

STUDIES ON X-RAY EFFECTS.

V. EFFECT OF SMALL DOSES OF X-RAYS OF LOW PENETRATION ON THE LYMPHOID TISSUE OF MICE.

BY WARO NAKAHARA, Ph.D., AND JAMES B. MURPHY, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

PLATES 3 TO 5.

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The destructive effect of x-rays on the lymphoid tissue was early noted in the study of the biological effects of this agent. The stimulating action of this agent on the circulating lymphocytes was first observed in this laboratory and was applied experimentally in the study of x-ray effects on spontaneous tumors of mice.¹ The earlier work was carried out with the old type of gas tube, with which it was difficult to regulate the amount and character of rays used, and therefore no attempt was made to standardize the dosage. This difficulty has largely been overcome by the use of the Coolidge tube. The stimulating dose for rabbits has been reported.² The work of Russ, Chambers, Scott, and Mottram³ confirms our earlier observation on the stimulative action in mice.

In our experiments with rabbits a histological study paralleling the blood counts confirmed the general nature of the stimulation by showing a marked increase in the number of mitotic figures in the germinal centers of the lymphoid organs of these animals.⁴ As mice are the animals used most extensively in our experiments, it was regarded as important to duplicate the histological study of the

¹ Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800.

² Thomas, M. M., Taylor, H. D., and Witherbee, W. D., *J. Exp. Med.*, 1919, xxix, 75.

³ Russ, S., Chambers, H., Scott, G. M., and Mottram, J. C., *Lancet*, 1919, i, 692.

⁴ Nakahara, W., *J. Exp. Med.*, 1919, xxix, 83.

lymphoid organs of the latter animals after a stimulating dose of x-rays. With this end in view, the following experiments were undertaken.

Methods.

In the analysis of the artificial stimulation of the lymphocytes the reaction in the lymphoid germinal centers must be studied first. The spleens and lymph glands from the stimulated animals were fixed with Conroy's 6-3-1 fluid and stained with Heidenhain's iron-hematoxylin. The reaction was analyzed mainly from the standpoint of the frequency of mitosis.

EXPERIMENTS.

Experiment 1.—Seven normal white mice were placed in a glass jar and exposed to the following dose of x-rays: spark-gap $\frac{3}{8}$ inch between points, milliamperage 25, distance from the target to the back of the animal approximately 8 inches, time 20 minutes. The top of the jar was covered to shield the animal from the heat of the tube. The mice were killed 24 hours, and 3, 5, 7, 10, 12, and 14 days, respectively, after the treatment and the lymphoid organs examined histologically.

24 hours after treatment numerous degenerated cells, with pycnotic or fragmented nuclei appeared in the spleen (Fig. 1). The Malpighian bodies were small and inconspicuous. The general histological condition simulated somewhat the picture described by Heineke⁵ and Warthin⁶ after a massive dose of x-rays, but the changes were not so extensive. No appreciable amount of pigment was seen, but there was a moderate number of mitotic figures distributed irregularly. The necrotic changes were not so marked in the lymph glands as in the spleen, but mitotic figures were almost totally absent from the former.

3 days after treatment the necrotic cells were found to be decreased in number. The Malpighian bodies of the spleen were more or less evident but showed few mitotic figures.

After 5 days, and up to 14 days, the general histological condition of the lymphoid organs was about normal. There was no increase in the number of mitotic figures.

⁵ Heineke, H., *Mitt. Grenzgeb. Med. u. Chir.*, 1905, xiv, 21.

⁶ Warthin, A. S., *Physician and Surg.*, 1907, xxix, 1.

It must be concluded that no lymphoid stimulation occurred in the mice used in the preceding experiment. The dose of x-rays used was one which yielded evidences of lymphoid stimulation without appreciable destruction in rabbits.

Experiment 2.—Three normal white mice were treated in the same way and with the same dose of x-rays as the mice in Experiment 1, except that the time of exposure was reduced to 10 minutes. These animals were killed 24 hours, and 4 and 8 days, respectively, after the treatment and the lymphoid organs studied.

A considerable increase in the number of mitotic figures in the nodule of the spleen (Fig. 2) was seen at 24 hours and at 4 days after treatment with x-rays. The rough estimate of the average number of these figures in a section of one nodule of the spleen was five or six at these periods. This is in striking contrast with the normal condition, in which mitotic figures in a splenic nodule are few. No other special change was observed, except an appreciable increase of pycnotic cells in the pulp at 24 hours after x-rays. This, however, was not so conspicuous as in the previous experiment.

The lymph glands showed an equally pronounced increase in the mitotic figures at the corresponding period (Fig. 3), and figures were also seen frequently in the medulla.

8 days after the x-ray treatment the spleen and lymph glands, as regards the number of mitotic figures, were normal in appearance, but gave the impression of having more abundant lymphoid elements.

Experiment 3.—Five normal white mice were x-rayed as before. In this experiment, however, the time of exposure was reduced to 5 minutes, the other factors remaining unchanged. The mice were killed 24 hours, and 4, 7, 10, and 14 days, respectively, after the exposure.

Histological examination of the lymphoid organs showed no marked change, as regards the number of mitotic figures, throughout the periods studied. Moreover, the evidences of destruction which were so marked in the first experiment and much less so in the second one were not present.

From the three series of experiments described we may say that the indication is that stimulation of lymphoid tissue in the mouse may be effected by a 10 minute exposure to a small dose of x-rays. To

establish this point the following additional experiments were made, triplicating Experiment 2.

Experiment 4.—Three normal mice were exposed to x-rays of the same quality as in the previous experiments for 10 minutes. They were killed 24 hours, and 4 and 8 days, respectively, after the treatment.

Experiment 5.—Five normal mice were treated with the 10 minute dose as before and were killed 24 hours, and 3, 5, 7, and 10 days, respectively, after treatment.

In the mice killed at 24 hours and 4 days after treatment in Experiment 4 and in those killed 24 hours and 3 days after treatment in Experiment 5, an abnormally large number of mitotic figures was observed in the lymphoid centers, just as had been observed in Experiment 2. The other mice of this series showed no such change. The initial destruction was the same as that observed in Experiment 2.

DISCUSSION.

The uniformity of the changes, as shown in the three experiments, both in extent and period of occurrence, cannot be considered as a mere coincidence. It is to be concluded, therefore, that the small dose of x-rays employed in these experiments is capable of stimulating to proliferation the lymphoid tissue of mice.

It is of interest to note in this connection the apparent relation between the extent of cellular destruction and the degree of cellular stimulation, as shown in Table I. It would seem from these observations that a certain amount of destruction is followed by proliferation, which occurs very slightly, if at all, after too much or too little destruction. While no definite conclusion should be drawn on this point from our small series of experiments, they seem to be of sufficient interest to be reported.

TABLE I.

Time of exposure.	Destruction.	Stimulation.
<i>min.</i> 20	+	±
10	±	++
5	—	—

From our present knowledge of the subject it is safe to say that the quantitative increase of the lymphoid elements in the body is mainly due to the hyperactivity of the lymphoblastic tissue of the lymphoid organs. Because of this activity hypertrophy of lymphoid organs, especially of the Malpighian bodies, and lymphocytosis in the blood may both result. Theoretically, as regards the number of these cells thrown into the circulation, it is conceivable that individual animals may react differently even with an equal stimulation of the lymphoid organs. Cases have been observed in which the blood lymphocytosis was due apparently to the mere emptying of the lymphoid organs without a corresponding actual increase of the lymphoid cells having taken place; and the histological studies just described have certainly given more nearly uniform evidence of stimulation than have blood counts.

CONCLUSION.

A dose of x-rays governed by the following factors induces a stimulation of lymphoid tissue in mice: spark-gap $\frac{7}{8}$ inch, milliamperage 25, distance 8 inches, time of exposure 10 minutes. Within 4 days after this dose there appeared an abnormally large number of mitotic figures in the lymphoid tissue of spleen and lymph glands, indicating an acceleration of the proliferative activity of the tissue.

EXPLANATION OF PLATES.

PLATE 3.

FIG. 1. A splenic nodule, 24 hours after a 20 minute exposure to the small dose of x-rays. Note the abundance of pycnotic cells.

PLATE 4.

FIG. 2. A splenic nodule, 24 hours after a 10 minute exposure to the small dose of x-rays. *M*, mitotic figure.

PLATE 5.

FIG. 3. A nodule of the mesenteric lymph gland, 4 days after a 10 minute exposure to the small dose of x-rays. *M*, mitotic figure.

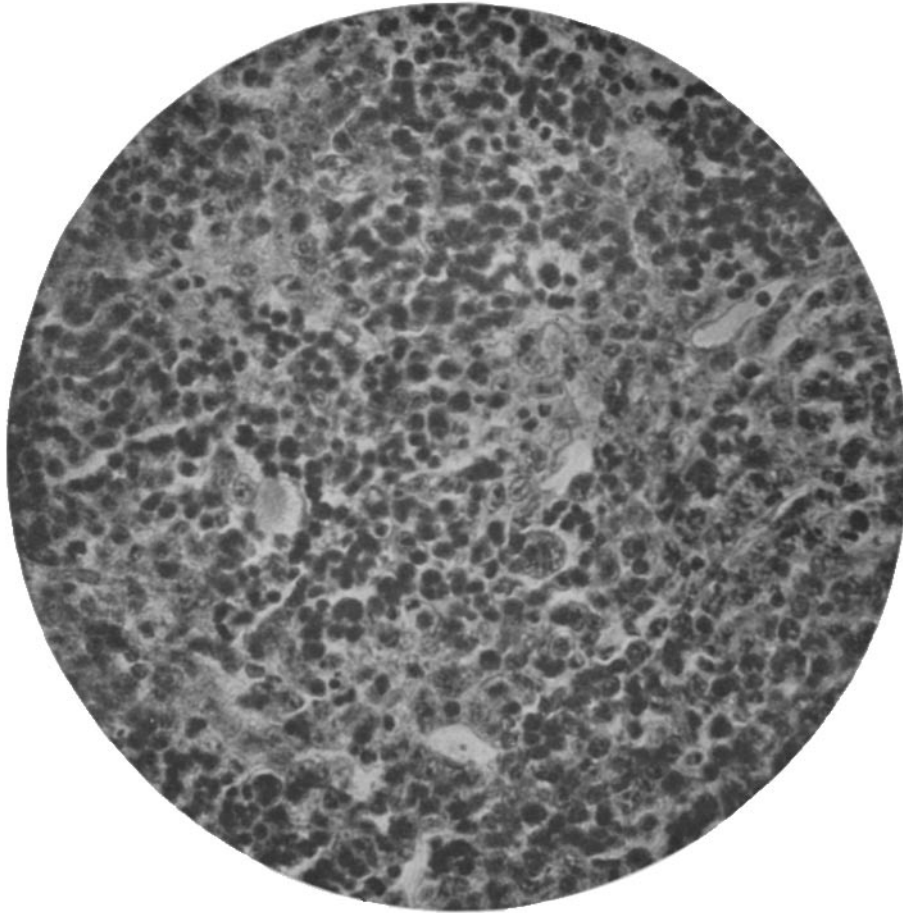


FIG. 1.

(Nakahara and Murphy: Studies on x-ray effects. V.)

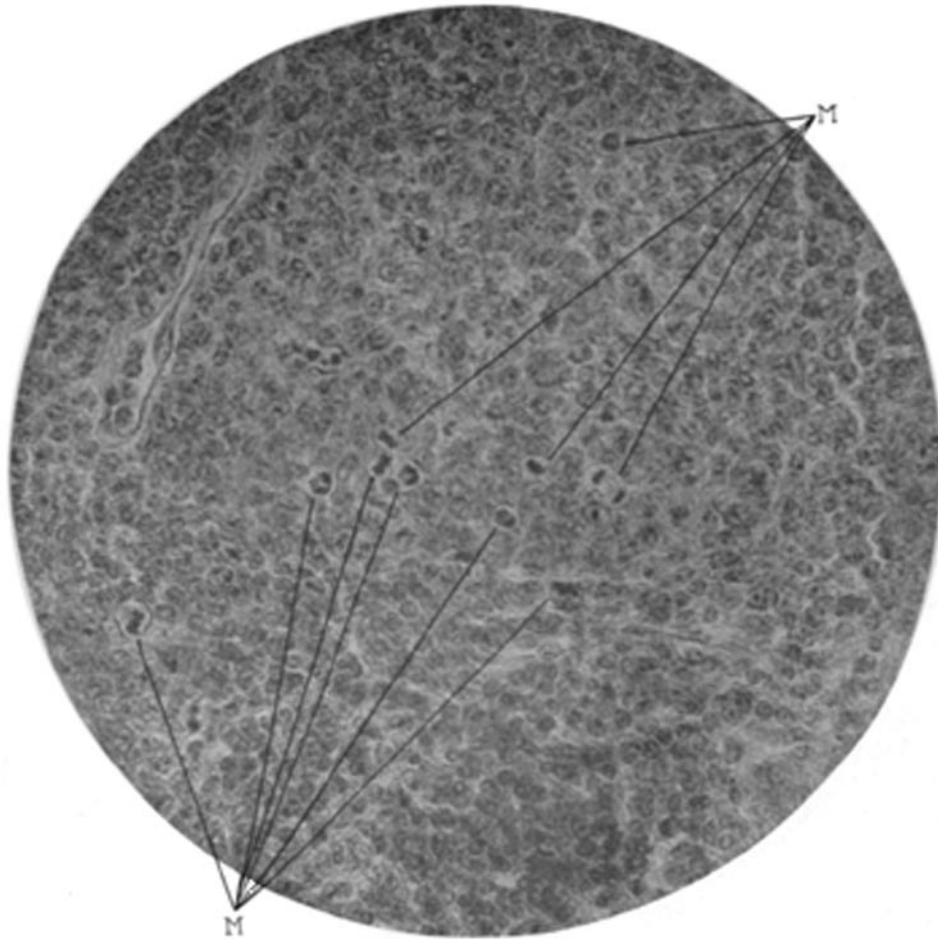


FIG. 2.

(Nakahara and Murphy: Studies on x-ray effects. V.)

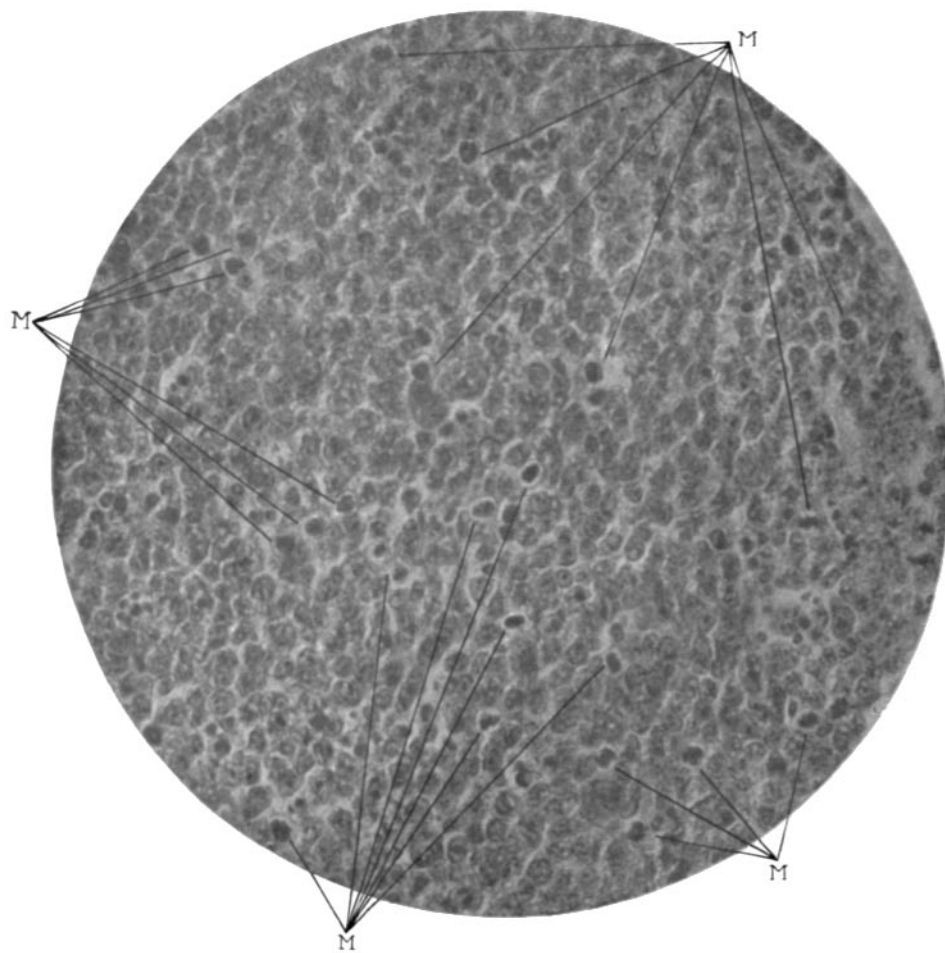


FIG. 3.

(Nakahara and Murphy: Studies on x-ray effects. V.)