the use of fission. Fission is essentially inexhaustible; and when nuclear reactors operate properly they are environmentally remarkably benign. Risks are largely associated with malfunctions of the reactor system. Man's long-term commitment to nuclear energy can be better guaranteed if certain technologies are developed. These include sharp separation of long-lived actinides from other fission products and rationalized siting of reactors. Residual environmental insult imposed by fission energy can be ameliorated if cures for radiation-induced disease can be developed.

A-4-4 *Macrophage Functions in Immune Responses to Tumors.* H. COTTIER, M. W. HESS, H. U. KELLER, E. PEDRINIS, AND B. ROOS, Department of Pathology, University of Bern, Switzerland.

Macrophages seem to be instrumental—at least in certain model systems—in both the afferent and the efferent limb of the immune response to tumor cells, and appear to play an important role in nonspecific resistance to neoplasia. Under certain conditions, macrophages exhibit the ability of specific immunological, i.e. antigen-directed, and non-immunological target cell recognition. The effector role of macrophages in tumor cell destruction has recently been given renewed

emphasis. These cells can be activated by specifically sensitized or non-specifically triggered T lymphocytes, and/or by non-specific agents. They may become "armed" by "cytophilic arming factor" or in conjunction with specific antibody coating the target cells. They can also be triggered to "firing" toxic factors. Encouraging effects with regard to tumor immunotherapy have been obtained by local or systemic administration of anaerobic corynebacteria or BCG, alone or in combination with chemotherapy and/or radiotherapy.

D-3-1 *"The Stem Cell; a Radiobiological Quark?"* PATRICIA J. LINDOP, Department of Radiobiology, Medical College of St. Bartholomew's Hospital, Charterhouse Square, London, EC1M 6BQ, Great Britain.

Interpretation of the changed kinetics in different cell populations of the irradiated haemopoietic system led to the concept of a haemopoietic stem cell pool. This was stringently defined; and then looked for. The stimulus of the stem cell concept, and its use in model building of irradiation effects, led to ingenious quantifiable techniques for *in vivo* or *in vitro* growth, isolation and identification of the stem cell.

A few workers are convinced they have seen a stem cell but even for those who have not, the research data led to a more quantitative understanding of haemopoietic damage, adaptation, and possible therapy.

It is relevant therefore to look at the preliminary results for looking for a stem cell—or apply the concept to postirradiation changes in other cell populations, as in the lung cartilage and tumour tissue.

By analogy with the elementary particle physicist hunting the quark of his theoretical physics colleagues, the radiobiologists can search for stem cells, with the expectation of useful cell kinetic data for other normal and tumour tissues, whether the stem cell is found, or remains a concept.

E-4-3 *The Middle Atlantic Neutron Therapy Association Clinical Trial.* CHARLES ROGERS, Virginia Commonwealth University Medical College of Virginia, Richmond, Virginia 23298.

MANTA, a consortium of radiologic oncologists in the Middle Atlantic States and physicists at the Naval Research Laboratory has been established to investigate the use of fast neutrons in the control of (some) malignant tumors. Radiobiological experiments have indicated that fast neutrons may have an advantage over conventional forms of radiotherapy in controlling tumors locally. In preparation for clinical radiotherapy trials, extensive measurements have quantified the various physical characteristics of the NRL cyclotron-produced neutron beam. Techniques have been developed for the accurate determination of the delivered dose at depth in tissue, which account for the relatively small component of gamma-ray "contamination," as well as the major component of fast neutrons. A collimator system has been designed to attain the field definition necessary for patient treatment. New techniques common to nuclear physics experiments have been utilized to obtain needed neutron dosimetry information and a dose control and monitor unit for the NRL beam has been developed and used clinically. The relative biological effectiveness of this neutron beam has been studied with several *in vivo* and *in vitro* systems, to guide the selection of appropriate radiotherapeutic dosages. A clinical pilot study employing these techniques and this information is currently in progress. The long-range plan is a full-scale clinical radiotherapy trial to test the effectiveness of neutrons as compared to conventional radiations in the treatment of malignant diseases. The progress of the pilot study phase, to date, will be presented.