Studies on Thymus of the Mammal. IX. Histochemical Study of Irradiated Mouse Thymus.*† (22740)

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The increased amount of lipids in the thymuses of C57 mice after exposure to a single total-body dose of x-rays was described by Smith and Holst(1). Thymuses normally contain little or no lipid. However, they become heavily loaded with fat 24 and 36 hours after irradiation when the evidences of injury are greatest. They return to a normal condition in 7-9 days. The mediastinal lymph nodes do not undergo a similar severe reaction. In the present investigation attention was directed to the study of acid phosphatase. In normal thymuses there is little indication of activity of this enzyme but after irradiation the changes in the number and distribution of cells showing sites of acid phosphatase activity correlate in general with lipid deposition.

Material and methods. BAlb/c male mice, 30-40 days old from the Roscoe B. Jackson Memorial Laboratory were used. The thymus, spleen and mesenteric lymph node were removed from control animals and from mice 2, 4, 8, 12, 24, 36 hours and 3, 5, 7 and 9 days after exposure to irradiation. The mice killed at 12 (in part), 24, and 36 hours had received doses of x-ray of 410 r; the radiation factors were 250 K.V., no filter, 3 min., 50 cm TSD.‡ The animals sacrificed 2, 4, 8, 12 (in part) hours and 5, 7 and 9 days received 434-455 r, gamma rays from radioactive cobalt (42 cm aluminum filter).§ Gomori's method(2) was used for the demonstration of sites of activity of acid phosphatase. The thymus, spleen and mesenteric lymph node were removed from each animal, fixed in ice-cold acetone and embedded in paraffin together; sections were cut at 8 μ and mounted on the same slide. The paraffin was not removed from the sections(3) and the incubation time in the substrate was usually 12 hours because this lengthened time brought out more clearly the slight reaction in the normal thymus. For cytological studies the period of incubation was reduced to 3 and 6 hours. The technic was controlled by incubating the sections in the buffer without the substrate. The two lobes of the thymus were often treated differently for comparative studies; (1) fixed in formal-calcium(4), embedded in 25% gelatin, sectioned on the freezing microtome and colored in oil red O; (2) fixed in acetone, embedded in paraffin and stained in periodic acid-leucofuchsin and methylene blue. Estimations of the amount of lipids were made independently by 2 or 3 persons by assigning values of + to ++++.

Observations. In studying the response of the thymus to injury the reactions of the mesenchymal and epithelial derivatives have been particularly noted. The nucleus of the former is characterized by a pattern of coarse chromatin; the latter by a large oval, vesicular nucleus, thin nuclear membrane and a fairly large bleb-like nucleolus to which is attached one or more small karyosomes. Thin cytoplasmic processes of the epithelial cells are well seen in Fig. 7 and 8.

The cortex of the normal thymus of the young BAlb/c mouse is less reactive than the medulla in all the technics used. In general there is no difference in the cortex between a subcapsular and an inner zone. There are few or no macrophages containing ingested cells, lipids or positive periodic acid-leucofuchsin granules. Only a few linear adventitial (mesenchymal reticular) cells along the capillaries contain lipids or give a positive re-

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action for acid phosphatase (Fig. 1). In the normal thymic medulla it is estimated that there are about 30% more lipids than in the cortex. Similarly there are more cells showing sites of acid phosphatase activity (Fig. 1). Macrophages with phagocytosed cells are scarce and small. These contain lipids and are blackened in the technic for acid phosphatase.

As soon as 2 hours after irradiation, even though the general appearance of the thymus was normal, the cells were visibly affected. In the cortex there was a limited amount of pycnosis. Phagocytosing macrophages were loaded with lipids and showed a positive reaction for acid phosphatase (Fig. 2).

At 4 hours after exposure the inner two-thirds of the cortex was more heavily laden with lipids than the subcapsular zone or medulla. In this region there were more lymphocytes and macrophages containing fat than at 2 hours and there was more evidence of acid phosphatase activity. Fig. 7 and 8 illustrate the characteristic appearance of the mesenchymal and epithelial reticular cells in the Gomori technic.

Degenerative changes were clearly evident 8 and 12 hours after irradiation. Localized cortical areas of pycnotic or fragmented nuclei gave little or no indication of acid phosphatase activity. In the inner cortical zone there were large macrophages loaded with ingested cells; these contained much lipid and gave a positive reaction for acid phosphatase (Fig. 3). In contrast to the cortex, the medulla showed little pycnosis, relatively less fat and fewer cells stained in sections treated for acid phosphatase.

At 24 hours post irradiation, the depleted and diminished cortex was heavily laden with lipids (about twice as much as at 12 hours) and stained a dark brown in the technic for for the demonstration of acid phosphatase (Fig. 4). The many loaded macrophages and epithelial cells were both colored in this reaction. Although there was a somewhat increased number of medullary cells which contained lipids and showed a positive reaction for acid phosphatase, the medulla was clearly less affected than the cortex.

Both cortex and medulla were estimated to be one-third to one-half more heavily laden with lipids 36 hours after exposure than at 24 hours and the cortex still stained deeply brown in the acid phosphatase technic. However, the removal of injured lymphocytes was indicated by the decreased amount of pycnosis and the greater number of vacuolated macrophages.

Thymuses examined 3, 5 and 7 days after irradiation showed that regenerative processes had started. At these times the amount of visible lipids and the evidence of acid phosphatase activity were diminished (Fig. 5). By 9 days the thymus was essentially normal (Fig. 6).

Discussion. Changes in lymphatic tissue after irradiation have been described mostly in relation to changes in the nuclei(5). More recently biochemical and histochemical technics have been applied to reveal other modifications in lymphatic organs after exposure to x-rays; for example, nucleic acids, Weymouth, Delfel, Doell, Steinbock and Kaplan(6); alkaline and acid phosphatases, Doyle(7,8); adenosine triphosphatase, Ashwell and Hickman(9); lipids, Smith and Holst(1).

The present study was concerned principally with the presence and distribution of histochemically demonstrable acid phosphatase in the thymus during its degenerative and regenerative phases after irradiation. The spleen and lymph node were similarly treated and examined for comparison (unpublished data). Acid phosphatase activity in the normal thymus is meager when compared with that evident in the red pulp and rim of the white pulp of the spleen or with the diffuse lymphatic tissue, medullary cords and sinuses of the mesenteric lymph node. This great difference probably results from the presence of many actively phagocytic cells in the spleen and lymph node and their scarcity in the normal thymus. As soon as 2 hours after irradiation there was an evident increase in acid phosphatase activity as the mesenchymal reticular cells became enlarged and rounded in both cortex and medulla (Fig. 2). They were, indeed, active macrophages with ingested lymphocytes. In these preparations

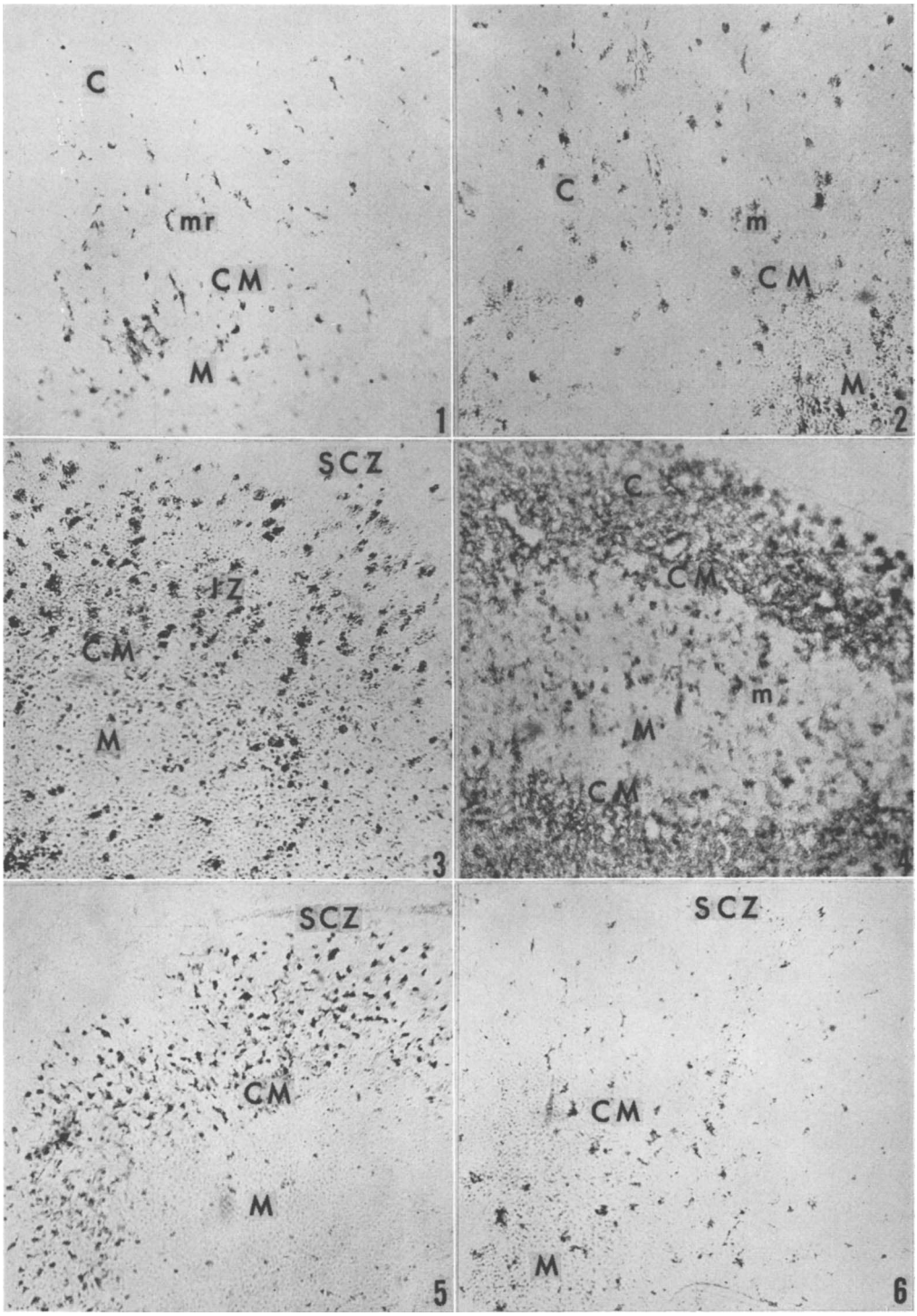

PLATE 1

Thymus of the mouse BAlb/c; fixed in acetone; Gomori's lead sulphide method for demonstration of sites of acid phosphatase activity; 12 hr incubation. × 100.

C, cortex; CM, cortical-medullary line; er, epithelial-reticular cell; IZ, inner zone; M, medulla; m, macrophage; mr, mesenchymal reticular cell; SCZ, subcapsular zone.

FIG. 1. Control. Linear mesenchymal reticular (adventitial) cells stained in cortex; slightly more stained cells in medulla.

FIG. 2. 2 hr after total-body irradiation. Development of macrophages seen as large round cells in cortex and medulla; few linear cells seen in cortex.

FIG. 3. 12 hr after irradiation. Large loaded macrophages more crowded in cortex than in medulla.

FIG. 4. 24 hr after irradiation. Narrowed cortex loaded with stained cells. Large macrophages seen clearly at edge of subcapsular zone. Vacuolated macrophages appear as empty spaces.

FIG. 5. 5 days after irradiation. Cortex, increased in width, contains more stained cells than medulla but macrophages are smaller.

FIG. 6. 9 days after irradiation. Thymus is essentially normal in appearance.

the lymphocytes which stained were near the blackened phagocytic cells. This quick mobilization of macrophages in the thymus is indicative of the rapidity with which thymic cells react to damaging influences. Kindred (10) noted that after the use of mustard gas the cortical macrophages of the thymus were active on the first day.

The indications of greatest activity of acid phosphatase were at the height of injury 24 and 36 hours after the sublethal dose of x-rays or gamma rays, the period also of the greatest deposition of lipids. The most vulnerable part of the thymus was the cortex; its inner zone was the first to show marked signs of injury. The intense staining at these hours (Fig. 4) was due to reactive macrophages, the epithelial cells and the coloring of the associated lymphocytes. Areas where there were dense accumulations of pycnotic or fragmented nuclei were ones where macrophages were scant or lacking. As compared with the cortex the medulla was much more stable during this period. In other technics this relatively quiescent medulla results in the appearance of the so-called inverted thymus.

Summary and conclusions. 1. In the normal thymus the relatively few mesenchymal

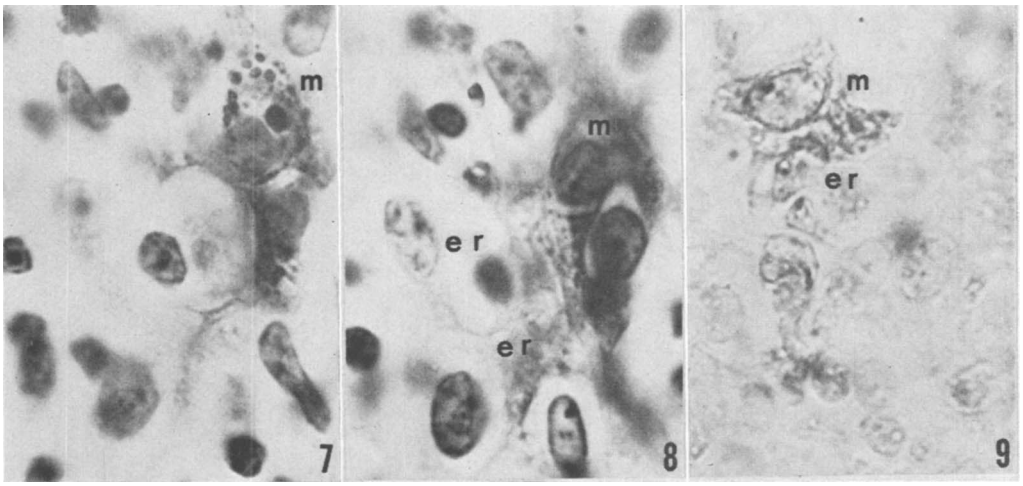

PLATE 2

Thymus of the mouse BAlb/c; fixed in acetone; Gomori's lead sulphide method for demonstration of sites of acid phosphatase activity; 6 hr incubation. × 1500.

FIG. 7 and 8. Medullary cells, 4 hr after irradiation. Same cells are photographed at 2 levels. Nuclei are stained with hematoxylin. Intimate association is seen of mesenchymal reticular cells with chromatic nuclei and stained inclusions in the cytoplasm and of epithelial cells with vesicular nuclei and weakly stained web-like cytoplasm.

FIG. 9. Medullary cells, 5 days after irradiation. A group of cells which include a macrophage with stained inclusions. Epithelial cells are barely visible. In this preparation only cells with stained cytoplasm had colored nuclei; lymphocytes and other cells with no evidence of acid phosphatase activity had unstained nuclei.

reticular cells give a positive reaction for acid phosphatase in acetone fixed sections. There are fewer reactive cells in the cortex than in the medulla. 2. As early as 2 hours after irradiation the mesenchymal reticular cells are mobilized to become active macrophages which stain deeply in the acid phosphatase technic. These become more numerous until at 24 and 36 hours after irradiation the involuted cortex is loaded with macrophages containing ingested lymphocytes or full of vacuoles. At these hours the browned cytoplasm of the epithelial cells is seen as the stroma of the depleted cortex. At 3, 5 and 7 days there is a gradual diminution in number and size of reactive cells. At 9 days the appearance of the thymus is essentially normal.

1. Smith, C., and Holst, E. A., *Anat. Rec.*, 1953, v116, 123.

2. Lillie, R. D., *Histopathologic Technic,* 1948, Blakiston, Inc., N. Y.

3. Goetsch, J. B., Reynolds, P. M., and Bunting, H., *J. Nat. Cancer Inst.*, 1952, v13, 255.

4. Baker, J. R., *Q. J. Micr. Sc.*, 1944, v85, 1.

5. Bloom, W., *ed. Histopathology of Irradiation from External and Internal Sources,* 1948, McGraw-Hill Book Co., Inc., N. Y.

6. Weymouth, P. P., Delfel, N. E., Doell, R. J., Steinbock, H. L., and Kaplan, H. S., *J. Nat. Cancer Inst.*, 1955, v15, 981.

7. Doyle, W. L., *Am. J. Anat.*, 1950, v87, 79.

8. ———, *Symposium on Cytology,* Mich. State College Press, 1951, 1.

9. Ashwell, G., and Hickman, J., *J. Biol. Chem.*, 1953, v201, 651.

10. Kindred, J. E., *Arch. Path.*, 1947, v43, 253.

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Alterations in Calcium Metabolism in Cancer Patients Treated with 6-Diazo-5-Oxo-L-Norleucine.† (22741)

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6-diazo-5-oxo-L-norleucine (DON), an antibiotic isolated from culture filtrates of a Streptomyces, has been reported by Clarke *et al.* (abstract) to inhibit the growth of sarcoma 180. It is also inhibitive in mouse leukemias and a spectrum of transplantable tumors. In combination with 6-mercaptopurine (6-MP) inhibition of transplantable tumors is synergistic. Patients with cancer metastatic to bone may demonstrate hypercalcemia and hypercalcuria secondary to the osteolysis incident to tumor growth. If the tumor growth is inhibited, as, for example, by local irradiation, the calcium changes may be reversed(1) with the cessation of bone breakdown. Hypercalcemia and hypercalcuria secondary to osteolytic cancer have been corrected also by the administration of adrenal steroids(2) and other compounds. These changes have been regarded as sensitive measures of decreased osteolysis secondary to a reduced rate of growth of tumor in bone(3).

In the course of clinical trials with DON, 11 patients with hypercalcemia and/or hypercalcuria secondary to osteolytic cancer were studied in order to detect any possible tumor inhibitory effects of the compound as measured by the sensitive changes in calcium metabolism. Marked effects on calcium metabolism were seen and it is the purpose of this paper to report these changes.

Material and methods. Six patients had carcinoma of the breast, 2 metastatic carcinoma of unknown origin, 2 bronchogenic carcinoma and one adrenal cortical carcinoma. Hypercalcemia occurred 13 times in these 11 patients. Treatment with DON alone was employed in 8 instances of hypercalcemia and the combination of DON and 6-MP was used in 5 instances. One patient who was normocalcemic but hypercalcuric was treated with DON alone. All but one patient were con-

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