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Morphological and Functional Changes in the Rat Heart after X Irradiation: Strain Differences

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The hearts of mature male rats of the Wistar and Sprague–Dawley strains were locally irradiated with single doses of 17.5 and 20.0 Gy of X rays, respectively. These two dose levels had previously been shown to result in a comparable latent period between irradiation and the death of rats of these two strains from cardiac failure. Morphological changes in the myocardium and modifications in cardiac function were assessed in the animals at 28, 70, and 100 days after irradiation. The first radiation-induced change which was observed in the myocardium was a rapid decline in capillary density and a loss of alkaline phosphatase activity by the capillary endothelial cells. The capillary density was reduced to ~50% of that of unirradiated control values at 28 days and to ~40% of the control values between 70 and 100 days after irradiation. The loss of enzyme activity was also detected at 28 days. Examination of histological sections showed an increase by 70 days in the areas with negative enzyme activity up to ~70% of the myocardium. The reduction in capillary density and the loss of enzyme activity occurred before any marked pathological changes were seen in the myocardium. The pathological lesions seen in the myocardium at 100 days after irradiation were qualitatively and quantitatively the same in the two strains of rat. Measurements of cardiac output in Sprague–Dawley rats showed a gradual decline in output after irradiation; however, measurements in Wistar rats showed a progressive increase in cardiac output over the same period of time. It was shown by rubidium extraction that there was an increase in the percentage of the total cardiac output distributed to the ventricular muscle of Sprague–Dawley rats, while similar measurements in Wistar rats showed no significant change. In spite of the marked strain differences observed in cardiac output and rubidium extraction, blood perfusion per gram of ventricular muscle was apparently not modified in both strains of rat after irradiation. These findings indicated that the correlation between morphological effects after irradiation and the functional expression of damage is highly complex.

INTRODUCTION

In the last few years, independent studies have been carried out in laboratories in Oxford (*1, 2*) and Neuherberg (*3–5*) to examine the response of the rat heart to X irradiation. These studies were conducted along slightly different lines: a physiological approach was undertaken in Oxford, while morphological changes were studied in Neuherberg.

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The morphological studies carried out in Wistar rats showed an early reduction in the capillary density of the myocardium after local heart irradiation. A substantial reduction in capillary density was observed before any obvious histological damage to the myocytes was apparent (*3*).

At the Oxford laboratory, sequential assessments of cardiac output in Sprague–Dawley rats showed a gradual but progressive decline in cardiac function after local heart irradiation (*1*). The decline in function was found to be significant from ~16 weeks after irradiation. ECG and histological studies have shown myocardial changes as early as 8 weeks after irradiation in this strain (*2*).

A comparison of the histological changes seen in the myocardium of both strains has demonstrated that the lesions observed after irradiation were qualitatively similar (*2, 3*). Both strains of rat developed focal myocardial degeneration and necrosis with only very slight connective tissue reactions. However, for the same irradiation dose, the severity of the damage observed was greater in Wistar rats than in Sprague–Dawley rats. The time course of development of radiation-induced heart disease also appeared to be different in the two strains: damage appeared earlier in the Wistar rat. In studies undertaken recently in the Neuherberg laboratory, Wistar and Sprague–Dawley rats were irradiated concurrently using the same technique (*4*). The results from these studies showed that the mean latent period between irradiation and death from cardiac failure was ~500 days in both Wistar and Sprague–Dawley rats after single doses of 17.5 and 20 Gy X irradiation, respectively. A mean latent period of ~500 days was also observed in the Sprague–Dawley rats at the laboratory in Oxford that were irradiated with 20 Gy using a slightly different technique (*1*). Irradiation of Wistar rats with 20 Gy shortened the latent period significantly to 205 days. Therefore the single doses of 17.5 and 20 Gy of X rays can be considered to be isoeffective because of the latent period for the development of late radiation-induced damage to the heart.

In view of these interesting findings and their important radiobiological implications, a collaborative study has been undertaken which has formed part of the collaborative program of the European Late Effects Project Group (EULEP). The overall objective of the study is to examine the time- and dose-related morphological and functional changes in the microvasculature of the rat heart and their relationship to late cardiac radiation damage. In addition, it was hoped to establish whether the morphological and functional changes showed the same strain difference as the latent period between local heart irradiation and the death of rats. This paper reports some preliminary findings.

MATERIALS AND METHODS

The experimental protocol for this study is summarized in Table I. Mature male Wistar and Sprague–Dawley rats 14–20 weeks old were used. Animals were irradiated in each laboratory with a single dose of either 17.5 (Wistar rats, Neuherberg) or 20.0 Gy (Sprague–Dawley rats, Oxford) of X rays. The irradiation techniques used in the two laboratories have been described in detail elsewhere (*1, 3*). Briefly, in the Neuherberg laboratory, Wistar rats were irradiated with 300-kV X rays, through a single lateral field, at a dose rate of 2 Gy/min (*3*). Before irradiation the position of the heart was located with the help of a radiograph. At the laboratory in Oxford, Sprague–Dawley rats were irradiated using an anterior–posterior field with two parallel-opposed 250-kV X-ray units, at a dose rate of 1.2 Gy/min (*1*). The heart was located using X-ray fluoroscopy prior to irradiation. A dose variation of ~3% was achieved across the heart. In both

TABLE I

Experimental Protocol for the Study of Radiation Effects in the Heart of Rats of Two Different Strains

	Neuherberg	*Oxford*
Animals:	Male Wistar rats	Male Sprague-Dawley rats
Radiation dose:	17.5 Gy	20.0 Gy
Time of observation:	28, 70,[a] and 100 days	28, 70,[a] and 100 days
Methods:	Quantitative morphology (2 rats of each strain per time)	Functional assay (5–6 rats of each strain per time)
	(i) Volume and length density of the capillary network—point counting using a squared lattice. Alkaline phosphatase as a marker for capillary endothelial cells.	(i) Coronary blood flow to the myocardium—rubidium (^{86}Rb) extraction technique.
	(ii) Percentage area of the myocardium which showed degenerative changes or negative alkaline phosphatase activity.	(ii) Cardiac output—external counting technique with ^{99m}Tc-pentechnetate as the tracer, determined immediately prior to the measurement of myocardial blood flow.

[a] An additional group of unirradiated animals was used as controls, 70 days after sham irradiation.

laboratories, the dose to the heart of each animal was monitored during irradiation using an ionization chamber placed in the beam.

Time-related morphological and functional changes were assessed at 28, 70, and 100 days after irradiation. Similar studies were carried out in a group of unirradiated animals of each strain 70 days after sham irradiation. The measurements of cardiac function were carried out at the Oxford laboratory. Groups of five or six animals of each strain were used at each time point. Wistar rats were sent to the Oxford laboratory and kept there for approximately 1 week before any functional measurements were made. For the morphological studies two animals were used at each time point. Fixation of the tissues of the Sprague–Dawley rats was carried out by perfusion in the laboratory at Oxford, taking advantage of the expertise from the Neuherberg laboratory. The subsequent preparation of histological sections and the morphological studies were carried out in the Neuherberg laboratory.

Quantitative morphology. Details of the perfusion–fixation technique, tissue processing, and the quantitative morphological method have been described previously (*5*). Briefly, at the time of sacrifice, the animals were deeply anesthetized, the thorax was opened, and the descending aorta was cannulated. The heart was fixed by retrograde perfusion with formalin/alcohol (perfusion pressure 130 mm Hg). After fixation, the atria were removed and the ventricles were cut into six blocks. Two or more 0.5-mm slices of ventricular tissue were taken at random from each block. After the slices were embedded into glycolmethacrylate, 4-μm-thick sections were cut, and the alkaline phosphatase of the capillary endothelial cells was selectively stained using the diazo coupling reaction (*6*). For the quantitative morphological assessments, each stained histological section was magnified using a slide projector, and the outline of the tissue sample was traced. The areas of enzyme loss and myocardial degeneration were identified microscopically and were marked on the outline drawing of each specimen. The area of damage was expressed as the percentage of the total area of all samples from the same heart.

The volume density and length density of the capillaries for each histological section were evaluated at a magnification of $\times$1000 by point counting using a squared lattice test grid (*7*). The volume density (V_v)

was defined as $V_v = \Sigma P_{cap}/\Sigma P_{myoc}$, where P_{cap} is the number of test points falling on a capillary lumen or an endothelial cell and P_{myoc} is the number of test points falling on myocardial tissue. It represents the relative volume of tissue occupied by capillaries. The length density (L_v) was given as $L_v = 2\ \Sigma cap \cdot m^2/\Sigma P_{myoc} \cdot d^2$, where cap is the number of capillaries transsected per field, P_{myoc} is the number of test points falling on myocardial tissue, m is the magnification, and d is the distance between test points. It represents the total length of the capillary network per cubic millimeter of myocardium. In the evaluation of the volume and length densities, capillaries were identified by the staining of capillary endothelial cells. For each heart specimen, 80 fields, randomly selected from areas showing positive alkaline phosphatase activities, were used for counting.

Physiological measurements. The nutrient blood flow to the myocardium of the ventricles was assessed using the ^{86}Rb-extraction technique (*8*). Before measurements were undertaken, animals were fasted overnight (~18 h) but were allowed free access to water. Rubidium-86 in the form of ^{86}RbCl (Amersham International) was diluted with saline to a concentration of ~20 μCi/ml. With the animal anesthetized, the femoral vein was exposed and a bolus of 0.5 ml of ^{86}RbCl solution, total activity ~10 μCi, was injected. At 90 s after intravenous injection the animal was killed by decapitation. The heart was dissected out and the atria were removed. The ventricular cavities were drained and rinsed with saline to remove any residual blood. The ventricles were then dried with tissue paper and weighed. The total activity of ^{86}Rb was determined by counting the whole organ in a NaI well-counter. The counter was connected to a multichannel analyzer (Nokia LP4800) set to count for an interval of 40 s. A minimum of 10,000 counts was accumulated over this period. The total dose of ^{86}Rb injected was calculated from counts obtained from a diluted sample of 0.5 ml of ^{86}RbCl solution.

Just prior to the measurement of extraction in ^{86}RbCl cardiac muscle, the cardiac output of each animal was determined using an external counting technique as described previously (*1*). Briefly, a bolus of ^{99m}Tc-pertechnetate was injected into the femoral vein of the anesthetized rat. The activity–time curve was recorded for 40 s over the heart using a NaI detector. This detector was connected to a multichannel analyzer (ND-62, Nuclear Data) operating in the multiscalar mode (0.1-s counting intervals). At the same time, the heart rate of each animal was recorded using a human ECG monitor (Hewlett Packard 78304) coupled to a scope memory (Model VK-12-2, Seltek Instruments Ltd.) and a chart recorder.

RESULTS

Morphological Studies

The time-related changes in the capillary volume density (V_V) and the capillary length density (L_V) in the myocardium of the ventricles of both strains of rat after irradiation are shown in Fig. 1. The material for Sprague–Dawley rats at 28 days was lost due to technical problems. However, the data shown are sufficient to illustrate the similarities in the reduction in the density of capillaries in the two strains of rat that were irradiated with doses that resulted in a comparable latent period between irradiation and the death of animals from cardiac damage (*1, 3, 4*). In the first 28 days after irradiation there was a substantial reduction in both V_V and L_V to ~50 and ~60% of their respective values in unirradiated animals. There was a further slight decline in L_V at later times. Both V_V and L_V seemed to reach a plateau at ~40% of the control values after 70 days.

The time-related changes in the percentage of the myocardium showing a loss of alkaline phosphatase enzyme activity and myocardial degeneration are shown in Fig. 2. Again, the similarities in the responses of rats of the two strains to X-ray doses associated with the same latent period for the development of late cardiac damage are apparent. Areas negative for alkaline phosphatase were found 28 days after irradiation and had increased to ~70% of the total areas after 70 and 100 days. Myocardial degeneration was seen after 70 days. The degenerative changes were very slight and involved <7% of the total area of myocardium examined. The lesions were character-

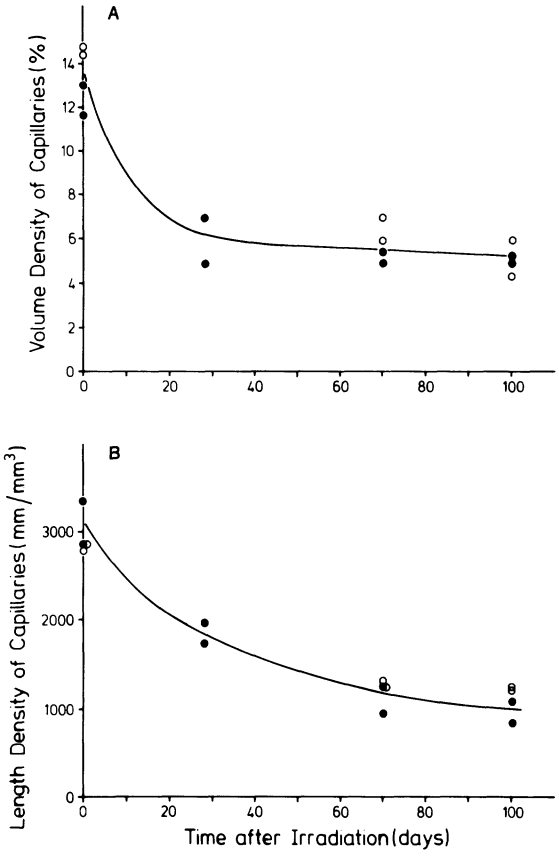

FIG. 1. Time-related changes in (a) capillary volume density and (b) capillary length density in rat myocardium after a single dose of X rays. ●, Wistar rats; ○, Sprague–Dawley rats.

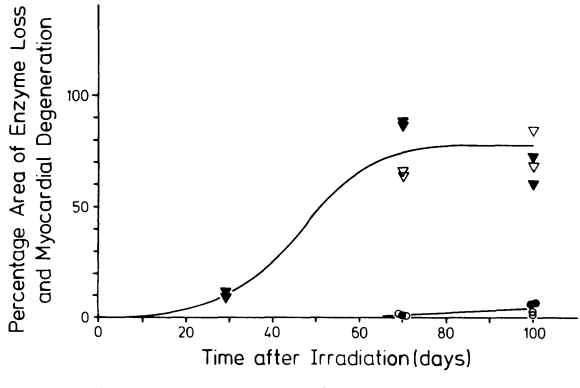

FIG. 2. Time-related changes in the percentage area of enzyme loss and myocardial degeneration after irradiation in two strains of rat. Solid symbols, Wistar rats; Open symbols, Sprague–Dawley rats. ▼,▽, enzyme loss; ●,○, myocardial degeneration.

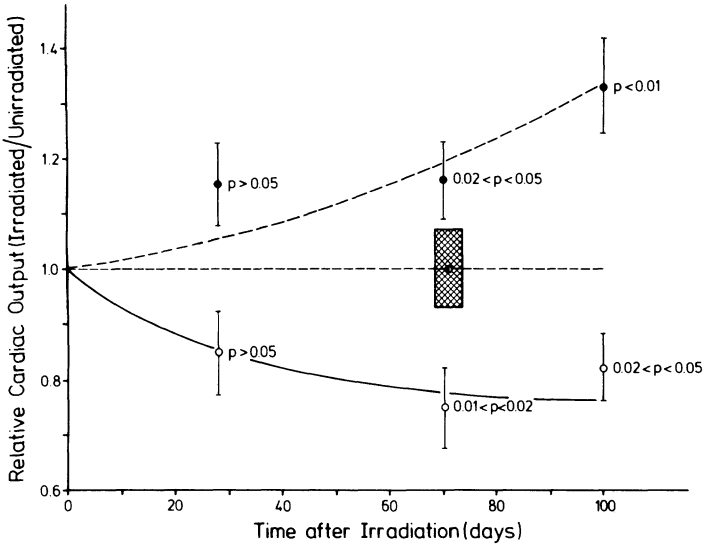

FIG. 3. Variation in cardiac function with time after local heart irradiation. 'P' values shown were calculated according to Student's t test. ●, Wistar rats; ○, Sprague–Dawley rats; ▩, control values ±SE for both strains of rat; error bars indicate ±SE.

ized by the loss of striations in muscle fibers, necrosis, and vacuolation of myocytes. The onset of myocardial degeneration followed the reduction in capillary volume and length density and coincided with the time when areas of enzyme loss had reached a maximum.

Physiological Studies

The time-related changes in the mean relative cardiac output (irradiated/"70 days" unirradiated animals) for both strains of rat after local heart irradiation are shown in Fig. 3. The Sprague–Dawley rats showed a gradual decline in cardiac output with time. The reduction in cardiac output was significant from 70 days after irradiation ($P < 0.05$). The mean cardiac output of these animals at 70 and 100 days was ~80% of that measured in unirradiated animals. The reduction in cardiac output observed in Sprague–Dawley rats was not seen in Wistar rats. The latter showed a gradual increase in cardiac output with time up to 100 days after irradiation. The cardiac output at 100 days was increased by ~30% and was significantly above that of unirradiated animals ($P < 0.01$). For both strains of rat, the mean heart rates of irradiated animals were not significantly different from their respective unirradiated controls ($P > 0.1$).

The time-related changes in the percentage ^{86}Rb extracted per gram of ventricular tissue, E_{Rb}, were also different in Sprague–Dawley and Wistar rats after local heart irradiation (Fig. 4). The E_{Rb} measured in Sprague–Dawley rats was increased to 5.28 ± 0.52% at 28 days after irradiation. The E_{Rb} then decreased but remained significantly higher than the value of 2.58 ± 0.03% for unirradiated animals ($P < 0.01$). For Wistar rats the E_{Rb} for irradiated animals was not significantly different from that of the unirradiated group throughout the period of the study ($P > 0.05$).

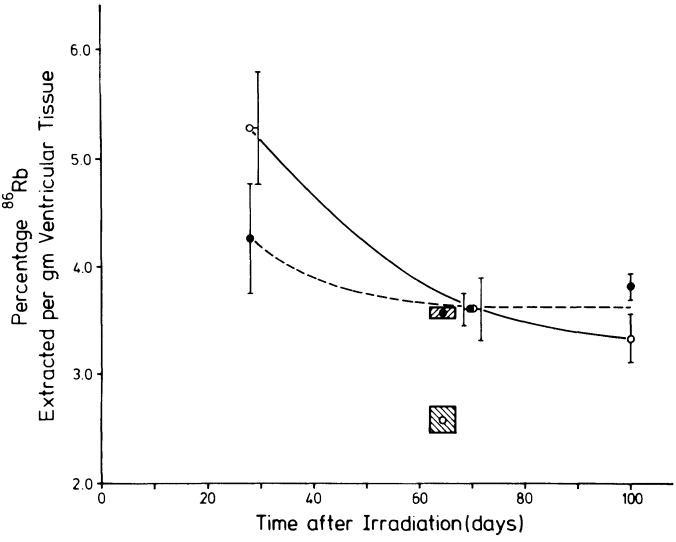

FIG. 4. Variation in the percentage of rubidium extracted, per gram of ventricular tissue after local heart irradiation. ●, Wistar rats; ○, Sprague–Dawley rats. Boxes indicate unirradiated control values for Wistar ▨ and Sprague–Dawley rats ▧ ±SE. Error bars indicate ±SE.

Blood perfusion to ventricular tissue, calculated from the data given in Figs. 3 and 4, showed that relatively normal blood perfusion per gram ventricular tissue (F_v) was maintained in both strains of rat (Fig. 5). For Wistar rats the F_v was not significantly different from that of unirradiated animals ($P > 0.05$), although there was the suggestion of an increased perfusion after 100 days ($P < 0.02$). In Sprague–Dawley rats,

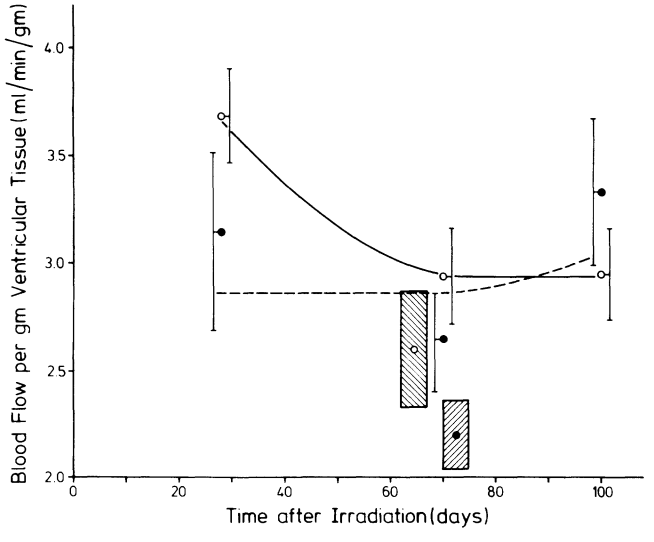

FIG. 5. Time-related change in ventricular blood perfusion after local heart irradiation (for key see legend to Fig. 4).

there was a significant increase in F_v after 28 days ($P < 0.01$). However, at 70 days and 100 days the F_v in irradiated Sprague–Dawley rats was not different from that in the unirradiated group ($P > 0.1$).

DISCUSSION

The present study was designed to examine the morphological and functional changes in the heart of Wistar and Sprague–Dawley rats irradiated with single doses of X rays which resulted in the same latent period between irradiation and the death of rats from heart failure. The morphological changes produced in the myocardium of both strains were qualitatively and quantitatively similar. The first sign of radiation-induced damage was a rapid decline in the density of the capillaries in the myocardium and a loss of the alkaline phosphatase activity of capillary endothelial cells. The reduction in capillary density was substantial (~50%) in the first 28 days, and it appeared to reach an even lower value from 70 to 100 days after irradiation. There were parallel changes in the capillary volume and length densities, suggesting that there was no major modification in capillary shape or diameter. The proportion of the capillaries in the myocardium that are normally perfusing with blood at any one time is uncertain. Measurements of heart function and the appearance of the myocardium in histological sections suggested that this sharp reduction in the number of capillaries at 28 days produced no significant functional changes or pathological lesions in the heart. Nevertheless, the substantial reduction in the capillary density at 28 days after irradiation would be expected to lead to a reduction in the functional reserve of the heart.

The staining of endothelial cells for alkaline phosphatase served two purposes. First, it was used to detect and quantify the loss of enzyme activity occurring within well-defined foci in the myocardium. The enzyme staining could also be used to identify obliquely cut capillaries in enzyme-positive areas for morphometric analysis. Thus the present finding of a reduction in the size of the capillary network was related only to an otherwise unchanged area of the myocardium. However, earlier rough estimations of the capillary density throughout the heart suggested that the areas excluded from the present analysis were at least as severely affected (*5*).

Damage to the myocardial parenchyma, i.e., the myocytes, was seen only after a 50–60% reduction in the capillary density of the myocardium and when there was a loss of alkaline phosphate activity in capillary endothelial cells involving ~70% of the myocardium. With the light microscope the lesions seen in the myocardium were characterized by the loss of striation in muscle fibers, necrosis, and vacuolation of myocytes. The infiltration of inflammatory cells into damaged areas was also seen occasionally. There was only a very slight reaction in connective tissue. These lesions were seen in both strains of rat. Since myocytes are postmitotic cells, this observation suggested that the minor degenerative changes in the myocardium after irradiation of the heart in this study were secondary to the reduction in the size of the capillary network. Similar changes have been reported in the myocardium of dogs (*9*) and of rabbits (*10*) after irradiation.

The results of the morphological studies in mature unirradiated Sprague–Dawley and Wistar rats indicated that there were no obvious significant morphological differences, in terms of both the capillary volume and capillary length densities in the

TABLE II

A Comparison of the Cardiac and Body Weight-Related Factors in Mature Sprague–Dawley and Wistar rats

Parameter	*Sprague–Dawley*	*Wistar*	P[a]
Cardiac output (ml/min/kg)	202.4 ± 11.8	170.4 ± 8.0	$P < 0.05$
Heart rate (beats/min)	375 ± 24	310 ± 29	$P < 0.05$
Percentage rubidium extracted per gram ventricular tissue (%/g)	2.58 ± 0.14	3.57 ± 0.18	$P < 0.01$
Blood perfusion per gram ventricular tissue (ml/min/g)	2.58 ± 0.22	2.20 ± 0.16	$P > 0.1$
Weight of ventricles (g)	1.141 ± 0.023	0.798 ± 0.024	$P < 0.001$
Body weight (g)	493.7 ± 11.7	360.9 ± 9.9	$P < 0.001$
$\frac{\text{Weight of ventricles}}{\text{Body weight}}$ (%)	0.231 ± 0.004	0.221 ± 0.002	$P > 0.05$

[a] Student's t test. Values shown are the means of six animals.

myocardium. However, consistent strain differences were observed in the weights of the hearts and parameters of cardiac vascular function in unirradiated animals of comparable age (Table II). The mean body weight and the mean weight of the ventricles showed that Sprague–Dawley rats were physically larger than Wistar rats. However, when the weight of the ventricles was expressed as a percentage of the total body weight, the two strains were remarkably similar ($P > 0.05$): the ventricles were ~0.2% of the total body weight. The mean cardiac output of unirradiated Sprague–Dawley rats was slightly but significantly higher than that of Wistar rats ($P < 0.05$). This could be attributed to the slightly higher heart rate of the Sprague–Dawley rat. The percentage of the cardiac output distributed to the ventricles, as indicated by the percentage of rubidium extracted per gram of ventricular tissue, E_{Rb}, was significantly higher in Wistar rats than in Sprague–Dawley rats ($P < 0.01$). However, when the ventricular blood perfusion was expressed in ml/min/g of ventricular tissue, there were no significant differences between the two strains of rat ($P > 0.1$). The differences in cardiovascular parameters listed above, which nevertheless resulted in a comparable resting blood perfusion rate for the myocardium in the two strains of rat, might provide an explanation for the observed variations in the cardiovascular function of the heart of Wistar and Sprague–Dawley rats after local irradiation.

The heart is in continuous activity, and because of this almost all the available oxygen in the coronary arterial blood is extracted by the muscle cells. The overall effect is that the myocardium, unlike skeletal muscle, cannot counteract for an oxygen debt. Thus an adequate blood supply to the cardiac muscle needs to be maintained for the heart to remain active. Measurements of the blood perfusion of the ventricles, F_v, in unirradiated Sprague–Dawley and Wistar rats of a similar age have been shown to be the same. After irradiation, when the size of the capillary bed was reduced, the cardiovascular system of both strains of rat adapted to restore the blood perfusion of the ventricles to relatively normal levels. This was apparently achieved in a slightly different way in each strain. In Sprague–Dawley rats, to compensate for the reduction in the capillary density of the myocardium, blood perfusion to the

ventricles was maintained by an increase in the percentage of the cardiac output distributed to the heart. This occurred despite a slight reduction in the cardiac output after irradiation. However, in Wistar rats the blood perfusion of the myocardium was maintained by an increase in the cardiac output. These findings seemed to indicate that it was the functional parameter that was lowest in irradiated animals (Table II) that was increased after irradiation. The mechanisms responsible for these differences remain uncertain.

These differences in physiological adaptation by the cardiovascular system to compensate for the same radiation-induced reduction in the size of the capillary bed of the myocardium in the two strains of rat were unexpected. There were no significant changes in the body weight in these two strains throughout the course of study. Thus differences in radiation response as indicated by the changes in cardiac output, which is measured in ml/min/kg, could not be attributed simply to the changes in body weight after irradiation. They reflect a true difference in the cardiac output of the heart in these two strains after irradiation. In fact, the changes in cardiac output observed in Sprague–Dawley rats and Wistar rats were mediated by a corresponding change in the stroke volume, since the heart rates of irradiated animals were not significantly different from those of unirradiated animals over the period of the study.

The proportion of the cardiac output that is distributed to the heart in irradiated and unirradiated animals was measured using the rubidium extraction technique (*8*). It has been shown that the initial distribution of radioactive rubidium after an intravenous injection is proportional to the regional blood flow (*8*). There is then a period of 6–90 s over which there is a plateau level of activity in the tissue before the radioactive rubidium gradually becomes uniformly distributed throughout the potassium pool within the body. During the initial plateau period, the ratio of the organ uptake of rubidium to the total radioactivity administered will be equal to the fraction of the cardiac output that perfuses that organ. The validity of the extraction technique applied to the measurement of regional blood flow is dependent on the permeability of the vasculature to the tracer. The extraction time should be extremely short in comparison with the blood transit time through the capillary bed; i.e., the tracer is said to be freely diffusible. It has been suggested (*11*) that the permeability of the blood vessel wall may be limited, even for a freely diffusible tracer, and hence extraction may not be directly proportional to flow when blood flow is modified. This may be particularly true when the blood transit times are greatly increased and approach the tracer extraction time. Changes in permeability of the vasculature to rubidium after irradiation were not detected in the mouse (*12*). Thus this is unlikely to be a factor under the conditions found in the present series of experiments, since extraction was increased only in Sprague–Dawley rats. This increase was small and only to within the range normally found in Wistar rats, i.e., ~4% per gram of ventricular tissue. This may represent an upper limit of extraction and hence indicate why the cardiac output must be increased in Wistar rats in order to preserve relatively normal perfusion in this rat strain when the size of the capillary bed was reduced. The success of the compensatory mechanisms in both strains of rat was reflected in the very minor focal pathological lesions that had developed after 100 days, given the marked reduction in the vascularity of the myocardium. The relationship of the present findings in the first 100 days after irradiation to the eventual development of late effects and

the death of animals of both strains from cardiac failure after a latent interval of ~500 days is uncertain.

Recently it has been shown that the cardiac output in Sprague–Dawley rats continues to decline with time after irradiation until death (*1*); however, the time at which myocardial perfusion is significantly decreased is unknown. No similar information is available for Wistar rats. From this study, it is not clear whether the cardiac output in Wistar rats remains elevated throughout the life span of the animals after irradiation. Thus the contribution of the differences in physiological adaption to the development and to the clinical expression of cardiac injury in the two strains of rat after irradiation and its effects on the peripheral systems must be investigated.

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