STUDIES ON X-RAY EFFECTS.

III. CHANGES IN THE LYMPHOID ORGANS AFTER SMALL DOSES OF X-RAYS.

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PLATES 8 AND 9.

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The observations of previous investigators have emphasized the fact that x-rays are preeminently and selectively destructive to lymphoid cells (Heineke\(^1\)). Thomas, Taylor, and Witherbee\(^2\) have shown that small doses of x-rays will bring about a stimulation of the circulating lymphocytes, confirming the earlier observations made in this laboratory.\(^3\) As in the experiments on the effect of heat on the circulating lymphocytes, there was a fall immediately after exposure, followed by a more or less gradual rise.

The histologic findings in the lymphoid tissues of mice treated with heat have already been reported,\(^4\) and it has been shown that the lymphocytosis induced by heat is due to the proliferation of certain cells in reaction to the extensive destruction of the tissue by this agent. \textit{A priori}, since x-rays are known to be destructive to lymphoid tissue, the lymphocytic changes observed seemed to be due to a similar cause. The experiments to be reported here were undertaken in order to determine this point.

\(^3\) Murphy, Jas. B., and Morton, J. J., \textit{J. Exp. Med.}, 1915, xxii, 800.
\(^4\) Murphy, Jas. B., and Sturm, E., \textit{J. Exp. Med.}, 1919, xxix, 1.
\(^5\) Nakahara, W., \textit{J. Exp. Med.}, 1919, xxix, 17.
Six normal rabbits of the same color (white), and of approximately the same age, were treated with the same dose of x-rays as was used by Thomas, Taylor, and Witherbee (spark-gap \(\frac{3}{4}\) inch, milliamperage 25, distance 8 inches, time 20 minutes). The animals were killed at intervals, and tissues were taken (a) immediately, (b) 48 hours, (c) 4 days, (d) 7 days, (e) 10 days, and (f) 14 days after treatment. This experiment has been duplicated with six brown rabbits. In the second experiment, however, animals were killed two by two (a) 24 hours, (b) 3 days, and (c) 14 days after they were x-rayed, these periods being found to be critical from the result of the first experiment.

For fixation, Carnoy’s chloroform-alcohol-acetic (6: 3: 1), was easy to use and gave good results. Its use saved much time by dispensing with the necessity of passage through the grades of alcohol. Some tissues were fixed in Zenker’s fluid for control. The staining was done almost exclusively with Heidenhain’s iron-hematoxylin, though a few sections were stained by the ordinary method of hematoxylin and eosin.

Results.

Spleen.—The general histologic condition of the spleen immediately to 24 hours after the treatment is approximately normal. Necrotic cells are extremely scarce in both the nodule and the pulp. The number of mitotic figures in the germinal center of the nodule varies; as a rule, it shows in section a few, sometimes several, and more rarely ten. In the normal spleen of the adult rabbit the germinal centers contain but few mitotic figures, and frequently none. The excessive abundance of mitotic figures after treatment may therefore be taken to indicate the stimulation of these cells.

Sections taken 48 hours after treatment show a condition apparently identical to that just described. The number of necrotic cells in the pulp seems to be slightly larger if anything, but not large enough to be regarded as abnormal.

4 days after the treatment the signs of stimulation become most marked. While the general histologic condition remains unchanged,
the number of mitotic figures in the germinal center of the nodule is decidedly increased. The germinal center is seen in the section to contain usually several, but sometimes about ten, mitotic figures (Figs. 1 and 2). A comparison of this with the normal condition, in which the proliferative activity in the germinal center is limited, seems to warrant the conclusion that the small dose of x-rays acts as a stimulant to the proliferation of the lymphoid cells.

Sections were taken on the 7th, 10th, and 14th days after treatment. The general histologic condition of all the sections was found to be normal, with the exception of a slightly enhanced mitosis. In the sections taken on the 7th day the number of mitotic figures in the germinal center of the nodule was seen to be smaller, and the tendency to decrease was manifested even more markedly in the sections taken later (on the 10th or 14th day). Even in the later sections, however, the proliferative activity of the cells, judged from the standpoint of mitosis, seemed slightly above the normal.

Lymph Glands.—In addition to the mesenteric lymph gland, cervical, axillary, and inguinal glands were taken for comparison. The changes in these glands were found to be so nearly uniform in character that a general description will apply to all of them.

The most striking deviation from the normal in the histology of lymph glands immediately after the treatment is the great abundance of mitotic figures in the nodule. The dividing cells are not localized, but are found all over the nodule. Some mitotic figures are seen in the internodular spaces in the cortex and also in the medulla, where, as a rule, very few if any division figures are normally present. The whole tissue was seen to be almost entirely free from degenerating cells. Some evidences of necrosis were, of course, observed here and there, but they were no more conspicuous than those seen in normal tissue.

48 hours after treatment the signs of stimulation of the cells were even more evident than immediately after treatment. All nodules contained excessively large numbers of dividing cells, each of the ordinary sized nodules showing in a section at least ten, and not infrequently as many as twenty mitotic figures (Figs. 3 and 4). The general condition of the gland is similar to that immediately after the treatment, except that there is a slight increase of necrotic cells, mainly in the pulp spaces.
Mitotic figures appear slightly less numerous in the tissue taken 4 days after treatment. The nodule usually shows several mitotic figures in a section. These conditions may still be taken as slightly abnormal. The occurrence of the dividing cells is more or less localized in the nodule, and in many instances they constitute rather typical germinal centers.

In sections taken 7, 10, and 14 days after the treatment, conditions are similar to those just described. The slightly excessive occurrence of mitosis as late as the 14th day is well shown in many nodules in the form of actively proliferating cells of the germinal center.

DISCUSSION.

As far as the results of lymphocyte counts show, the nature of lymphocytosis induced by heat and of that brought about by x-rays is indistinguishable, since the lymphocytic changes are exactly parallel in both cases; i.e., always with a characteristic fall preceding the marked rise. The idea that the phenomenon of lymphocytosis is of the same nature, regardless of whether the agent used for its production is heat or x-rays, seems probable, especially when we recall the results of Heineke and Warthin, who have shown that the effect of x-rays on lymphoid tissue is, in the main, similar to that of heat, as described by us in another paper. They used the x-rays, however, in different dosage from ours, and did not, furthermore, make observations during the critical period after the x-ray treatment, when an excessive multiplication of the cells may possibly take place. Notwithstanding the apparent similarity in the nature of the two phenomena which have been pointed out, the results of the experiments described in the present paper show conclusively that the lymphocytosis induced by the small dose of x-rays is due to the primary stimulative effect of the agent and hence is fundamentally different in nature from the similar lymphocytic change induced by heat, which is a sort of regenerative phenomenon.

Throughout the course of the experiment no indication has been observed that suggests the injurious effect of the dose upon any of the

Warthin, A. S., Physician and Surg., 1907, xxix, 1.
lymphoid tissues examined. On the other hand, mitotic figures were seen to become gradually more abundant after the treatment. In the spleen this enhanced proliferative activity of the cell reached its height about 4 days after the treatment, and the more or less distinct indications of the stimulation persisted in the germinal center up to the 14th day after the treatment. In the lymph glands the stimulative change is distinctly manifested earlier and is more extensive than in the spleen.

These histologic findings are in harmony with the results of the blood cell counts, which show that the increase of lymphocytes becomes most pronounced about 1 week after the treatment. If the lymphocytosis is due to the stimulation of the lymphoid tissues, the latter should show the change before the former becomes evident, and this is apparently what takes place.

SUMMARY AND CONCLUSION.

1. The small dose of x-rays applied to the rabbit has no appreciable destructive effect on the lymphoid tissue.

2. Indications of stimulation of the lymphoid tissue appear immediately after the treatment, become most pronounced in 2 (in lymph glands) to 4 (in the spleen) days, and persist, in a slight degree, up to the 14th day.

3. These facts suggest that the lymphocytosis induced by the small dose of x-rays is due to a primary stimulative effect upon the lymphoid tissue of the animal.

EXPLANATION OF PLATES.

PLATE 8.

Fig. 1. A splenic nodule, 4 days after the treatment, showing extensive stimulation. × 350.

Fig. 2. The same, showing mitotic figures (M) in higher magnification. × 1,000.

PLATE 9.

Fig. 3. A nodule of the mesenteric lymph gland, 48 hours after the treatment, showing intense stimulation. × 350.

Fig. 4. The same, showing mitotic figures (M) in higher magnification. × 1,000.
(Nakahara: Studies on X-ray effects. III.)