Morphological Changes in Thymus of Rats Following Whole-Body Exposure to Massive Doses of Radiation.* (22053)

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During the course of studies on the biochemical effects of ionizing radiation we observed that more thymic tissue was present in young rats after exposure to over 10,000 r of X-radiation than in comparable animals which had received lesser dosages. Following this observation, a study was made of the histological changes in thymus glands of rats given massive doses of radiation and attempts were made to elucidate the nature of the response. The present communication contains the results of experiments which have clearly shown that massive, whole-body irradiation induces only minimal cellular destruction in the thymus, in contrast to the more pronounced cytological changes that follow exposure to smaller dosages. In addition, they provide evidence to indicate that exposure of the head region plays an important role in the pathogenesis of the paradoxical cellular response in the thymus.

Materials and methods. For the studies the following general plan was used. Randomly selected groups of 5 to 10 rats were exposed over the entire body to X-ray or gamma radiation in doses between 400 and 30,000 r. The animals of these and all subsequent groups were sacrificed 24 hours after the completion of the exposures. The thymus, spleen, and adrenal glands were weighed, fixed in 10% formalin or Bouin's solution and, together with a number of pituitary glands and brains, were studied in detail by histological means. In a study of the localization of the tissues that govern the thymocytic response, groups of rats were exposed locally over the mediastinal region to graded doses of radiation and other groups of animals were given radiation over the body with the head shielded. To investigate the temporal aspects of this biological response, groups of rats were first given a small dose of total-body radiation and at varied times thereafter were re-exposed to massive doses. Twenty-five normal rats, together with groups of anesthetized and non-anesthetized irradiated animals were sacrificed for control purposes. Young female rats of the Sprague-Dawley strain were used. All were approximately 3 months of age and had weights of 200 ± 20 g. They were maintained throughout the experimental period in air-conditioned quarters with free access to Rockland rat pellets and water. X-radiation was administered at a rate of 320 ± 20 r/min. by a Keleket Therapy Unit operated under the following conditions: 250 KV, 15 ma, and filtration of 0.25 mm of Cu and 1.0 mm Al. Gamma radiation was administered from a Co^{60} source at a rate of 1,000 ± 50 r/min. To obtain localized exposure of the thymic area, the animals were anesthetized (sodium pentobarbital, 35 mg/kg) and irradiated within a 0.25-in. thick lead cylinder which had a single 1.0 by 0.5 in. aperture over the mediastinal region. To restrict gamma radiation to the body, the head was shielded with 0.62 in. of lead and the rays were collimated through concrete. There was less than 25% scatter of radiation to the head, with the highest dosage. The radiation fields and dose rates were calculated in air with phantoms in position. Measurements were checked with a high range Co^{60} rate meter, a 2,500 r Victoreen thimble chamber, $FeSO_4$, and one- and 2-phase chemical dosimeters.

Results. Single-dose, massive, whole-body irradiation. The mean weights of the thymuses of groups of rats at 24 hours after total-body exposure to doses of X-radiation from

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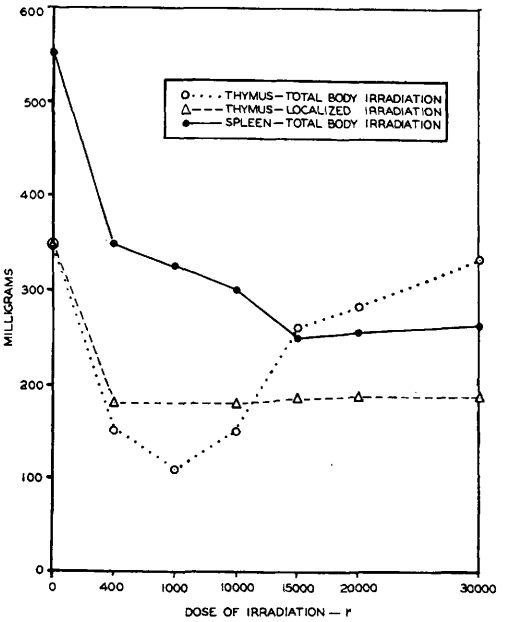

FIG. 1. Thymic and splenic weights in rats 24 hr after X-radiation of entire body and localized to mediastinal area. Each point is mean wt of organs from 5 to 10 animals.

400 to 30,000 r are shown graphically in Fig. 1. Following exposure to 400 r the thymus decreased within a 24-hour period to less than one-half its normal weight (mean weights, 361 to 149 mg). The weight loss was slightly greater after exposure to 1000 or 10,000 r. However, the administration of doses greater than 10,000 r was regularly followed by significantly less weight loss, with 30,000 r inducing only a 35 mg alteration in the mass of the thymus. A very close correlation existed between the weights of the thymuses of rats exposed to gamma radiation and those of animals administered the same quantity of X-radiation (Fig. 2). By contrast, the weight loss that was incurred in the spleens of these animals was regularly marked after all dosages, while the weights of the adrenal glands were not altered significantly from the normal. The weights of the thymus in anesthetized and nonanesthetized control groups were essentially identical.

In histological sections, the thymic tissues of rats exposed over the entire body to massive doses of X- or gamma radiation showed a conspicuous preservation of lymphocytes as compared with tissues of animals exposed to lesser dosages (Fig. 3). The preservation of cells was more evident in the cortex than in the medullary regions. In general, a very close correlation existed between the degree of cellular destruction, the width of the cortical layer, and the weight of the gland. The nuclei of many of the lymphocytes were pyknotic; nevertheless, the nuclear membranes remained intact and the chromatin material was particulate, being neither fused nor fragmented. Although karyorrhexis and phagocytosis were prominent features in the spleens of the same animals and in animals exposed to smaller dosages, they were inconspicuous or absent in the thymuses of rats exposed over the entire body to massive doses (Fig. 4). The adrenal and pituitary glands, as well as the central nervous system, with the exception of the granule cells of the cerebellum, showed no consistent morphological alteration attributable to radiation, the findings differing from those previously observed in monkeys(1).

Studies of the mechanism. When the mediastinal region of rats was exposed to localized X-radiation, the degree of cytological change

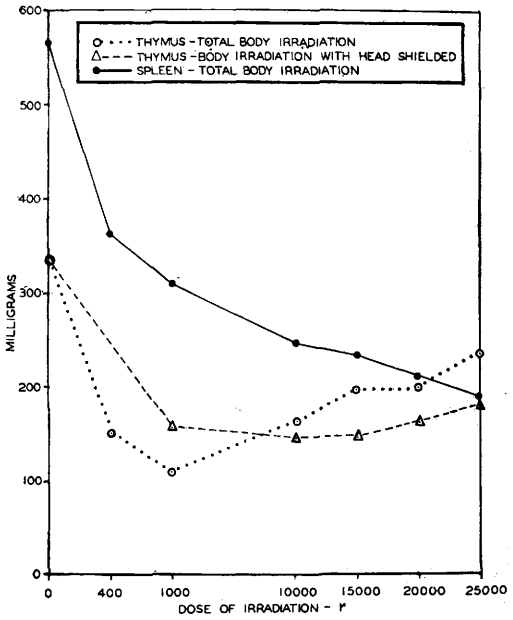

FIG. 2. Thymic and splenic weights in rats 24 hr after gamma radiation of entire body and localized to the body with the head shielded. Each point is mean wt of organs from 5 to 10 animals.

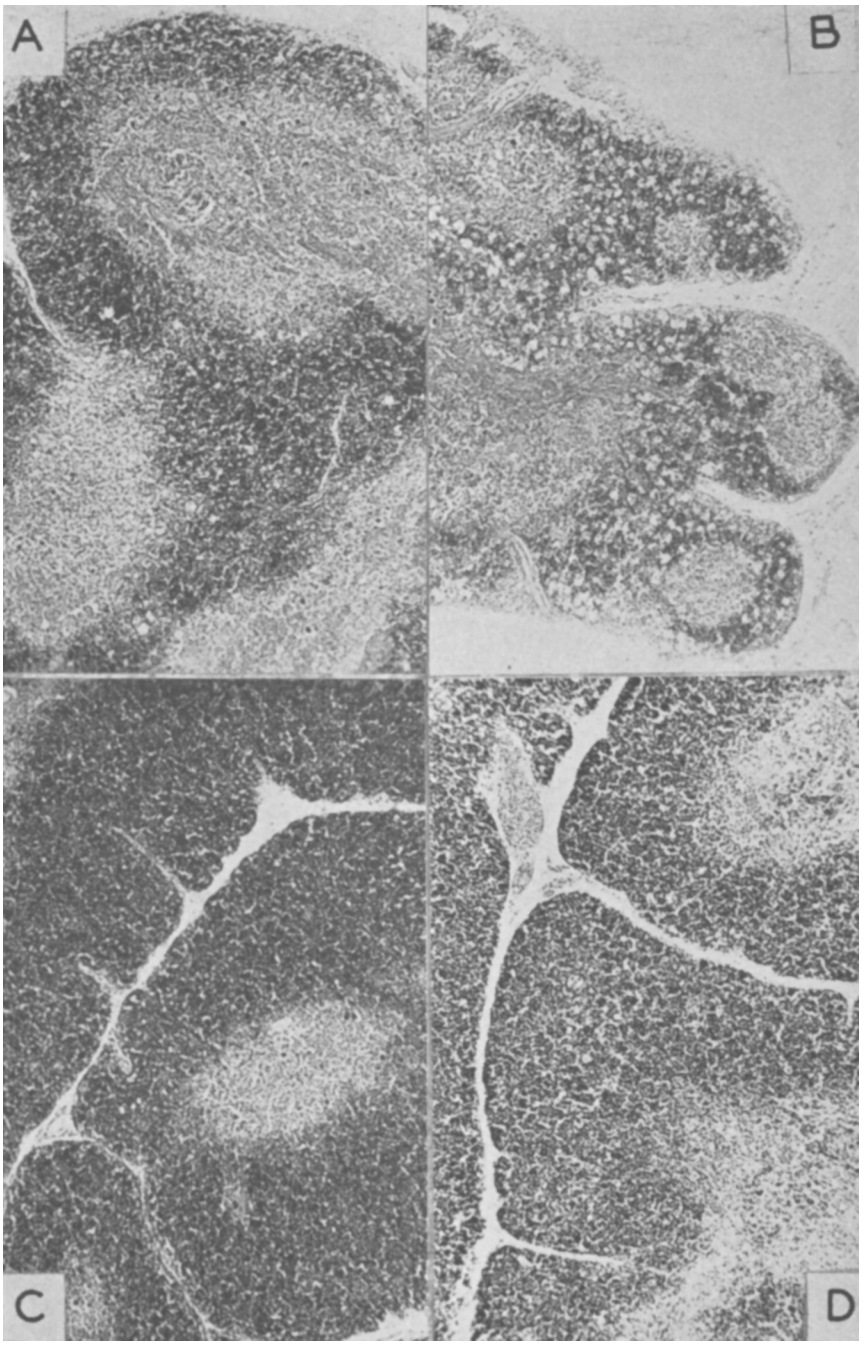

FIG. 3. Thymus of rats 24 hr after total-body exposure to X-radiation. Hematoxylin and eosin stain. × 50. A. (400 r exposure.) Cortex is narrowed, markedly depleted of lymphocytes, and has an avg thickness of .16 mm (normal = .52 mm). B. (10,000 r exposure.) Cortex is almost devoid of lymphocytes and has an avg thickness of .14 mm. C. (20,000 r exposure.) Cortex is of approximately normal thickness and is densely populated with lymphocytes. It measures on the avg .49 mm across. D. (30,000 r exposure.) Cortex is highly cellular and measures approximately .50 mm in width.

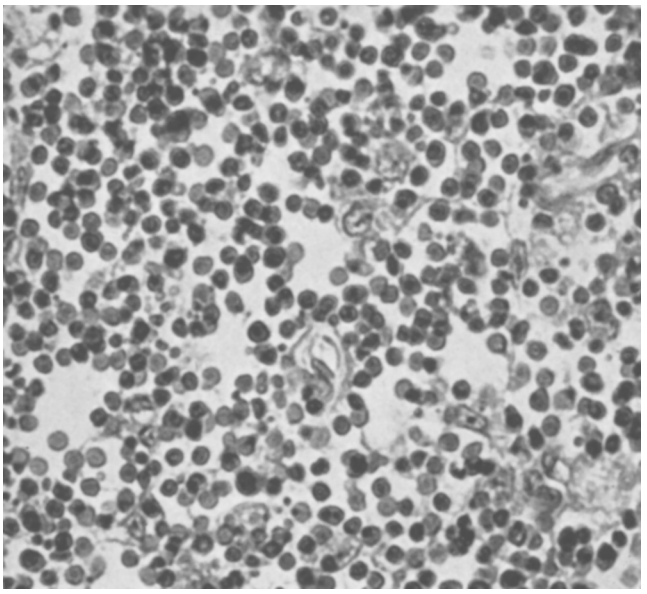

FIG. 4. (Higher magnification of Fig. 3D.) Lymphocytes in cortex are approximately normal in concentration although many are pyknotic. Karyorrhexis and phagocytosis are inconspicuous. Hematoxylin and eosin stain. × 800.

and weight loss in the thymus was essentially constant after all dosages above 400 r (Fig. 1). This finding provides evidence that the cytological response of the thymic lymphocytes to massive, whole-body irradiation is modified by the exposure of tissues outside the mediastinal area.

When the body alone was exposed to high intensity radiation with the head shielded, the degree of cytological destruction and weight loss in the thymus was significantly increased as compared with that induced by total-body exposure to the same dose of radiation (Fig. 2). This finding suggests that, during massive whole-body irradiation, exposure of the head plays an important role in the pathogenesis of the unusual cellular response in the thymus.

In groups of rats exposed to 400 r of whole-body radiation and then re-exposed within one hour to 19,600 r, the weight loss and cytological change in the thymus approximated that in animals exposed to a single, uninterrupted dose of 20,000 r. When the interval between exposures was lengthened to 6 hours, the weight loss in the thymus was markedly increased and equalled or exceeded that in animals exposed to a single dose of 400 r (Fig. 5).

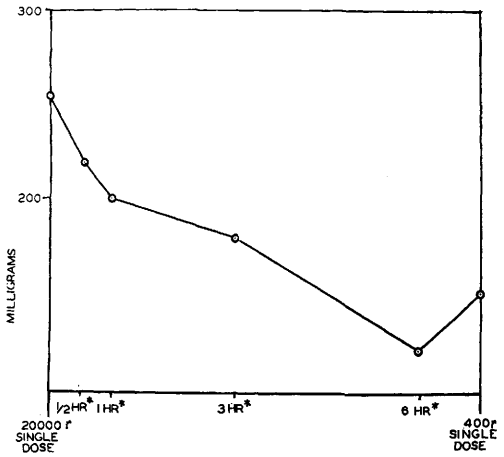

FIG. 5. Thymic weights in rats 24 hr after divided doses of 20,000 r of total-body X-radiation. Each point is the mean wt of organs from 5 to 10 animals. Asterisk indicates time lapse between administration of an initial dose of 400 r and a subsequent dose of 19,600 r.

Summary. The weight loss and degree of cytological alteration in the thymuses of rats was less after whole-body exposure to quantities of X- or γ radiation above 10,000 r than after exposure to smaller dosages. Administration of 10,000 to 30,000 r of localized radiation to the thymic region, or to the body with the head shielded, was regularly followed by

significantly greater cellular destruction and weight loss in the thymus than occurred after whole-body exposure to the same dose of radiation. Whole-body re-exposure to a massive dose of radiation within one hour after an initial total-body exposure to a small dose was regularly followed by less weight loss and cellular destruction in the thymus than occurred after total-body exposure to the small dose alone. The findings show that exposure of the head during massive whole-body irradiation markedly influences the cellular response in the thymus.

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Effects of Oxidized Fatty Acids on Ascites Tumor Metabolism. (22054)

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The unsaturated fatty acids of cells and mitochondria have been shown to be oxidized by ultraviolet light(1,2) and by aerobic incubation(3). The oxidation products of these fatty acids quite probably alter cellular activities as indicated by the findings that the unsaturated fatty acid esters, methyl linolenate and methyl linoleate, inhibit the activity of certain oxidative enzymes in homogenates and mitochondria(2,3); depolymerize deoxyribonucleic acid (DNA)(4); and inhibit cell division(5,6). The relation of oxidized fatty acids to enzyme action is further examined in the present study of the action of oxidation products of linolenate and linoleate on the respiration and glycolysis of Ehrlich ascites tumor cells.

Methods and materials. Ehrlich ascites tumor growing in A-strain mice was taken from the abdominal cavity 7 days after injection. Blood-free tumor cell suspensions were prepared by centrifuging the ascitic fluid first for 10 minutes at 100 x g. The cells were then resuspended in Ringer solution and centrifuged at 2 x g for 15 minutes. The supernate which contains erythrocytes and platelets was discarded and the tumor cells resuspended in Ringer solution. This procedure was repeated 3 times. For testing the effects of oxidized fatty acids, aqueous extracts containing the oxidation products were made from pure fatty acid esters (Hormel) oxidized by irradiation with ultraviolet light. Samples of 0.5 g of the esters were irradiated for 90 minutes in dishes 3 cm in diameter placed at a distance of 30 cm from a Hanovia Utility Model mercury arc rated at an intensity of more than 250 microwatts/cm^2 at 50.8 cm distance for wavelengths of 3130 Å and shorter. 0.5 g of the irradiated fatty acid ester was dissolved in 10 ml of petroleum ether and extracted against an equal volume of distilled water with shaking for 20 minutes. After separation of the petroleum ether the aqueous phase was centrifuged under fresh petroleum ether to remove any suspended droplets of ether. The aqueous phase was then placed under vacuum for 20 minutes to remove traces of ether. Two volumes of the aqueous phase were diluted with one volume of 3 x isotonic Ringer solution and varying amounts of this mixture were added to Warburg flasks to give the desired amount of oxidized material as determined by the thiobarbituric acid (TBA) color reaction(7). The weight of oxidized ester cannot be calculated but is of the order of micrograms. The respiratory and glycolytic measurements were made by standard Warburg technics. Lactic acid formed aerobically was measured by the method of Barker and Summerson(8). Nitrogen was determined by digestion and nesslerization. Analysis of the unsaturated fatty acids of tumor cells was made on lipid of several grams of packed cells extracted with a 3:1 alcohol-ether mixture for 12 hours in a Soxhlet type extraction appara-