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Studies on Thymus of the Mammal. X. Regeneration of Irradiated Mouse Thymus.*† (23024)

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The source of cells which appear in the cortex of a thymus regenerating after accidental involution has been questioned because the number of observed mitoses has been considered insufficient to account for the reconstitution. Murray(1) states that repopulation of the cortex after total-body irradiation proceeds outward from the medulla. Both Murray and Baillif(2), in a study of thymuses involuted after chlorazol black E injections, consider the possibility that new lymphocytes may be derivatives of the epithelial reticulum. However, in comments on epithelial origin of the thymic lymphocytes, Downey(3) suggests that "It is possible that these conclusions can be explained by a failure to distinguish between the epithelial and mesenchymatous portions of the thymic stroma." In the present investigation, the appearance of dividing cells in thymuses regenerating after total-body sublethal dose of gamma rays (400 r) was studied in animals receiving an injection of colchicine previous to their sacrifice. Cells in mitosis appeared all through the cortex and the evidence indicates that they are mesenchymal reticular cells and medium-sized lymphocytes.

Materials and methods. Most of the animals used in these experiments were CAF 1 male mice, 4-5 weeks old from the Roscoe B. Jackson Laboratory. Four were of the BALB/c strain. The series studied included normal animals and mice which had been irradiated 24, 36, 52 hours, 3, 5, and 7 days previously. The dose was 400 r, Cobalt 60: aluminum filter; 38 cm from source. Time of exposure varied from 9′ 3″ at the beginning of experiments to 10′ 3″ at the end. In general, thymuses used for counting cells were fixed in Bouin's fluid and stained in hematoxylin and eosin. May-Grünwald and Giemsa solutions were also used to stain both Bouin and Helly fixed material for the study of cell types. Sections were cut horizontally at 5 μ. All mitotic counts were made from animals injected subcutaneously with 1/20 cc of freshly prepared 0.5% solution of colchicine 6 hours before they were killed by cervical dislocation. As the purpose of these studies was to determine distribution of dividing cells in the regenerating thymus, time of injection had to be determined by the hour of irradiation (9 a. m.). The 24 hour irradiated mice were injected at 3 a. m., the 36 hours, at 3 p. m. and the 52 hours, at 7 a. m. All others were injected at 9 a. m. except three 3-day mice, at 7 a. m. Counts of resting and dividing cells were made with a net micrometer (56,000 cu. μ, 6x7 rows) under oil immersion at a magnification of 970 times.

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TABLE I. Effect of a Single Total-Body Dose of 400 r Gamma Rays Followed by Injection of Colchicine on Numbers of Thymic Cells in Unit Areas.

Mice	No.	Mean No. of cells ± S.E. Cortex	Mean No. of cells ± S.E. Medulla	Difference between means: Cortex No. ± S.E.	Cortex t	Cortex P_t	Medulla No. ± S.E.	Medulla t	Medulla P_t
Non-irrad.	5	4155.2 ± 88.16	2852 ± 149.28	2788.8 ± 169.04	16.50	<.001	324.4 ± 168.38	1.93	>.05 <.10
24 hr	5	1366.4 ± 144.24	2527.6± 77.91	34.2 ± 188.75	.18	>.80 <.90	306.6 ± 113.5	2.70	>.025 <.05
36 "	5	1400.6 ± 121.81	2834 ± 82.54	304.6 ± 153.85	1.98	>.05 <.10	52.8 ± 170.95	.31	>.70 <.80
52 "	5	1705.2 ± 93.95	2886 ± 149.71	43.3 ± 108.75	.40	>.60 <.70	384.5 ± 219.93	1.75	>.10 <.20
3 days	6	1748.5 ± 54.67	2501.5± 161.12	1192.7 ± 114.16	10.45	<.001	452.5 ± 189.08	2.39	>.025 <.05
5 "	5	2941.2 ± 100.23	2049 ± 98.95	855.6 ± 164.62	5.19	<.001	438 ± 176.77	2.48	>.025 <.05
7 "	4	3796.8 ± 130.61	2487 ± 146.48						

TABLE II. Effect of Single Total-Body Dose of 400 r Gamma Rays Followed by Injection of Colchicine on Numbers and Percentage of Dividing Cells in Unit Areas in Thymus.

Mice	No.	Mean No. of mitoses ± S.E. and % Cortex Mean ± S.E.	Cortex %	Medulla Mean ± S.E.	Medulla %	Difference between means: Cortex No. ± S.E.	Cortex t	Cortex P_t	Medulla No. ± S.E.	Medulla t	Medulla P_t
Non-irrad.	5	303 ± 18.58	7.3	14.2 ± 1.45	.5	295.8 ± 18.65	15.86	<.001	6.4 ± 3.59	1.78	>.10 <.20
24 hr	5	7.2 ± 1.65	.5	20.6 ± 3.28	.8	8.6 ± 3.07	2.80	>.02 <.025	5 ± 4.04	1.24	.25
36 "	5	15.8 ± 2.59	1.1	25.6 ± 2.36	.9	19.6 ± 8.21	2.39	>.025 <.05	13 ± 9.11	1.43	>.10 <.20
52 "	5	35.4 ± 7.79	2.1	38.6 ± 8.80	1.4	130.6 ± 32.12	4.07	>.001 <.005	26.4 ± 17.33	1.52	>.10 <.20
3 days	6	166 ± 31.16	9.5	65 ± 14.93	2.6	433.6 ± 73.25	5.92	<.001	28.6 ± 1.73	1.66	>.10 <.20
5 "	5	599.6 ± 66.3	20.4	36.4 ± 8.67	1.8	207.1 ± 80.18	2.58	>.025 <.05	4.9 ± 13.37	.37	>.70 <.80
7 "	4	392.5 ± 45.08	10.2	31.5 ± 10.18	1.3						

TABLE III. Effect of a Single Total-Body Dose of 400 r, Gamma Rays, on Percentage of Mitoses in 7 Successive Zones in Thymic Cortex from Capsule to Medulla.

Mice	No.	% of mitoses from capsule to medulla						
		1	2	3	4	5	6	7
Non-irrad.	5	13.3	9.8	8.6	7.3	5.8	4.2	2.4
24 hr	5	.37	.64	.71	.59	.58	.39	.39
36 "	5	2.6	.86	.98	.77	1.7	.46	.53
52 "	5	2.4	1.9	2.6	2.6	2.5	1.9	.8
3 days	6	11.9	10.8	10.2	10.1	9.0	8.3	6.8
5 "	5	19.6	21.1	20.6	21.9	20.7	19.9	18.6
7 "	4	10.7	12.6	12.2	11.5	10.0	8.3	6.5

Ten fields were chosen in cortex and medulla which were representative of each thymus as to cellular content and density of population. Sections were taken either 50 or 150 μ apart depending on the size of the thymus. In the cortex of the involuted thymuses, the region counted extended from capsule to medulla. However, if the width of the cortex in normal thymuses and the ones regenerating after irradiation was greater than the area of the net micrometer, 2 fields were counted: one just beneath the capsule and the other next to the cortico-medullary boundary. The data for successive zones were averaged so that they were comparable to those obtained when single fields were counted.

Observations. Seven and three-tenths % of cortical cells in the normal mouse thymus were in division. The most frequent type of arrested metaphase was the "ball" with scattered appearances of the "exploded" and "star" forms(4). There was a gradual decrease in numbers of mitoses toward the medulla (Table III). Dividing cells in the cortex were often grouped in cord-like arrangements or in small foci. Mitoses in the medulla were scarce.

Twenty-four hours after irradiation the thymus is characterized by a depleted and narrow cortex in contrast to a more densely populated and relatively more extensive medulla, the so-called inverted thymus. The number of cortical cells was significantly reduced (Table I). The density of medullary cells, on the contrary, was not essentially altered. In the most severely affected thymus, about 1/5 of the cortical cells remained and only 2 mitoses were seen in 10 fields examined. It was estimated that 1/3 of the undamaged cells were either mesenchymal reticular cells or medium-sized lymphocytes. In the medulla the percentage of dividing cells was 0.8%. All through these experiments the number of medullary mitoses was low though somewhat higher than in the normal organ.

The character and number of resting cells in the thymus 36 hours after irradiation had changed only slightly; there were more vacuolated cells and less debris in the cortex. As can be seen (Table III) the distribution of mitoses in this group was more irregular than in any other.

The thymuses 52 hours and 3 days after irradiation were still of the inverted type. The marked increase in percentage of dividing cells at 3 and 5 days post-irradiation was followed by an abrupt rise in total number of cells (Fig. 1, Tables I and II). By the 7th day, the number of cortical cells approached the normal and the percentage of mitotic fig-

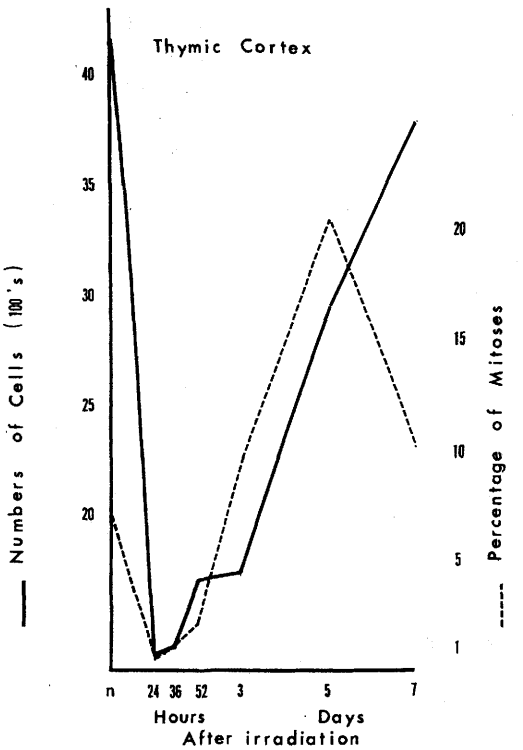

FIG. 1. Effect of single total-body dose of 400 r, gamma rays followed by injection of colchicine on number and percentage of dividing cells in a unit area of thymic cortex.

ures had decreased. Increases in the number of cells between the 3rd, 5th and 7th days were statistically significant (Table I).

In the irradiated thymuses, most of the dividing cells were in the subcapsular or middle cortical zones (Table III). They were diagnosed as either mesenchymal reticular cells or medium sized lymphocytes; only occasionally was an epithelial cell in the cortex thought to be in mitosis and none was seen in division in the medulla.

The choice of fields in the medulla can profoundly affect the counts so that a cautious interpretation of statistical analyses has to be made. It is evident from Tables I and II that there was no clear effect of irradiation on fluctuations of medullary cells. In the 5 and 7 day series when the transition was being made from the inverted thymic pattern to the normal one of a more densely packed cortex, the indistinctness of the cortico-medullary border made the determinations of the limits of the medulla difficult. To demonstrate the importance of the choice of the field in the medulla 7 days after irradiation, counts were made in 2 thymuses in 2 regions of different densities. The following averages were secured: 2253 cells, 30 in mitosis, 1.3%; 3133 cells, 290 dividing, 9.6%. The data included in Tables I and II for the 7 day series were from the less dense, more characteristic medullary regions.

Discussion. In previous studies of the cellular effects of the administration of colchicine and of irradiation, the two agents were either given together(5) or the drug was injected prior to raying(6). The purpose of the present experiments in which colchicine followed irradiation was to discover where and when mitoses appeared in the regenerating thymus.

In both normal and irradiated thymuses mitoses were seen all through the cortex but fewer near the medulla. After irradiation the significant depression in numbers of cortical cells was accompanied by a similar sharp drop in the percentage of dividing cells. Even at 24 and 36 hours after irradiation by a sublethal dose, when the evidences of destructive influences were still predominant (loss of lymphocytes, presence of pycnotic and fragmenting nuclei and active macrophages, accumulation of lipids(7), strong positive reaction for acid phosphatase(8)), some cells continued to divide. At 5 and 7 days after exposure the number of cortical cells rapidly approached the normal and the peak of the curve for the mitotic cells came at the 5th day. At these times, too, the thymus was recovering its normal appearance by a diminution in the amount of lipids and by less evidence of a reaction for acid phosphatase.

The sensitivity of the cortical cells of the thymus to irradiation by a sublethal dose and the rapid recovery are well shown in Fig. 1. The difference between the average number of cells in the non-irradiated thymuses and that of the 24 hour post-irradiated thymuses and the differences between the means for the 3, 5 and 7 day ones are all statistically significant while the differences occurring during the destructive phase from 24 hours to 3 days are not (Table I). In contrast none of the variations in the counts of medullary cells within the conditions of these experiments were judged significant. The picture of the inverted thymus appears due more to the contrast of a thinly populated cortex with a relatively more densely packed medulla than to any real increase in the number of medullary cells. This comparatively stable quality of the medulla in regard to numbers of cells and percentages of mitoses correlates with its less marked reactions after irradiation as indicated by the presence of fewer lipids(7) and of less acid phosphatase than the cortex (8) during the degenerative stages.

It is believed that the cells repopulating the cortex have not migrated from the medulla to any extent as described by Murray (1) but have arisen *in situ* for the regeneration of this part of the thymus is associated with a marked increase in numbers of dividing mesenchymal reticular cells and medium-sized lymphocytes. Occasional mitoses were judged epithelial in nature but there was no reason to think that any lymphocytes were derived in this way.

In evaluating the data obtained from enumeration of colchicine mitoses, certain

factors which might influence the results must be considered: variations in state of the thymus, choice of fields, diurnal mitotic rhythm, strain of mice, time of action and dosage of the drug.

The condition of the thymus at times of irradiation and of colchicine injection is unknown. Some differences among individual as well as between different regions in the same thymus would be expected.

Diurnal variations in mitotic rhythm have been described in the epidermal(9) and respiratory(10) epithelium of mice. In the present study, none of the variations could be attributed to the time of day injections were made. Strain differences in mitotic rates have also been reported(10) but these were not found in counts from CAF 1 and BALB/c mice in the same series.

The interval chosen for action of colchicine was 6 hours, the one suggested in general for mammals(11,p.379), and the dose (1/20 cc of a 0.5% solution per animal) was within the range used by other investigators(11).

It has not been possible to correlate number of mitoses in the thymuses from normal noninjected mice with these results from animals receiving colchicine. The methods which have been used have been too dissimilar to make comparisons valid.

Summary. 1. The numbers of resting and dividing cells in the cortex of the mouse thymus were significantly reduced 24 hours after a single total-body dose of 400 r gamma rays followed by an injection of colchicine. Increase in the number of mitoses between 52 hours and 3 days postirradiation preceded the rise in the number of cells. The greatest number of mitoses (20.4%) appeared on the 5th day after exposure. 2. The fluctuations in the number of resting and dividing cells after irradiation in the medulla followed no regular pattern. 3. The evidence presented supports the view that the cells repopulating the thymic cortex after irradiation arise *in situ*.

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