STUDIES ON X-RAY EFFECTS.

VIII. Influence of Cancer Inoculation on the Lymphoid Stimulation Induced by Small Doses of X-Rays.

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The studies carried out in this laboratory have shown that immunity to cancer, whether natural or induced, is attended by lymphoid stimulation.[1] X-rays have been employed to secure the stimulative effect on the lymphoid elements;[2] when of sufficient amount, they have been found to increase[3] the resistance of mice to cancer transplants[4] made at the height of the reaction. On the other hand, if the cancer inoculation is made immediately after the x-ray treatment, no unusual degree of resistance is exhibited.[5] Hence it would appear that the early inoculation of cancer must have in some way interfered with the development of the defensive mechanism. The present study has been planned to elucidate this point.

EXPERIMENTAL.

Experiment 1.—Twelve normal white mice were given the following dose of x-rays (Coolidge tube): spark-gap $\frac{7}{8}$ inch, milliamperage 25, distance 8 inches, and time of exposure 10 minutes. The manner of applying the dose was the same as in experiments previously reported. As soon after the treatment as possible the animals were inoculated subcutaneously in the left groin with fragments of a Bashford adenocarcinoma, No. 63. Of these mice, six were killed for histo-

[1] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 204.

[2] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800.

[3] Nakahara, W., and Murphy, Jas. B., *J. Exp. Med.*, 1920, xxxi, 13.

[4] Nakahara, W., and Murphy, Jas. B., *J. Exp. Med.*, 1921, xxxiii, 429.

[5] Murphy, Jas. B., *Proc. Nat. Acad. Sc.*, 1920, vi, 35. Murphy, Jas. B., Nakahara, W., and Sturm, E., *J. Exp. Med.*, 1921, xxxiii, 423.

logical study 48 hours and the other six 4 days after the inoculation. No difference was noted at autopsy between the two groups of mice killed at different periods.

In three of the six animals in each group the spleen and lymph nodes were found at autopsy to be smaller than is usual in normal mice. The mesenteric node, which is the largest lymph node in the mouse, was in a few instances as small as the normal inguinal, or even the axillary node.

Microscopically, a considerable number of pycnotic cells were found in these organs, but the deposit of pigment in the spleen was never conspicuous. The most striking feature was the almost complete suppression of the proliferative activity of lymphoid cells in half of the animals examined, only a few mitotic figures being found in each section. This suppressed activity was equally evident in the two groups, and apparently was independent of the size of lymphoid organs. In the remaining half of the animals, mitotic figures were found more frequently, but in no instance was there any sign of an activity above normal.

Experiment 2.—Eleven normal white mice were given the same dose of x-rays as before; six were killed 48 hours and five 4 days after the treatment, without having been inoculated with the cancer.[6]

The increase in the number of mitotic figures in the lymphoid tissue was evident in the majority of cases; namely, in nine out of eleven animals. The remaining two animals showed only slight signs of lymphoid proliferation, judging by the limited number of mitotic figures found.

Experiment 3.—Eleven normal white mice were treated with the same dose of x-rays. 7 days later the animals were inoculated subcutaneously in the left groin with fragments of Bashford Adenocarcinoma No. 63. Six of these mice were killed after 48 hours and five after 4 days; *i.e.*, 9 and 11 days after the x-ray treatment.

No unusual macroscopic feature of the lymphoid organs was noted at autopsy. There was some variation in the histologic condition of these organs. In six of the eleven animals, there was an extensive stimulation of lymphoid tissue as evidenced by the large number of mitotic figures in the tissue. The mitotic figures were found abundantly, not only in the area of the germ centers, but also in the pulp. The nodules were more or less enlarged. There were few pycnotic cells, and pigment was almost entirely absent. In four other animals, the stimulative reaction was similar in kind but somewhat less pronounced. One animal was exceptional in that it showed almost no signs of cell proliferation.

Experiment 4.—Six normal white mice were treated with the same dose of x-rays as those above but were not inoculated with cancer. Three were killed 9 days and three 11 days afterwards.

The spleen of four out of the six mice was found at autopsy to be abnormally dark in color and, except in one animal in which it was below normal, of usual size.

[6] This was a repetition, for the sake of comparison, of an experiment previously reported.[3]

Histologically, the dark spleens showed a great accumulation of blood in the pulp, which was sparingly supplied with lymphoid cells. In contrast to the findings in Experiment 3, mitotic figures were in no instance abundant in the lymphoid tissue of spleen or of the lymph nodes. Cells with pycnotic nuclei were numerous, except in one specimen in which the number was small.

For a further comparison, the four preceding experiments were repeated in a single experiment which included groups subjected to the various experimental conditions of the individual experiments described above.

Experiment 5.—Twenty-five normal white mice, divided into four groups, were given the same dose of x-rays as in the previous experiments.

Group 1: eight mice were inoculated with Bashford Adenocarcinoma No. 63 immediately after the treatment with x-rays. They were killed 3 days later.

Group 2: seven mice were killed 3 days after the x-ray treatment without having been inoculated with cancer.

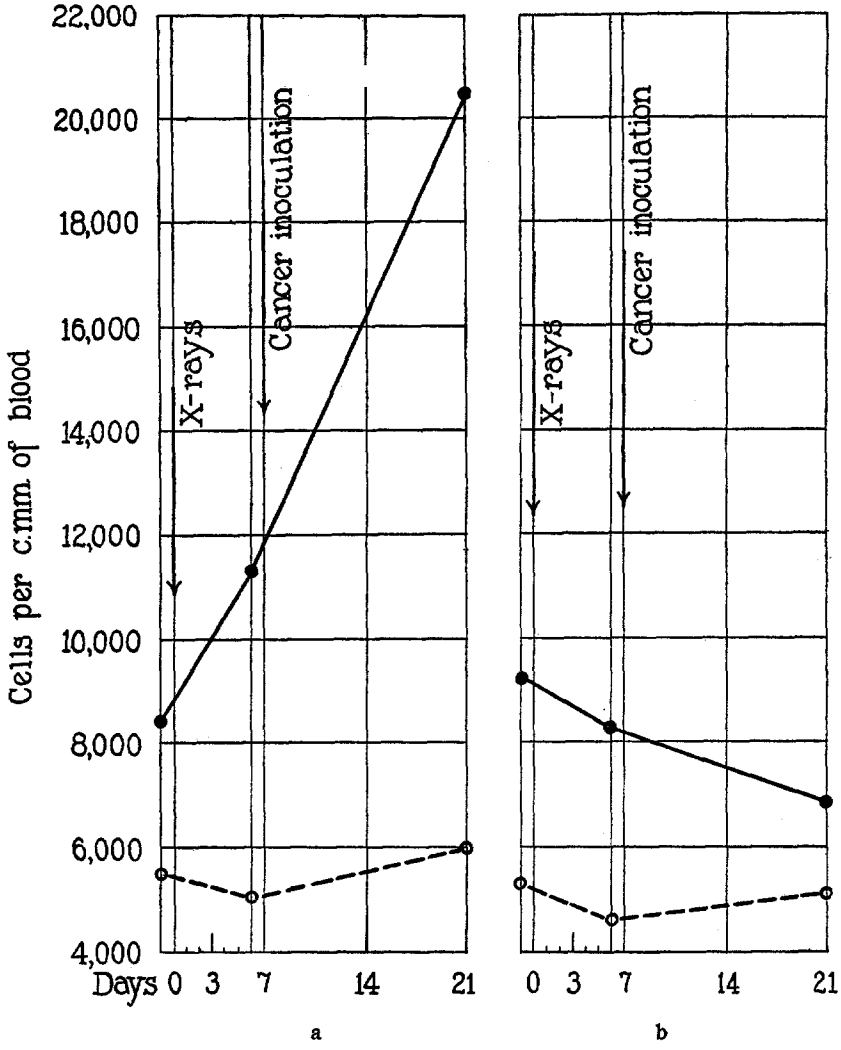
Group 3: six mice were inoculated, 7 days after the treatment with x-rays, with the same strain of cancer and were killed 3 days after the inoculation; *i.e.*, 10 days after the x-ray treatment.

Group 4: four mice uninoculated with cancer were killed 10 days after the x-ray treatment.

Of Group 1, all the mice except one individual showed suppression of the usual proliferation of the lymphoid elements. Of Group 2, all showed definite signs of increased proliferation of the same elements. In Group 3, three mice showed evidences of extensive stimulation, while the remaining three had a less marked reaction. Group 4 showed little signs of lymphoid proliferation.

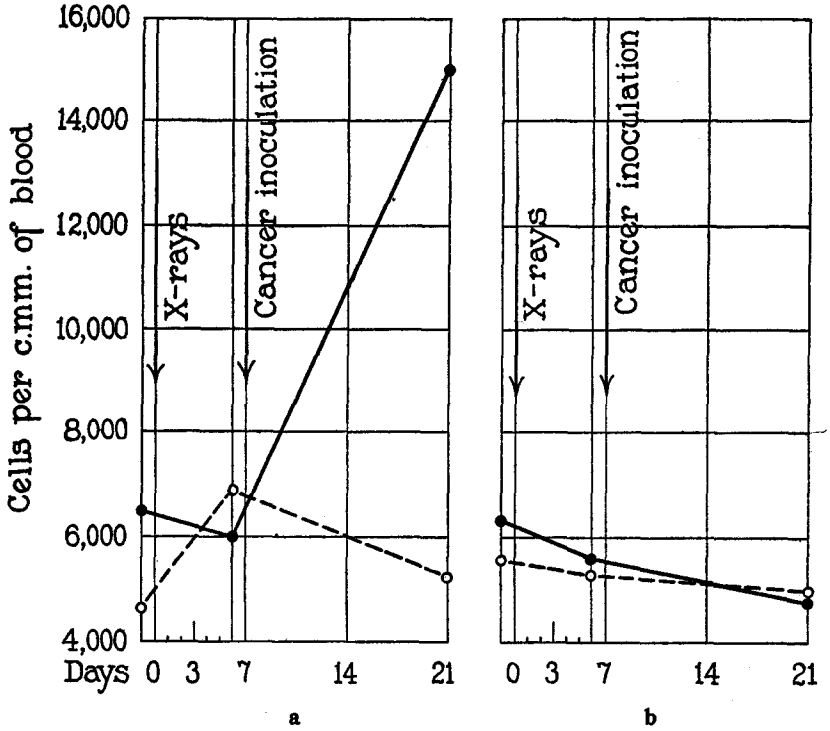
Hence, the results of this experiment are in agreement with those of the four experiments immediately preceding.

The following conclusions can be deducted from the results. (1) Cancer inoculation made immediately after a stimulative dose of x-rays interferes with the lymphoid reaction and little or no stimulation results. (2) Cancer inoculation made at the height of the stimulation augments the lymphoid reaction, and the proliferative activity of the cells continues longer than in animals which have been stimulated but have not received the cancer inoculation. (3) A proportion of animals given the stimulative dose of x-rays fails to react. It is of considerable interest to ascertain whether mice which fail to react are susceptible to cancer inoculations. To determine this point we have used the blood counts to ascertain the absence or presence of a stimulative phase.



TEXT-FIG. 1, *a* and *b*. Experiment 6. Composite curves of white blood cell counts on mice x-rayed and inoculated with cancer 7 days later. (*a*) Composite curves from fourteen mice proved to be immune. (*b*) Composite curves from ten mice proved to be susceptible. ———— Lymphocytes. ---------- Polymorphonuclear leucocytes.

Experiment 6.—Blood counts were made on twenty-four mice which were then given an exposure of x-rays similar to that used in the preceding experiments. 7 days later a second count was made, followed by the inoculation of each animal with a graft of the Bashford mouse cancer, and 14 days later a third count. Fourteen of the twenty-four animals resisted and ten responded to the cancer inoculation.



TEXT-FIG. 2, *a* and *b*. Experiment 7. Composite white blood cell counts on mice x-rayed and inoculated with cancer 7 days later. (*a*) Composite curves from the immune mice. (*b*) Composite curves from the susceptible mice. ———— Lymphocytes. ---------- Polymorphonuclear leucocytes.

In the immune mice (Text-fig. 1, *a*) the average number of lymphocytes per c. mm. of blood before x-ray treatment was about 8,300, the number of polymorphonuclear leucocytes being about 5,400. 6 days after the treatment (1 day before cancer inoculation) lymphocytes and polymorphonuclear cells were about 11,400 and 5,000 respectively. 2 weeks after the cancer inoculation the lymphocytes had, however, risen to approximately 20,000, while the polymorphonuclear cells showed but little change, being about 6,000.

In susceptible mice (Text-fig. 1, *b*) the average number of lymphocytes per c. mm. of blood before the dose of x-rays was approximately 9,600, the average number of polymorphonuclear cells was 5,300. A slight decrease in the white cells was noted 6 days after the treatment, the lymphocyte and polymorphonuclear leucocyte counts being 8,000 and 4,600 respectively. 2 weeks after the cancer inoculation the lymphocyte count was 7,000, the polymorphonuclear cell count 5,000.

Experiment 7.—The preceding experiment was repeated with twenty-six x-rayed mice, only nine of which resisted the cancer inoculation. Blood counts were made in the same way as before.

The average number of lymphocytes per c. mm. of blood in immune mice (Text-fig. 2, *a*) before x-ray treatment was about 6,500, and of polymorphonuclear leucocytes about 4,500. 6 days after the x-ray treatment the lymphocytes showed no material change, but the polymorphonuclear leucocytes went up to about 6,500. 2 weeks after the inoculation of cancer there was a rise of lymphocytes to about 15,000, while the polymorphonuclears decreased to about 5,000.

In susceptible mice (Text-fig. 2, *b*) the average number of lymphocytes and polymorphonuclear cells per c. mm. of blood before exposure to x-rays was about 6,000 and 5,000 respectively. No material change in these numbers was observed 6 days after x-rays. 2 weeks after cancer inoculation, however, the lymphocytes and polymorphonuclear cells were slightly decreased in number, the former being about 4,000 and the latter about 4,500.

DISCUSSION.

A comparison of the experiments described leads to the conclusion that if cancer inoculation is made immediately after the stimulative treatment with x-rays, no lymphoid stimulation occurs such as would regularly occur if the cancer inoculation were not made. On the other hand, if cancer inoculation is made 7 days after the x-rays are given, thus allowing the stimulation to develop before the inoculation, there is in the majority of cases what might be called a second stimulation of lymphoid tissue. It is significant, in connection with these facts, that while only little resistance to the transplantation was discovered when cancer was inoculated immediately after the x-rays, evidence of increased resistance appeared when inoculation was postponed until the 7th day.[5]

Attention is drawn in this connection to the parallelism existing between the lymphoid reaction accompanying the immunity to cancer grafts induced by physical agents (x-rays) and that induced by a biological agent (homologous blood). We have already shown

that mice immunized to cancer by means of an injection of defibrinated blood show an increase in the number of mitotic figures in the lymphoid tissue. Such mice, when inoculated with a cancer graft 10 days after the injection exhibit a second stimulation of the tissue,[7] as well as a marked blood lymphocytosis.[1] The experiments reported indicate a corresponding effect brought about by small doses of x-rays. The blood counts on the animals after the cancer inoculation show that only the animals presenting an increase in the lymphocytes prove to be resistant to the cancer. However, it should be stated that the blood counts were not made at a time to show the primary stimulative effect of x-rays, since this reaction, as previously shown, is of short duration. Hence it appears that as a result of the primary stimulation the animals have acquired the ability to react more strongly to a second stimulation; namely, the cancer inoculation.

SUMMARY.

Mice treated with small doses of x-rays and inoculated with cancer immediately afterwards, show a marked suppression of lymphoid proliferation. If, however, the cancer inoculation is made 7 days after the exposure to x-rays, thus permitting the primary lymphoid stimulation known to occur soon after the x-ray treatment to arise, a second stimulation takes place in a large proportion of mice thus inoculated.

Changes in the blood of mice x-rayed and inoculated with cancer 7 days afterwards show that the state of resistance to cancer inoculation is attended by blood lymphocytosis, as is the case in all other varieties of immunity to transplanted cancer so far studied.

[7] Murphy, Jas. B., and Nakahara, W., *J. Exp. Med.*, 1920, xxxi, 1.