maturation in the irradiated stunted rats. We have also reported a lack of response in growth to administration of growth hormone and/or thyroxine. Gonads, thyroids, and adrenals are heavier in comparison to body weight in the stunted head-irradiated rat (2). The present experiments show that the anterior lobes store as much hormone per unit of tissue weight as the controls. Studies of the blood level of somatotropin or of serum sulfation factor would be of value in confirming that hormonal release mechanisms are intact; the bulk of the evidence, however, suggests that well-known endocrine mechanisms are not involved in the stunting in the head-irradiated rat.

Summary. The injection of homogenates of the pituitaries of rats stunted by 600 R of X-irradiation administered to the head and of nonirradiated controls into hypophysectomized immature female rats resulted in comparable responses in terms of tibial epiphyseal width, gonadal weight, and thyroidal ^{131}I uptake. An exception occurred in 121-day-old females in which the pituitary homogenate of the stunted animals produced about twice the gonadal weight in the assay animals as did those of controls. These findings combined with those of earlier experiments, suggest that the permanent stunting resulting from head X-irradiation is not the result of hormonal deficit.

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Morphological Changes following Irradiation of a Segment of the Rat Thymus* (32934)

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Considerable evidence indicates that the thymus has a significant role in the development of peripheral lymphoid tissue and in the development of immunological responsiveness. Although the thymus has been studied for almost a century, many aspects of the population kinetics of the various types of thymic cells are controversial. One approach to the study of these problems has been to follow the pattern of regeneration of the thymus after experimental involution produced either by irradiation or by increased adrenal cortical activity. This approach, however, gives information only about the potentialities of the surviving cells which are primarily considered as epithelial derivatives of the thymus. The developmental potentialities of the mesodermal derivatives of the thymus and their role in the regeneration process cannot be adequately assessed since these cells are markedly reduced.

In this first paper in a series of studies of thymus cellular kinetics a method is described for producing by irradiation a narrow band of involution in the rat thymus bounded by essentially normal thymic tissue, and the pattern of regeneration of this involuted tissue is described.

Materials and Methods. Irradiations were performed with a Philips X-ray apparatus operated at 200 keV and 18 mA with 0.5-mm Cu and 1.0-mm Al filters. The animals were irradiated 30 cm from the source at a dose rate of 80 R/min. A 7-mm thick lead shield with a rectangular opening of 5 × 25 mm

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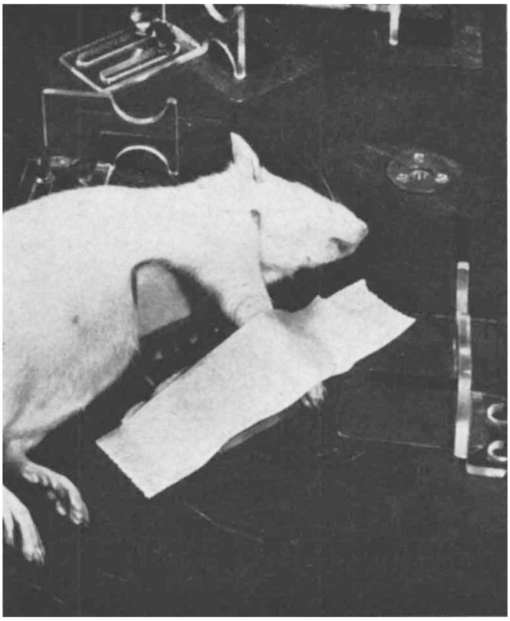

FIG. 1. The lead shield for local thymus irradiation. To the right the collimator opening for segmental irradiation and the lucite strip used for positioning the animal. To the left, the animal in position. The other collimator openings seen are for whole thymus irradiation. The X-ray beam is delivered from below.

was used to irradiate a part of the thymus. The animals were irradiated under sodium pentobarbital anesthesia. They were placed with the left side down on the lead shield with the upper part of the thorax over the rectangular opening. The X-ray beam was delivered from below the animal. To insure irradiation of comparable areas, animals were placed with their forefeet through holes in a lucite strip fastened perpendicularly to the lead shield at the end of the opening, and perpendicular to the longitudinal axis of the opening. This arrangement for irradiation is illustrated in Fig. 1. A 5-mm wide area through the middle of the thymus was irradiated by this procedure.

The thymuses of a group of 38 male Buffalo rats were irradiated with 400 R. The rats weighed 150–160 gm. Several rats were sacrificed daily for 9 days after thymus irradiation. Ten unirradiated animals served as controls. The thymus was removed at sacrifice, weighed, fixed in Bouin's fluid, and prepared for histological examination.

The number of cells in a unit area, $30 \times 15\ \mu$ square, was counted in the cortex and medulla of the shielded and irradiated areas of the thymus. The average number of cortical cells per unit area was calculated from the cell counts in 20 unit areas measured by moving the unit area from the subcapsular cortical area to the medulla and back. The total number of mitotic figures and pyknotic cells was also counted in 300–400 unit areas, and the mitotic and pyknotic indices were calculated. Similar counts were made of the medullary area, but only 16 square units were counted, since in some cases the medullary area was very narrow. Only the number of small lymphocytes was counted in the medulla.

Results. The weight of the irradiated thymus fell to a minimum (about 58% of the controls) by the third or fourth day after irradiation. The weight then gradually increased, and by the ninth day it was almost normal.

During the first few days after irradiation a pale, sharply outlined zone across the thymus corresponding to the irradiated area could be observed macroscopically, but by the third day the irradiated area of the thymus was less obvious macroscopically, although some narrowing and constriction of the area was often observed. The lobules in the irradiated area were smaller than in the shielded area of the thymus. By the ninth day after irradiation no obvious macroscopic differences between the irradiated and unirradiated areas of the thymus could be observed.

In stained sections of the thymus as shown in Fig. 2, a sharply outlined band corresponding to the irradiated area was distinctly visible during the first 5 days because of the differences in the uptake of dye in the relatively acellular irradiated portion of the thymus.

On the first day the outer portion of the irradiated cortex contained much cellular debris and many pyknotic thymocytes. The

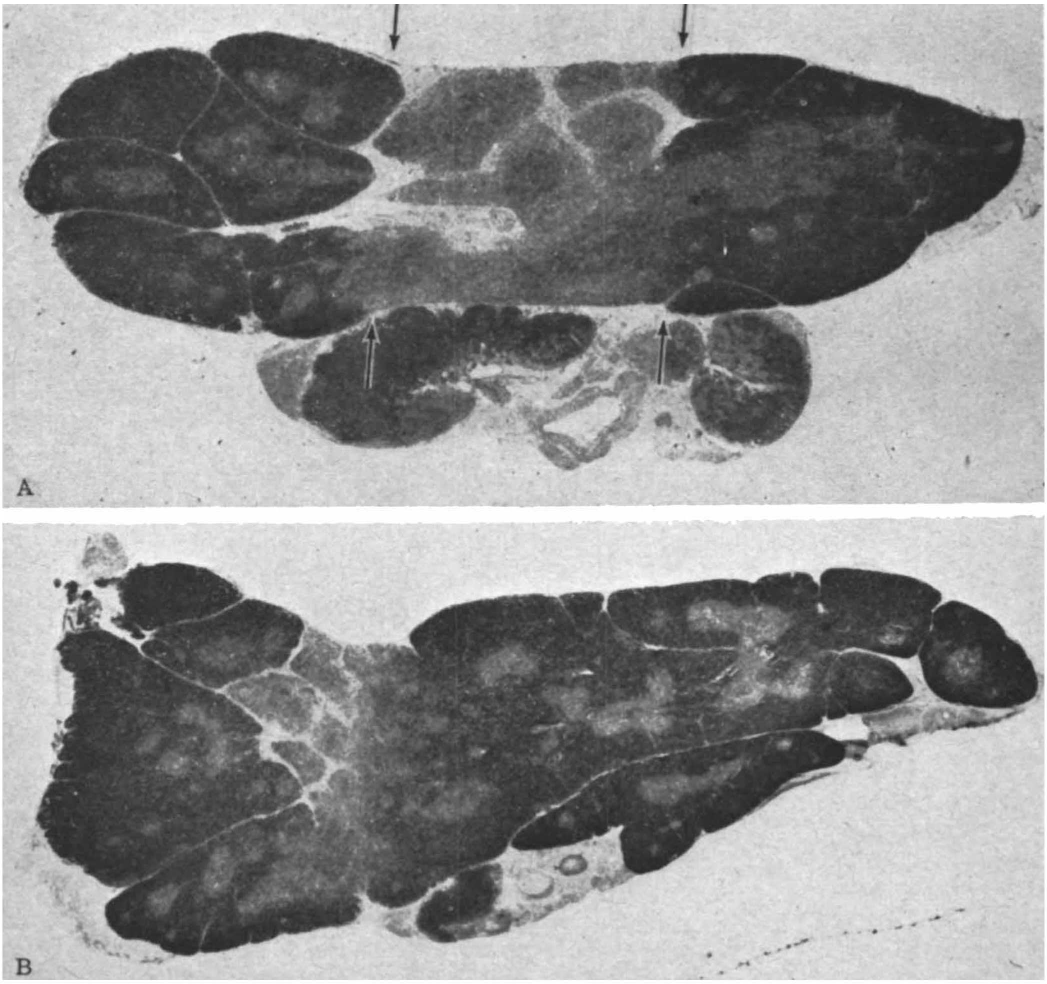

FIG. 2A. Segmentally irradiated rat thymus with adjacent lymph node 2 days after 400 R. The thymus irradiated area is depleted of cortical lymphocytes. Arrows point to the border between irradiated and unirradiated tissue. Some of the lobules are divided into irradiated and unirradiated areas. Marked edema of the interlobular tissue in the irradiated field is evident. The lymph node shows essentially normal cellularity. B. As in A, 5 days after irradiation; extensive repopulation of the irradiated segment has occurred.

inner portion of the cortex contained very few lymphoid cells. Mitotic figures were not observed in the cortex at this time. Dilated capillaries and clumps of dead cells gave the cortex a moth-eaten appearance. Small thymocytes of normal appearance were present in the medulla. Little debris was found in the medulla except for some accumulations in small cavities or capillaries at the border between the medulla and cortex. Even microscopically the border between irradiated and unirradiated tissue was quite sharp, following an almost straight line, sometimes dividing individual thymic lobules into irradiated and unirradiated areas. A transitional zone about 200 μ wide of decreasing damage separating the irradiated and unirradiated tissue was seen. The cellularity, mitotic and pyknotic indices of the unirradiated part of the thymus were similar to control thymuses (Table I).

By the second day after irradiation the cortex contained mostly stromal cells. Scattered mitotic figures could be found through-

TABLE I. Number of Cells and Pyknotic and Mitotic Indices in the Cortex of the Irradiated and Shielded Area of Rat Thymus at Different Intervals after 400 R Irradiation.[a]

	Irradiated area			Shielded area		
Day after irradiation	Cells in 20 unit squares	Pyknotic index[b]	Mitotic index[b]	Cells in 20 unit squares	Pyknotic index[b]	Mitotic index[b]
0				210	0.79	0.43
1	160	60	0	190	0.66	0.61
2	60	1.9	0.58	230	0.58	0.54
3	47	1.0	0.25	200	0.69	0.75
4	120	0.59	1.2	200	0.58	0.68
5	110	0.57	1.5	200	0.54	0.51
6	120	0.63	1.6	220	0.86	0.41
7	200	0.42	0.84	190	0.55	0.51
8	—[c]	0.49	1.2	210	0.64	0.48

[a] All values are the average of 2 or 3 animals.

[b] Number of pyknotic or mitotic cells per 100; 2000–3000 cells counted.

[c] Irradiated area not visible.

out the cortex. Many dilated vessels were observed which were probably capillaries and lymphatics. A few of these dilated vessels contained cellular debris, but most of them were devoid of debris or cells. Cellular debris was observed in the center of some of the Hassal's bodies. The border between the irradiated and unirradiated thymus was even sharper than the day before. Where a small portion of a lobule had projected into the irradiation field, it was depopulated, although the rest of the lobule was histologically normal (Fig. 3A).

The irradiated area was about the same on the third day as on the second day after irradiation. There were, however, fewer dilated capillaries than on the previous days, and some of the lymphatics and perivascular spaces were filled with apparently normal thymocytes. The irradiated medulla contained about as many small thymocytes as the medulla of unirradiated tissue, but many more small thymocytes than in the irradiated cortex, resulting in the appearance of the "inverse pattern" (Fig. 3B) i.e., the cellularity of the medulla is much greater than that of the cortex (1).

Relatively large depopulated areas around the center of the irradiated band with little evidence of regeneration were still evident on the fourth and fifth days after irradiation (Fig. 3C and D). Whole lobules could be found without cortical lymphoid cells, but with many lymphocytes in the medulla maintaining the appearance of the inverse pattern. Other lobules were divided into regenerating and depopulated areas. It is of interest and probably of considerable importance that the repopulating area of such lobules was always next to the shielded area. The border between the irradiated and unirradiated thymus was dominated by large basophilic cells, many of which were in mitosis. These cells extended from the unirradiated to the irradiated area of the thymus along the outer edge of the lobule. There was no evidence that repopulation occurred through an extensive migration of small thymocytes from the unirradiated thymus either across the interlobular septa or across an individual lobule (Fig. 3D).

The entire irradiated area was repopulated in 2 of 3 rats, primarily with large and medium lymphocytes, by the sixth day after irradiation. In one animal the cortex of some lobules in the center of the irradiation field was still depopulated. By the ninth day after irradiation the involuted area could not be distinguished from the shielded area of the thymus, except for a relatively smaller medulla and a slight narrowing of the thymus in the area corresponding to the irradiated band.

Discussion. This experiment indicates that it is technically feasible to irradiate a small portion of the normal thymus and produce an area of radiation damage bounded by normal thymic tissue. This preparation allows the study in the adult of the pattern of repopulation of the stromal epithelial elements of the thymus in close approximation to normal thymic tissue containing large numbers of mesodermal lymphoid cells.

Morphologic observations indicate that the pattern of destruction and repopulation of the irradiated portion of the thymus are

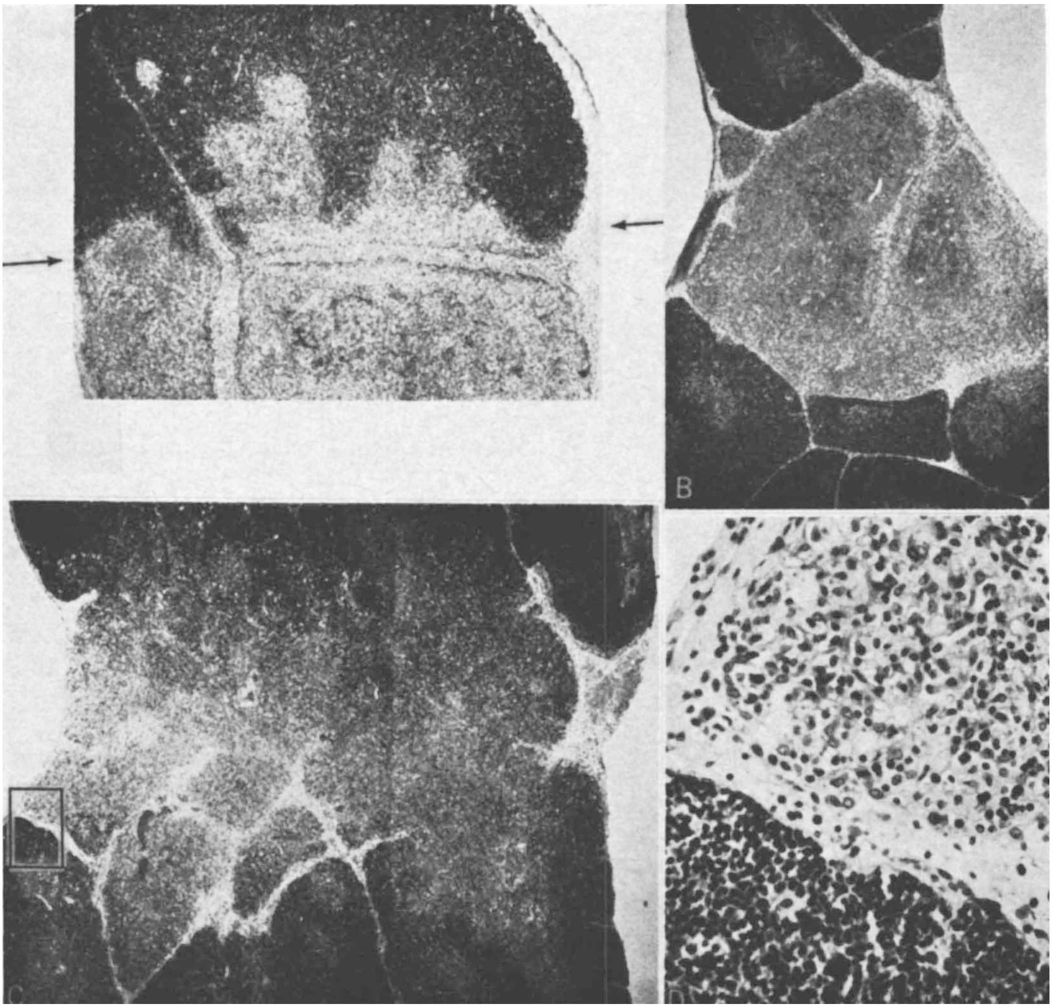

FIG. 3A. Segmentally irradiated rat thymus 2 days after irradiation with 400 R. Shielded area at the top, irradiated area at the bottom. Arrows mark the border between irradiated and shielded areas. To the left, the border passes through the middle of a lobule. To the right it passes close to the edge of the lobule marked by the interlobular septum. The lightly stained fields in the shielded area are medullary tissue. B. Segmentally irradiated rat thymus 3 days after irradiation. The border between depopulated and populated tissue follows the border of the lobules except for the lobule below to the right. The density of lymphocytes in the irradiated medulla is about the same as in the medulla of the shielded area but higher than in the irradiated cortex giving the inverse pattern. C. Same as in Fig. 2B, with higher magnification. Some of the lobules in the center of the field are still depopulated. Others are repopulated in the area adjacent to the shielded part of the thymus. Boxed-in area is Fig. 3D. D. Higher magnification of the framed area of C. Note the marked differences in thymocyte population of the 2 areas separated by the interlobular septum.

similar to that described when the entire thymus is involuted after either whole body irradiation or irradiation of the anterior half of the animal (1–4).

The cellular debris produced as a result of radiation damage was removed from the irradiated area within 2 days. The cellular debris disappeared most rapidly from the inner portion of the cortex, as has been reported by other workers (1). By the fourth day after irradiation, repopulation of the irradiated area is obvious, and by the ninth day the irradiated area is essentially undistinguishable from the unirradiated area. The origin of the cells responsible for the repopulation is unknown. Mitotic figures are not observed on the first day after irradiation, but are present from the second day on. These dividing cells, initially randomly distributed throughout the cortex, may simply be cells surviving the irradiation or cells which have migrated in from either the blood stream or the neighboring normal thymic tissue. The fact that the repopulation of individual lobules generally started in the area adjacent to the shielded thymus does suggest that there may be some influence from the unirradiated thymus; however, the irradiation dose to this area may be less than in the center of the field, and more cells may survive the irradiation. Regardless of the source of these dividing cells, the morphological observations indicate it is unlikely that any significant repopulation results from an extensive migration of small thymocytes from the unirradiated to the irradiated area even when the zone of irradiation transects an individual lobule. This is particularly interesting in view of the fact that irradiated lymph nodes in the same animal are essentially repopulated by small lymphocytes within 2 days after irradiation (Fig. 2A). This suggests that either the small thymocytes are not motile or, if motile, they are prevented from massive migration into the involuted area possibly because of some anatomical or physiological barrier.

The initial depopulation of the irradiated zone of the thymus is a direct effect of the irradiation, since not only is the morphological appearance of the unirradiated portion of the thymus indistinguishable from the thymus of unirradiated controls, but their mitotic and pyknotic indices are also the same.

Summary. A method is described for producing a narrow region of irradiation damage in the rat thymus bounded by normal thymic tissue. The mitotic and pyknotic indices of the shielded area of the thymus are comparable to those in normal thymic tissue indicating that the shielding is effective. The pattern of repopulation of the irradiated thymus is described. The source of the cells responsible for the repopulation is unknown; however, it is evident that an extensive migration of small thymocytes from the unirradiated area of the thymus into the irradiated area does not occur.

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