

## STUDIES ON LYMPHOID ACTIVITY.

## V. RELATION BETWEEN THE TIME AND EXTENT OF LYMPHOID STIMULATION INDUCED BY PHYSICAL AGENTS AND THE DEGREE OF RESISTANCE TO CANCER IN MICE.

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Two methods have been described by which a definite stimulation of the circulating lymphocytes, accompanied by a hyperactivity of the lymphoid centers, may be brought about. It was first noted that small doses of x-rays would induce this condition,<sup>1</sup> but the reaction was of short duration<sup>2</sup> as compared with the stimulation occurring in cancer-immune mice following inoculation of cancer.<sup>3</sup> The stimulation induced by x-rays is preceded by a period during which evidences of the destructive action of this agent on the lymphoid centers are present, and is followed by a period during which the stimulation phase alone is present. By the 4th day the proliferative activity is at its height and then quickly subsides.

The amount of stimulus produced by dry heat<sup>4</sup> is much greater in extent and of longer duration than that seen after exposure to x-rays. Immediately after the heat application there are also marked evidences of cell destruction in the lymphoid centers, but the stimulation following is more prompt and of greater volume as judged by the blood pictures.

<sup>1</sup> Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800. Thomas, M. M., Taylor, H. D., and Witherbee, W. D., *J. Exp. Med.*, 1919, xxix, 75.

<sup>2</sup> Nakahara, W., and Murphy, Jas. B., *J. Exp. Med.*, 1920, xxxi, 13.

<sup>3</sup> Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 204. Murphy, Jas. B., and Nakahara, W., *J. Exp. Med.*, 1920, xxxi, 1.

<sup>4</sup> Murphy, Jas. B., and Sturm, E., *J. Exp. Med.*, 1919, xxix, 1.

We have described the close relation existing between lymphoid stimulation and the resistant state to cancer inoculation. If this relation is quantitative in nature, it would be expected that mice whose lymphoid cells are stimulated by x-rays would show a definite degree of resistance but less than that exhibited by animals following heat stimulation. This fact is borne out by our figures on 102 mice heated 1 week before inoculation, which showed an average immunity of 60.3 per cent, controlled by 83 normal mice inoculated with the same tumors showing an average immunity of 16.5 per cent. On the other hand, 144 mice, x-rayed from 3 to 7 days before inoculation, had an immunity of 37.5 per cent; they were controlled by 137 normal mice inoculated with the same tumors, having an average immunity of 10.4 per cent.

If the hypothesis is true that stimulation of the lymphocytes of mice definitely reduces the number of takes of cancer grafts, it would be of importance to know whether there is a difference in resistance percentage when the tumor inoculation is made at varying times after the stimulus is administered. The following experiments were planned to test this point.<sup>5</sup>

*Immunity after Exposure to X-Rays.*

Mice of about the same age were divided into three lots. Two of these lots were given a dose of x-rays governed by the following factors: spark-gap  $\frac{3}{8}$  inch between points, milliamperage 25, distance from target to back of animals 8 inches, and time 10 minutes. One lot received this dose 7 days and the other immediately before they were inoculated with a tumor (Bashford 63). At the same time the third group was inoculated with the same material and weekly measurements were made of the resultant tumors.

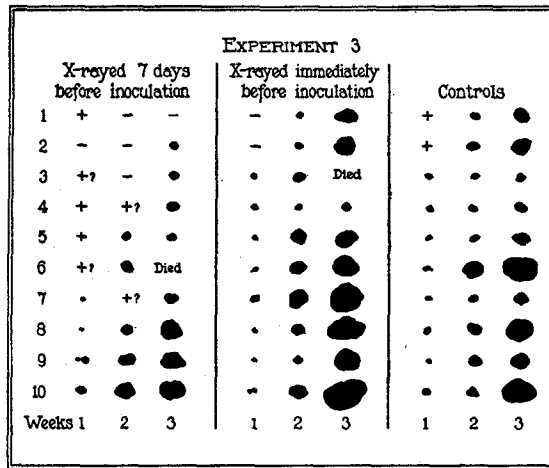
Table I gives the results of six experiments, the percentage of immune mice being estimated for a period 3 weeks after inoculation (Text-figs. 1 and 2).

<sup>5</sup> A preliminary report of this work was made before the National Academy of Science (Murphy, Jas. B., *Proc. Nat. Acad. Sc.*, 1920, vi, 35).

TABLE I.

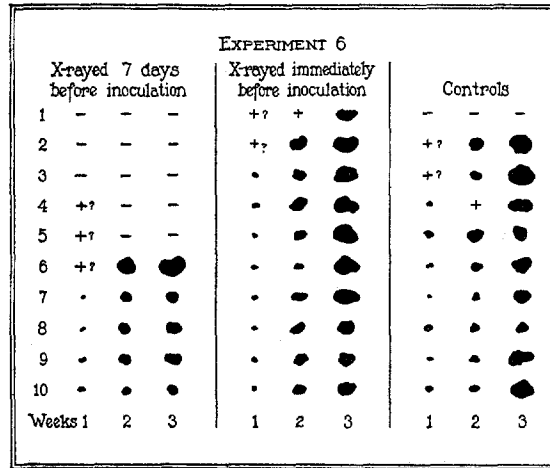
Experiment No.	Group 1.	Group 2.	Group 3.
1	11.1 per cent ( 9 mice).	0.0 per cent (10 mice).	0.0 per cent (10 mice).
2	30.0 " " (10 " ).	12.5 " " ( 8 " ).	10.0 " " (10 " ).
3	10.0 " " (10 " ).	0.0 " " (10 " ).	0.0 " " (10 " ).
4	50.0 " " (10 " ).	10.0 " " (10 " ).	20.0 " " (10 " ).
5	40.0 " " (10 " ).	11.1 " " ( 9 " ).	11.1 " " ( 9 " ).
6	50.0 " " (10 " ).	0.0 " " (10 " ).	10.0 " " (10 " ).

Group 1 was made of mice given a dose of x-rays 7 days before cancer was inoculated. Group 2 animals were x-rayed immediately before the cancer inoculation. Group 3 comprised the control mice inoculated with the same cancers. 175 mice were used for these tests.



TEXT-FIG. 1. The effect of x-rays on the immunity to transplanted cancer when administered 7 days and immediately before inoculation.

From the foregoing experiments it is seen that the resistance of mice inoculated with cancer immediately after a stimulating dose of x-rays is no higher than that of normal mice, and on the average is somewhat lower. On the other hand, the mice inoculated with cancer a week after a stimulating dose of x-rays show a consistently higher degree of resistance which reveals itself both in the number of takes and in the rate of growth of the tumor.



TEXT-FIG. 2. The effect of x-rays on the immunity to transplanted cancer when administered 7 days and immediately before inoculation.

#### *Immunity after Exposure to Dry Heat.*

These experiments with x-rays have been paralleled with similar tests with heat as the agent for stimulating the lymphocytes.

Three groups of mice were used. Group 1 consisted of animals which had been heated 1 week previous to the inoculation with cancer. Group 2 mice were heated immediately before the cancer inoculation, and Group 3 consisted of the normal controls inoculated with cancer. The mice to be heated were placed in an enclosed cage 3 inches above an electric heating lamp. A thermometer was placed half an inch below the bottom of the cage. The temperature was allowed to rise to 55°C. before the animals were put in the cage and then they were left in for 5 minutes, the temperature rarely rising above 63°C. during this time.

The results of the three experiments are given in Table II, the figures representing the immunity percentage 3 weeks after inoculation with cancer (Text-fig. 3).

TABLE II.

Experiment No.	Group 1.	Group 2.	Group 3.
7	66.6 per cent immunity.	40.0 per cent immunity.	20.0 per cent immunity.
8	55.0 " " "	35.0 " " "	10.0 " " "
9	50.0 " " "	30.0 " " "	10.0 " " "

Group 1 was made up of mice heated 1 week before inoculation. Group 2 mice were heated immediately before inoculation, and Group 3 comprised the control mice. The results are based on 118 mice.

EXPERIMENT 8									
Heated 7 days before inoculation, 55% immune			Heated immediately before inoculation, 35% immune			Normal controls, 10% immune			
1	-	-	-	-?	-	-	+	-	-
2	+	+	-	+	-	-	+	+	-
3	-	-	-	-	-	-	.	.	.
4	+	+	-	.	-	-	.	.	.
5	-	-	-	.	-	-	.	.	.
6	-	-	-	+	+	-	.	.	.
7	+	-	-	.	-	-	.	.	.
8	+	+	-	.	.	.	.	.	.
9	+	-	-	.	.	.	+	.	.
10	+	-	-	+	.	.	-?	.	.
11	+	+	-	.	.	.	.	.	.
12	.	.	.	.	.	.	+	.	.
13	.	.	Ulcer	.	.	.	+	.	.
14	+	.	.	+	.	.	+	.	.
15	+	+	.	-	.	.	+	.	.
16	+	+	.	.	.	.	-	+	.
17	+	+	.	+	+	.	+	.	.
18	.	.	.	-?	.	.	+	.	.
19	+	.	.	-?	.	.	-	.	.
20	.	.	.	.	.	.	-	.	.
Weeks	1	2	3	1	2	3	1	2	3

TEXT-FIG. 3. The effect of dry heat on the immunity to transplanted cancer when exposure to heat was done 7 days and immediately before inoculation.

The foregoing experiments indicate a definitely higher resistance in the mice inoculated immediately after heating than in the controls, while the mice heated a week prior to inoculation show an even more pronounced degree of immunity. The blood count after the heat treatment showed a sharp but very transitory drop in the number of circulating lymphocytes, followed within 24 hours by a marked increase and a continuous rise in these cells lasting for a week or more. Histological examination of the lymphoid organs of heated

animals showed that by 48 hours after the treatment these organs contain a larger number of mitotic figures in the germinal centers than is normally seen. The height of the reaction, judging from the blood pictures and the condition of the spleen and lymph nodes, occurs about the 7th day after the exposure to the heat. The degree of immunity to the transplanted cancer seems to vary directly with the amount of stimulation of the lymphocytes existing at the time of or immediately following the inoculation.

#### SUMMARY.

It has been shown that resistance to transplanted cancer follows stimulation of the lymphoid tissue when the stimulation is induced by either heat or small doses of x-rays. In this paper we have attempted to determine whether the degree of immunity had a quantitative relation to the amount of the stimulation. Fortunately, the two methods at our disposal give stimulation of markedly different characters. The small dose of x-rays gives a sluggish lymphoid cell reaction of short duration with a definite latent period between the treatment and the evidence of marked stimulation, while after heat a short period of depression is followed by a sharp stimulation continuing over a much longer period. The cancer inoculation into groups of mice made immediately after exposure to x-rays shows little resistance, while the inoculation made at the height of the stimulation phase shows a definite increase in the immunity. Animals inoculated with cancer immediately after the heat treatment exhibit a pronounced immunity, but not so marked as that shown when the inoculation is made at the height of the stimulation.

The amount of resistance shown when the cancer inoculation is made at the height of the moderately stimulating effect following exposure to x-rays, is much less than that seen when the inoculation is made at the height of the heat effect when the degree of stimulation is much greater. When the lymphocytosis sets in after the tumor graft is established only a slight effect is noted. All these results together are taken to indicate that the degree of immunity is dependent on the amount of lymphoid stimulation existing either at the time of or following soon after the cancer inoculation.