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Radiation and Environmental Biophysics

1A–0-8 Tumour Responses

COMBINED USE OF RADIOTHERAPY AND CHEMOTHERAPY IN THE TREATMENT OF CANCER

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Therapy involving a combination of radiation and cytostatic drugs has been applied for more than two decades in the treatment of cancers.

One type of association could be called spatial cooperation; for example, chemotherapy is used to eliminate microscopic deposits of tumor cells situated outside the irradiated volume or radiation is used to eliminate cells beyond the reach of the chemotherapeutic agent, i.e. in sanctuaries such as the brain or the spinal cord. This type of association has proved to be useful in many neoplastic diseases such as malignant lymphomas, acute leukemias and embryonal tumors.

In a second type of association, one attempts to achieve increased local effects of radiation through the addition of chemotherapeutic cell kill and/or chemotherapeutic enhancement of the radiation effect. In such regimens radiation and drugs may be given either concomitantly or sequentially. Concomitant administration has, in general, been very disappointing. Complementing or overlapping toxicity of drugs and radiation have often resulted in an enhancement of the detrimental effect on the normal tissues and a beneficial effect in terms of tumor response has seldom if ever, been observed. It has not been possible up to now to exploit either cell synchronisation or cell recruitment in clinical situations.

Results of sequential treatments are more encouraging. The problem is to find the proper time interval between the administration of drug and radiation in order to avoid cross toxicity. With adequate scheduling, it appears that some drugs can be given at their full single-agent levels in conjunction with local irradiation of a tumor with radiation doses that are close to the tolerance level for radiation alone.

A better understanding of drug-radiation interactions at the cellular level is required in order to fully exploit these associations. New schemes of treatments should also be introduced in order to take advantage of all the possibilities of these combined treatments.

1A-1

COMBINED ACTION OF IONIZING RADIATION (GAMMA OR FAST NEUTRONS) AND CIS- DIAMINEDICHLORO-PLATINUM (II) ON A SOLID TUMOR: EXTERNAL MEASUREMENTS AND ISOBOLOGRAM ANALYSIS

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Cis- diaminedichloroplatinum (II) (DDP) is clinically used for tumor therapy, but it's action is not yet fully understood.

4 µg DDP per g body weight was given in mice bearing Adenocarcinoma EO 771 in a hind leg, alone or 15 minutes prior to gamma or cyclotron neutron irradiation (mean energy 6 MeV). The effects were assayed in vivo, and 125-IUdR was used for tracing DNA precursor incorporation and cell loss in euoxic and average tumor cells.

Tumor growth delay was enhanced by a factor of 8-10 for gamma and neutrons and DMF's were identical for IUdR incorporation 24 hours after treatment. Both effects showed supra-additivity for combined treatment. - Comparing loss of tracer from the tumor with the rate of tracer incorporation, DDP affected euoxic cells mainly during recovery, but caused lethal radiosensitization in the average, largely hypoxic cell population.

DDP is a potent adjunct to therapy with different LET radiation causing distinct effects in euoxic cells (repair inhibition) and hypoxic cells (radiosensitization).

1A-2

Split-Dose Recovery and Repopulation in a Slowly Growing Mouse Carcinoma.

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The response of a slowly growing adenocarcinoma (T_d at the time of treatment ca. 10 d) to single and fractionated radiation was tested in situ, using regrowth delay. The fractionation schedules were 5F/8d, 5F/27d, 10F/9d, and 10F/27d. All doses were delivered to clamped tumours to exclude reoxygenation, the lowest dose per fraction being 10 Gy . The total doses required to produce a regrowth delay of 120 d increased from 55 Gy of single irradiation to 82 Gy (5F/8d), 101 Gy (5F/27d), 109 Gy (10F/9d), and 122 Gy (10F/27d) This decline in efficacy reflects that more dose was wasted by raising the number of fractions from 5 to 10 than by expanding the treatment time from 8.5 to 27 d. The average dose recovered per fraction was 4.8 Gy between the fractionated schedules,and 6.3 Gy when the daily treatment regimes were compared with single dose irradiation.
Estimates of repopulation from regrowth delay data are ambiguous in that a clear distinction between proliferation during treatment, i.e. repopulation, and post-treatment changes in proliferation cannot be made. This is also true when net delay time from the termination of treatment is quantitated rather than gross delay time, thus taking the last dose fraction for a test dose. Tentative measurements from net delay curves gave dose differences of 27 Gy for the 10F-schedules and 29 Gy for the 5F-schedules, amounting to a dose equivalent of repopulation of 1.5 Gy/d during the extra 18 d of treatment time. A similar figure, i.e. a "repopulated" dose of 6 Gy/4d, was obtained in another experiment where single anoxic test doses were given 3 or 7 d after a priming ambient dose of 10 Gy. A dose equivalent of 1.5 Gy/d would call for a doubling time during repopulation of less than 2 d which is much shorter than the T_d but close to the T_{pot} of the tumour at the time of treatment.

1A-3

COMPARED RADIOSENSITIVITY OF A TRANSPLANTED HUMAN MELANOMA USING γ RAYS, 50 MeV NEUTRONS AND NEON IONS. EFFECT OF MISONIDAZOLE.

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Human melanomas are generally considered as clinically radioresistant. Using human melanoma Nall transplanted into nude mice, we have previously shown that this radioresistance might be, at least partly, explained by both a high proportion of hypoxic cells (85 %) and a repair of potentially lethal damage (PLD). This repair has been found to occur in a comparable extent with γ rays and 50 MeV neutrons. We have got with misonidazole a sensitizing effect of a comparable magnitude : ER = 1.6 and 1.5 for γ rays and 50 MeV neutrons respectively. Furthermore, no repair of PLD could be detected with misonidazole at a concentration of 1 mg/g.

In the perspective of using high LET particles for deep-seated tumors, heavy ions (such as carbon and neon ions) seem to be of particular interest especially regarding their depth-dose distribution. Among the different biological properties of the heavy ions, some of them are well known at present (RBE - OER) ; in contrast, few data are available dealing with repair of PLD.

Radiosensitivity of melanoma Nall was measured with neon ions at the Bevalac (Berkeley - U.S.A). Tumors were irradiated in the middle of spread-out Bragg peak of 10 cm. The RBE has been found to be 3.7, a value which is higher to that obtained with 50 MeV neutrons. Likewise, a repair of PLD is observed, the magnitude of which is comparable to that described with γ rays and 50 MeV neutrons. Finally, the ER of misonidazole is 1.4 when plating is immediate ; in addition, repair of PLD does not occur when misonidazole is injected before irradiation.

1A-4

COMPARISON OF LOCAL RADIOTHERAPY AND SURGERY ON EXPRESSION OF METASTATIC DISEASE IN EXPERIMENTAL TUMOUR MODELS.

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Cells from three different mouse tumours and a rat rhabdomyosarcoma have been inoculated into the foot pads of syngeneic animals or their F1 hybrids. The tumours in the foot pad spontaneously metastasized to lymph nodes and/or lung, although the distribution between lymph node metastases and lung metastases differed among the various tumours.

Following local radiotherapy with doses of 45 to 80 Gy significantly more mice died with metastases than following local surgery of the tumour-bearing foot if the 2661 carcinoma was treated. No significant difference in incidence of mice and rats dying with metastases was observed after these treatments of the C22LR osteosarcoma and the Lewis lung carcinoma in mice and the R1 rhabdomyosarcoma in rats.

The metastases enhancing effect of local radiotherapy for the 2661 carcinoma seems not to be due to incomplete killing of tumour cells in the foot, since the other two mouse tumours are less radiosensitive than the 2661 carcinoma. Scattered radiation, received during local irradiation in shielded parts of the mouse, was not responsible for the metastases enhancing effect. Furthermore, neither the presence of an irradiated tumour-free leg nor the presence of an irradiated tumour mass influenced the incidence of mice dying with metastases. However, if amputation of the tumour bearing foot was accompanied by irradiation of another tumour-free leg together with transplantation of an irradiated tumour into the amputation wound, the incidence of mice dying with metastases was significantly increased, although not as high as when local radiotherapy was administered. Evidently, the presence of irradiated tumour together with an irradiated leg promoted the outgrowth of tumour cells, which were present outside the tumour bearing foot at the time of treatment.

The differences in response, i.e., a metastases enhancing effect of radiotherapy or not, among the various tumours studied could not be attributed to differences in radiosensitivity, immunogenicity, metastases localization or Révesz effect of the various tumours.

1A-5

Effects of hyperthermia and radiotherapy on a transplantable murine mammary carcinoma during development.

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The sensitivity for heat and X-irradiation of a transplantable murine mammary carcinoma during development was studied using tumour growth delay as parameter.
In the range 0.1 - 0.4 cm3 there is a progressive decrease of the radiosensitivity correlated with the tumour volume. Administration of hypoxic cell sensitizer (0.5 g/kg i.p.) did not affect the radiosensitivity in small tumours (0.1 cm3) but clearly enhanced the radiosensitivity in the larger tumours (0.4 cm3).

In the same range of tumour volumes we could not demonstrate differences in sensitivity to heat. Moreover misonidazole had no significant effect on the thermosensitivity of the tumour.

When the heat was applied immediately after irradiation an increase in tumour growth delay was observed. This increase was independent of tumour volume, and the extra delay (about 4 days) was the same as the delay caused by heat alone. The combined treatment after administration of misonidazole caused an extra delay of about 12 days, again independent of the tumour volume but significantly larger than the effect of heat alone.

From the observed effects we may conclude that hypoxia, which caused radioresistence in large tumours (0.4 cm3), has no influence on the thermosensitivity. In this model misonidazole has a large effect with the combined irradiation-heat treatment where-as it has no significant effect in the case of treatment by heat alone.

1A-6

Radiation response of a murine lymphosarcoma.

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The radiosensitivity of a murine lymphosarcoma in vivo has been studied using X-ray fractions of 2 Gy given according to various radiation schedules. The sarcoma is a very rapidly progressing tumour of lymphosarcomatous type causing death of the animals within 8 days after inocculation of 10^6 malignant cells.
Cytofluorometric observations and ^{3}H-TdR incorporation had revealed a 2 Gy induced arrest of 6 hr in DNA synthesis and a concomittant prolongation of the S-phase. Therefore, we started with two X-ray doses applied with a 6 hr time interval at various days after inocculation. The animals were irradiated on approximately 2/3 of their body (200 kV, 15 mA) leaving the part caudal to the spleen non-exposed.
Only irradiation on the first 3 days caused a slightly enhanced survival of the animals; the 3rd day exposure turning out to be most effective by showing a 30 per cent increase.
A third irradiation with 2 Gy 6 hr after the second or additional exposures on day 1 or 6 did not cause a better survival.
Also total-body irradiation failed to show an improvement.
The results are discussed in the light of the present knowledge of this highly malignant tumour.

1A-7

Influence of postirradiation growth kinetics on response to fractionated X-irradiation in Carcinoma NT

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The effect of various X-ray doses on the composition and growth kinetics of the spontaneous mouse mammary carcinoma NT have been studied. For each dose, the delay in growth of individual tumours was proportional to their preirradiation growth rate. A dose of 1 Kilorad delayed growth to double their treatment volume by 6 days in tumours that doubled every 4 days before irradiation. Tumour growth ceased 3 days after the irradiation, at which time the irradiated tumours had one third the amount of proliferative tissue and twice the amount of necrosis and blood space found in controls. The labelling index (LI) and mitotic index (MI) were reduced to one third and one sixth of control levels respectively. By day 6 the tumours began to grow again. They contained more proliferative tissue than controls but the LI and MI were still low. By day 8 the tumours were growing at their preirradiation rate and the excess necrosis and blood space had been lost. The effect of a dose of 500 rad, which delayed tumour growth by 1½ days, is being studied in a similar fashion. Second doses of 500 and 1000 rad were given at daily intervals after the first. The changes in tumour sensitivity to reirradiation will be discussed in relation to the changes in volume, cellular composition and kinetics.

1A-8

Effects of irradiation on inoculated brain tumours of the rat: radiographic, microangiographic and histological findings

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The development of experimentally induced brain tumours in 45 BD - IX rats was studied by means of several neuro-radiological methods, and the findings as to the localisation of the tumours and their size were confirmed by histological control. The tumours were chemically induced (ethylnitrosurea), malignant, mixed gliomas, which had been transplanted intracerebrally as a suspension of 1o ooo, i.e. 2o ooo cells. The growth of the tumour was observed in definded intervals from the 14th day after the implantation onwards until the prefinal stage by means of contact-radiography and partly in vivo after selective angiography of the internal carotid artery. The vascularisation of the tumour, its size, localisation and extension as well as its relation to neighbouring cerebral structures was studied in a group of tumours which did not receive any treatment. The results of this control group were compared with a group of identical tumours receiving radiotherapeutic treatment.

The radiation induced changes were all examined by means of the same neuro-radiological and histological methods in a total of 64 tumour carriers. The radiotherapeutic treatment consisted of opposite-field irradiation of the skull with 8 MV ultrahard x-rays of a linear accelerator. Given varying fractionation schemes, the NSD ranged between 13oo and 17oo ret. The vascularisation of the tumour, the modalities of spreading and the criteria of malignancy are described under qualitative and quantitative aspects and are compared with the findings of the control group.

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1A–9-14 Normal Tissue Responses

The late effects of X-irradiation on the rectum of the rat

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In radiotherapy of gynecological and other tumors in the true pelvis the rectum is often the critical and dose-limiting normal tissue. To study the acute and late reactions of the rectum in an animal model we developed a method to irradiate about 1 inch of the rectum of rats in the intact animal without exceeding the tolerance of other organs. The acute radiation response was a transient haemorrhagic proctitis, the late response was a pronounced fibrosis of the submucosa which finally caused stenosis and fatal rectal obstruction. Animals were irradiated with single and fractionated doses and dose response curves of fatal rectal obstruction were obtained. For single irradiation the dose to cause 50 % lethality from rectal obstruction (ED-50) was 19 Gy. For 4f/4d ED-50 was 24.5 Gy, for 2f/8d ED/50 was 25 Gy and for 4f/4 9d ED/50 was 27.5 Gy. Thus the rectum exhibits ELKIND recovery as well as slow repair. The results of 5f/4 9d are only preliminary, but ED/50 is definitely higher than 40 Gy.

1A-10

The effects of graded doses of fission neutrons and X-rays on the gastric fundic epithelium and gastric secretion in mice.

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Male mice were exposed to whole-body irradiation with either fast fission neutrons of 1 MeV mean energy or 300 kVp X-rays at centre-line dose-rates of 0.1 and 0.3 Gy/min, respectively. The mice received an isologous bone-marrow transplantation and a terramycin treatment to decrease acute lethality. Three weeks after irradiation stomachs were collected for histological study and secretion tests were done. Cells were counted in a unit area of mucosa. Secretion was measured in anesthetized pylorus-ligated animals.

The number per unit area of mucosa of mucous neck cells reached a peak value of 10 times the original value at 3.4 Gy neutrons and 11.5 Gy X-rays. The RBE for this effect is equal to 3.4.

Both the number of parietal cells per unit area and the histamine stimulated acid secretion decreased to near zero between 2.8 and 3.6 Gy neutrons and between 9.0 and 13.0 Gy X-rays. The RBE-value for the decrease of the parietal cell count is equal to 3.6 and for the decrease of the acid output 3.7. However, in the lower dose-range the acid output tended to increase, whereas the number of parietal cells per unit area showed the opposite.

Both the number of zymogenic cells per unit area and the histamine + insulin stimulated pepsinogen secretion decreased to zero between 1.0 and 3.2 Gy neutrons and between 2.0 and 11.0 Gy X-rays. The RBE-value for the zymogenic cell depletion is 3.6 and for the decrease of the pepsin output 3.7.

The number of glandular tubules per unit area decreased after neutron doses $\geq$ 2.4 Gy and after X-rays doses $\geq$ 10.0 Gy. The RBE for the depletion of glandular tubules decreased from 4.3 for a slight depletion to 3.3 for a 90 percent depletion.

1A-11

Quantitative assessment of pig skin response to non-uniform irradiation

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Non-uniform irradiation of pig skin is being studied to measure non-stochastic effects so as to provide radiation protection criteria. Strontium-90 sources, having a range of diameters from 1 mm to 25 mm, are being employed. A range of doses is being used that gives rise to a moist desquamation in the first wave acute reaction and dermal necrosis in the second wave reaction. We are examining the hypothesis that the first wave reaction is related to radiation damage to the basal layer of the epidermis, which in turn has an effect on the superficial papillary plexus of vessels, and is expressed as erythema and desquamation. The second wave reaction is thought to be related to damage to the deep plexus of vessels at the base of the dermis and is expressed as late atrophy and, for doses in excess of normal tissue tolerance, as dermal necrosis.

The Oxford skin scoring system is being used to quantitate the biological effects. Biopsy specimens are being taken to assess individual pig's skin thickness so that the skin scores can be correlated with the different depth dose data that are pertinent to the particular biological effect observed. Preliminary data from the pilot study will be used to illustrate the problems associated with this type of quantitative investigation.

1A-12

THE ACUTE EFFECTS OF X-IRRADIATION ON NORMAL AND GRAFTED PIG SKIN

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Surgically damaged skin is generally considered to be more radiosensitive than normal skin. In clinical practice, therefore, free-skin grafts are usually excluded from an irradiated field receiving a full therapy dose. However, this apprehension appears to have a very poor scientific basis. The present study was designed to compare the functional and morphological effects of X-irradiation on free skin grafts and normal skin in the pig.

Split thickness free-skin grafts were prepared on the backs of 3 month old pigs, using an electrodermatome. The grafts were approximately 0.07 mm thick. When bleeding on the graft donor site was controlled, the graft was replaced on to its original bed and sutured into position.

Skin blood flow measurements, using an isotope clearance method, were carried out at regular intervals for 2 to 21 days after surgery. Isotope clearance from the free-skin graft was compared with that in normal skin on the opposite side of the pig.

When the graft was well established (21 days after surgery) areas of both grafted and normal skin were irradiated with single doses (1800 to 2340 rad) of 250 kVp x-rays. The radiation reaction of the grafted and normal skin was recorded, using a skin scoring system, for the first 20 weeks after treatment. Isotope clearance studies were carried out at 3, 6, 12 and 26 weeks after irradiation.

The functional studies showed that re-vascularisation of the skin graft starts 3-4 days after surgery. Isotope clearance was faster in grafts than in normal skin between 10-21 days post-operatively. Following irradiation, the skin reaction of the grafted areas was less than that recorded in normal skin receiving the same dose. Isotope clearance in the irradiated grafted skin was faster than in normal skin. It would appear that established skin grafts are less sensitive to radiation than normal skin. This may be related to an increased vascular density in the graft.

1A-13

HISTOLOGICAL EXAMINATION OF THE EFFECTS OF DIFFERENT FORMS OF FRACTIONATED X-RAY IRRADIATIONS IN NORMAL ORGANS AND TRANSPLANTED TUMOURS

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A single dose of 50 Gy or 4 x 12,5 Gy, 10 x 5 Gy and 30 x 1,67 Gy X-ray irradiation were applied to the left upper abdominal area of rats and mice. During fractionated irradiation the accumulated dose of 50 Gy was given in two weeks. In the case of 30 x 1,67 Gy irradiation during 2 x 5 working days three irradiations were delivered daily /ultra-fractionation/. One, 3, 7 and 14 days after the last irradiation spleen, lymph nodes, vertebral bone marrow, small intestine, liver and the left kidney were processed histologically, though no histological damage was demonstrable in the two latter organs after either type of irradiation.

In rats, based on the cell number, with increasing the number of fractions the injury decreased in the minority of cases. In the white and red pulp of the spleen, sometimes in lymph nodes and on days 7 and 14 in the bone marrow, too, a higher cell number was observed after 10 x 5 Gy than after 4 x 12,5 Gy. In case of ultra-fractionation the decrease of bone marrow-cell count and at the early intervals the damage of the small intestine were less expressed. Similar but less explicit results could be demonstrated in the small intestine of mice as well, though in the other organs the decrease of injury with increasing number of fractions was exceptional.

After applying the same types of fractionated irradiation in transplantable F 180 Crocker mice-tumours the histological damage of the tumour seemed to increase with increasing number of fractions.

1A-14

EFFECT OF ^{60}Co-γ-IRRADIATION ON NORMAL AND DYSTROPHIC (VITAMIN E DEFICIENT) MUSCLE

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Radiation sensitivity of enzymes depends on their intracellular state, i.e. whether they are bound to cell structure or present in soluble form.This has been found in relationship with numerous enzymes, among others with acetylcholinesterase. Our recent results suggested that the alteration of enzyme activity in vitamin E deficiency induced muscular dystrophy possibly is consequence, at least partly, of a change in relationship of enzymes to muscular structure.Investigations have been carried out therefore to determine if this change is to be observed in the radiation sensitivity of the enzymes.The radiation sensitivity studies have been supplemented with the use of a non-ionic detergent, Triton-X-100 that is being widely used for the study of membrane bound enzymes.
It has been found that on the effect of increasing doses of ^{60}Co-γ-irradiation on the acetylcholinesterase activity in muscle homogenates of normal rabbit shows an initial increase which is followed by a decrease.No increase was observed after Triton-X-100 has been added or in homogenates originating from dystrophic muscle.Asimilar increase of acetylcholinesterase activity was found in the sarcoplasmic reticulum of normal muscle on the effect of low radiation doses.Activation was found to be smaller or even absent in dystrophic muscle preparations.The difference of radiation sensitivity in normal and dystrophic muscle suggests that alteration of enzyme activity observed in vitamin E deficiency is, at least partly, due to a structural damage of the muscle.

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1B–1-8 Hematology

1B-1

Acute and delayed effects of total-body irradiation on the granulocytic progenitor cell compartment in dogs.

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We have shown that in dogs exposed to acute total body X-irradiation (TBI) the concentration of granulocytic progenitor cells (CFU-C) in the blood was significantly reduced for about 35 days after 40 R and for more than 90 days after 80 R. Determinations of the CFU-C in bone marrow aspirates of the 80 R-irradiated dogs, on the other hand, revealed a lesser reduction on the first two days and an earlier repopulation of this pool, reaching slightly subnormal levels about day 30. In the present studies some experiments were directed to the mechanisms that may be responsible for (1) the quantitative differences in the early decrease between circulating and bone marrow CFU-C and (2) the differences in the regeneration pattern between both CFU-C pools. Results: Since the in vivo results suggested differences in the radiation sensitivity between circulating CFU-C and bone marrow CFU-C, survival curves for both populations were determined after in vitro irradiation. In fact, circulating CFU-C proved to be much more radiosensitive (D_0 = 25 ± 1 rad) than CFU-C derived from the bone marrow (D_0 = 61 ± 2 rad). According to these findings the differences in the acute response between blood and bone marrow CFU-C to TBI most probably are a consequence of their different radiosensitivity. - The spleen of normal dogs has been shown to contain large numbers of "blood" CFU-C under normal physiological conditions. This led us to study the role of the spleen in the long term repopulation of the blood CFU-C pool after 80 R TBI. A first increase in the CFU-C concentration commenced on day 17 in both, normal as well as splenectomized animals. However, whereas in the latter group nearly normal values were reattained between day 40 and 50 and a definite normalization occurred about day 90, the CFU-C in the irradiated normal animals remained at the 30 % level of normal only. These results show that the spleen of the dogs is involved in the mechanisms influencing the reconstitution of the blood CFU-C pool after small doses of TBI.

1B-2

Reparative processes in bone-marrow, spleen and blood of mice irradiated protractedly with the dose rate 4,8 Gy/day.

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The histological and cytological changes in bone-marrow, spleen and the changes in peripheral blood of mices till 60 days after the outset of continuous irradiation with the dose rate 4,8 Gy/day /500 R/ up to total doses 9,6 Gy /1000 R/ and 19,2 Gy /2000 R/ were evaluated. In the bone--marrow at the 9^{th} day many mononucleare cells of morphologically lymphoid type appeared and later an intensive reparation of granulocytopoiesis occured which already reached the control level at the 21^{th} - 28^{th} day. In the spleen an intensive reparation of erythropoiesis and megakaryocytopoiesis were observed. The very intensive ectopic spleen erythropoiesis reflected in an increase in spleen weight which was statistically significant beginning the 14^{th} day. The number of peripheral blood granulocytes, erythrocytes and thrombocytes reached the normal values between the 21^{th} - - 28^{th} day. The agranulocyte counts increased till the day 14 up to 50 % of normal values and remained on this level till the day 60 after the outset of irradiation. Total accumulated dose 19,2 Gy caused irreversible changes in hemopoiesis and the death of all animals till the day 12.

1B-3

PERSISTANCE OF STEM CELL DEFECTS IN THE HEMATOPOIETIC SYSTEM OF MICE FOLLOWING GAMMA-IRRADIATION. K.-H. v. Wangenheim, G.E. Hübner, L.E. Feinendegen
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Slow regeneration of hematopoietic tissues following transfusion of irradiated bone marrow suspensions into lethally irradiated mice suggested that surviving stem cells suffer from genetic injury which slows down the proliferation rate in their progeny.
Thus, mice were whole body irradiated four times with 450 rad at intervals of 21 days. After two months of recovery the mice were killed and appropriate numbers of nucleated bone marrow cells were injected into groups of 12 - 14 lethally irradiated recipients. Half of the recipients of each group were injected with 10 uCi of the thymidine analogue 125-iodo-deoxyuridine (125-IUdR) at day 3, the other half at day 5 after transfusion. From the increase in 125-IUdR incorporation into splenic DNA during the interval of 48 hours a doubling time is calculated which approximates, under the conditions used, the doubling time of proliferating cells.
The doubling time of the irradiated bone marrow cells was 17.9 $\pm$ 0.9 hours as compared to the unirradiated controls with 12.0 $\pm$ 0.5 hours. In similar experiments with single exposures of 500 rad the improvement of cell doubling time was found to be slow and a defect was still measurable after a recovery period of 7 months.
It is concluded that genetic injury is maintained in stem cells and that the elimination of these cells, at the advantage of undamaged or less damaged stem cells, is impeded due to peculiarities of the hematopoietic cell renewal system.

1B-4

RESIDUAL RADIATION EFFECT IN THE MURINE SPLEEN, DEMONSTRATED BY 125-IODO-DEOXYURIDINE LABELING. G.E. Hübner, K.-H. v. Wangenheim and L.E. Feinendegen.
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To demonstrate residual radiation effect, mice were whole body gamma-irradiated with 450 rad. Following recovery for 35 days these mice and unirradiated controls were injected intraperitoneally with the thymidine analogue 125-iodo-deoxyuridine (125-IUdR). During the following 50 - 74 days the incorporated activity was measured in the spleen at intervals of 2 - 5 days.
The resulting retention curves exhibit three components: During the first 10 days after 125-IUdR injection there was a rapid decline (first component). Thereafter a second component followed with a steeper decline in the control than in the experimental group. At days 25 - 28 and 40 - 45, respectively, both slopes entered into the third component of the curve with the slowest turnover, identical for both the irradiated and non-irradiated mice, with a half-time of 35 days. Subtraction of the component 3 from component 2 results in a half-time for the second component of 5.6 days for the control and 9.8 days for the irradiated group.
It is concluded that the first component of the retention curve is due to maturation and migration of labeled hematopoietic cells from the spleen and that the third component may reflect the slow turnover rate of reticulo-endothelial cells. The residual radiation effect measured in the second component of the retention curve seems to be due to a prolonged turnover rate of long lived cells, perhaps lymphocytes, and possibly hematopoietic stem cells.

1B-5

IDENTICAL RECOVERY OF RESPONSIVENESS TO ERYTHROPOIETIN AFTER SINGLE AND SPLIT EXPOSURE AS ESTIMATED BY COMPARISON OF SLOPES

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Responsiveness to Erythropoietin,known to be a specific estimate of quantified bone marrow RBC output if appropriate dose-response relationship is established, has been studied.Female Whistar white rats were irradiated with spaced doses of Co-60 gamma rays (650,800 and 950 R). Thirty days survivors were divided in two groups.The first one was injected with constant dose of standardized powdered Erythropoietin and assessed using 19 hours Fe-59 uptake.The other survivors were re-irradiated with 300 R and 30 days later injected with the same amount of Erythropoietin, fasted and bioassayed.
Response to Erythropoietin reveals exponential decrease dependent upon radiation dose with highly significant linearity in both groups of survivors indicating that recovery follows identical pattern. The only difference relates to the intercept of plotted curve (Response to Erythropoietin),due pressumably to manifold excess of cellularity of the bone marrow while compared between conditionning and repeated exposures.
Correlation coefficients are .999 and.971 respectively with t-value for the slope difference of .2 (p .05).
These data point 1) to the fact that constant amount of Erythropoietin results in constant fraction of maximal response for a given animal, no matter how the responsive units are figured in Erythropoiesis regulation, and 2) recovery of responsiveness following repeated exposure increases Erythropoietin responsive cells in the marrow.

1B-6

The efficacy of cyclophosphamide in combination with supralethal total body irradiation followed by bone marrow transplantation in the treatment of acute myelocytic leukemia.

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Today, cyclophosphamide (Cy) followed by supralethal total body irradiation (TBI) is a well-known combined modality treatment in patients with leukemia. A bone marrow transplant should be available to overcome the severe aplasia. The efficacy of this treatment regimen was investigated in a rat model for human acute myelocytic leukemia (BNML). Studying various treatment schedules, the calculated log cell kill appeared to be greater than expected from a pure additive effect of Cy and TBI. E.g.: after 100 mg Cy/kg i.p. followed by 450 cGy TBI q 24 h x 3 a 9 log cell kill was calculated and a 7 log cell kill was expected. Evidence will be presented that this is in fact a virtual discrepancy caused by the cell kill assay procedure. Data point to altered kinetics of proliferation of residual leukemic cells (smaller growth fraction, prolonged doubling time), which is possibly due to treatment-induced changes in the bone marrow microenvironment.

1B-7

^{226}Ra induced changes in haemopoietic cells of different marrow sites related to the surrounding bone structure and the absorbed radiation energy.

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The development of experimental assay systems for haemopoietic cells now makes possible local investigations of long-term effects of irradiation. Spatial and temporal assessments of the number of multipotential colony forming cells (CFU-s) and of granulocyte committed colony forming cells (CFU-c) after internal contamination with ^{226}Ra, are used to measure radiation damage in bone marrow. The biological damage is then related to the physical features of contamination with the α-emitter. Mice were injected with the "standard" radionuclide ^{226}Ra, at doses of 230 kBq ^{226}Ra/kg and 660 kBq ^{226}Ra/kg. 4 hrs,1,3,10,24,100 and 300 days after injection, marrow cellularity and the response of CFU-s and CFU-c was assessed in marrow sites surrounded by trabecular bone (distal end of femur, lumbar vertebrae, sternum) and in the femoral shaft where axial marrow and marrow cells near cortical bone were separately examined. The retention of ^{226}Ra was also measured in the surrounding bone sites at corresponding time-intervals and morphometric analyses of the marrow sites and their surrounding bone structure were performed. This allowed to calculate the radiation dose absorbed in the considered marrow sites. The response of CFU-s and of CFU-c was characteristic for each marrow site, e.g. 300 days after injection of 660 kBq ^{226}Ra/kg, the number of CFU-s and of CFU-c was reduced in comparison with the non-contaminated controls : the reduction factors were in the axial marrow of the femoral shaft resp. 1.8 and 1.7, in the peripheral marrow of the femoral shaft resp. 5.3 and 4.0, in the distal femoral end 3.2 and 2.3, in the lumbar vertebrae 2.6 and 2.0, and in the sternal marrow 3.8 and 2.7. These differences could be explained by 1) differences between the marrow sites in the percentage of marrow which absorbed α-particles 2) unequal ^{226}Ra concentrations retained in the surrounding bone structures of the considered marrow sites, although 300 days after injection no differences in ^{226}Ra concentrations were observed anymore, 3) the surface area of bone per unit volume of bone was not the same in the various surrounding bone sites. The first significant changes in CFU-s response occured in marrow sites which contained a high number of mitotically active CFU-s (femoral distal end, peripheral marrow of the femoral shaft). Fewer alterations were noticed after injection of 230 kBq ^{226}Ra/kg than after 660 kBq ^{226}Ra/kg. After 230 kBq ^{226}Ra/kg the observed changes in bone marrow cellularity and in the number of CFU-s and CFU-c were oscillations above the control levels, whereas after 660 kBq ^{226}Ra/kg depressions below the control level were observed.

1B-8

RADIATION-INDUCED RELEASE OF PLATELET SEROTONIN "IN VIVO"

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Two groups of female and male Wistar rats were submited to 800 rad of whole-body X-irradiation or to sham irradiation. Three hours later the rats were exsanguinated by aortal puncture under aether anaesthesia and the blood was collected in a plastic syringe containing anticoagulant. After separation of platelets, their serotonin concentration was determined by direct spectrofluorimetric method. In irradiated female as well as male animals a statistically significant decrease of platelet serotonin concentration was shown. This finding lends support to the theory of radiation-induced increase of membrane permeability which occurs "in vivo" after lethal whole-body exposure.

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Radiation and Environmental Biophysics

1B–9-14 Effects on Plants

1B-9

SYNERGISTIC EFFECT OF IONIZING RADIATION AND RADIOTOXINES

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Previous ideas of the role of biologically active substances, produced in the irradiated organism (radiotoxines), in the development of radiation damage were mainly based on experiments with intact, native structures (the genome) of cells (experiments on parabionts, partly shielded organisms, native cells, etc.). They were justified in the case of the additive character of direct action of ionizing radiation and the effect of radiotoxines. However, it is known that ionizing radiation and chemical mutagens produce sometimes a synergistic effect. Two methods were used to study the effect of radiotoxines on plant tissues subjected to direct action of ionizing radiation. The revealed synergism indicates that the role of radiotoxines in the development of radiation damage is more important than it was supposed before.

1B-10

THE EFFECT OF ANOXIA AND LET ON THE MODE OF STERILISATION OF IRRADIATED FERN SPORELINGS

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Sporelings of the fern Osmunda regalis have been used to study a "reversion" phenomenon, in which a proportion of cells sterilised by radiation do not divide and die but remain persistently green and physiologically intact. The sporelings were irradiated at 72 hours after plating, when there were about 7 cells in the prothallus. The proportion of sporelings irradiated in air which shows no dead cells in the prothallus (all cells green), increases with increasing dose to a maximum of about 80% of prothalli, with a slight decline at high doses. Sporelings irradiated in nitrogen and then returned to air show a similar shape of dose-incidence curve, but displaced to higher doses.

In view of the interest in the problem of radioresistant chronically-hypoxic cells, and in the role of differentiation in the radiation response of mammalian tumours, the following have been studied and are discussed:

(a) the effect on the reversion of storage of the sporelings in nitrogen or very low oxygen concentrations before or after X-irradiation.
(b) irradiations with 14.7 MeV neutrons.
(c) the correlation between the incidence of dead cells and survival of the sporelings.

1B-11

Potentially lethal damage repair (PLD repair) after ^{60}Co gamma-irradiation of an unicellular eucaryotic photosynthetic cell, *Chlorella pyrenoidosa*.

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The effects of five different levels of intensity of illumination on the survival rate after irradiation have been studied on cells taken from mass liquid cultures at plateau-phase (resting cells C_r) or at the end of exponential-phase (C_e-cells).

Level 0 is darkness. The delay between the seeding of the cells and the appearance of the colonies varies from 10 days for level 1 to 4 days for level 4.

The ratios S_d/S_o and S_i/S_0 define the extent of PLD repair. S_0 is the survival rate obtained with cells exposed to level 4 at the end of irradiation until the counting of the colonies. S_d is obtained with cells exposed 24 or 12 hours to levels 1, 2 or 3, then to level 4 until the counting. S_i is obtained with cells exposed to levels 1, 2 or 3 at the end of irradiation until the counting.

For C_r the extent of PLD repair increases from 1 to 2 when the first illumination decreases (S_d/S_0) or when growth continues at low level (S_i/S_0). Therefore, there is a correlation between PLD repair and cycle time. For C_e, S_d/S_0 is close to 1, but S_i/S_0 reaches 2 as for C_r.

1B-12

EFFECT OF IRRADIATION OF SEEDS ON THE TRANSCRIPTION AND STRUCTURE OF PLANT CHROMATIN

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Seeds of pea (Pisum sativum) and barley (Hordeum vulgare) were irradiated with 0-300 Gy doses of Cobalt-60 gamma radiation and germinated in the dark. The buds of one week old etiolated seedlings were used for experiments. The wet weight of buds were decreased in proportion to the absorbed doses. Although there was no changes on irradiation in the composition of chromatin (the mass ratios of DNA: RNA: histone: non-histone proteins), the polyacrylamide gel electrophoresis pattern showed, that the quantity on H1 histon was decreased on irradiation and there was a change in the ratio of H1 subfractions. The DNase II enzyme digested faster the chromatin of the irradiated sample than the non-irradiated one. These findings indicate radiation induced structural changes in the chromatin. There was no difference in the rate of in vitro transcription of DNA isolated from control and irradiated chromatins. On the other hand the K_m values, calculated from the in vitro transcription kinetics of chromatin, showed a gradual decrease on increasing dose of irradiation. These results suggest that the radiation alter the chromatin by loosening its structure, therefore the RNA polymerase can binde more tightly to the DNA and this may be the reason for the decreasing K_m.

1B-13

COMPARATIVE RADIOSENSITIVITY OF PROLIFEROUS AND RESTING ROOT MERISTEM CELLS OF Pisum sativum L.

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Variety of duration of root meristem mitotic cycle being estimated autoradiographically occured mainly at the expense of G-1 phase during gamma irradiating pea seedlings with growth-stimulating and growth-inhibiting doses. In so doing the number of cells present in this phase either decreased or increased respectively. Statistical analysis of hystograms of Feulgen-stained G-1 cell distribution according to optical density of nuclei with 545 nm wave length allowed to estimate the variety of their number occurings mainly at the expense of the nuclei absorbings light weakly that is those in the early G-1 phase.

Estimate of survival of gamma irradiated synchronically dividing meristems showed the cells in the early G-1 phase has the highest radiosensitivity (LD-90/10 made up 1400-1500 rads in comparison with 600-700 rads for the most radiosensitive G-2, M and late G-1 phase). Estimate of cell radiosensitivity of quiescent centre being present in G-0 phase which identified cytophotometrically as early G-1 showed them losing ability for proliferation with 1500-1600 rads. Closeness of values of letal doses for the cells of early G-1 and G-0 phases allows us to suppose that these physiological conditions of both are highly similar to each other and we can agree with Steel's opinion (1970) that both in plant and animal cells populations one may single out two variants of existence of resting cells. In the first case they are a part of G-1 phase (the early G-1 phase). Decreasing or increasing cycle duration occurs at the expence of changing the time of cells in this period. In the second case the resting cells are out of the cycle though they maintain potential possibility to proliferation (the G-0 cells of quiescent centre). The role of the latter seems to consist in filling up the pool of proliferous cells when injured.

1B-14

Radiation induced mutagenesis in *in vitro* cultivated plant cells

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Radiation induced mutagenesis in *in vitro* cultivated plant cells has been studied with respect to the effect of dose, delayed plating and the quality of the radiation on the frequency of induction of resistance to cycloheximide.
The following observations will be discussed:

- The dose effect relationship for mutation induction after irradiation with X-rays is linear quadratic and nearly mirror shaped to that for cell survival. No phenomenon comparable to the increased plating efficiency after low dose irradiation (Werry and Stoffelsen, Int. J. Radiat. Biol. 35, 293-298, 1979) could be detected.
- The dose effect relationship for mutation induction after irradiation with fission neutrons is nearly exponential and mirror shaped to that for cell survival.
- Delay of plating results in a substantial decrease of mutation frequency concomittant with a substantial increase in cell survival. It may be concluded that during the delay of plating repair of potentially mutational damage occurs in addition to the repair of potentially lethal damage.

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Radiation and Environmental Biophysics

1C–1-11 Cellular Effects

1C-1

The Response of Mammalian Cells to d-Be^{9} Neutrons Generated by a 4 MV Van de Graaff Accelerator

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V79 Chinese hamster cells and WHFIB murine tumour cells have been irradiated with neutrons generated by accelerating deuterons on to a beryllium target by the Gray Laboratory Van de Graaff accelerator. The machine is operated at 4 MV with a beam current of about 500 μA. This produces neutrons having a broad energy spectrum, the mean being ~2 MeV and the maximum being ~8.5 MeV. Cells were irradiated in suspension at a distance 45 cm from the target at a neutron dose rate of approximately 0.50 Gy/min. The cells were positioned just inside a large aperture in a collimator constructed of steel and of boron-loaded paraffin wax. With this configuration, the gamma-ray contamination was about 12 per cent of the total dose.

For V79 cells the survival curve parameters were: n = 1.4 Do = 0.8 Gy, OER = 1.7. The values for WHFIB cells were n = 1.7, Do = 0.50 Gy, OER = 1.6. The values for 250 kV X-ray irradiation were n = 6, Do = 1.6 Gy, OER = 3.1 for V79 cells, and n = 25, D = 1.20 Gy, OER = 2.7 for WHFIB cells.

The survival curve for V79 cells exposed to a single dose of X-rays and then immediately to a series of neutron doses was identical to that for neutrons alone, implying that cells which had received all the sublethal X-ray damage they were capable of absorbing were not then more sensitive to neutrons. The survival curve for cells exposed to X-rays after a priming dose of neutrons had a reduced shoulder relative to that for X-rays alone. This implies that the neutrons caused some sublethal damage. This is consistent with the observed small, but significant shoulder to the neutron survival curve.

Results will be reported on the interaction between X-ray and neutron damage following a period for recovery from sublethal damage between the two types of radiation.

1C-2

STUDY OF BIOLOGICAL EFFECTS OF FAST NEUTRONS PRODUCED IN THE CYCLOTRON OF THE "RUDJER BOŠKOVIĆ" INSTITUTE

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Synchronous and asynchronous mouse L929 cells in culture were used as a system for examination of radiobiological properties of fast neutrons produced by our cyclotron. An internal cyclotron beam of 14 MeV deuterons incident on the aluminum target was used to produce a continuous energy spectrum of neutrons with mean energy of 5 MeV. Gamma irradiation was carried out at the cobalt-60 source giving 1,25 MeV mean energy gamma rays. In our experiments relative biological effectiveness (RBE), oxygen enhancement ratio (OER), repair of sublethal damage and age response of L929 cells to neutron irradiation were studied. RBE decreased with increasing of dose. OER of fast neutrons was 1.2 as compered to 2.8 for gamma rays. Split dose experiments using three doses of gamma rays (4, 6.6 and 10 Gy) and fractionation intervals of 8 hours indicated the accumulation and repair of sublethal damage for doses of 6.6 and 10 Gy. Split dose experiments with comparable doses of fast neutrons (0.8, 2.6 and 5.2 Gy) showed repair of sublethal damage only for the dose of 5.2 Gy. The repair was lower for a factor of 2 as compared to the corresponding dose of gamma rays. The study of the age response of cells to gamma rays showed dose-dependent increase of the ratios between sensitive and resistant phases of cell cycle. Age response of cells irradiated with fast neutrons however, changed significantly less as compared to gamma rays, but was also dose dependent.

1C-3

BIOLOGICAL PROPERTIES OF THE NEUTRONS PRODUCED BY THE IRB CYCLOTRONE: CHEMICAL PROTECTION

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A series of experiments has been undertaken in order to test the biological properties of neutrons produced in the cyclotrone of the Institute "Ruđer Bošković" (IRB) in Zagreb. Protective effect of AET (2-amino ethyl-iso-thiouronium bromid hydrobromid) on survival of L cells irradiated by fast neutrons generated in the IRB cyclotrone were studied by employing the single cell clonal growth method. For comparison the protective effect of AET after gamma irradiation has also been studied. The most important findings that have emerged from these experiments can be summerized as follows: (1) Protective effect of AET was present after gamma irradiation only. (2) The amount of protection was dependent on AET concentration in the growth medium. (3) No protective effect was found after neutron irradiation. These findings are in agreement with the generaly less efficient protection of this compounds after high-LET irradiation.

1C-4

Thiopyronin-sensitized Photodynamic Effect on RNA-and Protein Metabolism in Yeast

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We describe here the efficiency of the photodynamic effect with thiopyronin on RNA and protein metabolism in vivo in yeast cells.

In photodynamically treated cells synthesis of new free and membrane bound ribosomes is inhibited, but there is only a slight influence on polyA-mRNA synthesis. We find that the relative amount of polyA-mRNA in the membrane fraction does not change compared to untreated controls, while binding of polysomes on membranes seems to be prevented. The protein synthesis in vivo is inhibited immediatly. After irradiation with low dosis of visible light and after a certain time of reactivation one can find an increase of polysomal polyA-mRNA and ribosomes on the membrane. The longer the time of reactivation the more ribosomes are to be found attached to the membranes. This event obviously indicates reinitiation of protein synthesis. At that time synthesis of new free ribosomes starts.

These results affirm that RNA synthesis in the nucleus is not directly photodynamically inhibited ,but the inhibition of ribosome synthesis may be due to the inhibition of protein synthesis.

According to these and other results , we can say that thiopyronin instead of furocoumarins can be recommended as a non-mutagenic and non-cancerogenic compound in skin therapy.

1C-5

Studies on the repair defect(s) of Ataxia telangiectasia cells.

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Primary fibroblasts derived from patients suffering for the hereditary disease, ataxia telangiectasia (AT), are abnormally sensitive to ionizing radiation and have been claimed to be deficient in repair of damage in DNA; e.g., Paterson et al. found some AT-strains(ex-) partially defective in repair replication after anoxic γ-radiation; other AT-cells were normal. We have initiated a study with AT and normal cells on the molecular processes involving DNA damage and repair.

Paterson et al. have developed a method to measure the number of γ-ray induced lesions in DNA of mamalian cells, by treatment with damage-specific endonucleases (or glycosylases) present in extracts of Micrococcus luteus. With this method the removal of these lesions was studied in normal and AT3BI cells, but no difference in the rate of repair was observed. AT cells show an increased level of both spontaneous and radiation-induced chromosome aberrations; possibly this might be due to a (partial) deficiency in the repair of single- and/or double-strand breaks in DNA. Since the effects may involve only a small fraction of the breaks, in our recent studies much effort has been given to measure single-strand breaks with high accuracy. They were determined after moderately high doses (2-15 krad) of γ-radiation and after low doses (up to 400 rad), following various post-irradiation incubation periods. After 2-15 krad, in normal human fibroblasts, a rapid and slow repair of single-strands breaks could be distinguished, representing two different types of lesions. It appeared that the induction of the rapidly repaired single-strand breaks can be prevented by the presence of cysteamine during irradiation. Both types of single-strand breaks were also observed in irradiated AT-cells ; they were repaired at the same rates as in normal cells.

After irradiation at doses up to 400 rad, in normal cells more than 50 percent of the radiation induced single-strand breaks was repaired within 2 minutes after irradiation, whereas preliminary results with AT-cells indicate that here some 3-6 minutes are needed to reach this percentage.

Ref. : Paterson M.C., B.P. Smith, P.H.M. Lohman and A.K. Anderson
Nature, 260 (1976) 444-447

1C-6

Cell cycle kinetics of synchronous and asynchronous tumour cells in vitro after irradiation with x-rays and α-particles during the cell cycle.
Analysis of flow microfluorometric data.

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Synchronous Ehrlich ascites tumour cells in vitro were irradiated with x-rays and α-particles at various points in their cell cycle. The radiation-induced perturbation of the cell cycle during the following two generations was measured by the method of flow microfluorometry. The main effects after irradiation were a small retardation of S-phase and the well known G2-block, depending both on dose and time of irradiation during the cell cycle. Retardation of S-phase was minimal after irradiation during G1- and early S-phase but increased after irradiation during late S-phase. The duration of the G2-block, corrected by retardation of S-phase, was small and remained constant after irradiation during G1- and S-phase but increased after irradiation during G2-phase. Both effects could be observed with x-rays and α-particles. The RBE for both effects was RBE = 3.5.
The relative number of cells in the G1-, S- and G2+M-phase from microfluorometric data after irradiating synchronous and asynchronous cells could be described by a simple mathematical model. Parameters of this model were the cell cycle dependant retardations of the different phases and the dose dependant number of cells which do not divide after irradiation.
The possible relevance of cell cycle kinetics in the survival of cells and on repair of PLD during the cell cycle will be discussed.

1C-7

ALTERATIONS IN LECTIN-BINDING CAPACITY OF MAMMALIAN CELLS UPON IONIZING RADIATION

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Earlier we have already demonstrated that human fibroblasts /WI 38 cell line/ had bound more ^{3}H-concanavalin A in the first hours after X-irradiation than the non-irradiated controls. The phenomenon proved to be dose-dependent. Recently, the radiation-induced changes of the lectin-binding capacity of plasma membranes of various blood cells from irradiated mice and from human blood irradiated in vitro were studied. The unirradiated erythrocytes, lymphocytes and platelets bind the lectin in increasing rate of this order, respectively. In addition, age-dependency in lectin-binding of the different subpopulations of erythrocytes and platelets was observed. Ionizing radiation in the dose range of 0,25 to 3,5 Gy revealed the similar effect as for the cultured cells, i.e. very early, temporary and dose-dependent changes in the amount of bound radiolabelled lectin. Sensitive alterations were also detected on CHO cells during continuous exposure to tritiated water and ^{3}H-thymidine. - As the lectin-binding technique may provide a practical method for the early indication of the extent of reaction in irradiated organisms, investigations were made to fix the supramolecular structure of plasma membranes in the various phases of the development of altered receptor-function by applying glutaraldehyde. The experimental data on these observations will be presented.

1C-8

RADIOGENIC DISSOCIATION OF MOST TIGHTLY BOUND CHROMATIN PROTEINS OF EHRLICH CARCINOMA CELLS

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Eukaryotic chromatin contains a small group of nonhistone proteins, which are extremely tightly (possible covalently) bound with DNA. X-irradiation of Ehrlich ascites carcinoma cells (5-20 Gy) leads to the dissociation of these proteins from DNA. DNA-protein crosslinks can be observed at supralethal doses of gamma-irradiation only.

1C-9

The influence of Arabinofuranosyl nucleosides on the repair of PLD in EAT-cells.

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Arabinofuranosyl nucleosides (ß-araA and ß-araC) have been found to be potent inhibitors of DNA synthesis.This inhibition takes place via inhibition of DNA dependent DNA polymerases α and ß and is competitive with respect to dATP and dCTP respectively.
We have studied the action of these drugs on the radiation sensitivity of a line of EAT-cells grown in suspension.Experiments have been performed when the cells where in the plateau phase of growth where they show the highest ability to repair potentially lethal damage (PLD). This was measured by keeping the cells after irradiation in the plateau phase for some hours before dilution and plating in fresh medium to measure the survival.Complete inhibition of the repair of PLD (detected by delayed plating) by 12 μM ß-araA was found.At higher concentrations a further decrease in survival was observed presumably due to additional inhibition of repair processes which would normally take place in cells plated immediately after irradiation.Since this damage is also lethal if unrepaired it can be classified as potentially lethal.This additional repair inhibition results in a decrease of the shoulder width of the survival curve (Do remaining unchanged),a finding indicating that the shoulder is probably produced by some repair of potentially lethal damage.Eventually exponential curves were obtained at high concentrations of ß-araA (120 μM).
If the drug was added various times after irradiation at a concentration of 120 μM it was found to inhibit the repair of lesions which were unrepaired at the time of its addition. The kinetics of the repair processes responsible for the formation of the shoulder could be estimated in this way.The repair took place with a time constant (t_{50}) of about one hour.It showed a temperature dependence,which indicates its enzymatic nature.
The high specificity of the action of ß-araA, exclusively restricted to the DNA polymerases, and its highly inhibitory activity on the repair processes taking place in cells after irradiation indicate that DNA polymerization is required for these repair processes.
Qualitatively similar results have been obtained with ß-araC too, whereas α-araA was found to be completely inactive.

1C-10

Influence of exogenous DNA on mitotic cycle of cells damaged by radiation and other mutagens

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Time parameters of mitotic cycle of irradiated meristem cells were studied and the possibilities for their modification by postirradiation treatment with syngeneic DNA were assessed. A population of Vicia faba primary root meristem cells x-irradiated with 1.5 Gy was used for these studies. The irradiated roots were grown in tap water and in a 10/ug/ml solution of syngeneic DNA. For determining duration of the individual cell cycle phases the method of accumulation of diploid and tetraploid metaphases at various intervals after a 30-min treatment with 0.1 % colchicine was used. The mitotic index and frequency of individual mitotic phases were determined in parallel experiments. Duration of one mitotic cycle in controls at 20 °C was found to be 15 h 5.88 min. After in vivo irradiation of cells with 1.5 Gy the mitotic cycle was prolonged by about 1/2, thus equalling 23 h 11.20 min. Due to postirradiation treatment with syngeneic DNA the mitotic cycle was practically balanced with the control value, lasting 15 h 56.27 min. Basing on accumulation of diploid C-metaphases the rate of entry of cells into division was determined and duration of the actual mitosis and of its individual phases was calculated. Irradiation of meristem cells was found to result in a prolongation of not only the length of mitotic cycle (1.54x), but also of the length of mitosis (2.34x) and of the interphase (1.44x). Of the individual mitotic phases it was primarily the prophase that was prolonged (2.51x). In the experimental group treated with syngeneic DNA after irradiation, both the duration of the mitotic cycle and the length of the actual mitosis receded back to control values.

Analogous assessment of the repair effect of syngeneic DNA was made in the case of its application to a cell population damaged by chemical mutagens.

1C-11

Rabbit Skin Tissue Culture, A New Biologic Model.

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Skin stripped off rabbit's ear is cultured for 4 days at 37 °C. The stripps are prepared in small rectangular partions and placed on glass rods in culture medium. Epidermal cells of the skin specimens grow in culture over the subdermal layer. Irradiated skin stripps are grown in culture with non irradiated partions as control. The extent of epidermal growth is dependant on the dose. Irradiation is given in-vivo, as in vitro. The system has the advantage that it tests primary radiation reaction in a quasi in-vivo situation, vascular effect is thus eliminated. Dose/time relations are tested, other possibilities are discussed.

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Radiation and Environmental Biophysics

1C–12-14 Transplantation and Immunology

1C-12

SUCCESSFUL TREATMENT OF LETHALLY IRRADIATED RHESUS MONKEYS BY BONE MARROW TRANSPLANTATION ACROSS MAJOR HISTOCOMPATIBILITY BARRIERS.

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A major limitation to the treatment of radiation victims by bone marrow transplantation is the risk of Graft-versus-Host disease (GvHD), which restricts donor choice to siblings identical for the major histocompatibility complex (MHC). These are only available in about 30% of the cases. Studies in rodents and monkeys have shown that acute GvHD can be prevented by elimination of immunocompetent lymphocytes from the graft. In mice, delayed GvHD, evoked by lymphocytes which develop from donor precursor cells, can be further mitigated by prior elimination of the gastrointestinal microflora of the recipients. In a study with rhesus monkeys various donor/recipient combinations were investigated for take and resulting GvHD after transplantation of stem cell enriched, lymphocyte depleted bone marrow fractions, prepared by density gradient centrifugation. All monkeys were given total body irradiation (TBI; 850 cGy, 300 kV X-rays) and kept under reversed barrier nursing during the aplastic phase. Under these conditions the effect of matching for MHC-products could be clearly demonstrated. No take failures and no GvHD occurred in recipients of stem cell fractions from MHC-identical sibling donors, while only 40% of the recipients of stem cell fractions from unrelated mismatched donors showed a take, resulting invariably in lethal GvHD. (Half)sibling donor/recipient combinations mismatched for one MHC-haplotype gave no better results than unrelated mismatched controls. In unrelated monkeys, matching for the MHC A/B-locus antigens both improved takeability and decreased the risk of GvHD. Matching for MHC D-locus products had no beneficial effect. It was subsequently demonstrated that the effect of MHC-differences on takeability could be abolished by giving TBI in two fractions of 700 cGy X-rays, separated by 72 hours. The effect of MHC-differences on delayed GvHD could be largely eliminated by gastrointestinal decontamination of the recipients. If decontamination was added to the protocol of stem cell fractions from unrelated A/B-matched and haploidentical sibling donors the monkeys showed no or transient signs of GvHD (n = 5) and survived recontamination as stable chimeras up to more than 2 years. These results indicate that bone marrow transplantation across MHC barriers in an outbred species like man does not result in lethal GvHD if the graft is depleted from immunocompetent lymphocytes and the recipient subjected to gastrointestinal decontamination.

1C-13

Genetic control of the radiation-induced enhancement of the immune response.

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The production of antibodies may be increased by a whole-body irradiation. Different mechanisms have been proposed to explain this phenomenom and, in particular, a preferential inhibition of the regulatory T suppressor cells.

We studied rats from two inbred strains which, in the same experimental conditions, behave differently. A dose of 4 Gy was delivered by caesium source to the animals 48 hours before an intraperitoneal injection of 5 micrograms of dinitrophenylated ovoalbumin and 10^{10} Bordetella pertussis organisms. Antibody titres were obtained by the passive cutaneous anaphylaxis technique.

LOU/M rats did not present any increase of antibody production after irradiation by comparison with the unirradiated controls. On the contrary, OKA rats gave a radiation-induced enhancement of antibody production in the same experimental conditions.The same increase of antibody production was also found in (LOU/M x OKA) F1 hybrid rats, but not in the test cross rats LOU/M (LOU/MxOKA) N2 which displayed no enhancement.

These results strongly suggest that the radiation-induced enhancement of the immune response is under a genetic control which we are presently studying.

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1C-14

RADIOSENSITIVITY OF HELPER T CELLS.

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Irradiation of HRBC-primed mice impairs the ability of their spleen cells to help the anti-TNP antibody response of syngeneic normal cells immunized in vitro with TNP-HRBC. This radiation effect on helper T cell activity increases with time after injection of HRBC.

At different priming times,the curve describing the remaining helper activity as a function of the radiation dose shows the presence of two components,one radiosensitive,the other radioresistant.The radioresistant helper T cell subpopulation decreases with time after priming.

Cell separation and recombination experiments indicate that the two T cell subpopulations interact in the generation of helper activity.

Experiments are in progress to assess the type of functional alterations resulting from depletion of the radiosensitive subpopulation of helper T cells.

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Radiation and Environmental Biophysics

1D–1-14 Molecular Effects

STRUCTURAL DEPENDENCY OF MEMBRANE LIPIDS WITH REGARD TO RADIOSENSITIVITY

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In order to investigate the relative radiosensitivity of the most important naturally occurring fatty acids, model lipid systems were prepared and X-irradiated under different conditions. Liposomal and micellar models have been used. The liposomes were prepared from phospholipids extracted out of biomembranes. The highest radiosensitivity of these liposomes was found at concentrations of about 0.5 mg lipid per ml. Micelles were investigated below and above the critical micell concentration (CMC). A sharp rise in radiosensitivity occurs above the CMC as is known from earlier studies.
High oxygen enhancement ratios were obtained in both systems, especially at low dose rates, suggesting the operation of slowly progressing chain reactions initiated by ionizing radiation.
All cis-5,8,11,14-eicosatetraenoic acid (20:4, arachidonic acid) and all cis-4,7,10,13,16, 19-docosahexaenoic acid (22:6) were the most vulnerable species in the liposomal structures. When micelles prepared from pure fatty acids were used, it appeared that cis,cis-9,12-octadienoic acid (18:2, linoleic acid) was most easily destroyed by X-irradiation. All cis-6,9, 12-octadecatrienoic acid (18:3, γ-linolenic acid) and arachidonic acid were more resistant. It was found that the fatty acids in mixtures (mixed micelles)had different radiosensitive properties as compared to the fatty acids structured in micelles of one species.
It is concluded that the degree of unsaturation of a fatty acid is not necessarily determining the radiosensitivity of the individual fatty acid. Structural situations of the lipid in membranes, permitting different degrees of contact between the fatty acyl chains are probably very important for the propagation of the damaging radiation induced oxygen dependent chain reaction.
In liposomes the phospholipids are structured in bilayers just as found in biomembranes. It is assumed that (20:4) and (22:6) are the most radiosensitive species in the biomembranes of living cells.

1D-2

PERMEABILITY CHANGES OF THE PLASMA MEMBRANE OF TUMOR CELLS AFTER LOW DOSES OF IRRADIATION.

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Polyunsaturated fatty acids as arachidonic acid (20:4) and docosahexaenoic acid (22:6) were relatively vulnerable to X-irradiation compared to saturated fatty acids as measured in a model membrane system of liposomes (1).

In vivo experiments with lymphosarcoma-bearing mice show a rapid incorporation of intraperitoneally injected radioactive labeled fatty acids in the tumor phospholipids. At different times after injection, the mice were irradiated with low doses of X-rays (5 Gy). A sharp rise in the specific radioactivities of the lipids in the subcellular fractions of the tumor cells was seen shortly after irradiation. Within one hour the increase was back to control level. A second dose gave a repeatable effect. Both fatty acids, [^{3}H]-palmitic acid (16:0) and [^{14}C]-arachidonic acid (20:4), simultaneously injected, behaved in a similar way. Directly after irradiation the free fatty acids accumulate in the soluble fraction of the tumor cells. This temporarely increase took place before the rise in the specific radioactivities in the phospholipids of the membranes. So far we conclude to a non specific increase in the plasmamembrane permeability of the tumor cells to both types of fatty acids after X-irradiation.

In vitro incubations of the lymphosarcoma cells and also of lymphoma cells (L5178Y) at 37°C with the labeled fatty acids mentioned, also showed that the specific radioactivity in the lipids was raised after X-irradiation, although less than found in vivo. Also here, there was essentially no difference with respect to 16:0 and 20:4. When the tumor cells were incubated with radioactive fatty acids, washed and irradiated with 5 Gy of X-rays a decreased label incorporation in the phospholipids was observed. Up to now these experiments lead us to the suggestion that the polyunsaturated fatty acids of the membrane phospholipids are well protected against low dose irradiations when the cells are held under proper nutritional conditions. This confirms recently obtained results on the protection against radiation induced lipid peroxidation in the liver cell membranes (2). The irradiation probably enhances temporarely the in- and outflux of metabolites such as fatty acids. The latter compounds seem to be rate limiting in the de novo synthesis of membrane phospholipids of these cells.

1) Konings A.W.T., Damen J. and Trieling W.B., Int. J. Radiat. Biol., 35 (1979) 343-350.
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1D-3

Radiation sensitivity changes associated with membrane fatty acid composition

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We have investigated the role of unsaturated fatty acids in modifying the sensitivity of bacterial and mammalian cells to ionizing radiation. E. coli K1060 an auxotroph unable to synthesize unsaturated fatty acids was used to study the effect of membrane fluidity on survival and DNA damage. Oleic and elaidic acids were incorporated into the bacteria and the temperatures of irradiation chosen to give maximum differences in survival between the 'liquid crystal' and the 'gel' states. Maximum sensitization was achieved when the bacteria were at ice temperatures at the time of irradiation. Both oleic acid and elaidic acid grown bacteria were able to repair DNA strand breaks equally as well down to background levels. This extensive repair was seen after irradiation conditions which gave very big differences in survival. We conclude that DNA breaks per se are not responsible for cell death and possibly another vital macromolecule may be the site of primary lethal damage. Alternatively misrepair to the damaged DNA may be the event leading to cell death.

We have in addition changed the unsaturated fatty acid composition of a lymphoid mammalian cell line. Cells were grown in medium from which the majority of the lipid had been extracted and replaced with one of a number of unsaturated fatty acids. Unlike the results with bacteria the increased content of these acids in the cell did not increase the sensitivity under oxygenated conditions of irradiation but markedly reduced sensitivity when irradiation was in anoxia.

1D-4

EFFECT OF GAMMA-RADIATION ON MEMBRANE-BOUND RIBOSOMES ISOLATED FROM RAT LIVER

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Authors exposed isolated rat liver microsome and ribosome suspension to ^{60}Co-gamma irradiation /0,25 - 5 kGy/ in air atmosphere and investigated the aminoacid incorporation activity of the particles. They stated that the sensitivity of ribosome function to radiation changes both qualitatively and quantitatively in the presence of endoplasmic reticulum. The increased radiosensitivity of translational regulatory factors acting in the membrane significantly contribute to the qualitative change.

The particle concentration, the temperature and the frozen or melted state of suspension during exposition decisively effect the ratio of radiation induced inactivation.

Preliminary whole-body irradiation did not change the sensitivity of ribosomes to radiation, in vitro. The sensitivity to radiation of ribosomes isolated from various rodents /rat, mice, rabbit, guinea pigs/ showed insignificant difference, while that of microsomes is significant.

Microsomes, isolated from malignant transformed liver cells of rat carry on protein synthesis more intense than that of the normal, showing however, increased sensitivity to radiation.

1D-5

Effects of sublethal gamma irradiation and exercise on lipid peroxides and lipofuscin in rat liver and muscles.

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Ionizing radiation is leading to the formation of lipid peroxides. The product of their decomposition, malondialdehyde (MDA), forms stable lipofuscin like pigments (LFP), noxious to cells. The effect of irradiation and of exercise on MDA and LFP content in liver and muscle was studied in view of the detrimental sequels of exercise performed on the 3rd postirradiation day, as reported last year.

First, we estimated LFP concentration in rat liver homogenate and mitochondria up to 18 days from whole body irradiation (400 R). In the homogenate, LFP content increased abruptly by 50% of the preirradiation value during the 1st postirradiation hour. A slow increase continued up to the 7th postirradiation day (200%) and then slowly decreased (160%) above the controls on day 18. LFP in mitochondria increased during the 1st h. after irradiation also by about 50% but since then, LFP steadily decreased to reach 54% of initial value on day 18.

Next, MDA and LFP were estimated in rat liver and skeletal muscles, samples of which were obtained from control animals, 30 min. after swimming for 30 min., 30 min. after irradiation and from rats forced to swim on the 3rd postirradiation day. Liver MDA increased significantly in swimming (65%) or irradiated (60%) rats, while the combination of these factors led to a non-significant increase (27%). Liver LFP increased to a smaller extent in all groups. In muscles of swimming rats MDA increased by 175% and in irradiated animals by 300% of control level. Muscle LFP produced a slight increase in swimming or in irradiated rats and the combined regimen presented a still smaller increase.

These results suggest that 1/ liver concentration of MDA is proportionate to LFP deposits 2/ in muscles, exercise as well as irradiation produce large ammounts of MDA with a lower transformation into LFP 3/ the combination of exercise and irradiation produces less LFP in comparison to the isolated effects of these loads.

1D-6

BIOCHEMICAL AND PHYSICO-CHEMICAL CHANGES OF THE MEMBRANES AFTER IRRADIATION AND POSSIBILITY OF THEIR REPARATION

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The X-ray irradiation causes the regular changes of composition and structural viscosity of membrane lipid,connected with the rate of their peroxidate intensification under irradiation fecilitates the removel of lipids from the membrane, decreases concentration of less oxidable fraction of phospholipids and makes phospholipid phase more rigid.

By methods thin-layer and gas-liquid chromatography the quantitative content of the liver microsomal lipids of irradiated mice with the antioxidant was stadied. The decrease in lipid oxidative reactions (antioxidant) was found to resalt in the increase of the phosphatidylserin and phosphatidylinositol content in cell membranes. These lipids were most unsaturat and most oxidabl. The addition of antiosidant in vivo and in vitro was found to extrusion of these fractions from the membranes and to decrease in their proportion. It suggests that the oxidable lipids have the advantage in the utilisation and exchange rate and the interraction with the lipid carrying protein as compared the unoxidable lipids.

In earlier peroods after radiation we obserwed a correlation between the changes of activity and cooperative properties of membrane-bound ensymes and the content of lipids- their allosteric effectors, which may be important for X-ray disease. Synthethic antiosidant, intribiting lipid oxidation normalise membrane lipid composition and thus recover the functional activity of the membrnae.6The reparation of the membrnae in shown to be significant for cell and organism survival.

1D-7

FURTHER INVESTIGATIONS ON THE INTERACTION OF GLUTATHIONE WITH ORGANIC RADICALS.

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Previous observations on this subject have shown that reduced glutathione (GSH) can repair OH-induced hydroxyalkylradicals by a hydrogen transfer mechanism. At fixed GSH concentration and radiation dose, the per cent repair appeared to be constant with different concentrations of single aliphatic alcohols, where the repair was always complete and carbohydrates, where the repair was less than 100%. Moreover the rate constant for the repair reaction for carbohydrates, which according to the mechanism should be first order in GSH, showed significant deviations from the theoretical kinetics.

Such deviations have been now shown to be mostly apparent due to the characteristics of the kinetics of GSSG formation, either directly from GSH radiolysis, or from the repair reaction. In particular it appears to be important to take into account competing radical-radical reactions influencing the observed yield of repair.

1D-8

Activity of Prostaglandin-synthetase in the Liver, Brain and Testis of F_1-mice ($CBAxC_{57}Bl$) after γ-irradiation

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Activity of microsomal prostaglandin-synthetase in the liver, brain and testis supernatants at various time after irradiation of mice with lethal dose (950 r) was studied. Microsomes and supernatants were incubated with 1-^{14}C arachdonic acid, labeled products were extracted by ethylacetate and analyzed by thin-lower chromatography. Activity of enzyme was evaluated by the amounts of prostaglandins $F_{2\alpha}$ and E_2 synthetized. 15 min after irradiation of mice the activity of enzyme in the tissues was increased. At later times (60 min, 6,24,72 hrs) the activity of enzyme in the liver and testis was decreased and in the brain was sharply increased. Possible role of hypophysis-adrenal system and of endogeneous inhibitors of biosynthesis in activation and inhibition of enzyme activity was discussed.

1D-9

EFFECT OF IONIZING RADIATION ON ACTIVITY OF ADENYLATE CYCLASE IN LIVER OF RAT EMBRYOS

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A comparative study of the effect of ionizing radiation on basal and catecholamine-stimulating activity of adenylate cyclase (ATP pyrophosphate-lyase (cycling), EC 4.6.1.1.) (AC) in liver of 20-day rat embryos under in vivo and in vitro conditions (a membrane fraction and plasma membranes) has been made.

The activity of basal AC is found to increase after exposure of the membrane fraction or embryos to 2.5 Gy of γ-rays. No changes in the activity of the enzyme are observed after irradiation of isolated plasma membranes under similar conditions. The share of the mitochondrial fraction in the radiation effect of AC activation is shown. The contribution of the indirect effect of radiation to modifying of the AC activity is discussed.

γ-irradiation of isolated plasma membranes with doses 100-1000 Gy is shown to inhibit the AC activity. This effect is more pronounced when AC is stimulated with isoproterenol. Indirect data suggest that the observed inhibition of AC activity may be due to radiation-induced changes in the lipid phase of membranes.

1D-10

Reduction reactions with native and irradiated proteins.

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The rate-constants for the reaction of the hydrated electrons with native bovine serum albumin (bSA) were measured at different pH-values and in the presence and absence of formate and ethanol. Preirradiation of bSA in the absence of scavengers does not produce a change of the rate-constant, but in the presence of ethanol and formate the reduction became faster with the preirradiation dose. The electron transfer from formate radicals to the disulfide bridges of native bSA is only 10 times slower than the reduction by e^-_{aq}, while with native ribonuclease (RNase) the velocity of these two reactions differ by two orders of magnitude. In both cases with bSA as well as with RNase the reduction reaction became faster with the preirradiation dose. Especially with bSA there is a great increase of the yield of S-S-radicals after preirradiation. Though the redox potentials of formate and ethanol radicals are very similar, the reactivity of the ethanol radicals is comparingly low, but it is also increased by preirradiating the proteins.

1D-11

RADIOLYSIS OF TYROSINE, GLYCYLTYROSINE AND POLY-TYROSINE IN ALKALINE SOLUTIONS

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We have shown previously that radiolysis of water solutions of tyrosine and glycyl-L-tyrosine at pH lower than that of OH group ionization in the absence of oxygen results in dityrosine formation. Present communication summarizes results obtained during radiolysis of free and peptide-bound tyrosine /glycyltyrosine and poly--tyrosine/ in alkaline water solutions, pH $\geqslant$ 10.8 at concentrations equivalent to $2 \cdot 10^{-3}$M of tyrosine. Samples saturated with nitrogen, N_2O or air, were irradiated with ^{60}Co gamma rays at the dose rate of 160 cGy.s^{-1}. Radiolysis products were separated by gel filtration and were analysed with amino-acid analyser. Absorbance and fluorescence spectra were also measured. The results obtained demonstrate that at pH higher than 10.8 radiation-induced dityrosine structures are formed mainly when solutions are oxygenated. The radiation yield of dityrosine at these conditions is much higher than in deaerated, neutral or acid solutions.

1D-12

EXPERIMENTAL DATA ON BIOCHEMICAL INDICATORS OF RADIATION INJURY IN MAN

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Present investigations are based on the methodology and experience of previous experiments on rats. Human control urinary pseudouridine and deoxycytidine excretion values were determined in more than 100 healthy subjects (age between 18 - 30)using HPLC separation. Mean normal values for pseudouridine were found to be slightly above literature data (160 - 270 μmol/L). The distribution of excretion values showed three maxima representing three distinguishable sub-population of the subjects. No significant differences in excretion values were found according to sex or age. These findings lead to the conclusion that diagnostic use of the examined parameters invariably requires the knowledge of the actual individual control level.
Excretion of the two mentioned nucleosides was followed in more than 30 breast cancer patients during a postoperative X-ray therapy. These patients are supposed not to have malignant cells to such an extent that they would be able to alter significantly the excretion pattern of nucleosides. Therapeutical irradiation was performed with 200 kV, 20 mA X-rays filtered by 0.5 mm Cu, in three sets, with 3 and 6 months' interruptions, of 5-10 irradiations each. Applied doses were between 10 and 20 Gy per set, and 4 samples were taken during each set including self-control. Excretion values are found to be elevated in irradiated patients; the extent and the tendency of the increase are expected to be of prognostic value.

1D-13

THE EFFECT OF IONIZING RADIATION ON THE TRANSPORT OF CALCIUM IONS IN MITOCHONDRIA

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The influence of radiation on the Ca^{2+} ion transport in the mitochondria isolated from the rat liver 24 hr after γ-radiation of animals with 1000 r is studied. It is found that the character of the registered changes of Ca^{2+}-transporting systems in the mitochondria of irradiated animals strongly depends on the time of storage of the isolated mitochondria. Radiation causes a disturbance in the function of Ca^{2+}-transporting systems which is reflected by changes of the following parameters:

1) Ca^{2+}-capacity (maximal amount of Ca^{2+} ions accumulated by mitochondria) immediately after isolation exceeds the control value, 5 hr after it returns to the control level and 8 hr after it decreases by 15-20% as compared to the control;

2) the time of Ca^{2+} retention in a matrix, right after isolation of mitochondria, increases two-fold as compared to the control, 2 hr after it approaches the normal value and 4 hr after decreases by 20-25% as to the control;

3) the rate of the Ca^{2+}-H^{+} ion exchange in the mitochondria of irradiated animals is lower than in the control, but then it shows gradual increase and 8 hr after it exceeds the control by 20-25%;

4) the radiation does not practically affect both the content of endogenous calcium in mitochondria and the efflux of endogenous calcium from mitochondria under the conditions of the membrane potential being decreased.

1D-14

THE γ-INDUCED DEMETHYLATION OF THYMINE IN AQUEOUS SOLUTION AND IN THE SOLID STATE

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Evidence is presented for the γ-induced demethylation of thymine in aqueous solution and in the solid state.
The demethylation of solid thymine was measured qualitatively and quantitatively with specially purified thymine-(methyl-C-14). Analogous experiments with thymine-(methyl-3-H) and radio-gaschromatographic analyses provided proof for the formation of molecular hydrogen and methane.
The cleavage of methyl groups from thymine in aqueous solution depends on the concentration and on the dose rate. Here, methanol und methane were found radio-gaschromatographically.
The dose-independent G-values for the demethylation of thymine in aqueous solution are essentially smaller than the dose-dependent G-values for the demethylation of solid thymine. This surprising fact is probably due to the direct action of radiation on the C_5-C_7 bond of thymine in aqueous solution. Irradiations in the presence of radical-scavengers could test this assumption.
The attack of the methyl group by the water radicals seems only to induce the formation of hydroxymethyl-uracil.
The cleavage of methyl groups from thymine leads primarily to uracilyl-5-radicals, which can react with hydrogen atoms to form uracil. The formation of the RNA-base uracil from the DNA-base thymine is an important change in the DNA-code.
The results are discussed in connection with ESR-spectroscopic studies, and with respect to the dehalogenation of halouracils.

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Radiation and Environmental Biophysics

2A–0-8 Effects on DNA

2A-0

CHEMICAL NATURE AND BIOLOGICAL SIGNIFICANCE OF DNA DAMAGE CAUSED BY IRRADIATION IN VITRO

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Irradiation of DNA in aqueous solution and subsequent analysis of the damaged macromolecules provided a wealth of information on the chemical nature of the damage in terms of base and sugar modifications. However, relatively little is known about the biological consequences of specific chemical alterations. Some recent work in this field will be discussed; both inactivation and mutation will be considered.
Also the effects of a few sensitizing and protecting compounds present during irradiation will be discussed.

2A-1

Rate of Strand Break Formation in Poly-Uridylic Acid Induced by OH Radicals in Aqueous Solution

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The reaction of OH radicals with the potassium salt of poly-uridylic acid (poly U) in aqueous, O_2 free, N_2O saturated solution was studied measuring the change of conductivity as a function of time after application of an electron pulse (1 µs), and after ^{60}Co-γ irradiation. The results obtained varying dose and dose rate show that the reaction with OH leads to an increase in conductivity with first order kinetics. The rate constant at 18 °C and pH 7 is $k = 0.67\ s^{-1}$ (activation energy = 12 kcal/mol and frequency factor = $3 \times 10^{10}\ s^{-1}$). Surprisingly this increase in conductivity is due to an increase in free potassium ion concentration as measured by a K^+ sensitive electrode and not to a change in pH. As is shown by the effect of scavengers (tetranitromethan and p-benzoquinone) on the kinetics it is the lifetime of poly U radicals and not the diffusion of degradation products of poly U which governs the rate of the conductivity increase.

The rate determining step is proposed to be the formation of main chain breaks (strand breaks) of poly U which causes a shift in the equilibrium between free and poly U bound K^+ ions. In analogy to the mechanism for single strand breaks in DNA (1) it is postulated that it is mainly the C-4' radical in poly U which leads to chain breaks by a cleavage of the sugar-phosphate bond. The pH dependence of the rate of the conductivity increase is in agreement with this view.

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2A-2

Charge migration in rigid DNA-water systems; a pulse radiolysis conductivity study.

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The conductivity resulting from pulse-irradiation with 3MeV electrons of concentrated DNA-water mixtures has been studied at nanosecond timescale over a temperature range of -20 to -120°C, using the microwave absorption method.

In a sample of NaDNA (calfthymus, mol.wt. 2×10^7) which contained 110 molecules of water per nucleotide, the product of the free ion yield (G) and the mobilities of the charge carriers (µ) was found to decrease only slightly with decreasing temperature. A value of $G\mu = 3 \times 10^{-4}$ (± 40%) $m^2V^{-1}s^{-1}(100eV^{-1})$ was calculated from the conductivity signal at -40°C. The time dependence of the conductivity signal showed a combined pseudo first order (trapping) and second order (recombination) decay. The trapping rate constant at -40°C was found to be approximately $1.5 \times 10^7\ s^{-1}$.

On reducing the water content to 15-20 molecules of water per nucleotide (i.e. 50% w/w water) the Gµ-value dropped by a factor of 2 while the trapping rate at -40°C increased by a factor of ten. In a sample which contained about ten molecules of water per nucleotide no transient conductivity could be detected, which means that the signal was at least a hundred times smaller than in the more hydrated samples.

At -40°C a value for Gµ of $4 \times 10^{-4}\ m^2V^{-1}s^{-1}(100eV)^{-1}$ has been reported for pure ice, where the electron has been shown to be the major charge carrier. Comparison of this value and the values mentioned above suggests that the conductivity observed in the samples containing 110 and 15-20 molecules of water per nucleotide is also due to migration of electrons. Assuming the free ion yield in the sample containing 15-20 molecules of water per nucleotide to be approximately equal to the value for ice $(0.4\ (100eV)^{-1})$, an electron mobility of $4 \times 10^{-4}\ m^2V^{-1}s^{-1}$ is calculated.

2A-3

Formation and properties of the DNA-electron adduct.

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The pulsradiolysis technique has been used for the study of the reaction of the hydrated electron with DNA.

That this reaction really takes place, is spectrophotometrically evidenced by the rate of electron decay and the growth of an electron adduct.

The absorption spectrum of this electron adduct, which differs from the hydrogen and hydroxyl adducts, is not just a straightforward addition sum of those of the nucleotides.

Best fits are only obtained by assuming that, at least at longer times, the electron is located at the thymine moiety.

This, in turn, could be a clue for electron migration along the DNA chain.

2A-4

DNA chain elongation in Chinese hamster cells after ^{60}Co gamma irradiation

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Ionizing radiation seems to affect the DNA synthesis mechanism mainly by inhibition of initiation of new replicons. There are, however, only few studies on the effect on the DNA chain elongation. The aim of this work was to investigate, by using a sensitive method, if there was any inhibition of the DNA chain elongation in irradiated mammalian cells.

Cells were pulse-labelled with ^{3}H-thymidine and then incubated at 37^{o}C. After various periods when the labelled region was a distance from the replicating fork (DNA chain elongation rate - (μm/min) - times minutes), the cells were transferred to alkaline solution of low ionic strength. The further treatment was similar as the unwinding technique for analyzing DNA strand breaks.

The result is expressed as percentage of ^{3}H-activity found in the double-stranded fraction after hydroxylapatite chromatography. At short times after pulse-labelling most labelled DNA will be unwound and thus found in the single-stranded fraction but with increasing periods in 37^{o}C less and less labelled DNA will be unwound. After about 60 minutes a plateau is reached indicating the termination of the labelled replicons.

No indication of any inhibition of the DNA chain elongation could be seen from 10 to 90 minutes after irradiation with 0.25 to 50 Gy.

2A-5

Rejoining of DNA strand breaks induced by ^{3}H- or ^{125}I-decay in DNA at 0°C

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Decays of radionuclides incorporated into DNA as ^{3}H-thymidine (^{3}H-TdR) or ^{125}Iododeoxyuridine (^{125}IUdR) induce DNA strand breaks in mammalian cells. During the labelling period at 37°C many DNA strand breaks induced by ^{125}I-decays are unrepaired (about 30 % of all breaks) while breaks induced by ^{3}H-decays are more efficiently repaired although some breaks are found unrepaired at 37°C (about 3 % of all breaks).

The aim of this investigation was to study the repair of DNA strand breaks induced after an incubation period at 0°C. Cells were labelled with ^{3}H-TdR or ^{125}IUdR for 18 hours and incubated in non-radioactive medium for additional 3 hours. The cells were then transferred to small test tubes and chilled to 0°C. After 6 hours the cells were incubated at 37°C for various periods (repair-phase). Analysis of DNA strand breaks was performed using the DNA unwinding technique.

During the labelling period the cells accumulate a certain number of unrepaired DNA strand breaks (0-value) and after 6 hours at 0°C more DNA strand breaks are accumulated. When the cells are returned to 37°C these "new" breaks seem to be repaired within 30-60 minutes depending on the accumulated dose. In all experiments performed up to now there appeared to be an "over-shoot" - that is the cells show less DNA strand breaks after 30 minutes repair than the 0-value. This effect may depend on an inhibition of the initiation of new replicon or/and an increased rate of death of cells with high level of DNA strand breaks during the 0°C treatment.

2A-6

THE OXYGEN ENHANCEMENT RATIO IN CULTURED HUMAN KIDNEY CELLS EXPOSED TO ^{3}H INCORPORATED IN DNA

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The experiments aim at elucidating toxic effects on DNA by DNA bound ^{3}H-TdR.

Human kidney cells in culture were unifilarly labeled with 6-^{3}H-TdR. Cell survival and the induction of DNA single (SSB) and double (DSB) strand breaks were investigated under aerobic and hypoxic conditions. Decays were accumulated at temperatures between 0 and 4°C for time periods ranging from 4 days to 2 weeks.

In DSB measurements, the ratio of breaks under aerobic conditions and in the hypoxic state yielded an OER of 3.6 as expected from low LET radiation. In SSB measurements, 1.48 and 1.0 breaks per decay were found under aerobic conditions and 0.95 and 0.57 SSBs in the hypoxic state. The oxygen enhancement ratios thus are 1.56 and 1.75. There was no indication of repair effects.

The unusually low OER is assumed to be caused by the transmutation of ^{3}H to ^{3}He in the 6 position of TdR causing a high yield of SSBs also under hypoxic conditions.

2A-7

Determination of DNA double strand breaks in Ehrlich ascites tumour cells

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Usually DNA double strand breaks (dsb) are measured in the dose range between 100 Gy and 1000 Gy while cell survival studies are done at doses below D = 10 Gy. So an extrapolation over a dose factor of 100 is necessary for the correlation of both endpoints. This difference is diminished by the presentation of a method which permits the determination of dsb in Ehrlich ascites tumour cells at x-ray doses down to D = 5 Gy. The method, based on that of Appleby (1977), involves preparation of high molecular weight DNA by lysing cells and digesting away DNA associated components using a mixture of detergents and enzymes contained in a cut-down syringe. After such incubation the resulting DNA solution is cautiously layered onto the top of a neutral sucrose gradient and centrifuged at very low speed (ν = 20/s, t = 90 h) to avoid speed effects (Zimm 1976). Strand break values are calculated by a numerical comparison of the sedimentation profiles of unirradiated and irradiated samples with a FORTRAN program. The detection limit is about 100 dsb per cell. Data show the induction and repair of dsb under various conditions. The induction of dsb is linear with dose, having a molar induction rate of $(9 \pm 2)\cdot 10^{-12}$ dsb/Gy·(g/mol) for 30 MeV electrons, $(11 \pm 2)\cdot 10^{-12}$ dsb/Gy·(g/mol) for 140 kV x-rays and $(5 \pm 1)\cdot 10^{-12}$ dsb/Gy·(g/mol) for 4 MeV α-particles. The repair rates of dsb are dependent on the culture conditions and on the radiation quality. While more than 90 % of the dsb produced by x-rays are repaired within t = 8 h, most of the dsb stay unrepaired after α-irradiation, changing the relative biological effectiveness of $n_\alpha / n_x = 0.4$ for the induction of dsb to $n_\alpha / n_x \approx 3$ after repair.

References:

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2A-8

The effect of dose rate on the production of DNA double-strand breaks in eukaryotic cells.

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Yeast cells were irradiated with 30 MeV electrons at a high dose rate (130 Gy/min) and with 60-Co-gamma-rays at a low dose rate (0.55 Gy/min). In the high dose rate experiments cells were kept at 0°C during irradiation in order to inhibit repair processes. The initial number of double-strand breaks (dsb) shows a linear dependence of dose after irradiation at high dose rate, the frequency of induction is 6.8×10^{-12} dsb per g mol^{-1} per Gy. When irradiated cells are allowed to repair dsbs in a non-growth medium at 30°C for 72 h the linear relationship between initial dsbs and dose is converted into a quadratic relationship between irreparable dsbs and dose (5.15×10^{-16} dsb per g mol^{-1} per Gy^2). This quadratic term reflects the accumulation of dsbs which form irreparable dsbs.
In low dose rate experiments cells were kept at 30°C in a non-growth medium allowing repair during irradiation. The number of dsbs measured after irradiation at low dose rate is smaller than the number of irreparable dsbs observed after irradiation at high dose rate. The dsbs measured after irradiation at low dose rate comprise not only irreparable dsbs but they still contain a fraction of reparable dsbs. The number of irreparable dsbs produced by a dose of 2400 Gy applied at low dose rate is about one third of the irreparable dsbs induced by the same dose applied at high dose rate. This indicates that during low dose rate irradiation a drastic decrease of the quadratic term occurs.

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Radiation and Environmental Biophysics

2A–00-14 Cellular Effects

2A-00

The use of model systems for the interpretation of radiobiological data.

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Radiobiological data can be interpretated in a quantitative way by the use of mathematically formulated models for the production of radiation lesions and models for the expression of these lesions through biological pathways in the irradiated objects. All such models have to be made up by measurable parameters which for the interpretation of the biological data on a molecular level must have more than only a formalistic value.
As an example, starting with a Poisson distribution of radiation induced low numbers of lethal lesions in mammalian cells and including a quantitative model of repair of potential lethal damage using saturated and unsaturated enzyme systems it can be shown that the biological endpoint of cell reproductive death and its quantitative relation to absorbed dose, the "shoulder curve", can be explained quantitatively from this system of models. Comparison of the parameters used with separate experiments on the molecular level such as the repair time constants for the repair of DNA-lesions give more information on the mechanism of damage expression in living cells which can be applied also to other observed biological endpoints.

2A-9

DEPENDENCE OF THE QUADRATIC MODEL PARAMETERS ON DOSE RATE (γ RAYS) AND 50 MeV NEUTRONS : AN ANALYSIS BASED ON THE IN VITRO SURVIVAL CURVES OF 6 HUMAN CELL LINES.

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We have already shown that the γ rays survival curves of six human cell lines were adequately described by the quadratic model. Furthermore, a test of relevancy lead to the conclusion that out of the 4 mostly used models, the quadratic model was the only one that provided a set of parameters values for a given survival curve irrespective of the part of the curve analyzed.

In the present study, we try to verify if the traditional interpretation of the α, β parameters is in agreement with the dose rate effect we observe from our six cell lines. As expected, dose rate effect appears to vary widely from one cell line to another, but surprisingly α and β modifications do not comply with the theory. Not only a β component is always detectable, (even with the lowest dose rate used (0.11 $Gy.hr^{-1}$)), but also the α component undergoes a substantial reduction for some of the cell lines.

The same analysis is performed on the survival curves to 50 MeV neutrons. Once again, the effect of neutrons differs widely from one cell line to another. The main effect of 50 MeV neutrons was a priori expected to result in an increase of the α component. As a matter of fact, such a behaviour is observed in only three out of six cell lines.

In conclusion, although the quadratic model provides pertinent fittings for the human cell survival curves, additional hypotheses should be required to take into account the evolution of α and β with dose rate and neutrons. The interpretation of these variations may call for biological factors, such as repair mechanisms, that are inherent to each cell line but are lacking in this physically oriented model.

2A-10

The correlations between different radiation induced biological effects.

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If it is assumed that one basic type of radiation induced molecular lesion can be responsible for a variety of different biological effects, such as cell killing mutations, chromosomal aberrations, then a series of straight forward mathemetical equations can be derived which predict the correlations between two different end points. The only restriction on a comparison of the two end points is that they be measured in the same cell population in the same experiment. The mathematical equations will be derived and a variety of experimental data will be analysed to demonstrate the predicted correlations. Correlations will be shown between survival and mutations, survival and chromosomal aberrations, different aberrations, different mutations all in somatic cells and it will be shown that similar correlations can be found for effects induced in germ cells. The implications of the correlations for radiation biology will be discussed.

2A-11

Cell coupling and radiosensitivity of cells under tissue-equivalent organization (spheroids)

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It is shown that some cell-lines when cultured as multicell spheroids are more resistant against γ-ray induced killing than in monolayer- and single cell culture. A correlation could be established between the degree of this "contact resistance" (CR) and electrical coupling between cells through gap-junctions as measured by micro-electrode-technique. Moreover, spheroid cells exhibit a decreased frequency of radiation-induced mutations and chromosome aberrations. To elucidate the mechanism of CR several cell parameters were investigated in dependence of cell-organisation (single cells/spheroids). The results obtained allow the conclusion that alterations in cellular regulation experienced by communication-competent cells when cultured under tissue-equivalent conditions are the basis for understanding and further investigating the phenomenon of CR. The significance of the results for radiotherapy, genetics and cancerogenesis is discussed

2A-12

Cell Proliferation in Preimplanted Mouse Embryos after Neutron Irradiation in Vitro.

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Preimplanted mouse embryos were cultured in vitro from the 2-cell stage to blastocysts (about 100 cells per embryo). The embryos were irradiated with neutrons (0.06-1.0 Gy) in the G_2-phase of the 2-cell stage. The number of cells per embryo was determined at various ^{2}periods until 110 hours after irradiation. The distribution of cells in the cell cycle was measured by microscope cytofluorometry. Furthermore the occurrence of micronuclei as a measure of cytogenetic damage was evaluated.

After a dose dependent division delay the cells migrate through two cell cycles with the same rate as the unirradiated controls. Thereafter the proliferation rate decrease with dependence of dose, measured by the total number of cells and the number of S-phase cells. The dose effect curve for the formation of the micronuclei has been determined at various time periods. At early periods the curve is steeper after lower radiation doses than after higher doses. The shape of the curve is determined by two phenomena: The cytogenetic damage a) which is only expressed after mitosis and b) the radiation induced division delay. The micronuclei occurring after the first or second mitosis following irradiation can be interpreted as a consequence of chromatin breaks (possibly double strand breaks) and other types of chromosome damage (for instance single strand breaks) respectively.

The data will be compared with corresponding results after X-irradiation. RBE value have been calculated. They decrease with increasing doses and range from 9.0 to 2.5.

2A-13

INTERACTION BETWEEN X-RAYS AND CADMIUM IN THE PREIMPLANTED MOUSE EMBRYO

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Cadmium belongs to the about 30 substances which are definetely known to be carcinogenic to man. Moreover cadmium is a severe environmental toxic agent the relevance of which becomes more and more apparent.
Therefore it is of great interest to investigate whether there are synergistic or antagonistic interactions between cadmium and X-rays.
Test system was the preimplanted mouse embryo in vitro. The embryos were isolated in the G_2-phase of the 2-cell stage, transferred to a medium containing cadmium ($CdSO_4$ or CdF_2) or to control medium and irradiated immediately. The influence of X-rays and cadmium either alone or in combination was determined from the following parameters:
a) The microscopic visible development of the mouse embryos in vitro until 144 hours post conceptionem. b) The number of cell nuclei, the labelling index and the mitotic index at different stages of development. c) The cell cycle phases. d) The number of micronuclei.
All experiments performed so far (investigation of the microscopic visible development and of the number of micronuclei) indicate that the effects of X-rays and cadmium are additive.

2A-14

Neuronal maturation defects in the brain of mice following X-irradiation during fetogenesis

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The alignment of neuronal processes in the cortex layer Va as well as the diameter of total cerebral cortex and corpus callosum were studied in juvenile mice after intrauterine X-irradiation (25-200 R) on day 13 p.c. Microvideoanalysis of silver impregnated histological slides (Bodian stain) revealed deviations in the alignment of neuronal processes with a linear correlation to the exposure level. Diameter diminution of corpus callosum, being four times stronger than in the cortex, showed linear dose relationships too. Contrary to fluctuating irradiation induced maturation responses, for instance observable during formation of myelin or tigroid substance, these structural defects are persistent, even after a 25 R exposure.

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2B–1-8 Normal Tissue Responses

SURVIVAL OF SPERMATOGONIAL STEM CELLS IN THE CBA MOUSE AFTER COMBINED EXPOSURE TO 1 MeV FISSION NEUTRONS AND HYDROXUREA

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Single neutron dose-response studies have demonstrated the presence in the CBA mouse testis of about 2000 radioresistant (D_0 80 rad) colony-forming stem cells. After surviving a dose of 150 rad neutrons, these cells rapidly increase their radiosensitivity, as revealed by their very low survival after a second 150 rad dose given at various intervals up to 72 hours later. The second dose survival showed a cyclic pattern: a decrease to very low levels during the first 16 hours after the initial dose, a rise to higher levels from 40 hours on and a second decrease starting at 56 hours. To investigate whether the fluctuations in stem cell survival are correlated with a synchronized progress of surviving stem cells through their first post-irradiation cell cycle, we replaced the second neutron dose by an injection with the S-phase specific cytotoxic agent Hydroxurea (HU). The pattern of stem cell survival found confirmed the synchronisation. It can be concluded that the neutron survivors 1) were not in the S-phase of their cell cycle at the neutron exposure, 2) successively entered an S-phase between 36 and 52 hours after the neutron dose and left it about 20 hours later, 3) had an intermediate radiosensitivity in the S-phase and 4) were radiosensitive in the preceding (part of the) G_1-phase.

HU given before neutron irradiation resulted in a sharp decline of stem cell survival down to 3% of the level after irradiation alone when the treatment interval was prolonged to 16 hours. This indicates that the great majority of the potential neutron survivors were in an S-phase of their cell cycle at 16 hours before irradiation. It may not be excluded however that the great amount of stem cell killing in the HU-neutron experiments was caused by a triggering by HU of radioresistant slow-cycling cells into rapid proliferation, accompanied by a sharp increase of their radiosensitivity.

2B-2

DEPOPULATION AND REPOPULATION OF THE SEMINIFEROUS EPITHELIUM IN THE MOUSE AFTER 1 MeV FISSION NEUTRONS

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Spermatogenetic cells have different sensitivities for irradiation, spermatocytes and spermatids being much more radioresistant than the earlier cell types in the line of spermatogenesis: the A, In, and B spermatogonia. Due to these differences a progressive depopulation takes place in the seminiferous tubules after irradiation; the immediate disappearance of spermatogonia is followed in time by an outflow of more mature cell types, which complete their differentiation, but are not succeeded by new ones.

Repopulation starts from a category of radioresistant stem cells, which give rise to colonies which refill the tubules by longitudinal outgrowth. This outgrowth was followed after irradiation with a dose of 100, 150, 300, and 350 rad neutrons with the use of stereological methods up to 30 weeks.

The study on the growth rate of the colonies up to five weeks after a dose of 100 rad was complicated by the depopulation process (especially the shrinkage of the tubules), which dominates the repopulation. From five weeks on the outgrowth of the colonies was strongly inhibited by collision of the colonies.

The growth rate up to five weeks after 150 rad, eight weeks after 300 rad and 30 weeks after 350 rad was almost similar and almost linear in time. Apparently longitudinal clonal growth is independent of the dose, i.e. independent of the number of surviving stem cells. From five weeks on after 150 rad the outgrowth of the colonies was inhibited by collision. A growth retardation was also found from eight weeks on after the higher dose of 300 rad, which however cannot be explained by collision of the colonies, since less than 10% of the tubules showed repopulation at that time after irradiation.

During repopulation of the epithelium the stem cells are more radiosensitive than they were prior to irradiation. After a first dose of 150 rad a slow return to the normal radioresistancy was observed, which seemed to run parallel with the refilling of the tubules by a new epithelium.

The differentiation of the spermatogenetic cells in the colonies was found undisturbed after 100 and 150 rad. After 300 and 350 rad the appearance of the round spermatids was retarded for one week.

2B-3

Murine Spermatogonial Regeneration after Exposure to X-Rays and 15 Mev Neutrons.

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The seminiferous epithelium represents a unipotential compartmental system whose single cell compartments show extremes in radiosensitivity. The regenerative stem cells (spermatogonia) are radioresistant. The testis has, thus, been proposed as a model for radiobiological inter-comparisons (GERACI et al, 1977). Studies on murine spermatogonial regeneration have commonly been restricted to observation-times which do not exceed 1 to 2 generation cycles (GC = 35 days) and, therefore, fail to render a comprehensive impression of the regenerative process. The objective of this report is to show, comparatively, the indices of regeneration (RI) of spermatogonia as a function of dose and time.

NMRI-mice were whole-body-irradiated with D-T-neutrons at doses ranging from 0.5 to 2 Gy. The reference radiation was 150 kV X-rays; matched doses ranging from 1.5 to 6 Gy were used. Mice were sacrificed periodically up to 150 days post-irradiation (i.e. at least 4 GCs); the testes were removed and processed for histology. RI was evaluated in HE-stained serial sections. 200 tubuli were scored for each point.

A biphasic pattern of regeneration was recorded. A cursory/abortive rise was followed by permanent regeneration. Differences in the patterns of regeneration after neutron and X-irradiation were noted. The higher RBE which is generally attributed to fast neutrons holds true only for the cell-sterilising effect. The final repopulation after neutron-irradiation was found to be more efficient than that after X-irradiation.

The radiobiological implications with regard to possible qualitative differences in radiaton action at the cellular level shall be discussed.

Reference : GERACI, J.P. et al: Radiat. Res. 71, 377-386 (1977).

2B-4

Long Term Cellular Recovery from Radiation Damage in Rat Thyroid; Studies on Cell Survival and Chromosome Aberration Frequency.

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Administration of an antithyroid drug, goitrogen, stimulates cellular hyperplasia and results in at least a 4 to 5 fold increase in gland weight in normal animals. If the observed weight increase is expressed in terms of cell survival, a dose of 5.5 Gy X-rays reduces survival to about 80%. There is no significant change in survival when the time between irradiation and stimulation is delayed by up to 6 months.

5.5 Gy X-rays produces an initial chromosome aberration frequency, in terms of anaphase bridges and fragments observed at day 6 of goitrogenic stimulation, of about 25% above the control level. When the stimulation is delayed for 6-weeks post irradiation, this level is reduced almost to control values. There is no further change up to 6 months after radiation.

Neutron irradiation of 2.75 Gy produces an aberration frequency of 28% above control levels which does not decrease significantly until stimulation is delayed for 6 months after radiation. This reduced level is still considerably higher than control values.

Thus long term recovery in thyroid is observed by loss of chromosome aberrations after X-rays but this is not mirrored by the ability of thyroid cells to divide following goitrogenic stimulus. However, the loss of aberrations is not observed after neutrons to the same extent. This latter observation is similar to previously published results using rat liver.

2B-5

Development of the radiation-induced atheromatous plaque.

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Local irradiation of the carotid arteries of hypercholesterolemic rabbits results in the development of thick atheromatous plaques within two months after the irradiation. In order to obtain information about the onset of plaque formation and about the origin of the enormous quantities of lipid-laden foam cells, observations were started from a few hours up to one month after local exposure of the arteries to 1000 rad of X-rays.
This study was performed with light- and electron microscopic techniques.
From 8 h up to about 10 d after the irradiation monocytic cells were seen adhering to the endothelial cell layer and penetrating into the subendothelial space. These cells contain lipid vacuoles, they progressively change into lipophages or foam cells from 2 d post-irradiation.
Increased migration of smooth muscle cells from the tunica media into the subendothelial space was observed from about 20 d post-irradiation. These cells apparently replace necrotic lipophages; they show several lipid vacuoles.
In areas where neighbouring enlarging plaques become fused, endothelial cells of the original lining become enclosed and trapped within the resulting plaque.
It is not yet clear whether the increased smooth muscle cell migration is the only mechanism to provide cells for the enlarging plaques, or that a cell proliferation process is involved.

2B-6

EARLY VERSUS LATE EFFECTS OF X-RAYS IN THE CNS: A MORPHOLOGICAL SURVEY

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The origin of late effects caused by focal X-irradiation to the brain is investigated by means of microscopical assessment of vascular, glial and nerve cell components at various depth levels in the cerebral cortex of female adult rats irradiated at 3 month old. When the appropriate corrections are made for anaesthetic influence, cortex depth variations, cortex zone surveyed and volume of irradiated brain tissue, certain differential changes in the glial populations can be found to occur between grey and white matter.

In this communication, a detailed electron microscopic investigation of the modifications observed in the mid-cortex (Cajal large pyramidal cell zone) observed at 15 days and 3 months after irradiation with 6 different doses of X-rays (ranging from 2,5 to 60 grays) is also reported. Preliminary results for longer periods of time will be presented too. The systematic EM exploration reveals that the 3 glial cell types behave differently after X-radiation: in the long while, irreversible cell depletion appears to be restricted only to oligodendrocytes, the cells responsible for myelin production and also the major satellite elements to neurons. The possible relationships of such situation with the development of various aspects of the late damages in the RX brain, will be considered.

2B-7

DOSE-DEPENDENT CHANGES IN THE OCCURRENCE OF FOCAL VASCULAR ABNORMALITIES OF THE IRRADIATED RAT BRAIN.

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Current investigations on the radiation-induced changes of the rat brain are being performed by a cooperative European group* as a part of the "Eulep" program. This study deals with one part of the work of the group, i.e. the analysis of changes in the angio-architecture by means of a vessel-filling method that has especially been developed for this purpose. The entire blood vessel system is perfused with a mixture containing pigments, plastic latex and fixatives, and cleared sections of about 0.5 mm thick become available for examination.

The initial investigations showed that after a dose of 2000 cGy focal abnormalities appeared in the central regions of the brain after an average latent period of 17 months. Development of teleangiectasies were the first aberrations, and foci of telangiectasies could progress into sizable areas with highly abnormal vessels, surrounding a necrotic part. More recently investigations with doses of 2500 cGy and 3000 cGy have shown that similar changes develop, however, after a short latency period. Preliminary data indicate that after a dose of 3000 cGy vascular abnormalities may develop as early as 8 months after irradiation.

*The "Eulep" CNS/vascular group consists, besides the authors, of: W. Calvo (Ulm); G. Gerber, J. Maisin, H. Reyners (Mol) and A. Keyeux (UCL, Bruxelles).

2B-8

QUANTITATIVE STUDY OF MICROCIRCULATION CHANGES IN THE RAT BRAIN AFTER LOCAL IRRADIATION

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Vascular damage is one of the most probable causes of late postirradiation necrosis. Thus the dynamics quantitative parameters of rat brain capillary system was studied after local irradiation with 20-60 Gy of 200 kVp X-rays. The method of automatic analysis was used, which permitted to evaluate several quantitative parameters that could not be registered by conventional microscopy. In order to determine the time-related incidence and location of gross vascular lesions 2-12 months after irradiation, the vascular network was filled with pigment and coronal 15μ slices of the brain in the region between the optic chiasma and the posterior margin of bulbus olfactorius were automatically analyzed by the computerized cell analyzer PRJAC designed and constructed at the Institute of Biological Physics Acad. Sci. USSR. The vessel density, their mean cross section area and the total cross section area in each field of vision and the distribution of capillaries according to their size were determined in the brain cortex, nucleus caudatus, septum and corpus callosum. These brain regions differ in vessel density in decreasing order. The number of capillaries did not depend upon the age of animals. In irradiated animals the number of capillaries decreased and their mean size increased at the same time intervals. In the nucleus caudatus, septum and corpus callosum, localized regions of capillary dilation and deformity were seen microscopically. After irradiation with 30-60 Gy, in the corpus callosum were found necroses beginning at 6 and 4 months, respectively. So, the observed angiopathy manifested itself both in changes of the number of vessels and their size. The obtained results show that the radiation effect is the stronger expressed, the poorer was the original vascularity.

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2B–9-14 Effects on DNA

2B-9

Chemical Modification of the Radiation Sensitivity of Transforming DNA

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We have examined the actions and interactions of oxygen and sulphydryl compounds upon the radiation sensitivity of the biological function of purified Bacillus subtilis transforming DNA (tryptophan locus) irradiated under chemically defined conditions. The sensitivity of the DNA irradiated in dilute aqueous solution is less when irradiation is performed in 100% O_2 than when in 100% N_2, i.e. O_2 protects transforming DNA from inactivation with a dose-modifying factor of about 0.8 (Held et al., 1978). The sulphydryl-containing compound, dithiothreitol (DTT) protects the DNA against radiation-induced damage, and the dependence on DTT concentration indicates that this effect can be partially explained by the scavenging of OH radicals by DTT. At low DTT concentrations (micromolar range) this protection is roughly equivalent in N_2 and O_2. However, at higher DTT concentrations, the protection is much greater when irradiation is in N_2 than in O_2. For example, at 1mM DTT the protection factor (ratio of radiosensitivities of DNA in the absence and presence of DTT) is about 500 in N_2, but only about 50 in O_2. Thus, at this DTT concentration, the ratio of anoxic to oxic response ("O.E.R.") is about 10, as contrasted to a value of about 0.8 in the absence of DTT. These results can be explained in terms of the "hydrogen donation" hypothesis of radiation protection by sulphydryl compounds and will be discussed in relation to the effects of O_2 and sulphydryls on lethal damage in intact bacterial systems (Michael and Harrop, 1979).

The radiation response of transforming DNA is also affected by electron-affinic sensitizers other than oxygen. The interaction of some of these compounds with transforming DNA, and the effects of sulphydryl compounds on these processes will also be discussed.

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2B-10

Influence of anoxic sensitizers on the radiation damage in biologically avtive DNA.

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It is generally assumed that one of the most important target molecules of the cell is the DNA and it is therefore not unlikely that chemicals influencing the radiosensitivity of cells act on DNA.

If DNA is irradiated in dilute solution, the presence of a chemical, such as a sensitizer, will usually result in protection of the DNA, because virtually almost every chemical is a radical scavenger. This means that possible sensitizing properties may be overshadowed by the scavenging of radicals. However, by careful comparison with a simple competitor it is possible to differentiate between pure scavenging on one hand and sensitization or repair on the other hand.

Under the conditions of our experiments, in which biologically active ϕX174 DNA is used, PNAP and misonidazole act like ordinary scavengers, whereas metronidazole (flagyl) is involved in DNA repair reactions. Only with nifuroxim sensitization is observed. Also the effects of these radiosensitizers on the production of breaks and of base damage are studied.

2B-11

A DIRECT METHOD TO DETERMINE THE AMOUNT OF DNA-PROTEIN CROSSLINKS IN IRRADIATED EUCARYOTIC CELLS.

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A method to isolate practically protein-free DNA from cells and tissues has been developed. It consists of a deproteinization step where the proteins in the homogenates are hydrolysed using immobilized Proteinase K. The DNA in the deproteinized sample is then adsorbed to hydroxylapatite and isolated by stepwise elution using buffers of increasing ionic strength. The ratio of the DNA absorbance at 260 to 230 nm has been found to be a measure of the protein content in the isolated DNA. When DNA from irradiated cells is isolated using this procedure this ratio was found to decrease with radiation dose. Incorporation of S-35 methionine in the DNA-fraction indicated that the ratio is reduced due to crosslinking of proteins to DNA. The results show that crosslinked protein can be detected after doses of $\sim$10 rad (0.1 Gy) , and that this effect is not negligible when compared with other radiation effects on DNA in cells. The results also indicate that repair mechanisms exist for the crosslinked protein.

2B-12

MOLECULAR MECHANISMS OF THYMOCYTE INTERPHASE DEATH IN GAMMA-IRRADIATED RATS

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Degradation of thymocyte nuclear DNA in gamma-irradiated rats was studied. Regardless of irradiation dose, chromatin degradation begins after a 2 hr lag-period, reaches a maximum level in 6 hrs and does not change during the next 4 hrs. The amount of DNA fragments is determined by the quantity of perishing cells in which all the DNA is degraded rather than by the extent of chromatin breakdown in the whole thymocyte population. The chromatin fragments formed are nucleosomes and their oligomers with normal histone content and intact structure, as judged by their DNAse I cleavage products. Intranucleosomal DNA scission is negligible. The DNA breakdown is not accompanied by degradation of histones and non-histone proteins. Thus, DNase I and proteases do not participate in the postirradiation DNA degradation. Chromatin degradation induced by hydrocortisone in hormone sensitive thymocytes occurs in the same way. 3 hrs after irradiation of rats autholytic digestion of DNA with endogenous Ca^{++}/Mg^{++}-endonuclease in purified thymocyte nuclei in vitro accelerates. But the activity of isolated nuclear nucleases is unchanged at the same period. The sensitivity of chromatin preparations from irradiated thymocytes for exogenous nucleases does not also differ from control. Cycloheximide injection prevents postirradiation chromatin degradation in vivo, which is caused, at least partially, by the decrease of activity of the chromatin Ca^{++}/Mg^{++}-dependent nuclease. The data obtained show that thymus chromatin degradation in irradiated rats is rather a step of events leading to the cell death than a consequence of hydrolytic enzyme activation in died cells. DNA degradation is likely accomplished by Ca^{++}/Mg^{++} endonuclease but the process is initiated by another factor whose nature is yet unknown. It is supposed that DNA breakdown, which may be the result of realization of special genetic programm, is a general step of different forms of cell death.

2B-13

STUDY OF KINETICS OF DNA REPAIR AND ITS MODIFICATION IN CULTURED MAMMALIAN CELLS

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The rate of repair of DNA single-strand breaks (SSB) in Chinese hamster cells is shown to decrease shortly for 5 min after exposure to a dose of 200 Gy. At the G_1-stage this decrease is more pronounced ($\sim$10 fold) than that in asynchronous population containing 60 per cent of cells at the S-stage (5 fold). After irradiation at dose 15 Gy the rate of repair at the G_1-stage is 1.5 times lower than in the asynchronous culture, but it remains constant till the end of the process in both cases.

Postirradiation treatment of cells with cysteamine (MEA), caffeine-benzoate (CB) and caffeine sharply inhibits the repair of SSB at 5 min. This inhibition is reversible since washing of treated cells leads immediately to the resumption of the repair, but its rate is decreased with prolongation of treatment and increasing the concentration of agent. The efficiency of MEA and CB, which are weak electrolytes, is also pH-dependent. It is shown that the intracellular concentration of substances is the most important factor determining their efficiency. The presented data serve as the evidence for the existence of unspecific mechanism of the influence of the substances studied.

2B-14

DNA REPAIR IN LIVER CELLS OF γ-IRRADIATED MICE OF DIFFERENT AGE

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The level of unscheduled DNA synthesis of the liver cells of 22-month old mice remains, for 3 hr after γ-irradiation, by 25-30% lower than in 1.5-month ones. DNA from the liver of irradiated mice was first treated with the extract from *M. luteus* and then in the reaction with DNA-polymerase I. It is shown that the template activity of DNA from the liver of old mice is higher than that of DNA of young animals. This allows to suggest that repair of DNA damages in the liver cells of old mice occurs with a slower rate than in the liver of young mice. However the DNA polymerase activity in the extracts from the liver of mice of both ages is the same. The assay of the relaxation of nucleoid of the liver cells from the mice after their γ-irradiation has shown that the nucleoid of old mice cells is less relaxed than the one of young mice cells. A proposal is made that the rate of repair of DNA damages in the liver cells of old mice is reduced because of the limited access of the enzymes to the damaged sites of DNA.

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Radiation and Environmental Biophysics

2C–1-14 Sensitization and Protection

ON THE pH-DEPENDENCE OF THE EFFICIENCY OF RADIOPROTECTORS

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The pH-dependence of radiation-induced reproductive death of Chinese hamster fibroblasts (a 24-hour-old asynchronous culture B-II-d-ii-FAF-28 aneuploid strain 431) treated prophylactically with different chemical substances was investigated. It is established that in the pH range from 6,0 to 8.0 the protection effect of cysteamine increases with the increasing pH of the medium and that of caffeine-benzoate and thioglycolic acid decreases. In the same conditions the effect of caffeine does not change. Many of the extensively used radiomodifying agents are known to be classed with weak electrolytes. Based on the peculiarities of dissociation and penetration of such chemicals into the cell, it is shown that the revealed regularities result from inhomogeneous distribution of the studied substances between the medium and cells in the presence of pH gradient. The analysis of the data obtained enable the conclusion that the total intracellular concentration is the main factor determining the pH dependence of the efficiency of radioprotectors.

2C-2

DYNAMIC MODIFICATION OF TISSUE RADIOSENSITIVITY DURING THE COURSE OF TUMOR THERAPY (POLYRADIOMODIFICATION)

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The use of radiomodifying agents in the clinical practise is limited by their toxicity and diminution of the modifying effect in the case of fractionated irradiation. This obstacle could be partly overcome by polyradiomodification, by which we mean the dynamic modification of tissue sensitivity with the combination of agents with different mechanism of action, which in this case could be used in relatively smaller concentrations than in the case of separate use of each modality. The combinations of chemical protectors (WR-2721 and 5-metoxytryptamin) with the hypoxic gas mixtures or metronidazole as well as the combinations of short-term hyperglycemia with metronidazole or oxygen were investigated during radiation therapy of solid Ehrlich carcinoma. The protective effect of these substances can be greatly increased by application of mild total-body hypoxia. Hyperglycemia in combination with metronidazole or oxygen improved the efficacy of radiation therapy of tumors.

The advantages of different schemes of polyradiomodification will be discussed.

2C-3

THE REACTIONS OF CLONOGENIC CELLS IN CULTURE AND IN TUMOURS INDUCED BY RADIATION AND CHEMOTHERAPEUTIC DRUGS

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In culture cells and in mice solid tumours the proliferative activity and colony-forming ability after the action of radiation and methylnitrosourea (MNU) have been determined. The cloning efficiency doesn't depend on the proliferative activity and is identical in log- and plateau-phase of growth for culture and for dividing and resting cells in tumours. The cell population radiosensitivity, that determined was by the cloning efficiency, doesn't depend on the proliferative activity. But quiescent cells in culture and in tumours repair potentially lethal damages (PLD) induced by irradiation. The degree of repair increases when tumour masses and the fraction of resting cells increases. MNU induced the selective killing of resting cells. The plateau-phase culture cells and clonogenic cells in large tumours which has a large fraction of nondividing cells, are more sensitive to MNU. Cells in log-phase and of small tumours don't repair PLD induced by MNU. If the fraction of resting cells in culture and in tumours increases, the ability to PLD repair appears and increases too. The mechanisms of cells resistance to irradiation and action of chemotherapeutical drugs are discussed.

2C-4

CHEMICAL PROTECTION AGAINST X-IRRADIATION BY AN ORALLY ADMINISTERED COMPOUND

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The possibility of chemical protection against low LET ionizing irradiation has been studied since Patt (1949) and Bacq (1951) described protection by cysteine and cysteamine, respectively. Since that time a great number of compounds with protective activity have been reported. In many cases the margin between a protective and a toxic effect is small. Most compounds are not active when administered by oral route. The degree of protection is mainly well below a dose reduction factor (DRF) of 2. In the present report results obtained with an orally active compound will be described. It concerns N-(1adamantylmethyl)-α-mercapto-acetamidine hydrochloride and was obtained as compound WR 109342 AC from the Walter Reed Army Institute of Research.
In (C57BL x CBA)F1 hybrid mice the LD_{50} was about 225 mg/kg, when orally administered. When we gave an oral dose of 75 mg/kg 30 minutes before X-irradiation a DRF of 1.7 was obtained. Application at shorter or longer intervals before irradiation resulted in a smaller protective activity. A small but definite protection was still found when it was administered 4 hours before irradiation. Lower doses gave smaller protective activity. The compound itself is poorly soluble.
Investigations are performed to study whether a soluble compound is formed in the gut, which is taken up by the blood and which protects at the cellular level. Blood taken from mice 30 minutes after oral administration of the compound is tested for its protective activity in vitro on cultured cells.

2C-5

RADIOPROTECTIVE AND THERAPEUTIC EFFECT OF LEUCOTROFINA UPON RADIATION SICKNESS IN MICE.

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The radioprotective and therapeutic effect of Leucotrofina (a cell-free thymic extract) was investigated. The LD50/30 for the untreated control group was estimated of 570 rad, while after Leucotrofina treatment (5 u/day x 6, after irradiation) the value rose to 690 rad. From the LD50/30 data a dose reduction factor of 1.21 was calculated. The radioiron (59Fe) uptake the erythrocytes 3 days after irradiation with 100 rad.resp. 2 days after the radioiron administration for the unirradiated, untreated control group was estimated of 37.7 ± 3.21, for the irradiated control group of 10.5 ± 0.7%, for the pretreated (5 u. 60 min. before Rx) and irradiated group of 23.7 ± 0.5%, and for the irradiated and post-treated (5 u.60 min. after Rx) group of 21.7 ± 0.9%. The radioiron uptake in the spleen and bone marrow 9-days after whole body irradiation with 500 rad was estimated for the unirradiated untreated group of 7.01 ± 0.6% resp. 0.59 ± 0.07%, for the irradiated control group of 5.35 ± 0.3% resp. 0.64 ± 0.06%, and for the irradiated and treated (5 u/day x 6) group of 10.30 ± 0.5% resp. 1.01 ±0.06%. 9 Days after the whole-body irradiation of DBA mice with 500 rad the number of endogenous spleen colonies was of 8.15 ± 0.57, in the control group and significantly increased to 15.30 ±0.75 in Leucotrofina treated (5u/day x 6) mice. The amount of DNA in thymus, and the uptake of 3 H-thymidine into thymic DNA were strongly enhanced following irradiation with 500 rad in Leucotrofina treated (5 u/day x 6) A_2G mice. The recovery effect was also evident in the bone marrow after the application of Leucotrofina. However the mechanism of action of thymic extracts is still not clarified, it seems that the radioprotective and therapeutic effect of different thymic factors is mainly produced by stimulating the haemopoietic stem cells, by reactivating the energic or severely depressed haemopoietic and immune system.

2C-6

On the Biochemical Mechanism of Endogenous Radioprotection
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The evolution of tissular antiradical and antioxydant defense has been studied under different conditions in which an increase in radioresis--tance caused by biostimulation can be observed (burning in mice, injection of oestradiol to female mice, injection of $CoCl_2$ to rats, injection of phenylhydrazine to mice). For this purpose, the following substances and enzyme activities were determined: glutathione, GSH-peroxydase Glutathione-transferase, GSH-reductase, catalase, superoxyde dismutase, GSH peroxydase et glucose-6-phosphate dehydrogenase. All these parameters were measured in spleen and liver.
The only factor which regularly coincided with increased radioresistance was an increased content of reduced glutathione in spleen.

2C-7

CHEMICAL PROTECTION AGAINST GAMMA RADIOLESIONS ON RATS.
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The protective activity of NAD, FAD, Quinone was demonstrated on DNA and on erythrocytes in vitro. The highest DRF37 was obtained with a mixture of NAD, FAD and Quinone. The DRF37 obtained were 17,7 for DNA at 5×10^{-5} M of the mixture; 11,1 for rabbit erythrocytes and 3,9 for chicken erythrocytes, both at $2,5 \times 10^{-4}$ M of the mixture. The effect of these chemicals and that of ATP and mixtures of them on rats was studied. The D37 of the rats (Wisconsin) was 788 rads. The compounds studied, NAD, FAD and ATP were well tolerated. They were injected up to 400 mg/Kg body weight. Quinone showed a high toxicity, so it was not considered for in vivo experiments. Adult rats weighing 290 grs. approximately were anesthetized and the chemicals injected intraperitoneally, 15 minutes before whole body irradiation. The number of rats surviving 30 days was scored. Animals were irradiated with a 137-Cs source. The dose rate was 80 rad/min. A protective effect was obtained with FAD 40 mg/Kg and with NAD-FAD 20 mg/Kg (1:1). The effect of the mixture was higher than the effect obtained with higher concentration of NAD and FAD. For FAD 40 mg/Kg at 800 rad and 875 rad, the enhancement of the surviving fraction was 43,8% and 14% respectively. For NAD-FAD 20 mg/Kg. (1:1), the enhancement was 87,5 % and 42,8% respectively. When NAD-FAD-ATP 93 mg/Kg. (1:1:1) was assayed, the enhancement was 31,3% and 100%. FAD 40 mg/Kg and NAD-FAD 20 mg/Kg (1:1) showed a gain of doses giving a hundred percent surviving. The mixture NAD-FAD-ATP did not show this shift of the shoulder for higher irradiation doses, but it gave an increase of the surviving fraction at higher irradiation doses. Higher concentrations of these compounds were also assayed. Besides, some biochemicals indicators suchas plasmatic polyamines levels were studied on irradiated non protected and protected rats. The efficiency of these compounds for in vivo use is discussed.

2C-8

INVESTIGATION OF THE EFFECT OF LITORALON /gamma-L-glutamil-taurine/ COMBINED WITH OTHER TYPE OF RADIOPROTECTIVE AGENTS

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Authors have already reported /6th ICRR, Japan,1979/ on the low radioprotective effect of Litoralon /gamma-L-glutamil-taurin/ as against X-ray, gamma and mixed neutron-gamma radiation; this effect becomes apparent in LD_{50-70} range of radiation dose. Litoralon showed some beneficial effect even in treatment of early syndromes of reaction to radiation /primarily adynamia, development of the physical ability/. In this paper authors report that the radioprotective effect of Litoralon may be increased if combined with AET / S,beta-amino-etilizotiuronium Br.HBr., cystamin, cisteamin, etc./. It was proved that toxic side effects of AET have favourably been lessened by Litoralon. The moderation of the side effects manifesting in cardiotoxic symptoms predominate caused by AET in Litoralon treatment. It is all the more worthy of note as Litoralon cannot be compared to pharmacological antagonists investigated in this field. Authors suppose that this moderation may be caused by the special structure of the molecule as no decrease was observed neither in radioprotective nor in cardiotoxic effects when AET was given in combination with taurine.

2C-9

RADIOSENSITIZATION BY IOTHALAMIC ACID IN SINGLE CELL SYSTEMS.

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The radiosensitizing activity of 3-(acetylamino)-2,4,6-triiodo-5-[(methylamino) carbonyl] benzoic acid (iothalamic acid, ITA) has been tested on survival of E. coli B/r and V79-753B cells.
Bacterial experiments were carried out using pulsed electrons from a 12 MeV linear accelerator, and mammalian cells experiments using 250 KeV X-rays. Maximum sensitization was already achieved in bacteria at 10 mM ITA in phosphate buffer saline (PBS), while in mammalian cells the radiosensitivity steadily increased up to 200 mM ITA. The enhancement ratio (E.R.) in bacteria was much greater in aerobic than in anoxic cells, and was 1 in growing medium (G.M.). In mammalian cells the E.R. was the same in PBS as in G.M., as well as in aerobic and in anoxic conditions.Different results between bacterial and mammalian cells were also found when scavengers, such as glycerol, DMSO, and cysteine were present in addition to ITA: namely any sensitizing effect was abolished in bacteria, while in mammalian cells the same E.R. than in absence of scavenger was detected.
Possible mechanisms of those effects will be discussed.

2C-10

"Structure-Activity Relationship of Some Pharmacological Agents that Act as Hypoxic Radiosensitizers and/or Oxic Radioprotectors."

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Results from our laboratory have indicated that local anesthetics such as procaine or lidocaine are capable of modifying the response of mammalian cells toward x-irradiation. Whereas oxic cells are radioprotected, hypoxic cells are preferentially radiosensitized by these agents. In an attempt to seek a possible structure-activity relationship and to gain further insight into such paradoxical radiation modification afforded by these agents, we have proceeded to screen a series of clinically-used and related pharmacological agents using the *in vitro* murine L5178Y lymphoma cell system. Agents studied thus far include a series of local anesthetics, barbiturates, some tranquillizers, analgesics and psychopharmacological agents. The radiation-modifying effect of most of these compounds is in many ways similar to that resulting from treatment with local anesthetics. The efficacies of some of these compounds to radiosensitize hypoxic and/or radioprotect oxic cells are being compared with misonidazole and cysteamine, respectively. Possible mechanisms for the differential radiation modification exerted by these various subclasses of agents will be presented. Parameters to be discussed will also include electron affinity, solubility, partition coefficient, and the ability of some of these compounds to inhibit cellular oxygen consumption. This investigation is supported in part by grants CA15901 and CA19283 from the U.S. National Cancer Institute, Department of Health, Education and Welfare.

2C-11

EFFECTS OF MISONIDAZOLE AND IRRADIATION ON INITIAL TRANSPLANTATION GENERATIONS OF A MAMMARY ADENOCARCINOMA IN MICE

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Cell kinetic parameters of a mouse ("H"strain) mammary adenocarcinoma in third and fourth generations were estimated LI-18%, T_S-6, 7 hr, T_C-17, 0 hr, T_P26 hr, GF-65%, cell loss-88%. When in the leg mean tumor diameter was in the range of 3-8 mm a local gamma-^{60}Co irradiation was given. Tumor growth curves indicated an exponential shape. Experiments performed and results obtained are summarized:

Groups & Treatment	No.	TC	RG	T_D
1. Controls	15	-	-	9,5
2. Misonidazole 1x*	10	-	-	9,5
3. Misonidazole 2x**	10	-	-	9,5
4. 20 Gy	12	25	18	22
5. 2x10 Gy**	12	-	25	15
6. 20 Gy+Mison. 1x*	15	40	45	10
7. 2x10 Gy+Mison.2x**	12	17	37	11

No.=Animals number
TC =Tumor curability
RD =Regrowth delay (days)
T_D =Tumor doubling time (800-1600 mm^3) (days)
* =1 mg/g body weighth, i.p.
** =24 hr interval

Dose fractionation is more effective for tumor growth delay but not for tumor regrowth potential. Fractionation of the dose does not increase tumor curability (Groups 4,5). Combination of a single dose irradiation and misonidazole is more effective for tumor growth delay and curability than fractionation of irradiation and drug (Groups 6,7). Tumor regrowth potential in both treatment groups (6,7) is almost similar to those of the controls

2C-12

Radiosensitization of Hypoxic Bacteria by Radiosensitizers Containing Functional Groups other than Nitro

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It has been suggested that the neurotoxicity of nitroimidazole radiosensitizers may be associated with the nitro group or with the reduced intermediates of this group. This paper describes the use of strains of the bacteria E.coli and S.lactis to investigate a number of non-nitro compounds as potential radiosensitizers. Whereas most nitroimidazole compounds have a one-electron reduction potential, E_7^1 in the range -0.6 to-0.2V (NHE) compounds containing substituents with electron-withdrawing properties similar to the nitro group have proved to be much less electron-affinic. Most of the compounds have $E_7 < -1.0V$, although a potential of -0.75 V was measured for a trifluoroacetyl derivative. Thus it is likely that radiosensitization seen with some of these compounds is due not to the electron-affinity of the compound, but to the radiolytic production of toxic intermediates or products.

2C-13

Liquid-Flow Rapid-Mixing Studies on the Radiosensitization of Mammalian Cells

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A liquid-flow rapid-mixing technique using high dose-rate irradiation from the Gray Laboratory Van de Graaff accelerator has been used to study the time course of radiation effects in hypoxic Chinese hamster cells. The results from these studies yield information on the mechanisms involved in the modification of the radiation response by added chemicals. This technique has also been used to study the effects of the lipophilicity of radiosensitizers on sensitization in vitro. Data on radiosensitization by a highly efficient ortho-substituted 4-nitroimidazole will be presented and discussed in the light of proposed mechanisms of sensitization. For comparative purposes the time course of sensitization by oxygen in these cells has also been studied and the data will be compared with the results of similar experiments by Asquith et al., 1975.

2C-14

THE ROLE OF RADIOLUMINESCENCE IN THE EFFECT OF BETA RADIATION ON THE STIMULUS THRESHOLD OF MUSCLE.

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According to our previous experiments scintillations can be evoked by very weak beta radiation of incorporated tritium in the cross striated muscles of frog.

As it was found by others

- the same radiation or
- the illumination by light

decreased the stimulus threshold in muscles.

If the muscles are kept in solution containing tritium and radioluminescent chemicals the decrease of stimulus threshold is significantly larger than that caused by tritium only. These chemicals applied without tritium increased the stimulus threshold.

The results of recent experiments support the possible connecting role of scintillations in the mechanism of the stimulus threshold decreasing action of weak beta radiation.

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Radiation and Environmental Biophysics

2D–1-8 Dosimetry and Isotopes

2D-1

RECENT RESULTS OF ELECTRON TRACK STRUCTURE CALCULATIONS FOR RADIATION BIOLOGY

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So-called "low-LET" radiations as e.g. fast electrons, X-rays, and gamma quanta are of special fundamental as well as practical interest to radiation biology, -therapy, and to radiation protection. For the adequate specification of the radiation quality of such fields, for the promotion of our understanding of basic radibiological mechanisms set in motion by their interaction,etc. more information is needed on the microscopic pattern of relevant chemical activations produced by irradiation within cell nuclei and their constituing biomolecules.

To provide such basic physico-chemical data for primary electron track structures for further use in radiobiological theories, extensive Monte-Carlo simulation calculations have been performed for the model substance water vapour. The cross sections used in these programmes were carefully checked against experimental data wherever possible and meaningful in this context. Here results will be presented especially for the important energy region between 0.01 and 10 keV. Emphasis will be put on

- the stochastic energy density frequency distribution in small sites of 0.1 to 10 000 nm diameter,
- the yield of "clusters" and their frequency distributions,
- the yield of blobs, spurs, short tracks, etc.,and their frequency distributions.

These data will be compared to the respective more integral quantities LET and degradation spectra, and the advantages and disadvantages of their use in radiation biology will be discussed.

2D-2

A SECONDARY STANDARD IN NEUTRON DOSIMETRY AND A TRANSFER DOSIMETER

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The chlorobenzene-ethanol-trimethylpentane (CET) dosimeter is comparable with the well known secondary standard for absorbed dose measurements, the ferrous sulphate dosimeter. In some properties the CET-dosimeter is more favorable: reproducibility with commercially available chemicals, measurement by spectrophotometry at 552 nm, higher sensitivity (0,017 O.D. units per 100 rad cm^{-1}). The important advantage: exposed to the mixed neutron plus gamma field it measures directly the total tissue dose. The response of CET per rad im muscle tissue of neutron dose is nearly equal to the response per tissue rad of gamma dose for radiation energies up to 15 MeV. Based on the CET system the dosimeters in the form of 5 mm OD and 130 mm long sealed ampoules were developed and are commercially produced under the name "DL-M3". This is the standard personal dosimeter for nuclear accident or emergency dosimetry. In this case the readout of dose is performed with an accurate visual colorimeter which adds to the simplicity and reliability of measurement, especially in cases when standard dosimetry has to be performed by people not specialized in the field. Due to stability and high reproducibility of individual properties the precision of 1 to 2 % is easily obtained. Compared to other methods of standard dosimetry the time consumption and cost are lower. The factor of time economy is most important if high absolute accuracy is required and there is no time for checking of the instruments. The same is true if measurements are made at longer time intervals. In our case the dosimeter and the reader are completely stable. Here we present the results of error analysis relevant for accurate practical dosimetry and for intercomparison measurements by transfer dosimeters (i.e. dosimeters which are sent by post). Special care is given to errors introduced with the correction for the fading of the signal in cases of readout performed several days after irradiation. For transfer times of less than 15 days this error is below 1 percent. The dosimeter can measure doses up to 1500 rad and the resolution below 100 rad is few rad. The effect of neutron energy could not be observed within the experimental error of ± 10 percent. Further experimental work is in progress aimed to develop standard procedures in neutron, gamma and fast electron dosimetry.

2D-3

RADIATION QUALITY OF FAST NEUTRONS AND RBE OF HUMAN KIDNEY T CELLS IN A WATER PHANTOM

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The spatial differences of radiation quality in patients irradiated with collimated fast neutrons for radiation therapy are not well known, whereas the RBE in terms of survival of cells in culture, measured by different authors, does not show any significant change inside and outside the central neutron beam.

In order to extend such observation to in vivo tissue, the microdosimetric radiation quality and the RBE of human kidney T-cells as well as their spatial variation was measured in a water phantom. The fast neutrons had a mean energy of 6 MeV and were obtained by bombarding a thick Beryllium target with 14 MeV deuterons. The collimator defined a field size of 10 x 10 cm in 125 cm distance.

The saturation corrected dose average y^* decreased between the phantom surface and a water depth of 20 cm by only 12 %. The corresponding survival curves at these positions exhibited no change in RBE. At 5 cm water depth the microdosimetric spectra in the beam center and at a distance 5 cm from the beam boundary were significantly different. However, the survival curves obtained at these positions and the parameter y^* did not differ.

It can be concluded that doses below 1 Gy inside the beam have the same effect on the colony forming ability of cultured mammalian cells in the phantom as equal doses in the penumbra region. This experimental result is an agreement with theoretical considerations relating y^* and RBE.

2D-4

The UK Radium Luminizer Survey

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The use of paints containing radium to luminize watches and instrument dials became relatively widely practiced in the UK during the period 1939 to 1950. During the early 1960's a survey population consisting of some 1600 workers in this industry was set up. An interim analysis of causes of death in part of this group (~1100 women who started work during or after 1939) has revealed little evidence of the effects on bone, including osteosarcoma, which were observed in the US luminizer population[1], but during the last decade the survey population has shown an excess of breast cancer when compared with the UK population in general[2]. Evidence which suggests that this excess may be related to the higher background radiation from radium and radon experienced by these workers at their work places is examined.

The distribution of cancers other than breast cancer in the survey population is also examined in the light of recent claims that the ICRP estimates of risk factors[3] for certain cancers are under estimated by factors of an order of magnitude or more when applied to exposure of low dose rates[4].

The problems associated with interpreting the results of small epidemiological studies such as the UK luminizer survey in the context of radiological protection are also discussed.

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2D-5

STUDY OF Sr-90 CONTENT OF HUMAN TEETH

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In connection with the commencement of the first Nuclear Power Station in Hungary, we have been dealing with estimation of the present radiation exposure of the population, and with measurements of basic levels of environmental radioactivity. Activity concentrations of Sr-90 in environmental media and in human bone tissue due to nuclear weapon tests have been determined. For the latter, extracted teeth collected from various clinics and dentistries of Budapest, have been classified according to their relatively most intensive period of development, and their activity concentration of Sr-90 measured.

The data obtained so far indicate that the Sr-90 concentration of human teeth readily follows the changes of Sr-90 activity in the environment. Naturally, no Sr-90 can be detected in teeth cut before the nuclear weapon tests. Assessment of the bone marrow dose received from the Sr-90 content of the bones has been attempted.

2D-6

THEORETICAL AND EXPERIMENTAL STUDIES ON THE TOXICITY OF ^{123}I, ^{125}I AND ^{131}I IN THE THYROID GLAND.

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Refined dosimetric calculations for different iodine isotopes in the thyroid gland were performed by the use of Monte Carlo procedures. These procedures were applied on a microscopic scale to the beta spectrum and on a macroscopic scale to the gamma spectrum of the isotopes. The doses calculated to be received by follicular cells depended strongly on follicle size, thickness of the surrounding cell layer and thyroid weight. In order to verify the validity of these calculations the thyroid function was determined after irradiation originating from ^{123}I, ^{125}I and ^{131}I. Groups of mice were injected with various activities of these isotopes and a year later, the thyroid uptake of a tracer dose was determined. From the dose effect curves, the activity of each isotope required for suppression of tracer uptake to 20% of the control value was estimated. The calculated average dose in the cell layers was found to be more relevant for damage to the thyroid function than the calculated dose in the thyroid.

2D-7

RADIOTOXICITY OF THALLIUM-201 IN V79 CELLS.

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The uptake of thallium-201 in Chinese hamster V79 lung fibroblasts has been compared to that of potassium-42/-43 and rubidium-86. Because these intracellular cations are not bound to cellular elements, special methodology was developed for the rapid separation of cells from radioactive media. The rates of uptake of ^{201}Tl, ^{86}Rb, ^{43}K and ^{42}K were linear with increasing concentrations of radioactivity in the media reaching a plateau within 6 hr. Efflux of thallium after 18 hours of exposure was more rapid than that of rubidium and potassium.

The radiotoxicity of these monovalent cations to V79 cells was also determined. ^{201}Tl, the only Auger emitter, was most toxic. Both ^{42}K and ^{43}K had equal toxicities while ^{86}Rb was the least effective. The radioactive contents of whole cells at a surviving fraction of 37% for ^{201}Tl, $^{43}K/^{42}K$ and ^{86}Rb were 1.2, 1.8 and 3.8 pCi/cell, respectively. While the uptake of all these radionuclides was inhibited by Ouabain, only the toxicity of ^{201}Tl was sensitive to changes in intracellular concentrations. Thus, for the latter cation, as would be expected from the decay scheme, the microscopic distribution of energy within the cell is the determinant of radiotoxicity.

2D-8

REMOVAL OF RADIO-BARIUM BY CRYPTAND (222) FROM MAMMALS .

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Sr-90,Sr-89 and Ba-140 appear in Mattauchs curve of Uranium and Plutonium fission in the maxima, e.g. in highest amounts together with the corresponding isobars to about 4,8%,5,9% & 6,4% in reactor burn-up . All of these nuclides are bone-seeking elements and therefore hazzardous to man in the case of an accidental exposure . But very little attention is paid at present to the fact, that there exists no sufficiently developed remedy in order to remove these radionuclides, once incorporated into the human body . One of us has presented previously cryptand (222) as a possible means to remove Sr-90 from mammals with an extrapolation to man . (x) Here we should like to present cryptand (222) as a means even more potent for the removal of Barium-140 circulating in mammals. The responsible parameters, such as affinity of (222) towards Ba-140,(K_1), and the therapeutic range are given. Decorporation of Ba-140 is shown also in the context of its relatives Sr and Ra . By a treatment scheme, demonstrating the therapeutic effect e.g. the decorporation effect as dependent on the applied (222)-dose and the time of treatment start in rats, we try to estimate the efficiency of a probable (222)-treatment in radio-barium contaminated man . Extendebility and limits of a (222)-treatment are discussed .

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Radiation and Environmental Biophysics

3A–0-13 Mutagenesis

3A-0

Interspecies differences of radiation induced genetic effects
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Nuclear volume, DNA content and the capacity to repair damaged DNA are important among the factors which affect sensitivity of different species towards genetic effects of radiations. From the available data, quantitative comparisons between diverse species can only be made with two endpoints, namely chromosomal aberrations and 'point mutations.' A linear relationship between DNA content and frequencies of gene mutations induced by ionizing radiations in diverse organisms has been presented by Abrahamson et al. 1974. Though this relationship in general is attractive it has been subjected to criticisms on valid grounds. Results from in vitro studies involving rodent cells and human fibroblasts, indicate equal sensitivity (or within a factor of 2) for induction of point mutations for 6-thioguanine resistance ($HGPRT^-$) induced by X-rays. Chromosomal aberrations induced by ionizing radiations in diverse mammalian species can be compared from data obtained in somatic cells (peripheral blood lymphocytes) and germ cells (stem cell spermatogonia). The magnitude of interspecies differences obtained for induction of dicentric chromosomes by X-rays in peripheral blood lymphocytes is not different from variations present within the same species indicating an equal radiosensitivity for this end point. However, data for induction of reciprocal translocations in stem cell spermatogonia in diverse species (mouse, hamsters, rabbit, monkeys and man) exhibit a higher variability than the ones observed in lymphocytes. A major factor for this interspecies variability in response is due to differences in elimination of cells containing chromosomal aberrations (cell selection) from the time of induction until meiotic division.

3A-1

Chromosome aberrations and cell death

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A possible correlation between chromosome aberrations and reduced proliferation capacity or cell death was investigated.- Synchronized Chinese hamster fibroblast cells were irradiated (300 rad X-rays) in early G_1. Despite synchronization the cells reached the subsequent mitosis at different times. The rate of chromosome aberrations was determined in the post-irradiation division at 2-h intervals. The highest rate occurred in cells with a first cell cycle of medium length. - The colony-forming ability of mitotic cells was measured in parallel samples by following the progress of individual mitoses. The proportion of cells forming macrocolonies decreased with increasing cell cycle length, and the number of non-colony-forming cells, i.e. cell death, increased. Irrespective of various first cell cycle lengths and different rates of chromosome aberrations, the number of cells forming microcolonies remained constant. A correlation was found between cells without chromosome aberrations and the ability to form macrocolonies. However, cells with a long first cell cycle formed fewer macrocolonies.

3A-2

Characterization of radiation-induced mutants of cultured mammalian cells

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When mutants resistant to the toxic purine 6-thioguanine (TG) are isolated from mammalian cell cultures, they are usually found to have reduced activity of the purine salvage enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT). Somatic cell hybridization and pedigree analyses have shown that HGPRT activity is associated with the X chromosome in all mammalian species investigated.

Reproducible increases in the frequency of TG-resistant mutants can be obtained with ionising radiations (Thacker & Cox 1975, Nature 258, 429; Cox et al 1977, Nature 267, 425). Our preliminary characterization of radiation-induced mutants suggested that the majority had a complete lack of HGPRT activity and could have arisen by large changes in the X chromosome rather than from 'point' mutations involving only a few nucleotides (Thacker et al 1976, Mutat. Res. 35, 465; Thacker et al 1979, Int. J. Radiat. Biol. 36, 137; Cox & Masson 1979, Int. J. Radiat. Biol. 36, 149; Cox & Masson 1978 Nature 276, 629).

We have now isolated about 50 independent TG-resistant mutants induced by γ-rays in V79 hamster cell cultures, as well as a smaller number induced by high LET radiations, and 50 mutants occurring spontaneously (without treatment). A similar number of mutants was induced by the chemical mutagen ethyl methane sulphonate (EMS), which is known to cause 'point' mutations. The isolation experiments were designed to ensure, as far as possible, that no selection operated against any one mutant phenotype. Assay of HGPRT activity in cell-free extracts has shown that none of the radiation-induced mutants has activity, while some of the spontaneous and the EMS-induced mutants have partial activity. This supports our preliminary hypothesis, although we have found from chromosome banding studies that few mutants have visible X-chromosome changes, and so far no TG-resistant mutant has shown deficiency in another X-linked enzyme, glucose-6-phosphate dehydrogenase.

3A-3

Sister chromatid exchange /SCE/ in human lymphocytes exposed to low dose radiation during G_0

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In order to evaluate the induction of SCE by ionizing radiation, and to check the validity of linear extrapolation of data in radiation cytogenetics, the effect of a wide range /0,05-5 Gy/ of low-LET radiation /^{60}Co-gamma/ was tested on peripheral human lymphocytes.

Results obtained in course of investigation showed a multiphase dose-response function between induced SCE frequency and doses applied. A sharp, almost 3,5 fold increase in SCEs was observed after 0,1 Gy followed by a decrease between 0,1-0,25 Gy, while doses between 0,5-5 Gy exhibited a modest increase, showing a quasi-linear dose-relationship.

Furthermore, 45 normal persons and 30 individuals exposed to low doses of occupational radiation were tested by harlequin technique. There was found a significant difference between examined groups, in average 5,5 SCE/cell and 8,8 SCE/cell, respectively. However, the contribution of other mutagenic agents /chemicals, drugs, etc./ might not be excluded in the latter group by all certainty, based on our findings, the elevated SCE frequency can be ascribed partly to the low level radiation as well.

3A-4

Comparison of the effectiveness of different types of radiation for induction of cell reproductive death and chromosome aberrations in three cell-lines.

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The induction of impairment of clonogenic capacity and chromosome aberrations have been studied in three established cultured cell-lines (R-1M, RUC-2 and V-79). The experiments have been performed with cells in plateau phase cultures, which contain about 70 to 80 per cent of cells in G_0 or prolonged G_1 phase of the cell cycle. The irradiations were performed with ^{137}Cs gamma rays, 300 kV X rays and 0.5, 4.2 and 15 MeV neutrons. The studies of chromosome aberrations have been restricted to clearly observable chromosome aberrations i.e. dicentrics and centric rings.

The different types of cells show a large variation in sensitivity for induction of both types of effects. The dependence on radiation quality shows a comparable behaviour for the different cell types for both types of effect. The radiosensitivity is highest for 0.5 MeV neutrons, intermediate for 4.2 and 15 MeV neutrons and lowest for photons. Differences in effectivity between 300 kV X rays and ^{137}Cs gamma rays were only observed for RUC-2 cells for induction of both types of effect and for induction of chromosome aberrations at higher dose levels for V-79 cells.

With regard to cell reproductive death the R-1M cells show the highest sensitivity for all types of radiation whereas the results for RUC-2 and V-79 cells are similar. For induction of chromosome aberrations the R-1M cells show the highest sensitivity for photons, whereas for neutrons the highest susceptibility is observed for V-79 cells. For doses, only taking into account the linear coefficients of dose-effect relations, the effectivenesses per unit of dose for loss of reproductive capacity are higher than those for induction of dicentrics and centric rings by a factor of 2.5 to 6. These differences are larger than expected if all impairment of clonogenic capacity results directly from gross chromosome aberrations.

Maximum RBE values were smaller than 10 for all cell types at 50 per cent survival and 10 per cent chromosome aberrations for 0.5 MeV neutrons.

3A-5

Structural chromosome aberrations induced in human G_o lymphocytes by aluminium and carbon-characteristic ultrasoft X-rays

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Human G_o lymphocytes were irradiated with ultrasoft X-rays from aluminium and carbon targets. Chromosome aberrations were scored in predominantly first metaphase cells. Both radiation qualities were found to be effective in inducing structural aberrations, including dicentric (and polycentric) chromosomes, centric rings and acentric fragments as well as gaps and chromatid exchanges.

The lowest photon energy used in our experiments was 0.28 keV (i.e. carbon K X-rays). These produce within the irradiated cells monoenergetic photoelectrons which have ranges of $\lesssim$ 7 nm. The finding that these electrons are capable of efficiently inducing exchange aberrations (e.g. dicentric chromosomes) indicates that "primary lesions" in chromatin fibres can be produced by $\lesssim$ 280 eV (i.e. $\lesssim$ only 14 ionisations) confined to distances smaller than 7 nm.

3A-6

Chromosome analyses in human lymphocytes after 14-MeV (DT) neutron therapy.

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Chromosome analyses were carried out in peripheral lymphocytes of 17 patients with different tumors in various body regions. 4 females and 13 males, aged between 12 and 80 years, were irradiated by a 14-MeV (DT) neutron therapy facility at the University Hospital Hamburg-Eppendorf. Blood samples were taken before, during and after a partial-body exposure in different irradiation fields with doses of between 1.3 and 15.6 Gy ($D_n + \gamma$). The frequency of dicentric chromosomes showed a positive correlation with the therapeutical surface dose. Equivalent whole-body doses could be ascertained by using a calibration curve determined in an earlier in vitro experiment with 15-MeV (DT) neutrons. The dose estimation by means of dicentric chromosomes yielded a mean of 3 - 6 % of the surface dose depending on the irradiated body region. Despite partial-body exposure cytogenetic dosimetry is in principle feasible even for high LET radiation. However, it is necessary to take into account some correction due to different exposure sites of the body. These experiences obtained under controlled medical exposure conditions can contribute to an improved cytogenetic dosimetry after accidental or occupational partial-body exposure.

3A-7

Analysis of cell kinetics of human lymphocytes and aberration yields in first (MI) and second (MII) post irradiation divisions by FPG-staining.

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Under the culture conditions of our laboratory at the culturetime of 48 hr the frequency of MII cells in blood samples of 50 different unirradiated donors varied between 7 - 56 %. Beyond this, significant different MII frequencies (35 - 51 %) were present in blood samples from one and the same donor taken at different time intervals. These data hold for a BrdU-concentration of 11 µg/ml. They certainly do not reflect real cell kinetics without BrdU, because application of BrdU in concentrations down to 1 µg/ml leads to a pronounced increase of the MII frequency. In an irradiation experiment with 220 kV X-rays (50-400 rad) we found a dose dependent decrease of MII frequency.

In a separate experiment with a single dose exposure of 200 rad, chromosome analysis in MI and MII was carried out. The dicentric yield in MII cells is only one half of that observed in MI cells. A similar analysis of acentric fragments will be discussed.
From these experiments it can be deduced that in cases with rapidly dividing lymphocytes, i.e. high MII frequencies, lower aberration yields will be scored with conventional staining. Therefore it is suggested that calibration curves used for cytogenetic dosimetry should be established by means of a standardized FPG-technique.

3A-8

Increased chromosome sensitivity in Xeroderma pigmentosum cells with defective repair of ionizing radiation induced damages in DNA

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Frequency of chromosome aberrations has been studied in the cells of patient with Xeroderma pigmentosum form named XP II. According to previous results (Mikhelson et al., Tsitologia, 1974, 16, 2:203), XP II is characterized by defective rejoining of γ-induced single-strand breaks and decreased unscheduled DNA synthesis after both UV- and γ-irradiation.

Chromosome aberration rate after UV-irradiation in the XP II lymphocytes was increased to the same extent that in classical XP cells. After X- and γ-irradiation chromosome aberration rate was increased only in the XP II cells, but did not differ in classical XP cells from that in norm. Effect of chemical mutagens of "UV-tipe" (BMBA, 4NQO, AAAF) on chromosomes of classical XP and XP II cells was similare, but MMS, EMS and MNNG increased the chromosome aberration rate only in XP II cells. Spontaneous chromosome aberration rate was increased in both XP forms studied.

These findings show the decisive role of DNA repair in sensitivity of chromosomes in human cells and the community of DNA repair mechanisms during normal cell activity and in mutagen treated cells

3A-00

THE RADIOBIOLOGICAL BASIS OF SOMATIC RISK EVALUATION

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Since the publication of the BEIR and UNSCEAR reports in 1972, considerably more attention has been given to the somatic, than to the genetic risks associated with radiation exposure. The reason is that carcinogenesis is considered to be the main somatic risk of irradiation at the dose range involved in radiation protection. Knowledge on the carcinogenic effect of ionizing radiation on man has been increased over the past decades and is based on a large collection of data. For dose levels of a few hundred rads risk can be evaluated rather accurately. For lower doses of a few rads or tens of rads, however, the likely frequency of tumour induction is still quite uncertain.

Assumption that a linear dose response relationship holds down to zero dose /in other words that the biological effect is proportional to dose regardless of the size of dose/ has been disputed from both sides. Some scientists claim that there are much radiobiological and epidemiological evidences indicating that the linear hypothesis lead to a substantial overestimate of radiological risk. According to other scientists, certain data show that the linear hypothesis actually underestimates the risk. It is of the greatest importance, therefore, that in addition to the follow-up studies in selected human populations, appropriately conceived radiobiological experiments in model systems, at both cellular and organism levels, contribute to the exploration of the mechanism of radiation carcinogenesis, of the influence of dose rate, dose fractionation and linear energy transfer.

3A-9

An analytical approach to radiation induced translocations in mouse spermatogonia.

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Within the model proposed by Oakberg for the spermatogenesis in the mouse, two assumptions have been made to derive an analysis of the radiation induced effect. It is assumed that the stem cells A_s are made up of two populations with radically different radiation sensitivities, and it is further assumed that the same type of molecular lesion can be responsible for the formation of a translocation or for cell reproductive death. Using these assumptions mathematical equations can be derived to describe the effect of acute radiation as the average number of translocations induced in the survivors of the sensitive and resistant stem cells. The equations are fitted to experimental data. Unusual results have been found especially after 24 hour fractionation treatments and it has been suggested that the explanation for these results arises, because the first dose of radiation induces some of the resistant cells to move into the sensitive state. We assume that the progression of the resistant cells into a sensitive state is unaffected by the first dose, but that the progression of the sensitive cells into the resistant phase is delayed by the first dose and that the probability for delay is linearly proportional to the first dose. Using this assumption the mathematical equations can be modified to describe the effect of 24 hour fractionation. The mathematical predictions are compared with experimental data. The analytical approach will be used to discuss how the biology of spermatogenesis can influence the radiation induced effect, especially in the comparison of the hereditary effects in different species.

3A-10

Kinetics of micronuclei formation after X-irradiation of preimplanted mouse embryos cultured in vitro.

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Micronucleus formation was studied in preimplanted mouse embryos cultured in vitro after irradiation in the late G_2-phase of the 2-cell stage. The micronucleus test allows a rapid assesment of chromosomal damage (Countryman, P.L. and Heddle, J.A. 1976, Mut. Res. 41, 321). 15 hours after irradiation the embryos had developed to 4-, 5-, 6-, 7- or 8-cell embryos. At this time the formation of micronuclei depended on the X-ray dose and on the radiation induced division delay. In the embryos of the 4-cell stage (first mitosis post radiation) we observed micronuclei only after the relative high doses of 100 R and 200 R. Embryos which at the same time had already developed to the 8-cell stage (second mitosis post radiation) showed micronuclei over the whole dose range from 12.5 R to 200 R. In the 8-cell embryos the number of micronuclei reached a maximum. However after the higher doses (100 R and 200 R) only a small number of 8-cell embryos was present in the cultures whereas the number of 4-cell embryos was high due to the radiation induced division delay. Therefore the number of micronuclei per X-ray dose was relatively small after application of 100 R and 200 R. The dose effect curve was steeper in the lower dose range than in the higher dose range. -
39 hours after irradiation the shape of the dose effect curve had changed. We found a linear relationship at this time. The investigations underlined the high sensitivity of the embryos for this cytogenetic effect. Even a dose of 6 R doubled the formation of micronuclei 200 R caused a 30 fold increase (control value: 0.4 micronuclei per 100 cells). - Considering a period of 63 hours after irradiation the number of micronuclei per embryo increased with time. Micronuclei were apparently formed even after the third and further mitoses post radiation.
Possible mechanisms and the significance of micronuclei formation for the embryonic development will be discussed.

3A-11

RADIO-INDUCED GENETIC DAMAGE IN ASPERGILLUS NIDULANS : THE EFFECT OF RADIOSENSITISERS AND A TUMOUR PROMOTOR.

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Aspergillus nidulans is a eukaryotic fungus in which a variety of genetic damage can be examined in one cell system. In this study A. nidulans has been used to estimate the degree of chromosomal non-disjunction, mitotic crossing-over, gene conversion and point mutation caused by ionising radiations of different quality including fast electrons, soft X-rays, β-particles and α-particles. Depending on the genetical end-point being considered, the calculated RBE values for the radiations were markedly different. For example, following α-irradiation, the RBE values varied from 1.69 for mitotic crossing-over up to 150.36 for non-disjunction.

With respect to chemical modification of the response of A. nidulans to radio-induced genetic damage studies have been made using the tumour promotor 12-O-tetradecanoylphorbol-13-acetate (TPA) and two radiosensitisers, metrondiazole and misonidazole.

Conflicting data exist on the ability of TPA to induce sister chromatid exchanges in mammalian cells after initiation by chemical mutagens. Our study has shown that while TPA has no effect on the viability of the cells it does increase the frequency of gene conversion after initiation by Co-60 γ -rays. Moreover, no such increase in the frequency of point mutation was found.

In terms of radiosensitisation to electrons the enhancement ratios for misonidazole were always higher than those for metronidazole and varied from 1.64 for crossing-over on linkage group I to 2.96 for crossing-over on linkage group VIII, compared with 1.16 and 2.42 for metronidazole. The oxygen enhancement ratio again depended on the particular genetical system being assayed and varied from between 2.43 for non-disjunction to 8.33 for gene conversion.

3A-12

Sensitization of chromatid break- and gap-formation in DNA-repair deficient mutants of Drosophila melanogaster

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When metaphase chromosomes are analysed in the first mitosis following irradiation of cells in the preceding interphase chromatids may contain in addition to obvious breaks smaller non-staining regions without significant dislocation of the chromatid parts on eather side of the gap. Interpretations of the nature of gaps remained controversial so far and their impact upon the function of the chromosomes is not yet fully understood. It has been questioned if they are related to damages of the chromosomal DNA at all. In meiotic and mutagene sensitive (excision repair deficient) mutants of Drosophila (mei-9) the frequency of radiation induced chromosome aberrations in larval neuroblasts is significantly increased compared to the radiation effect in wildtype (repair competent) animals. This sensitization by a genetic defect in DNA repair metabolism affects the frequency of obvious breaks and of achromatic lesions classified as gaps. This suggests that formation of gaps is also initiated by damages of the DNA. Breaks and gaps may simply represent different manifestations of identical lesions modified upon chromatin condensation into microscopically recognisable discontinuities of various extent, or different primary damages which are normally repaired by a common pathway of genetically controled repair mechanism give rise to eather breaks or gaps when this mechanism fails to opperate.

3A-13

RADIATION SENSITIVITY OF HUMAN ATAXIA TELANGIECTASIA AND RETINOBLASTOMA CELLS: VARIOUS END-POINTS.

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Our recent studies (Scott and Zampetti-Bosseler, 1980) on mouse lymphoma cells of differential sensitivity to the lethal effects of X-rays showed a positive correlation between cell killing, chromosome aberrations (CAs), spindle defects and mitotic delay. We suggested that CAs and mitotic delay are derived from a common DNA lesion, that mitotic delay in G_2 leads to spindle defects but that cell death is caused primarily by CAs. Our present studies on human fibroblasts with different radiosensitivities support the hypothesis that CAs rather than spindle defects lead to cell death but not that CAs and mitotic delay share a common lesion. We were unable to confirm the elevated sensitivity of fibroblasts from a patient with retinoblastoma (RT), having a deletion in chromosome 13 (Weichselbaum et al., 1977), to either cell killing or aberration induction. However, cytogenetic observations of lymphocytes from six RT patients (non-deletion type) compared with age and sex-matched controls revealed a higher incidence of spontaneous sister chromatid exchanges (SCEs) and of radiation induced CAs and SCEs.

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Radiation and Environmental Biophysics

3B–1-8 Effects on DNA

3B-1

Radiolytic pathways in γ-irradiated DNA. Influence of chemical and conformational factors. (*)

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The structures and the reaction pathways of free radicals induced by γ-irradiation in numerous different DNA samples have been analysed by ESR. In particular, the possible involvement of the following chemical and conformational factors has been thoroughly probed: base composition (DNAs from different sources); denaturation and degradation (from highly polymerised DNA to single-stranded oligonucleotides); relative hydration degree (from DNA 2mg/ml H_2O, through 100% hydrated DNA to dry DNA); anoxic or oxic conditions (from totally deaerated to O_2 doped DNA solutions). This systematic analysis resulted in the production of a large number of extremely diversified ESR spectra. However, the application of the computer assisted technique developed in our laboratory to these composite spectra permitted their resolution in terms of linear combinations of the same four elementary patterns: a doublet, associated with thymine anions (T^-); a poorly resolved singlet, associated with guanine cations (G^+); an octet, associated with H-addition radicals to C-6 of thymine (TH) and an asymmetrical doublet, associated with peroxyl radicals (ROO•). Such a formal interpretation of the numerous experimental spectra, provided us with a quantitative description of single radical reaction kinetics and permitted us to reveal the following basic features of DNA radiolysis in frozen aqueous solutions.

- Neutral DNA solutions frozen at 77 K are phase-separated systems. Radiation damage to DNA can only be produced by the direct effects of radiation. Water radicals are formed in high density spurs in the solvent phase and become deactivated before diffusing to the clusters of solute molecules.
- Primary radicals on DNA are ions of both signs, randomly distributed among the constituent bases. Their net electric charge refers to the base π-electron systems.
- Electrons and positive holes migrate via the stacked bases. The final electron sink is the base thymine. The final positive hole sink is the base guanine.
- Increasing the temperature, G^+ does not react with the surrounding water molecules, whereas T^- does react to form H-addition radicals at C-6.
- In the presence of dissolved oxygen and at low DNA concentrations, the predominant secondary radical reaction is the formation of peroxyl radicals ROO•.
- No sugar-located free radical has been detected in the course of the present investigation.

(*) Contribution 1681, Radiation Protection Programme, Commission of the European Communities.

3B-2

ESR STUDY OF THE RADIATION - INDUCED RADICALS IN A SINGLE CRYSTAL OF DEOXYGUANOSINE 5' - PHOSPHATE (SODIUM SALT)

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In an irradiated single crystal of the sodium salt of deoxyguanosine 5' - monophosphate tetrahydrate (5'dGMP 2Na · $4H_2O$) six different radical species are distinguished. The three radicals stable at 77 K could not be completely spectroscopically characterized, because their ESR spectra are not well enough resolved in most of the crystal orientations. From the available date we conclude that one of the species is an anion on the guanine base, and that another species is of the π-cationic type. The third species remains unknown. The details of the arguments for the interpretation of the former two species is presented.

At room temperature three stable radical species different from those observable at 77 K are identified and spectroscopically characterized. One of them is the well known radical formed by a hydrogen-atom addition on $C_{(8)}$ of guanine. The second species, with a characteristic nitrogen-atom coupling, is interpreted to be the deprotonated cation. The third species is formed in the sugar moiety, by abstraction of a $C_{(5')}$ - H hydrogen.

The appearance of the radicals in the base moiety and the lack of any experimental evidence for the scission of the sugar-phosphate bond is discussed in terms of the electronic structure of the nucleotide.

3B-3

RECENT DEVELOPMENTS IN THE RADIATION CHEMISTRY OF DNA COMPONENTS

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The mutagenesis and the inactivation of living cells by ionizing radiation is due at least to a certain extent to the chemical alterations of the DNA bases. There are various types of damages produced when DNA is irradiated. In aerated solutions, an important event responsible for the degradation of the pyrimidine bases is the formation of hydroperoxides. However less in known about the alterations of purine constituents. We wish to report here a comparison of the radioinduced degradation of guanine and adenine constituents when DNA is irradiated. Analysis of the radiation of the DNA constituents is easier to perform and a lot of information can be gained by the study of 2'-deoxyguanosine and 2'-deoxyadenosine. The chemical reactions undergone by purine deoxyribonucleosides due to ionising radiation can be classified into four main categories : rupture of the N-glycosidic bond, C'5 - C'8 cyclisation, oxidation of the C-8 of the purine, isomerization and anomerization reactions of the sugar moiety. The corresponding radiation products have been identified both in aerated and deaerated solutions. They have been separated by high pressure liquid chromatography and characterized by 250 MHz NMR spectroscopy and mass spectrometry. Some of these defects have been isolated after gamma irradiation of DNA itself.

3B-4

PROTECTION OF ϕX174 DNA BY CLUSTERS OF METHYLENE BLUE AGAINST INACTIVATION BY OH RADICALS.

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Methylene blue bound to ϕX174 DNA protects the DNA better against inactivation by OH radicals than free methylene blue. We measured the protection in presence of t-butanol and we calculated it from a model describing the binding of methylene blue to DNA.

Methylene blue forms dimers and trimers in solution. Binding of methylene blue to DNA increases this effect and therefore still larger clusters exist near the DNA. The model for binding of the clusters includes only electrostatic effects and it is assumed that the protection is caused by scavenging of the radicals by the bound clusters.

The concentration dependence of the protection is described very well, whereas the magnitude of the protection cannot be predicted due to uncertainties in the rate constant of the reaction of methylene blue with OH radicals.

3B-5

STRUCTURAL ANALYSIS ON SINGLE-COPY DNA AFTER IRRADIATION

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Double-stranded DNA from gamma-irradiated bacteriophage T1 was checked for structural integrity by thermokinetics (i.e. melting and reannealing) and by cleavage with the single-strand specific nuclease S1. Compared to the reannealing behavior of DNAaseI degraded DNA samples, the reassociation rate constant k_2 was three- to fourfold reduced with DNA from gamma-irradiated phages. This was a clear hint, that structural lesions, different from single-strand breaks, were present in the irradiated DNA. Temperature of melting (T_m) was also reduced, dependent on the dose. Yet, the T_m of the reassociated DNA molecule returned to normal values, indicating that those structural lesions, interfering with kinetics, were not participating during subsequent melting.This behavior excluded any mismatch reaction during reassociation. Thus, we considered such lesions as "heteroduplex sites" in double-stranded DNA.
Further proof for "heteroduplex sites" was their quantitative excision with nuclease S1. The ratio of frequency of radiation-induced single-strand breaks to S1 nuclease-sensitive-sites was about 1 : 1. After removal of the heteroduplex sites from the gamma-irradiated DNA, The sample reanneals normal, but is considerably reduced in size. From molecular weight analysis, a rise in heterogeneity revealed a random distribution of the heteroduplex sites. Since the amount of base damages could be considered to be about three times more frequent than chain scissions, we have assumed that several base damages (defect bases or base loss) are associated in such a heteroduplex site. Probably, the direct absorption of energy in the DNA, packed in the phage head during irradiation induced such "hetreoduplex sites".

3B-6

The Effects of Ionizing Radiations on Hamster cells; 5 Types of DNA-breaks differentiated by Kinetics of Repair.

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Some of the most sensitive techniques to measure chain-breaks, alkaline elution (1) and the DNA-unwinding technique (2,3) involve alkaline treatment of the DNA to expose the breaks. It is then not possible to distinguish between different types of breaks, single strand breaks, double strand breaks or alkali labile bonds. Other means have to be used to identify biologically significant lesions; for example it has been shown that the chain-breaks remaining after a few hours of repair correlate to cell killing (4,5).

We have used the DNA-unwinding technique to measure chain breaks in irradiated hamster cells during an incubation period ranging from 0 to 30 hours. The first minute after raising the temperature from 0 to 37°C we observe an increase in number of breaks. This increase in breaks is cancelled in presence of catalase indicating that peroxides start to react with DNA when the temperature is increased. The repair then follows a first order kinetics giving two components with half lifes of 4 and 35 minutes, respectively. The remaining breaks are repaired at a much slower rate giving a minimum of breaks after 15-20 hours of incubation at 37°C. Below a critical dose, 10 krads for gamma rays and 5 krads for fast neutrons, the level of breaks is not significantly higher than in unirradiated cells. At this time, 15 -20 hours, new breaks start to appear in irradiated cells. These breaks are of unknown origin but might reflect degradation taking place in dead cells.

Cell survival and mutation in relation to DNA-damage and repair will be discussed.

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3B-7

Rejoining Kinetics of DNA Double Strand Breaks in Mammalian Cells

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Non-repaired double strand breaks are generally believed to be lethal to the cell. Evidence for the rejoining of these breaks is equivocal. In our attempt to investigate the rejoining mechanisms, we used the neutral elution technique (1) to study its kinetics. The technique allows us to make sensitive determinations of breaking and rejoining after the irradiation of cells with relatively low doses (1 krad).

V79 hamster cells rejoin DNA breaks introduced by Co-γirradiation with a half-time of 20 min. at 37°C. The rejoining follows at least 2nd order kinetics and reaches a plateau when 80-90 % of the DNA breaks are rejoined. The very fast kinetics suggest that double strand rejoining occurs not only in the G2 phase but rather throughout the cell cycle.

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3B-8

Effects of inhibitors and culture conditions on repair of double strand breaks in the DNA of Ehrlich ascites cells measured by the unwinding method.

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The DNA "unwinding" technique (Ahnström and Edvardsson 1974, Rydberg 1975) has been used to investigate the repair of radiation induced double strand breaks (dsb) in Ehrlich ascites cells. Advantage was taken of the biphasic nature of the disappearance of DNA unwinding points as a function of time after irradiation. A rapidly repairing component with a time constant (t_{37}) of about t_{37}=3o min gives way to a much slower repairing component (t_{37}= 2-9 h,depending on conditions). This second component has been shown to correspond with the repair of dsb as measured by velocity sedimentation (Bryant and Blöcher 1980). Extrapolation of data for repair of breaks as a function of time back to t= 0 enabled an estimate also of the number of initial breaks induced to be made. The repair of dsb is shown to be strongly dependent on the quality of the radiation and affected by inhibitors of DNA synthesis ß-ara A and ß-ara C.

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Radiation and Environmental Biophysics

3B–9-13/3C–1-4 Effects on Bacteria

3B-9

THE STABILITY OF RADIATION RESISTANCE OF SPOREFORMING BACTERIA

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The biological indicators of sporeforming bacteria with significant resistance against ionizing radiation is discussed.

A correlation could be drawn between irradiation and photoreactivation i.e., biological damage and the formation of pyramidine dimers. In irradiated spores the damage of their nucleic acid is not photoreactivable and no pyrimidine dimers are found. The dose effect relation gives evidence for the existance of repair mechanisms. For inactivation of macromolecules, viruses and spores the survival curves are generally one-hit, but with bacteria the curves are sorts of hybrid between the one-hit and many-hit curves.

The inactivation curves of sporeforming biological indicators irradiated in Co^{60} (0.2-1.4 Mrads), in liquide state under the aerobic conditions take a linear form. The population of biological indicators with high radiation resistance (B.sphaericus and B.cereus) after being passed and irradiated in liquide culture media shows sharp decrease of radiation resistance. The population of B.pumilus after the same treatment does not change radiation resistance related to its parent population.

3B-10

Photoreactivation after Ionizing Radiation: The Role of Concomitant UV Damage by Cerenkov Radiation

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It has long been established that the exposure of cells to visible light after potentially lethal doses of UV causes photoreactivation repair (PR) of the UV damage. Experiments to determine whether ionizing radiation-induced damage could also be repaired by PR failed to detect any such effect until the work of Myasnik and Morozov (1977) who showed that PR of γ-ray induced damage did occur in certain UV-sensitive strains of E.coli. They also found that PR of damage induced by X- or γ-rays depends on the type and energy of the ionizing radiation (Myasnik et al.,1980). The nature of this dependence suggested to us that damage by UV Cerenkov light produced by Compton electrons might be a contributing factor and calculation of the proportion of energy available as UV Cerenkov light showed that there was a sufficient amount of this light to account for the effect. To test this possibility we used 1.8 MeV electrons from a linear accelerator to irradiate suspensions of E.coli K12 AB2480 uvrA recA either with the electrons and their concomitant Cerenkov light, or with a similar flux of Cerenkov light only that was produced by stopping the electrons in 6 mm of high purity fused silica (Spectrosil) placed immediately in front of the cell suspension. We found that the photoreactivable fraction of the (electron + Cerenkov) damage was partly attributable to the (Cerenkov only) damage. The latter was almost fully photoreactivable. It is concluded that a large part of the photoreactivation that can be achieved after ionizing irradiation involves repair of damage induced by UV Cerenkov light produced in the suspending medium. Similarly, Cerenkov light photolysis may be important in other radiobiological and radiation chemical work where light-sensitive components are present.

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3B-11

RADIORESISTANCE OF E.COLI K12 REC⁻ MUTANTS HARBOURING R-FACTOR

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UV and Co-gamma radioresistance of a wild type E.coli K12 strain /E.coli AB1157/ and its rec⁻ mutants /recA⁻, recB⁻, recA⁻recB⁻ double mutants/ harbouring R46 R-factor was investigated. R-factor was transferred by conjugation into E.coli K12 strains.
The appearence of R46 in rec⁺ and rec⁻ E.coli strains was detected by conferring resistance to four antibiotics /ampicillin, streptomycin, tetracyclin and sulfonamides/, coded by R46. On the other hand R46 was detected by the method of Freifelder et al. By this latter method the chromosomal DNA can be separated from the covalently closed circular plasmid DNA making possible the detection of the above form of R-factor in previously R⁻ bacterium strain.
A well detectable increase in UV and Co-gamma radioresistance was found in recB⁻mutant harbouring R46. It seems that R46 R-factor is able to partly compensate the lack of the coding activity for exonuclease in recB⁻ mutant. At the same time R46 slightly increased the survival of the wilde type strain and had no effect on the survival of recA⁻strain and recA⁻recB⁻ double mutants.

3B-12

Radiosensitization of Staphylococcus aureus by p-hydroxybenzoic acid

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Selective sensitization of hypoxic cells to radiation without increased damage to normal cells has been demonstrated with certain electron-affinic substances. The implications to radiotherapy of the use of such radiosensitizers is well documented. Current interest in the search for new hypoxic radiosensitizers has been concentrated on substances whosemedicinal use is established and therefore, whose pharmacology, toxicology and pharmacokinetics is well documented. One particular compound anticipated, on the basis of its relatively high electron-affinity, to act as a radiosensitizer is p-hydroxybenzoic acid (PHBA) ($k_e^- = 4 \times 10^8\ M^{-1}s^{-1}$), whose esters are incorporated in many pharmaceutical formulations as antimicrobial preservatives.

In this study the effect of different concentrations of PHBA on the sensitivity of oxic and anoxic buffered suspensions ($_p$H 7) of Staphylococcus aureus to ^{60}Co gamma-rays has been examined. Slopes of dose-ln survivor curves have been used as the criteria for assessment of radiation response.

Increasing concentrations of PHBA from 10^{-6} to 5×10^{-5}M in anoxic bacterial suspensions produced no change in the radiation response characteristic of bacteria irradiated in anoxic buffer alone. However, further rises in PHBA concentrations to 10^{-2}M caused a marked increase in radiation sensitivity, with the maximal response very close to that for suspensions irradiated in the presence of O_2. The testing of higher PHBA concentrations has been confounded by problems of toxicity.

The testing of PHBA in oxygenated suspensions demonstrates that the enhancing effect of this agent and oxygen are not additive and that the sensitizing action of PHBA operates within the 'oxygen effect'.

Preliminary results indicate that esters of PHBA, like the parent compound , also possess radiosensitizing properties.

3B-13

Repair of photodynamic lesions produced by TP or 8-MOP and induction of repair processes by photodynamic effects of these dyes in Escherichia coli

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The ability to repair lesions produced by photodynamic action using UV-induced repair systems as well as the induction of repair processes by photodynamic effects was investigated in E.coli. After treatment with thiopyronine(TP) and visible light, the survival percentage of wild type cells is 20 times greater than that of rec A cells, indicating that TP produced photodynamic lesions are repairable. If UV-irradiated E.coli cells are infected with λ-phages treated with TP and light, induced repair system repairs TP-lesions of phage, so that phage mutation rates increase to 2-5 times. In E.coli cells pretreated with TP and light and infected with UV-irradiated λ-phages the viability of the phages is smaller than in untreated E.coli cells. This negative "induced repair" may be caused by a specific accumlation of TP in E. coli cells, by which the viability of the phages is decreased. The results obtained after TP-treatment are compared with those obtained after treatment with 8-MOP and the capacity of inducible repair as well as the effects of photodynamic action by TP and 8-MOP is discussed.

3C-1

UV-INDUCED DEATH OF E.COLI

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Fate of the prophage part of the E.coli chromosome was followed in the course of post-UV incubation. For this purpose, lambda cI857 ind prophage, which can be induced by heat but not by UV-light, was used. Over the entire dose range tested, survival of the prophage, if measured immediately after UV-irradiation, was higher than that of its host. This sensitivity difference was completely lost at the end of post-UV division delay, although the capacity of irradiated cells to support growth of the exogenous phage was fully preserved. The experiments on DNA synthesis kinetics showed that, just at the end of post-UV division delay, DNA synthesis abruptly slowed down. The analysis of the DNA synthesis curves strongly suggested that this change in the rate of DNA synthesis was due to the cessation of replication in the "doomed" cells. Thus both post-UV fate of the prophage and post-UV DNA synthesis kinetics implied that complete functional failure of the chromosome might be a specific cause of UV-induced cell death.

3C-2

REPAIR OF E.COLI AND LAMBDA DNA AFTER UV-IRRADIATION

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Repair-proficient E.coli cells lysogenic for the thermoinducible prophage lambda cI 857 ind red3 were exposed to UV-irradiation and then tested for colony- and plaque-forming ability. The results show that the bacterial cell is about 1.5 times more sensitive to UV-light if compared to the prophage that is irradiated in the bacterial chromosome and, on heat induction, repaired in the cytoplasm. Since the bacterial DNA is about 80 times larger than the phage DNA it is inferred that the host repair processes are about 50 times more efficient on its own chromosome than on the chromosome of the phage. UV-survival data for the lysogenic strains carrying mutations in the uvrA and/or recA genes were also analyzed in the same way and the main conclusion is that the recA-dependent postreplication repair does not operate on the phage DNA.

3C-3

W Reactivation is Inefficient in Repair of the Bacterial Chromosome

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UV-inducible "SOS" processes associated with W reactivation of phage lambda were studied for their effect on repair of lambda prophage integrated in the bacterial chromosome. For this purpose, lambda cI857 indred-lysogens were used. These lysogens, although non--inducible by UV light, can be induced by raising the temperature from 30^{o} to 42^{o}. If the W reactivation processes are involved in repair of the bacterial DNA, when the lysogens are incubated at 30^{o} after UV exposure W reactivation should be fully expressed and should also exert an effect on the bacterial chromosome and the prophage inside it. When heat--induction is delayed until the time at which W reactivation reaches its maximum, a considerable increase in phage survival might then be expected. The results presented in this report show, however, that the delayed induction had only a small effect on the survival of prophage in the wild-type strain (possibly attributable to excision repair) and no detectable effect on prophage in a uvrA strain. From these results we conclude that W reactivation is largely irrelevant to the repair of UV-damaged bacterial DNA.

3C-4

THE SELECTION AND REPAIR CAPACITY OF A BIOLOGICAL FILTER

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We have defined a "Biological Filter" as an intermediary (micro and/or macroorganism) between irradiated food and a human consumer . Food irradiation with sterilizing doses ($2x10^{6}$rad) could induce genetic damage in the ingesting organism. When irradiated food ($2x10^{6}$rad) is consumed in a direct form, the probability of genetic error was previously estimated through a mathematical model as 10^{-11}considering a selection and repair capacity, but when one or two biological filter are used, the risk was 10^{-13} and 10^{-15} respectively.

The selection capacity of Saccharomyces Cereviseae and small intestine of rat (in vitro) was studied as micro and macroorganism biological filter models respectively. For Saccharomyces Cereviseae we found that one third of the whole set of damaged molecules enter to the cells, by incubation in a labelled medium irradiated at $2x10^{6}$ rad and 10^{6} rad in a ^{60}Co gamma source. The selection capacity of rat small intestine was in vitro, 82% approximately.

DNA lesions were studied on mouse small intestine feeded with irradiated food (10^{6} and $2x10^{6}$ rad) supplemented F10 medium irradiated at 10^{6} and $2x10^{6}$ rad were used. DNA was extracted by a Marmurś modified technique and DNA lesions were estimated by CsCl density gradients. The efficiency of the use of one or two biological filter is experimentally demonstrated.

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3C–5-13 Carcinogenesis

3C-5

INFLUENCE OF DOSE FRACTIONATION ON LIFE SHORTENING AND CAUSES OF DEATH OF MICE EXPOSED TO IONIZING RADIATION

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Male mice of the BALB/c strain, 12 weeks of age, were given single γ-rays exposure given in 10 daily fractions at a dose rate of 140 rad/min in order to assess the amount of non reparable and reparable lesions. In order to obtain dose effect relationships, different groups of animals were irradiated with increasing doses of γ rays from 25 to 600 rads. The animals were inspected daily, holidays and weekends included, for their entire lifespans. Death was recorded when it occurred naturally, and the lifespan of the different groups of mice determined. Dead mice were autopsied and the causes of death determined. The causes of death were classified among the following 12 groups : thymic lymphoma ; non thymic lymphoma ; reticulum cell sarcoma B ; myeloid leukemia ; all leukemia ; lung carcinoma ; liver tumors ; all cancers others than leukemia ; sarcoma ; glomerulosclerosis ; non cancerous lung lesions. The preliminary results on the causes of death indicate that a treatment with 10 daily fractions of ^{60}Co γ rays increases significantly compared with the same dose of γ rays given in a single exposure the total incidence of leukemia and cancer.

3C-6

MAMMARY GLAND OESTROGEN RECEPTORS AND PLASMA PROLACTIN DURING OESTROGEN-ENHANCED RAT MAMMARY TUMOUR DEVELOPMENT; STRAIN DIFFERENCE IN THE RESPONSE TO OESTROGEN.

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The mechanism through which oestrogens and ionizing radiation act synergistically in the development of malignant rat mammary tumours is not completely understood. One way to explain the effect of oestrogens in mammary tumour development, is through an oestrogen-induced rise in plasma prolactin concentrations. On the other hand, it could be possible that oestrogens affect the synthesis or the availability of their own receptors in the mammary tissue, and thus modulate the sensitivity of the mammary tissue to oestrogens.

The aim of the present study was to detect possible changes in the oestrogen receptor content of the mammary tissue and the prolactin concentration in the plasma of rats exposed to low doses of exogenous oestrogen and/or radiation. In this respect, the nuclear oestrogen receptor content of mammary tissue was estimated in female Sprague-Dawley rats for a period of 14 months after administration of a cholesterol-paraffin pellet containing 2 mg oestradiol, and/or exposure to 2 Gy of X-rays. In addition, prolactin was estimated in peripheral plasma of the same rats as well as of Wistar and Brown Norway rats which were given the same amount of oestrogen.

In Sprague-Dawley rats, oestrogen treatment resulted in a prolonged decrease of the number of oestrogen receptors in the mammary tissue; a prolonged increase of the prolactin concentration of the plasma concomitant with the occurrence of pituitary tumours; and the loss of the oestrus cycle. Radiation had no additional effect on the parameters studied. Wistar rats showed an increase of prolactin similar to Sprague-Dawley rats, but Brown Norway rat responded to oestrogen treatment with a relatively small increase of the prolactin level. The basal concentrations of prolactin in the three strains did not differ. Interestingly, the Brown Norway rats are the least susceptible to mammary tumour development.

The results indicate that the enhancing effect of oestrogens on the development of radiation-induced mammary tumours is exerted either through a decrease in oestrogen receptor concentrations or through an increase of the concentration of prolactin in peripheral plasma.

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3C-7

INDUCTION OF MAMMARY GLAND TUMORS IN RATS FOLLOWING SINGLE AND MULTIPLE FRACTION IRRADIATION AND HORMONE ADMINISTRATION: MORPHOLOGICAL ASPECTS

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Investigations of mammary gland carcinogenesis in female WAG/Rij, BN/Bi and Sprague-Dawley rats have been in progress for several years. Among the goals of these studies, which employed single and multiple fraction irradiation with X-rays and monoenergetic neutrons, are the assessment of risk estimates for low dose exposures in an experimental animal model, and the influence of exogenous hormone administration on mammary tumor development. An important aspect of these studies is also to make a distinction between benign and malignant mammary tumors, the latter being most relevant in extrapolating the findings to the situation in man.

In this presentation, data will be presented on the numbers and histological types of mammary tumors found in the various experimental groups. The most common type of benign tumor found was the fibroadenoma, with adenomas and fibromas occurring less frequently. The most common malignant tumors were adenocarcinomas and papillary carcinomas. Cribriform and anaplastic carcinomas were seen less commonly, and sarcomas were rare. It will be shown that the numbers of induced tumors increased with increasing radiation dose, and that considerable differences in susceptibility for tumor induction existed among the three strains. Furthermore, it was found that malignant tumors constituted nearly 50% of all mammary tumors in WAG/Rij rats, but these were relatively rare in BN/Bi and Sprague-Dawley rats. Exogenous estrogen administration increased not only the proportion of rats with malignant tumors but also their absolute incidence in the WAG/Rij and Sprague-Dawley strains. Similarly, multiple mammary tumors developed more frequently in estrogen-treated rats than in their non-estrogen-treated counterparts. No apparent correlation was observed between the incidence of pituitary gland adenomas and mammary tumors in the various experimental groups.

In the accompanying presentation, data will be shown which emphasize the tumor prevalence curves as a function of time as well as the relative risks of the various types of irradiation.

3C-8

INDUCTION OF MAMMARY GLAND TUMOURS IN RATS FOLLOWING SINGLE AND MULTIPLE FRACTION IRRADIATION :DOSE-EFFECT RELATIONS AND RBE.

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Experimental studies on radiation carcinogenesis can provide a basis for risk-benefit analyses of occupational exposure and diagnostic procedures involving relatively small doses of ionizing radiation. The human data available for X- and gamma-irradiation all pertain to large doses. In order to extrapolate these data to the range of much smaller doses, the shape of the dose-effect curves for tumour induction must be evaluated.

The objectives of the present program on mammary carcinogenesis are to investigate the cumulative tumour prevalence in different rat strains over a wide dose range for single and fractionated irradiations, to determine the relative biological effectiveness (RBE) of neutrons with energies of 0.5, 4 and 15 MeV and to study the possible synergistic interaction of oestrogen administration and neutron irradiation, so as to gain insight into possible increased risk of women on oestrogen-containing contraceptive medication.

The animals were irradiated at an age of approximately 8 weeks. A total number of 7000 animals is included in the experiment. The animals were allowed to live out their natural life span and were killed when moribund. The probability of animals surviving without known mammary tumour as a function of time, has been calculated according to a life table analysis. Different subgroups, notably intact females, intact females with oestrogen (E_2) and hysterovariectomized females were included in the study.

The following conclusions can be formulated: 1) The number of tumours increases and the latency period for tumour induction decreases with increasing dose. 2) The latency period for non-irradiated controls without E_2 can be in excess of 22 months. In the hormone treated animals tumours appear earlier than in parallel groups without E_2. 3) There are considerable differences in susceptibility for tumour induction in the three rat strains. 4) At a level of 30% cumulative prevalence the RBE for 0.5 MeV neutrons varies between 8 and 25 and for 15 MeV neutrons between 2 and 4 for the three rat strains. 5) The results for the fractionated irradiations indicate that repair processes play a relatively more important role after the X-irradiations than after the neutron-irradiations. In the accompanying presentation the histological classification of the mammary tumours is discussed.

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3C-9

The significance of gene transfer - misrepair as a mechanism of radiation carcinogenesis.

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The gene transfer - misrepair hypothesis (1) postulates that fragments of DNA released from lethally damaged cells may contain specific onc genes. Such DNA fragments can penetrate into viable cells and if the recipient cells are in the process of DNA synthesis, e.g., repair synthesis as a result of sublethal damage by radiation, they have a certain chance of being incorporated at a site remote from the influence of controlling genes. As a result, that cell is transformed into a malignant cell. It has recently been demonstrated:

1) that DNA fragments can penetrate into cells and become incorporated in their nuclear DNA (transfection);
2) that fragments prepared from DNA of normal mouse tissues can transform 3T3 cells in vitro (2, 3).

In case such processes are of importance in radiation carcinogenesis, the linearity of the dose response curve at doses below 0.1 rad can be challenged, since at such doses the distance between damaged cells releasing DNA fragments and damaged cells capable of incorporating DNA fragments becomes too large to allow for equal chances of transfection as following exposure to higher doses of radiation.

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3C-10

CELL POPULATION KINETICS AND RADIATION CARCINOGENESIS OF THE THYROID GLAND IN SUCKLING RATS

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Cell population kinetics of thyroid follicular cells was studied in Wistar rats during the first 2 weeks after birth and compared with those of adult animals. In 10 days old rats the number of proliferating follicular cells (MI, LI) is up to 50 times higher than those of adult animals. In the former, 40% of the follicular cells are in the cycle (GF) with the median cell cycle time (T_C) - 79 hrs, S-phase duration - 8 hrs, G_2-phase - 3 hrs and G_1 - phase - 68 hrs. Three hundred rads X-rays given to the neck region of 10 days old rats lead to the development of more thyroid tumors within a shortent latent period, than the thyroid gland of adult animals has been irradiated with the same dose of X-rays. A subsequent treatment for a long time of irradiated animals with methylthiouracil increases the number of thyroid adenomas and carcinomas and shortened their latent period. The thyroid tumor induced were predominantly of follicular type. Papillary, trabecular and medulary tumor variants were observed in individual animals only.

3C-11

INDUCTION OF LUNG TUMOURS IN THE RAT BY Ir-192 IMPLANTS

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There is a distinct need for realistic animal models to explore new treatments for lung cancer in man. The method of implantation of iridium-192 wires in the rat lung was used with the aim of inducing transplantable lung tumours in rats as a model for human bronchial carcinoma. Ionizing radiation emitted by Ir-192 was used as carcinogenic agent. The half-life of Ir-192 is 74.5 d. The isotope was implanted in the form of wires of 5mm length and 0.3 mm diameter enclosed in tissue equivalent plastic tubing. Each rat was implanted with a wire which contained 0.27 mg Ra-equivalent of activity at the time of implantation.

Forty male WAG/Rij rats were implanted at the age of 4 weeks. They were anaesthetized with ether. The skin of the thorax was shaved and an incision of about 1 cm between two ribs was made. A lobe of the lung was brought outside the thoracic cavity and the wire was implanted using a hollow needle of 10 cm with an inner diameter of 0.7 mm. After implantation, the lobe was returned into the thoracic cavity and the skin was closed by two or three clamps.

In the observation period of 15 months, 30/40 animals developed tumours. Malignant hemangioendotheliomas occurred with the highest frequency (50 per cent). In 12 lungs, a squamous cell carcinoma was found. Several other types of tumours were found, e.g. osteosarcoma, pleomorphic sarcoma, neurofibrosarcoma, adenosquamous carcinoma and mesothelioma. In 8 lungs two tumours and in 1 lung 3 different tumours were found. Tumour fragments were transplanted into syngeneic hosts for propagation of the tumours.

These observations show that local irradiation with an implanted radioactive source is an efficient means of inducing malignant lung tumours yielding a relatively large number of squamous cell carcinomas. Transplantability of the squamous cell carcinomas was, however, adversely influenced by the high incidence of malignant hemangioendotheliomas in the tumour samples used for transplantation. The yield of transplantable squamous cell carcinomas in this series is 10 percent.

3C-12

Myeloid leukaemia in CBA/H mice following whole-body exposure to fission neutrons: An interim report.

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Male CBA/H mice (bred at Harwell) were given single brief whole-body exposures to fission neutrons in the special facility of the Low Flux Reactor at Petten. They were then maintained for life at Harwell. Cases of myeloid leukaemia have occurred after each of seven different doses from 2 to 200 rads. No example has been recognised in several hundred controls. The dose-response will be compared with that for low LET irradiation.

3C-13

TUMOR INDUCTION IN RHESUS MONKEYS AFTER X-RAY AND NEUTRON IRRADIATION

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Over the past 17 years experiments have been performed on the longevity of rhesus monkeys and the effects of bone marrow transplantation after irradiation with X-rays and fission neutrons (van Zwieten et al., 1978). Two groups of longterm surviving animals are studied consisting of 9 monkeys irradiated with fission neutrons in doses ranging from 2.3 to 4.4 Gy, and 20 X-irradiated monkeys which received doses ranging from 3 to 8.6 Gy. A control group of comparable age distribution comprises a total number of 21 untreated rhesus monkeys. In the neutron-irradiated group, 6 out of 9 monkeys died. In five of them tumors were observed which were classified as follows: osteosarcoma, malignant glomus tumor, renal adenocarcinoma, astrocytoma and glioblastoma. Of the 3 monkeys which are still alive, one has a malignant glomus tumor. In the X-irradiated group, 11 out of 20 animals died. In 6 of them tumors were observed which were classified as follows: osteosarcoma, malignant glomus tumor, renal adenocarcinoma and colonic adenocarcinoma. In the 21 untreated control monkeys, which are all still alive, no tumors have been observed up to the present. The number of monkeys with malignant tumors over the total observation period in years is 5/76 for the neutron-irradiated group, 7/186 for the X-irradiated group and 0/310 for the control group.
Based on the incidence results and the average doses received (7.2 Gy and 3.4 Gy for the X-irradiated and fission neutron-irradiated groups, respectively) risk values of $7/186 \times 7.2 = 52 \times 10^{-4}$ year^{-1}Gy^{-1} for X-rays and $5/76 \times 3.4 = 190 \times 10^{-4}$ year^{-1}Gy^{-1} for fission neutrons can be calculated. From these data, the RBE for tumor induction is approximately 4, which is lower than that previously reported (van Zwieten et al., 1978). This is due to the occurrence of tumors in monkeys of the X-irradiated group which died recently. The latency period of 11 years in the latter group is considerably longer than that of the neutron-irradiated group, namely 7 years.

Reference:
M.J. van Zwieten et al.: Longevity studies in rhesus monkeys after X-ray and neutron irradiation with special emphasis on tumour induction. In: Late biological effects of ionizing radiation, Vol. II. Vienna, International Atomic Energy Agency, 1978, pp.165-179.

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Radiation and Environmental Biophysics

3D–1-14 Cellular Effects

The interaction of radiation and adriamycin in Chinese hamster cells

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In combined modality treatment of Chinese hamster cells, interactions were observed between irradiation and adriamycin which were additive in V-79 cells but synergistic in B-14 cells. The radiosensitizing effect of adriamycin may last for hours and days and can even be transferred to the progeny of surviving B-14 cells. On the other hand, irradiation does not influence the sensitivity of V-79 cells to adriamycin, yet adriamycin given after irradiation interacts additively with radiation as well. Adriamycin completely inhibits the accumulation of sublethal radiation damage in V-79 cells irradiated several hours later but does not interfere with split dose recovery and the shedding of sublethal radiation damage.

3D-2

EFFECT OF ADRIAMYCIN ON THE REPAIR OF RADIATION DAMAGE IN CHINESE HAMSTER CELLS

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The aim of this study was to assess the effect of subsequent adriamycin administration on the cells ability to repair radiation damage. Exponentially growing V-79 monolayer cultures were used and X-ray survival curves at various time of drug administration were examined. The data show that the adriamycin-radiation interaction is substantially timetable dependent. Chinese hamster cells treated with adriamycin 5 to 7 hours after irradiation exhibit an exponential combined treatment survival curve, as a result of shoulder reduction. If the cells were irradiated twice, 7 and 5 hours before adriamycin treatment, the shoulder on survival curve reappeared. When the combined treatment survival curve is sigmoidal; i.e. if irradiation is performed 13 hours prior to adriamycin treatment, the shoulder of this curve disappears if second irradiation is delivered two hours after the first exposure. The observed results implied that adriamycin interacts with radiation influencing the repair of sublethal radiation damage.

3D-3

Bleomycin-induced Potential Lethal Damage in HeLa cells in vitro.

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Increase in survival of HeLa cells, exposed to various growth conditions after a single dose of bleomycin may be explained either by repair of potential lethal damage or by a decreased pharmacological activity of drug mobilised from the cell membranes, reflecting drug catabolism. Both theories have been tested and in the case of clonogenic assay following exposure in monolayer, the latter concept may be the more feasible.

Attempts to exploit this membrane bound drug pool by hyperthermia to enhance cell killing have not proved unequivically successful. However co-operative action of bleomycin and hyperthermia, used simultaneously to decrease cell survival has been readily demonstrable.

3D-4

Efficient G2-delay after very low doses (0.03-1 Gy) of alpha particles

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Previous experiments on cell cycle perturbations by beams differing in their ionization density revealed densely ionizing radiation to be especially effective in causing G2-delay in V79 cells during their first postirradiation cell cycle. A maximum RBE value of 8.3 was found for this LET-dependent effect at about 100 keV/µm. 4 MeV alpha particles as emitted by an Am-241 source (LET ∿110 keV/µm) were, therefore, used to investigate the phenomenon of G2-delay. Exponentially growing cultures of human and animal origin were irradiated under retainment of optimal growth conditions and afterwards analysed for cell cycle alterations by means of flow-cytofluorometry. A higher effectivity of high-LET alpha particles compared to low-LET Cobalt-γ-rays was found for all cell lines. G2-delay increased linearly with dose yielding G2-fractions above 70 % at doses less than 1 Gy of alpha particles. Even 0.03 Gy alpha-rays caused a measurable effect. At low doses (more than 50 % of the cells survive) G2-blockage was reversible reaching control values 12 hours after irradiation. At higher doses the effect became irreversible, most of the cells stayed at G2 even 24 hours after irradiation. In contrast to low-LET Cobalt-γ- or X-rays cells seemed to die without completing at least one mitosis after irradiation.
In order to learn about the target responsible for G2-delay the purine derivative caffeine with its specific affinity to damaged DNA was added just before irradiation. Then G2-delay was reduced considerably. Even at high doses (cell killing above 90 %) G2-blockage became reversible and most of the cells completed mitosis. This effect was concentration dependent. 2 mM caffeine, however, was enough to abolish G2-delay after doses up to 1 Gy completely. If caffeine was added after cells had already accumulated totally in G2-phase (about 10 hrs after 5 Gy) the block became transient. That means that cells which would have stayed in G2 until cell lysis occurred, regained the ability to complete mitosis. The reduction of G2 after addition of caffeine led to an increased cell killing effect demonstrated by a D_{37}-sensitization factor of 1.45.

3D-5

The effects of X-irradiation on the intermitosis time and the occurrence of cell death of Mouse Osteosarcoma cells.

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137 pedigrees of mouse osteosarcoma cells, irradiated to achieve 50% survival in a cloning assay, were constructed by time-lapse cinematography. The data were organized in cumulative frequency distributions of intermitosis times (α-plots) and cumulative frequency distributions of differences of cell cycle times of sibling pairs (β-plots). The slope of the semilogarithmic β-plot is a measure for the transition probability P of the cell cycle. The shoulder of the α-curve gives an indication for the distribution of the "B-phase" duration. The X-ray treatment causes an extension of the generation, in which the irradiation took place. This extension appears to be due to two effects. Cells, which presumably are in G1- or S-phase show only a small extension of the "B-phase", but the cells, that are lethally damaged show a decreased transition probability P. The G2-phase cells show a much more pronounced extension of the "B-phase", while the lethally damaged cells of this group cannot be distinguished by a decreased transition probability.
During the first post-irradiation generation, cells, whose parents received lethal damage in the G1- or S-phase, die preferentially. All lethally damaged cells, that pass into the second post-irradiation generation, show a decreased transition probability, regardless the age of the parent at the time of irradiation. However, the differences in "B-phase" durations in the irradiated generation have dis appeared in the first post-irradiation generation. Cells, whose grandparents were irradiated while in G2, die preferentially in the second post-irradiation generation.
The occurrence of cell death appears to be significantly correlated for sister cells.

3D-6

COMPARISON OF THE CLONOGENIC EXPRESSION AND TUMOUR GROWTH AFTER TRANSPLANTATION OF R-1,M CELLS INTO THE SYNGENEIC WAG/Rij RAT AND THE ALLOGENEIC BALB/c.nu MOUSE IN THE COURSE OF TD_{50} ASSAYS.

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The expression of the clonogenic capacity of R-1,M tumour cells may vary with the growth conditions provided by the assay system employed. This is evidenced by results of assays performed with cell suspensions containing R-1,M cells alone or admixed with either heavily irradiated R-1,M cells, designated as F(R-1,M)cells, or normal syngeneic MER-1 cells with a phagocytic capacity. Assays were done by employing the in vitro plating technique and the TD_{50} assay using WAG/Rij rats or BALB/c.nu mice.

A maximal colony forming capacity of 0.8 to 1.0 per R-1,M cell in suspension was observed with the in vitro plating technique and using medium enriched with F(R-1,M) cells. In vivo assays performed with the WAG/Rij rat and BALB/c.nu mouse revealed that the TD_{50} is about 6000 cells for each of these strains if only tumour cells are injected. Smaller values of TD_{50} were obtained by admixing tumour cells with F(R-1,M) or MER-1 cells, the smallest value being 10 cells which was observed when R-1,M + MER-1 cells were assayed in the BALB/c.nu mouse.

However, R-1,M tumours originating from inocula containing MER-1 cells plus fewer than 1000 R-1,M cells grow more slowly and even showed complete regression, in the BALB/c.nu mouse as compared to that observed in the WAG/Rij rat.

Finally, experiments designed for assessing the intrinsic radiosensitivity of in vitro irradiated R-1,M cells demonstrated that data on fractional surviving cells obtained with the two in vivo assay systems showed significant differences with respect to the initial slope of the survival curve as compared with corresponding data obtained by the in vitro plating technique.

3D-7

Proliferation Kinetics of Human Melanoma Cells after Hyperthermia and X-rays.
Part I: DNA Content and DNA Synthesis Rate of Human Melanoma Cells after fractionated Hyperthermia and X-irradiation.

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Human melanoma cells were exposed to hyperthermia (3 x 1 h, 42° C; 1 x 3 h, 42° C or 3 x 1 h, 44° C; 1 x 3 h, 44° C). The hyperthermia was combined with 400 R X-rays. The DNA content per cell culture was measured by a biochemical method. The DNA synthesis rate was determined by the incorporation of 3-H-thymidine into the DNA. The DNA content was taken as an equivalent for the cell number.
Immediately after 3 h, 42° C a slight growth delay was observed, afterwards the cells proliferated like the controls. 72 hours after treatment a slight cell loss occurred. X-irradiation combined with hyperthermia enhanced the cell loss. After fractionated hyperthermia (3 x 1 h, 42° C) the cell grew like the controls, but after the combination of fractionated heating and irradiation with 400 R cell loss was observed again. The recovery from heat damage after fractionation was deminished by the irradiation. This could mean, that the expression of thermotolerance was suppressed by X-irradiation. The DNA synthesis rate showed a depression after hyperthermia alone. After heating for 1 h, 42° C it had recovered 3 hours after treatment. After 3 h, 42° C the recovery took place after 9 hours. The return to the controls values was not changed by X-irradiation, but in contrast to heating alone no overshooting reaction occurred. The depression of the DNA synthesis could be related to the observed growth delay. Further investigations of the proliferation kinetics under these conditions will be reported by Dr. Zamboglou in the following paper.

3D-8

Proliferation Kinetics of Human Melanoma Cells after Hyperthermia and X-rays.
Part II: Cell Cycle Distribution of Human Melanoma Cells after fractionated Hyperthermia and X-irradiation

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In the same system presented by Dr. van Beuningen and after the same treatment further parameters were investigated. After staining the cells with ethidium bromide the DNA-distribution patterns were recorded with a microscope photometer (Leitz MPV 2) yielding the percentages of cells in the cell cycle phases. The DNA content of each nucleus and the comparison of the results of autoradiography on the same nucleus allowed us:

a) the determination of the cells which have a S-phase DNA content but are not labelled autoradiographically. Such cells had been found already 24 h after fractionated treatment by hyperthermia and X-rays (23 % of the S-phase cells were unlabelled).

b) the determination of the cells which have a G_1-phase DNA content but which are labelled autoradiographically. Those cells had been found 3 h after irradiation or combined treatment by irradiation and hyperthermia. About 25 % of the G_1-phase cells were labelled.

Furthermore the formation of micronuclei increases after irradiation (3 times 4 Gy) from 5 (controls) to 85 micronuclei per 100 cells. After combined treatment it increases to 120 micronuclei per 100 cells.

3D-9

ULTRASTRUCTURAL CHANGES OF A TRANSPLANTABLE MAMMARY CARCINOMA IN MICE AFTER IRRADIATION WITH A SINGLE DOSE OF GAMMA RAYS

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The radiobiological changes of the tumor cells of a transplantable mammary adenocarcinoma in mice were studied during the initial transplantation generations by variety of morphological (autoradiography, light microscopy, electron microscopy) and cytochemical (cytophotometry) metods. This transplantable carcinoma was derived from a spontaneous mammary tumor growing in "H" strain mice. Tumor cells from the third transplantation generation in the control non-irradiated animals were predominantly arranged in trabecular and microfollicular structures containing sometimes a secretory product. A lot of mitochondria, membane attached and free ribosomes were observed in the cytoplasm of tumor cells. The nuclear membrane indicated deep invaginations. Condensed chromatin in the nucleus was marginally situated and close conected with different in shape and structure nuclolli. First ultrastructural changes were noted 2 hrs after tumor irradiation with 20 Gy gamma rays (^{60}Co) and concerne predominantly the nuclear membrane and mitochondria matrix. In the next hours (6,9,24) a further increase in the cellular damage was found: swelling of nucleus and mitochondria, extension of perinuclear space, appearance of phagolysosomes, agregation and disagregation of chromatin structures and nucleolus.

3D-10

SPECIAL HEMATOLOGICAL FINDINGS FOLLOWING RADIOIODINE THERAPY FOR THYROID CARCINOMA

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Based on 391 patients of thyroid neoplasms hematological follow-up-studies in 46 cases, treated by overall J-131- dose of more than 500 mCi, were performed.
The following parameters were included: 1. Peripheral blood control, 2.Bone marrow aspiration, 3. Cell volume distribution size of leucocytes after preparative enrichment. If in dought a bone marrow szintigraphy was added.

Hematological examinations were carred out using standard clinical laboratory methods, observed over a 4 year therapy and correlated with time and dosage of administration of therapeutic J-131.
Except for one case developing an acute leukemia after having received 990 mCi J-131 during a period of 11 years we didn't find some variations of usual parameters in peripheral blood.
On the other hand the value of parameter 2 and 3 can be calculated as follows: Biopsy of bone marrow provides detailed information for detecting hy poplastic alteration being latent in circulating blood for some years, even of J-131 cumulation dose below 500 mCi. The analysis of destribution size of leukocytes represents a functional aspect after radiation induced injury of bone marrow.
The results are correlated with histological criteria of thyroid carcinoma and J-131 retention after therapeutic application of radioiodine.

3D-11

Change of UV fluorescence intensity in lymphocytes after incorporation of radiocaesium in rats

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After single incorporation of various Cs-137 amounts (0,74, 3,7, 9,25, 18,5, 37,0 and 62,9 kBq Cs-137/g body weight) the change of UV fluorescence intensity of peripheral lymphocytes (excitation of cells at 280 nm and measurement at 340 nm) was studied in rats. The measurement of fluorescence intensity of lymphocytes was made 1, 14, 28 and 90 days after radiocaesium application. In order to obtain another measure for internal radiation exposure, in addition the absolute number of lymphocytes was counted.
After application of more than 0,74 kBq Cs-137/g body weight, the results have so far shown an increase of UV fluorescence intensity which is significant from 3,7 kBq Cs-137/g body weight onwards. While UV fluorescence intensity of cells again is normalised within 90 days up to 18,5 kBq Cs-137/g body weight, this effect will remain unchanged over this period after application of 18,5 kBq Cs-137/g body weight. After application of larger radiocaesium amounts, UV fluorescence intensity of cells decreases to subnormal values after reaching a maximum value. The results obtained are compared with those of other authors obtained after chronic external irradiation.

3D-12

THE EFFECT OF IONIZING RADIATION ON RABBIT LIVER MITOCHONDRIA IN EMBRYOGENESIS.

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The effect of gamma radiation on the enzymatic activity and stability of liver mitochondria of 25-30 days old embryos (when the mother was irradiated), as well as adult rabbits of 1, 4, 24 and 48 hrs after effects with the dose of 200 R was studied. The activity of succinic oxidase, NADH and cytochrom-c-oxidase systems of mitochondrial respiratory chains fluctuates throughout embryogenesis. The stability of cytochrom-c-oxidase did not significantly change. The stability of NADH and succinic oxidase decreased during the early and late periods of irradiation.

Electrophoretic studies in PAAG within 1 hr after irradiation have shown the same electrophoretic and densometric pictures of polypeptide fractions of inner mitochondrial membranes from livers of normal and irradiated embryos as well as adult rabbits. Within 24 to 48 hrs after irradiation the thickness of protein patches in gels, as well as the densometric height, corresponding to high molecular weight (above 46.000 daltons) mitochondrial proteins were increased. In contrast to mitochondrial membrane proteins from adult organisms, embryos showed high molecular embryo specific fractions, the thickness of which increased after 48 hrs of irradiation.

Electrophoretic lability of proteins after irradiation during periods mentioned above did not change. We suppose that radiation changes taking place in the biosynthesis of some protein components from mitochondrial membranes are connected with the loss of links of mitochondrial protein synthesizing systems. The effect of irradiation is accompanied by unequal levels of enzymatic stability in the liver mitochondria from adults and embryos, which is connected with different functional states and specificity of the enzymes in the respiratory chain.

3D-13

ESR-STUDY OF RADIATION-INDUCED FREE RADICALS IN TISSUES OF HEALTHY AND TUMOR-BEARING ANIMALS AND THEIR POSSIBLE ROLE IN TISSUE RADIOSENSITIVITY

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The nature of radiation-induced damages in a number of tissues of healthy and tumor-bearing animals (hepatoma 22a) has been studied by ESR method. The present study examines the possibility of a relationship between the number and nature of primary damages and the different tissue radiosensitivity. The ionizing radiation effect on tissues is shown to result in the formation both radical products of water radiolysis (OH, HO_2) and free-radical products of the chemical cell components. The most important results are: (i) the establishment of the fact of DNA damage (formation of hydrogen-addition radicals of thymine) in animal tissues at the initial stages of radiation-chemical processes; (ii) the observation of the greater extent of DNA damage in the radiosensitive mouse tissues (spleen, thymus, bone marrow) as compared to the radioresistant ones (liver, kidneys, muscle, heart); (iii) the occurence of the DNA damage in the cells of hepatoma 22a, the tumor cell culture. Besides the water radiolysis radicals (OH and HO_2) and H-adduct radicals of DNA thymine bases, the radicals of protein nature (singlet with $\Delta H = 23$ Oe, $g = 2{,}003$) and the carbohydrate radicals (quintet with $a = 20$ Oe, quartet with $a = 16$ Oe and singlet with $\Delta H = 9$ Oe and $g = 2{,}005$) are shown to form in the irradiated tissue samples. In the hepatoma samples peroxide radicals of lipids are observed. For establishing the localization of radiation damages ESR spectra of subcellular structures and biomacromolecules have been studied. The comparative study of nature of the radiation damages in normal and pathological tissues has revealed centres which can be observed only in tissues of sick animals (hepatoma 22a and liver of the tumor-bearing animal). These paramagnetic centres have a singlet signal (ΔH-6 Oe and g=2,0005), its intensity depends on the period of tumor development. The studies of kinetic changes in intensity of this signal in hepatoma and regenerating liver (a system with active proliferation of nonmalignant etiology) have made it possible to conclude that its behaviour correlates with activity of cell proliferation in the liver and tumor tissues. The nature of this signal is presented as centres of type ($Fe^{3+}\ldots O_2^-$) which are intermediate active states of a number of enzymes of the oxidase type.

3D-14

ROLE OF SEX HORMONES IN DEVELOPMENT AND REPAIR OF RADIATION INJURI TO TESTIS

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In experiments on mice using tritium-labeled sex hormones it was established that exogenic androgens and estrogens penetrate into the testis where they combine with incretory cells. Testosteron is bound with spermatocytes-I, young spermatids and Sertoli cells, and estradiol with Leydig's and Sertoli cells. Sex hormones bound to germ and incretory cells change their DNP affecting the functional status of the genom. In experiments on irradiated animals it was shown that androgens and estrogens produce a modifying effect on the genom of testis cells at a time when abscopal effect of ionising radiation comes into play. Using exogenic hormones a cytophysiological correlation was rewealed between the genom activity of incretory cells of the testis and division and differentiation of germ cells. Testosteron and estradiol regulate mitotic and meiotic activity in spermatogonia and spermatocytes-I which is important for repair of radiation damages.

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4A–1-9 Bone and Bone-Seeking Radionuclides: Physiology, Dosimetry and Effects. Symposium of the European Late Effects Project Group (EULEP)

Bone tumour induction and its significance: An introductory outline.

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Bony tissue is among the less sensitive for tumour induction by ionizing radiation but is nevertheless of major significance in the practice of radiological protection. Alpha-emitting radionuclides, such as plutonium and other actinides, are preferentially taken up into bone from the circulating blood, and the relatively high local concentration in tissue, together with the relatively high efficiency of α-particles for damage, makes the possibility of induction of bone tumours a major criterion in regulating the intake of these materials into the body. Moreover the first recommended "dose limit" based directly on human experience of tumour induction (1941) was that for the α-emitter radium-226 in bone. Not only has this limit remained effectively unchanged for the past 40 years but it has also continued to provide a major reference point within the internationally accepted system of radiological protection.

Bone tumour induction also provides an important experimental model for studies of carcinogenesis by ionizing radiation and in general. It provides insights into the nature and anatomical localisation of the cells from which the tumours may originate, into the kinetics of tumour induction and its modifications by physiological and radiological variables, and into the principles by which experimental observations may be applied to the human species, since the experimental findings may be compared with well-established evidence about bone tumour induction in many by α-emitters in bone.

4A-2

Bone cells: A review.

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Bone marrow contains two main cellular systems, the stromal and hemopoietic systems, and these are derived from histogenetically distinct cell lines in the postnatal organism. Recently, evidence has accumulated to show that the two main functional cells in bone, the osteoblast and the osteoclast are components of the stromal and hemopoietic systems respectively.

The origin of the osteoclast from a blood-borne mononuclear cell has been demonstrated in experiments with cell markers, osteopetrotic animals and parabiotic systems. A substantial body of circumstantial evidence suggests that the osteoclast is most probably an end cell of the mononuclear phagocyte system.

Evidence for the origin of the osteoblast from the stromal cell system comes from work in three areas; transplantation of marrow to ectopic sites, regeneration of marrow tissue in an ablated marrow cavity and culture of marrow stromal cells. Studies in the first two areas provide good morphological evidence for the following conclusions. That marrow stromal cells and the soft connective tissue associated with bone surfaces are each capable of giving rise to both bone tissue and marrow stroma and that the formation of trabecular bone and the stromal tissue of the sinusoidal microcirculation appears to be necessary before hemopoiesis can be established. Studies in the third area using more recent techniques provide support for these conclusions. Furthermore in vitro/in vivo culture of stromal fibroblasts from marrow has demonstrated directly that these cells contain the stem cells of the osteoblast cell line.

Experiments performed to investigate whether there is interchange of cells between the stromal and hemopoietic systems or whether there is a pluripotent cell giving rise to both have proved negative. Currently, this is the best evidence that osteoblasts and osteoclasts are not derived from a common precursor in the postnatal organism.

4A-3

Construction and Reconstruction of Bone

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Bone is a late evolutionary development. Formed from embryonic mesenchyme it begins in fetal life as condensations, most of which become cartilage but some remain membranous. The signal for bone formation is vascularization. For endochondral ossification, after a first periosteal membranous cuffing, blood vessels invaginate the perichondrium and, preceded by cartilage cell-degeneration, invade the cartilage matrix, ultimately to approach the ends, there forming bone growth-plates. Linear growth depends on continued growth of cartilage in the plates, circumferential growth being mainly periosteal membranous. Bone-marrow forms in the lysed cartilage matrix, stroma from pericytes of the vessels and haematopoietic tissue by diapedesis of immigrant haemic stem cells from the liver through the now sinusoidal vessel walls.

Bone formed is either ephemeral (woven) or definitive (lamellar). To fashion the bone much ephemeral and some lamellar bone is resorbed here and more apposed there, ultimately to form an ivory outer cortex and a trabecular inner cancellation.

Even at maturity resorption and repair continue, especially in cancellous bone, on a care and maintenance basis to be greatly accelerated on damage, e.g. fracture.

Bone is mostly matrix, mineral, collagen fibre and glycoprotein and only sparsely cellular still dependent on vascularization.

Radionucleides may deposit in the mineral, as calcium substitutes (^{90}Sr, ^{226}Ra) or in matrix or its surface (^{239}Pu). Consequential death of osteocytes demands repair from osteogenic precursors, some of which may have been "transformed". Preliminary resorption is effected by osteoclasts derived from haematopoietic stem cells.

Histology of bones from mice bearing radionucleides indicates increased resorption and repair, with sporadic mis-repair, i.e. osteosarcoma, often multiple ranging from microscopic to massive.

4A_4

CYTOLOGICAL STUDIES OF PLUTONIUM UPTAKE IN BONE: THE EFFECTS OF BONE REMODELLING

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In common with other actinide and lanthanide elements most plutonium in the body is concentrated by the liver and skeleton. Within the skeleton plutonium is initially deposited onto bone surfaces. It is thus commonly referred to as a bone-surface seeking radionuclide. This notation distinguishes it from the bone-volume seekers such as strontium which are analogues of bone constituents and are deposited throughout the matrix.

Autoradiographic studies in rats using a low energy β-emitting isotope of plutonium, namely plutonium-241 have shown that subsequently, plutonium is either buried by the apposition of new bone onto contaminated bone surfaces or is removed during bone resorption. The plutonium that is resorbed is initially concentrated by osteoclasts where it remains for several days. It is then transferred to macrophages in the bone marrow. It remains in these cells for a much longer period producing a substantial bone marrow deposit of plutonium. However, eventually this plutonium also is lost from the cells and is redeposited onto bone surfaces. Consequently, there is a tendency with time for plutonium to become fairly evenly distributed throughout the bone and bone marrow.

4A_5

The spatial distribution of plutonium-239 in bone and its changes with time.

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The cells within bone most likely to be at oncogenic risk have been identified as (a) the haematopoietic stem cells of the marrow (b) the osteogenic precursor cells on or near endosteal surfaces, and (c) certain epithelial cells located close to bone surfaces (ICRP Publication 11).

Alpha-particles emitted by the industrially important radionuclide plutonium-239 have a range of less than 40μm in soft tissue and 20μm in bone mineral. To understand the toxicological effect of plutonium-239 on bone tissue it is necessary to have a quantitative measure of its distribution with a resolution considerably less than the range of alpha-particle.

The spatial distribution of plutonium-239 about the periosteal and endosteal surfaces of the ilium of the CBA mouse at various times after intravenous administration of the radionuclide is considered. Two different dose levels (50 nCi ^{239}Pu kg^{-1} body mass and 1 μCi kg^{-1} body mass) are reported and contrasted.

4A-6

Dosimetry of alpha-emitters in bone

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The paper describes the development and application of a novel technique for evaluating alpha-autoradiogramms. The method involves scanning the alpha-track distribution on cellulose-nitrate detector foils and determination of tissue structure from Alizarin-stained bone sections (150 µm) by means of a computer controlled microscope photometer. Geometrical alignment between the two samples is preserved during the measuring process. After combining the files containing the track density distribution with the corresponding digitized bone images on a random access storage medium, detailed information concerning the radionuclide distribution on a microscopic scale, dose rates, hit frequencies etc. are extracted by a sequence of FORTRAN-programms.
The potential of the method is demonstrated in a study dealing with the distribution of 239-Pu in the lumbar vertebra and distal femur of adult rats. The animals were injected (i.v.) with 1.8×10^4 Bq kg^{-1} and sacrificed after days 1,7,28 and 84.
Endosteal and periosteal dose rates in the distal femur and the lumbar vertebra are similar and typically about .01 Gy/d in the endosteal marrow 0-10 µm from bone surfaces. Dose rates are increasing up to the 84th day due to recirculating and redepositing 239-Pu. The periosteal deposition of activity is about 20 % of the endosteal one. Only about 2 % of the total activity in a section was found over 'deep' marrow, but with the tendency to increase up to 8 % at the 168th day. The distribution functions of dose rates reveal a rather non-uniform deposition of the radionuclide on bone surfaces and correspondingly a wide variation of dose rates.
The mean burial depth amounts to only a few microns up to day 84. The measured dose rate distribution is discussed in terms of hit probabilities for cell nuclei. Consequences and relationships of the results with regard to quantitative tumour models are indicated.

4A-7

BONE DOSE AND TUMOUR INDUCTION

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Incorporation experiments with short-lived radionuclides are very promising means to study in particular problems of dose response as a function of time factor. For this purpose long-term series experiments with mice were performed in order to study oncogenic effects of some short-lived bone-seekers in dependence on radiation quality, skeletal dose and its temporal and spatial distribution. Special emphasis was laid on the extensive variation of dose rate, keeping the total dose constant and using different treatment spans.

Ra-224 (3.6 d half-life) and Th-227 (18.7 d half-life) were used as alpha-emitting bone-seekers. They were applied with mean skeletal doses between 20 and 10 000 rad as single injections and between 120 and 2 000 rad with protracted application over a period up to 36 weeks. The results were also compared with those after single injections of the long-lived radionuclides Ra-226 and Ac-227.

Lu-177 (6.7 d half-life) was the representative for a short-lived beta-emitter. It was used with skeletal doses by single injections between 2 800 and 22 400 rad, and protracted application between 2 800 and 11 200 rad over a period up to 12 weeks. The comparison with the effects of the long-lived beta-emitter Sr-90 showed that the osteosarcomas were induced by similar total doses of the short-lived as well as of the long-lived radionuclide.

As a general rule it was shown for middle and higher doses that protracted application of a short-lived radionuclide (alpha- as well as beta-emitter) was able to cause significantly higher osteosarcoma incidences than the same activity given as a single dose.

4A_8

Influence of biological parameters on the induction of ^{90}Sr induced tumours

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Several biological variables such as species, strains and age variations are known to alter the spectrum of pathological lesions induced by ionizing irradiation. Data as regards in particular internal emitters are still scanty and not yet suitable for meaningful generalizations. In addition many factors such as hormonal dysbalances, metabolic disorders, infections and other diseases which are integrated parts of a heterogenous human population are hardly investigated at all. The objective of this report is to give some additional information on parts of this subject with special reference to internal emitters. ^{90}Sr has been used as a model in mouse experiments and the following biological parameters have been investigated: Sex, age at time of exposure, genetical constitution, hormonal balance and immunological response. In this particular report the osteosarcoma frequency has been used as an index of carcinogenicity.

As a general conclusion these experiments indicate that female mice were more prone to develop osteosarcoma than were males (1.11 vs 0.72 tumours/mouse; total material 680 mice). Furthermore it was found that the puberty period (75 days of age) was the most sensitive time for osteosarcoma induction followed by juvenile mice (25 days), middle aged mice (150 days) and old mice (300 days).
In seven different strains of mice the osteosarcoma incidence was investigated and found to vary with a factor 2 between the least and most sensitive mouse strains.

When ^{90}Sr was combined with oestrogenic hormones (polyestradiolphosphate) the tumour frequency was increased with a factor 2 whereas the induction time decreased approximately with 50 per cent. In addition when low doses of ^{90}Sr were combined with oestrogenic hormones an unexpectedly high incidence of pituitary tumours (40%) was observed in contrast to 1% and 7% when ^{90}Sr or oestrogen were given alone. When methylprednisolone was given once every second week in combination with ^{90}Sr there was a remarkable decrease of the tumour incidence as compared to when ^{90}Sr was given alone (0.16 vs 2.16 tu/mouse). The tumour induction time was prolonged with more than a factor 2. Finally it should also be mentioned that BCG-vaccination of ^{90}Sr-treated mice significantly reduced the osteosarcoma yield.

4A-9

BIOLOGICAL FACTORS AS ILLUSTRATED IN WORK WITH SHORT-LIVED ALPHA-EMITTERS

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With regard to the evaluation of the osteosarcoma risk at low dose level of internal alpha-irradiation it seems to be important to study the mechanism and stages of the tumour development and the possible effects of additional endogenous biological and exogenous promoting factors which may influence the tumour incidence and/or shorten the latency period.

- The occurrence of non-neoplastic proliferations preceding radiation-induced osteosarcoma was studied in an experiment with 100% tumour expectancy. In female and male C3H x $101/F_1$ mice such proliferations were observed with much higher frequency than large or intraosseous osteosarcomas. They occur within a relatively short time interval before osteosarcoma.
- Age disposition: The time distribution of age-related bone dysplasia in female NMRI mice and of spontaneous osteosarcoma may partly determine the time of occurrence of osteosarcomas, as shown in experiments with osteosarcoma risk less than 50%.
- Strain disposition: Several strains of mice (BALB/c, C57BL, NMRI) showed different sensitivity for osteosarcoma induction with regard to the tumour latency period. A mouse strain (101) with a high incidence of spontaneous osteomas did not show a higher sensitivity for osteosarcoma induction as compared to NMRI mice. In addition the sites of radiation-induced osteosarcomas did not correspond with the most frequent sites of the spontaneous osteomas.
- Aspects of tumour promotion in the field of radiation-induced osteosarcoma will be discussed.