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DOSE TO THE SHIELDED THYMIC REGION OF IRRADIATED MOUSE CADAVERS

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The use of lead shields in radiation biology experiments poses the question of the radiation-protecting efficiency of the shielding geometry. Several authors have discussed the relevancy of a shielding arrangement and a number of devices have been developed to ensure the required protective effect (CARSTEN & NOONAN 1959, CORP et coll. 1961, BLOMGREN & RÉVÉSZ 1968, CARSTEN & CRONKITE 1971, RAUCHWERGER 1972, JÄRPLID 1972, BOJTOR et coll. 1974). The generally used shielding arrangements give satisfactory protection against the primary beam only. The dose from scattered radiation to the shielded organs may, however, be a factor to be considered in biologic conclusions (CORP et coll., BOJTOR et coll. 1974).

Recently biologic experiments were performed concerning the mechanism of thymic involution and regeneration in cortisone-treated mice (HIESCHE et coll.). The experimental approach comprised the application of lead shields of various shapes and sizes to the thymic area of cortisone-treated mice during exposure with roentgen rays. In the course of the experiments it became desirable to obtain information about the radiation-protecting efficiency of the shielding arrangements.

This report presents data from dose measurements made with thermoluminescent dosimeters in the shielded thymic region of irradiated mouse cadavers.

Experimental method

Preparation of mice. Female CBA mice of about 20 g weight were given a heavy ether anesthesia. They were then rapidly fixed onto a plastic sheet—lying on their

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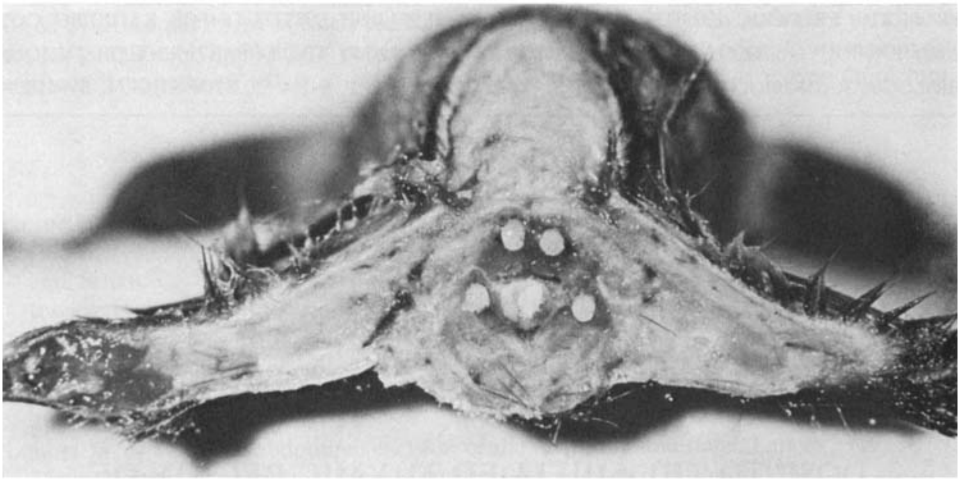

Fig. 1. Cross section of a frozen mouse with the TL rods loaded into the thymic lobes, near tissues and vertebra.

backs with legs extended—and plunged into a dry ice-acetone fluid mixture. By this method of deep freezing the native status of the mice could be retained at the expense of a slight loss of water content of the body (NIKL 1969). The bodies were then sawn up vertically at the upper limit of the sternum and at the position of the ilium. Five holes were drilled into the chest segment at the position of the thymic lobes, the vertebra, and the tissues at 4 mm distance at both sides from the vertebra. Three holes were made at 6 mm depth in the abdominal segment. Each hole was loaded with one piece of 6 mm × 1 mm LiF-Teflon rod (Fig. 1).

Irradiation geometry. Five frozen mouse cadavers were placed radially onto a 3 mm thick perspex plate of 23 cm diameter with their heads towards the center. To cover the thymic area, 4 mm thick and 18 mm × 24 mm or 12 mm × 12 mm lead plates were used (Fig. 2). The bodies were uniformly exposed to 200 R at 80 cm focus—surface distance. The exposure was continuously measured on the plate by a Farmer 0.3 cm^3 ionization chamber. Before irradiation the uniformity of the exposure rate was checked along the fictitious longitudinal axis of the animals, and the inhomogeneity within the radial distance occupied by an animal was found to be less than ± 1.5 per cent.

Radiation qualities. Radiation was generated using a Medicor THX 250 roentgen machine. It was operated at 200 kV, 15 mA with 0.5 mm Cu total filter.

In order to perform the calibration of the rods and the R/Gy conversion adequate to the proper radiation energies, an estimate was made of the radiation qualities outside and under the shield. The HVL of the incident primary beam was measured as

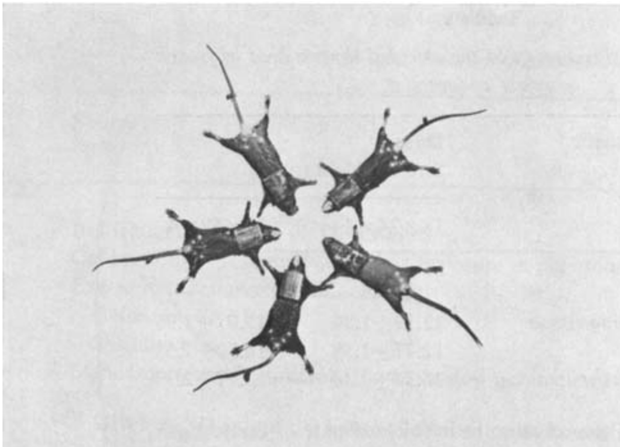

Fig. 2. Irradiation arrangement of the mouse cadavers supplied with 18 mm × 24 mm × 4 mm lead shields.

1.2±0.042 mm Cu, which corresponds to approximately 84 keV effective energy (JAEGER & HÜBNER 1974). Since the spectral composition of the scattered photons under the shield cannot be determined easily by measurement in the very small volume of a mouse (PUITE & CREBOLDER 1974), one is confined to approximate data from phantom measurements.

In the experiments, the radiation qualities under and outside the shields were calculated by applying the spectral distribution data reported by SKARSGARD & JOHNS (1961), BRUCE & JOHNS (1960) and HETTINGER & LIDÉN (1960). It follows from their results that in exposure of a mouse to deep roentgen rays, the effective energy of total —incident and scattered—radiation does not differ markedly from that of the incident, primary beam up to a few mm depth. Therefore, 77 keV was accepted for the effective energy of total radiation in the unshielded body. Considering the dose distribution from scattered radiation and the weighted means of scattered photon intensities at different energy intervals of the incident, primary beam, it follows that the photons occurring under the shield fall mostly in the 35 to 80 keV interval. Thus, 55 keV was taken as the effective energy of scattered radiation under the shield.

Calibration. A set of LiF-Teflon rods was annealed and selected and individually calibrated for 10 and 100 R of 84 keV roentgen rays. (No dose dependence was experienced in the individual factors.) For the calibration of TL response vs exposure, a teflon phantom of volume 22 cm³ was used. The exposure in the phantom was measured by a Farmer 0.3 cm³ thimble chamber attached to Dosemeter 2502/3. Before use, the chamber was calibrated in the proper energy interval at the National Office of Measures, Budapest, Hungary. The chamber and 10 rods were positioned at 4 mm depth in the phantom and exposed to 10, 50, 100, 200 and 250 R of 84 and 55 keV roentgen rays. The rods were read out using a 7100 TS reader (Teledyne

Table 1

Dose from scattered radiation to the shielded thymic area of frozen mouse cadavers*

Position of measurement	Dose Size of shield (mm^3)	
	$18 \times 24 \times 4$	$12 \times 12 \times 4$
Thymic region:		
Lobes + surrounding tissue	13.19 ± 1.84	17.07 ± 1.98
Thymic lobes	12.77 ± 1.58	16.70 ± 1.53
Vertebral bone marrow	18.34 ± 2.44	23.77 ± 2.79

* Expressed as per cent of dose in unshielded part of body. Each value is the mean ± SD. The SD includes the statistical errors from the sources listed in Table 2.

Isotopes). The TL response due to 55 keV roentgen rays showed a 5.3 per cent increase relative to that obtained at 84 keV, in agreement with the calculated sensitivity for LiF (CAMERON et coll. 1968).

Instead of plotting the TL response (Y) against exposure (X) as calibration curve, the corresponding equation $Y = a_0 + a_1X$ (where a_0 = regression constant and a_1 = regression coefficient) was calculated by linear regression in regard to both 55 keV and 84 keV roentgen rays. The regression line fitted the experimental points with correlation coefficient $r = 1.000014$. In experimental irradiations the exposure, X_i, received by an individual rod, was calculated by solving the calibration equation.

Results and Conclusions

Dose measurements. In two repeat experiments, mouse cadavers were exposed to 200 R roentgen rays under the conditions described. To investigate the possibility of calculating the mean from the total number of measurements obtained in the same position of the animals, the difference between the means of the TL responses of individual animals within a group was tested for significance. The q-test was applied at the following parameters: the number of the means, $k = 10$; the number of the measurements in each sample within a group, $n = 3$ (reference) or $n = 2$ (thymic lobes); the degrees of freedom for pooled variance (Σn_i-k), $df = 20$ (reference) or $df = 10$ (lobes). The q-values proved that no significant difference existed between the means of the TL responses of individual animals even at $p = 0.1$ level. Consequently, the evidence pointed toward acceptance of a calculation of the mean from the total number of measurements.

To convert exposure to dose absorbed in tissues of the thymic or abdomen region, the Gy/R factors of 0.928×10^{-2} (55 keV) and 0.943×10^{-2} (77 keV) were calculated

Table 2

Statistical errors in measurements

Source	Relative error* (per cent)
Individual calibration of TL dosimeters	±2.2
Calibration of TL response against exposure in phantom	±1.3
Exposure measurements in animals	
Reference	±1.3 to ±3.1
Shielded area	±3.4 to ±4.1
Standard error of estimating the regression parameters	±0.7

* Given as SD except the figure of the last row where SE is indicated.

and used (SINCLAIR 1969). The dose to the vertebral bone marrow was calculated approximately assuming that the marrow cavities are spherical with a diameter of 200 μm. Thus, the factor of 1.31×10^{-2} was used for conversion (SPIERS 1969).

The results are summarized in Table 1. The dose in the shielded thymic area is expressed in per cent of that measured at 6 mm depth in the unshielded abdomen. In two series of experiments the dose in the abdomen was 1.6158 ± 0.1071 and 1.6675 ± 0.0767 (mean ± SD) Gy, using the larger and the smaller shield, respectively, to cover the thymic area. The mean percentage dose under the shield was found to be markedly increased in the vertebral bone marrow as compared to in the thymic lobes and the surrounding soft tissues. An increase in mean percentage dose also accompanied a change from the larger to the smaller size shield.

When evaluating the dose to the thymic lobes and the dose to the tissues near the vertebra separately from each other, it should be noticed that, due to the scatter conditions in the very small volume of a mouse, no significant difference could be detected between them. For this reason it appears to be more realistic to speak about dose to the thymic region rather than to the lobes and tissues within this volume.

Precision of the measurements. Several sources of uncertainties in measuring methods exist. The most remarkable components among these are statistical errors coming from the dosimetry method applied (in the present case the dispersion of TL responses) and from the number of measurements. The statistical errors calculated are listed in Table 2. In the second column the standard deviations are indicated, except for the regression, where the standard error of the estimated parameters is given. The total inaccuracy owing to calibration procedure of the TL dosimeters was calculated to be ±3.5 per cent which includes the standard deviation of both the individual factors and the readout at various exposures.

The uncertainty of calibrating the ionization chamber was ± 3 per cent. For the inexactitude in R to Gy conversion, ± 2.5 per cent may be taken, if it is taken into consideration that the spectral distribution of scattered photons ranged from 35 to 80 keV. These factors of uncertainty were left out of consideration because they do not influence relative results.

For calculating the total inaccuracy, the additive rule of relative errors was employed (Rényi 1962). The percentage dose from scattered radiation in the shielded region was determined with an inaccuracy of at most ± 14 per cent, which includes the relative errors of both the reference and scattered dose measurements.

Conclusions. Given the present experimental conditions, the results allow the following conclusions: (1) In the irradiated mouse cadavers, the dose from scattered radiation in the organs and tissues of the shielded thymic area is relatively high as related to the dose in the unshielded abdomen, and it should, therefore, be considered a factor of biologic importance; (2) When the area of the lead shield is reduced by a factor 2/3, the dose from scattered radiation to the protected organs is markedly increased; (3) According to expectation, the dose from scattered radiation is not proportional to the size of the shielded area.

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SUMMARY

Frozen mouse cadavers were exposed to 220 kV roentgen irradiation with the thymic area covered with a lead plate sized 18 mm × 24 mm × 4 mm or 12 mm × 12 mm × 4 mm. LiF-Teflon rods were used for dose measurements. With the larger plate the mean dose from scattered radiation, in per cent of that in the unprotected abdomen, was 12.77 (thymic lobes) and 18.34 (vertebra). With the smaller shield the figures were markedly increased, 16.70 and 23.77, respectively. Since the percentage dose is relatively high, the amount of scattered radiation may have biologic significance.

ZUSAMMENFASSUNG

Gefrorene Mäuse-Kadaver wurden mit 220 kV Röntgenstrahlen mit einem Schutz des Thymusgebietes durch eine Bleiplatte von einer Grösse von 18 mm × 24 mm × 4 mm oder 12 mm × 12 mm × 4 mm bestrahlt. LiF-Teflon-Stäbchen wurden zu den Dosismessungen verwendet. Bei der grösseren Platte war die Mitteldosis durch Streustrahlung in Prozent des nicht-geschützten Abdomens 12,77 (Thymuslappen) und 18,34 (Vertebra). Mit der kleineren Abschirmung erhöhten sich die Ziffern markant auf 16,70 bezw. 23,77 Prozent. Da die prozentuelle Dosis relativ hoch ist, kann die Menge der Streustrahlung biologische Bedeutung haben.

RÉSUMÉ

Des cadavres congelés de souris ont été exposés á l'irradiation roentgen de 220 kV, la région thymique étant couverte avec une plaque de plomb mesurant 18 mm × 24 mm × 4 mm ou 12 mm × 12 mm × 4 mm. Des baguettes de Teflon-LiF ont été utilisées pour les mesures de doses. Avec la grande plaque, la dose moyenne provenant du rayonnement diffusé en pourcentage de la dose á l'abdomen non protégé a été de 12,77 (lobes thymiques) et 18,34 (vertèbres). Avec la petite plaque, ces nombres sont notablement augmentés (respectivement 16,70 et 23,77). Etant donné que le pourcentage de dose est relativement élevé, la quantité de rayonnement diffusé peut avoir une importance biologique.

REFERENCES

BLOMGREN H. and RÉVÉSZ L.: The effect of bone marrow protection on the cellular composition of irradiated mouse thymus. Exp. Cell Res. 53 (1968), 261.

BOJTOR I., HIESCHE K. D. and SCHNELL P. O.: Dose from scattered roentgen radiation to a protected part of a mouse phantom. Acta radiol. Ther. Phys. Biol. 13 (1974), 32.

BRUCE W. R. and JOHNS H. E.: The spectra of X-rays scattered in low atomic number materials. Brit. J. Radiol. (1960) Suppl. No. 9, p. 40.

CAMERON J. R., SUNTHARALINGAM N. and KENNEY G. N.: Thermoluminescent dosimetry. Chap. 4, p. 37. The University of Wisconsin Press, Madison, Milwaukee, London 1968.

CARSTEN A. L. and CRONKITE E. P.: Comparison of autologous marrow injection to shielding in lethal irradiation of the mouse. Proc. Soc. exp. Biol. 137 (1971), 948.

— — and NOONAN T. R.: Recovery from partial body X-irradiation as measured by the lethality of two exposures. Radiat. Res. 11 (1959), 165.

CORP M. J., LAW A. W. and MOLE R. H.: Abdominal irradiation and the thymus of the adult rat. The relative importance of the adrenal glands and of scattered radiation. Amer. J. Roentgenol. 85 (1961), 550.

HETTINGER G. and LIDÉN K.: Scattered radiation in a water phantom irradiated by roentgen photons between 50 and 250 keV. Acta radiol. 53 (1960), 73.

HIESCHE K. D., NIKL I. and BOJTOR I.: Thymic shielding in irradiated mice. An attempt at characterization of the intrathymic 'stem' cell. To be published in Acta radiol. Ther. Phys. Biol.

JAEGER R. G. und HÜBNER W.: Dosimetrie und Strahlenschutz. 2nd edition, p. 170. Georg Thieme Verlag, Stuttgart 1974.

JÄRPLID B.: Thymic shielding in irradiated mice. Acta radiol. Ther. Phys. Biol. 11 (1972), 536.

NIKL I.: The use and comparative study of dose measurement media in animal experiments. Acta biochim. biophys. Acad. Sci. hung. 4 (1969), 201.

PUITE K. J. and CREBOLDER D. L. J. M.: Energy dependence of thermoluminescent dosemeters for X-ray dose and dose distribution measurements in a mouse phantom. Phys. in Med. Biol. 19 (1974), 341.

RAUCHWERGER J. M.: Radiation protection by tibia-shielding in adult, weanling and suckling mice. Int. J. Radiat. Biol. 22 (1972), 269.

RÉNYI A.: Wahrscheinlichkeitsrechnung. Chap. III. Deutscher Verlag der Wissenschaften, Berlin 1962.

SINCLAIR W. K.: Radiobiological dosimetry. *In* Radiation dosimetry, Vol. 3, p. 624. Edited by F. H. Attix and E. Tochilin. Academic Press, New York 1969.

SKARSGARD L. D. and JOHNS H. E.: Spectral flux density of scattered and primary radiation generated at 250 kV. Radiat. Res. 14 (1961), 231.

SPIERS F. W.: Transition-zone dosimetry. *In:* Radiation dosimetry. Vol. 3, p. 840. Edited by F. H. Attix and E. Tochilin. Academic Press, New York 1969.