THE LYMPHOCYTE IN NATURAL AND INDUCED RESISTANCE TO TRANSPLANTED CANCER.

III. The Effect of X-Rays on Artificially Induced Immunity.*

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In previous communications it has been shown (*a*) that the normal chick embryo lacks the ability to destroy heteroplastic tissue grafts;[1] (*b*) that this lack of resistance disappears between the 19th and 21st days of incubation, and it is significant that the spleen develops at about this time;[2] (*c*) that the lack of resistance, seen during the early days of the incubation period, is replaced by a degree of resistance comparable with that observed in the adult animal if the embryo is supplied with a small bit of adult lymphoid tissue;[3] (*d*) that the natural resistance of adult animals to heteroplastic tissue grafts can be destroyed by the x-rays in proper dosage;[4] and (*e*) that the disappearance of tissue grafts in resistant animals is associated with an accumulation of lymphocytes about them.[5]

It is known[6] that mice injected subcutaneously with homologous living tissue cells, after an interval of about 10 days, are potentially immune to tissues subsequently inoculated from the same species; namely, mouse cancers. This potential immunity can be readily destroyed by exposing the animals to suitable doses of the x-rays in the interval between the immunizing dose and the cancer inocula-

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* A preliminary announcement of these experiments was made by Murphy and Taylor before the American Association for Cancer Research, New York, April 5, 1917, and was published in abstract form in the proceedings of this meeting (*J. Cancer Research*, 1917, ii, 504). Since this report a paper has appeared by Mottram and Russ, repeating and confirming these observations (Mottram, J. C., and Russ, S., *Proc. Roy. Soc. London, Series B*, 1917–18, xc, 1).

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[1] Murphy, Jas. B., *J. Am. Med. Assn.*, 1912, lix, 874.

[2] Murphy, Jas. B., *J. Exp. Med.*, 1913, xvii, 482.

[3] Murphy, Jas. B., *J. Exp. Med.*, 1914, xix, 513.

[4] Murphy, Jas. B., *J. Am. Med. Assn.*, 1914, lxii, 1459.

[5] For review of literature see Da Fano, C., *Z. Immunitätsforsch., Orig.*, 1910, v, 1.

[6] Imperial Cancer Research Fund, *Brit. Med. J.*, 1906, ii, 209.

tion. This finding was interpreted as being due to interference with the lymphoid blood crisis which has been shown to follow the tumor inoculation[7] and which has been thought to influence the subsequent resistance.

It is established that the potential immunity to cancer resulting from an homologous tissue injection is of the nature of a non-specific reaction, the resistance produced being directed toward a great variety of cancers and sarcomas as well as toward homologous normal tissues. Injection with one of the transplantable mouse carcinomata renders the animal resistant to that tumor alone and thus the reaction becomes specific. It seemed possible that while the lymphocyte might be a potent factor in bringing about the potential immunity, which is non-specific, after the resistance becomes specifically directed against a particular tumor, this cell might no longer play a part in the maintenance of the immunity. To test this point the following experiments were carried out.

Method.

Mice were immunized by an injection of homologous defibrinated blood beneath the skin of the back. 10 days later a bit of tumor (Bashford Adenocarcinoma No. 63) was inoculated into the left groin of each animal. A number of non-immunized mice were inoculated at the same time with the tumor in order to control its virulence. After the animals had been observed for a period of 3 weeks, the immune animals were divided and one group was subjected to small repeated doses of x-rays, the other being set aside for controls. A week later both groups were reinoculated in the right groin with the same tumor strain, the virulence of the strain being determined by simultaneous inoculation into normal mice. The x-ray dosage used in these experiments was one which previous experiments[8] had shown to be adequate to destroy the major portion of the lymphoid tissue without appearing to affect the general health of the animal.

Experiment 1.—Forty-nine white mice of the same approximate age, size, and weight, obtained from one source, at the same time, were injected beneath the skin of the back with 0.2 cc. of defibrinated mouse blood. 10 days later there was

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

[8] Murphy, Jas. B., and Ellis, A. W. M., *J. Exp. Med.*, 1914, xx, 397. Taylor, H. D., and Murphy, Jas. B., *J. Exp. Med.*, 1917, xxv, 609.

inoculated into the left groin of each a bit of Bashford Adenocarcinoma No. 63. At the end of the 3 weeks interval following inoculation, forty animals, or 81.6 per cent, were found to be immune. Of ten normal animals inoculated simultaneously as controls, nine, or 90 per cent, developed tumors. It is evident, therefore, that the tumor is freely transplantable to normal animals and that the resistance present in those previously injected with mouse blood represents an induced immunity.

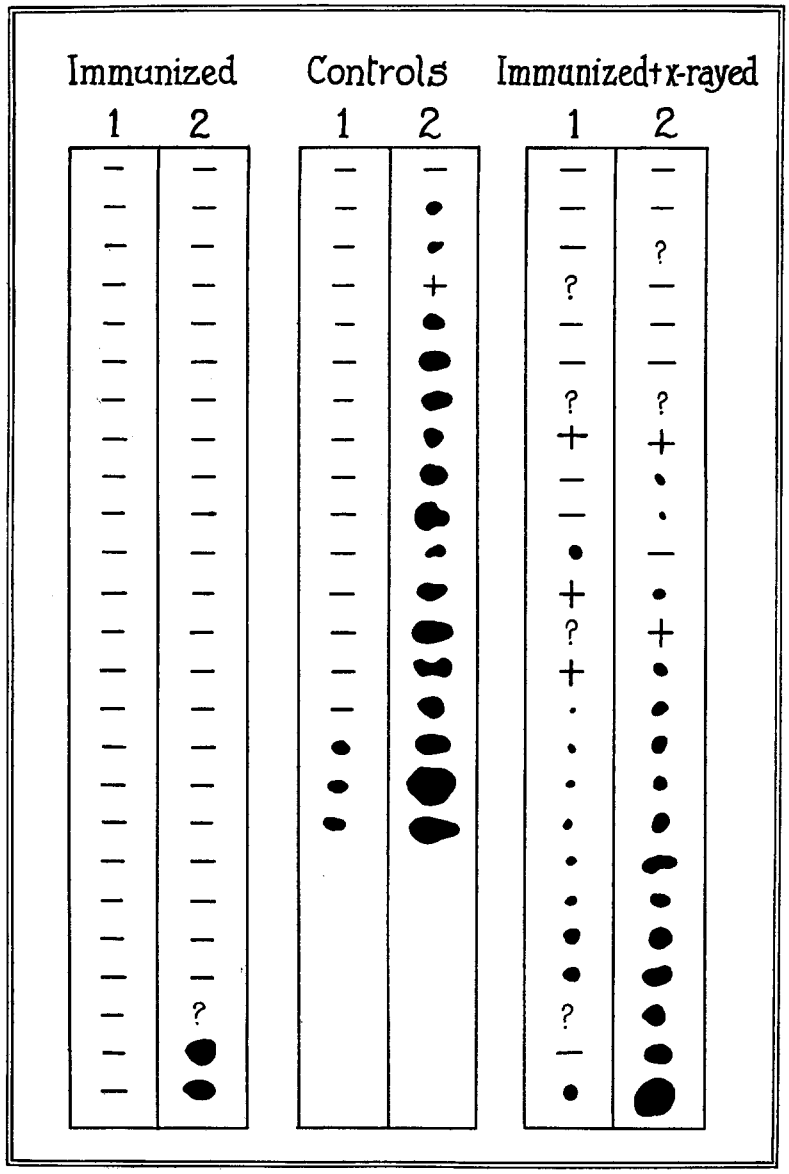
After a period of 3 weeks following the first inoculation with the tumor, the forty immune mice were divided into two groups. The first, comprising nineteen animals, remained untreated, while the second, comprising twenty-one animals, was exposed to the x-rays for 7 successive days. The dose of x-rays given at each exposure was as follows: a 2¼ inch spark-gap, 10 milliamperes, with a distance of 12 inches between the target of the Coolidge tube and the nearest point on the backs of the mice, time of exposure 2 minutes. After the seven exposures both groups of immune mice and eleven normal controls as well were inoculated with a later generation of the Bashford tumor in the right groin, with the results shown in Table I.

TABLE I.

Group.	Treatment.	Per cent of takes.
I	Immunized.	21.0
II	" and x-rayed.	52.4
III	Controls.	90.9

Of the animals artificially immunized against, inoculated with, and proven immune to a transplantable carcinoma, those which received no x-ray treatment before a second inoculation with the same tumor still showed a high degree of immunity, as only four of the nineteen, or 21 per cent, developed tumors. The second group of mice, which was x-rayed before the second tumor inoculation, showed a much smaller degree of immunity, eleven mice, or 52.4 per cent, yielding tumors. Of the controls, ten, or 90.9 per cent, developed tumors.

Experiment 2.—Fifty-two mice were immunized and inoculated with the mouse carcinoma in the manner indicated in Experiment 1; of these, fifty-one, or 98 per cent, proved to be immune. It happened that nine of the fifteen control mice developed tumors, so that 40 per cent showed natural immunity. During the 3rd week following the initial tumor inoculation twenty-five of the animals received seven daily x-ray treatments with a Coolidge tube, the following factors being used at each exposure: spark-gap 2¼ inches, milliamperes 10, distance from the target to the backs of the mice 12 inches, and the time of exposure 2 minutes.



TEXT-FIG. 1. Comparable sizes of the tumors in Experiment 2, as given in Table II. Column 1 shows the results 1 week and Column 2, 2 weeks after inoculation.

Twenty-five of the immune animals received no x-ray treatment. The two groups, fifty mice in all, together with eighteen control animals, were then inoculated in the right groin with a bit of a later generation of the Bashford tumor used in the primary inoculation. The results are shown in Text-fig. 1 and Table II.

TABLE II.

Group.	Treatment.	Per cent of takes.
I	Immunized.	12.0
II	" and x-rayed.	64.0
III	Controls.	94.4

Of the immune mice not treated with x-rays, only three, or 120. per cent, developed tumors. Of the immune animals x-rayed before the second inoculation, sixteen, or 64 per cent, developed tumors. There was tumor growth in seventeen, or 94.4 per cent, of the eighteen control animals.

TABLE III.

Group.	Treatment.	No. of mice.	Before x-ray treatment. Per cent of takes.	After x-ray treatment. Per cent of takes.
I	Immunized.	19*	10.5	73.3
II	Controls.	10†	90.0	90.0

* Fifteen surviving at second inoculation.

† Ten mice as controls for each inoculation of Group I.

Experiment 3.—As shown in Table III, nineteen mice were inoculated in the left groin with a bit of the Bashford tumor after having received, 10 days before the tumor inoculation, 0.2 cc. of defibrinated mouse blood. Two mice, or 10.5 per cent, developed tumors. Six doses of x-rays with the Coolidge tube were then administered on successive days, the daily dose depending on the following factors: spark-gap 2¼ inches, milliamperes 10, distance from target to mouse 12 inches, and time of exposure 1 minute. The fifteen mice which survived the period were then inoculated in the right groin with a later generation of the Bashford tumor and eleven, or 73.3 per cent, developed tumors. There was tumor growth in nine, or 90 per cent, of the ten animals used as controls to the first inoculation and the same percentage of growth in the same number of controls to the second inoculation.

Experiment 4.—Mice immunized and inoculated as indicated in the previous experiments were divided into two lots. After inoculation with the Bashford

tumor the first lot, consisting of nine mice, showed tumors in three, or 33.3 per cent. The second group of eight immune animals was given a series of x-ray treatments identical with those of Experiment 2, and 50 per cent developed tumors. There were 66.6 per cent of takes in the nine control animals (Table IV).

TABLE IV.

Group.	Treatment.	Per cent of takes.
I	Immunized.	33.3
II	" and x-rayed.	50.0
III	Controls.	66.6

Experiment 5.—Twenty-seven animals immunized as in the previous experiments were inoculated in the left groin with the Bashford tumor and to control further the immunity which was evident at this time were reinoculated with the same tumor. All proved to be immune after this rigid test. Seventeen of these immune mice gave 29.4 per cent of takes after a third inoculation with the same tumor to control the ten remaining ones which were also inoculated with this tumor after x-ray treatments on 3 successive days in the same manner as that employed in Experiments 1, 2, and 3, except that the time of exposure was 3 minutes for each of four exposures. Five of the second group, or 50 per cent, developed tumors. Four, or 40 per cent, of the ten control animals developed tumors (Table V).

TABLE V.

Group.	Treatment.	Per cent of takes.
I	Immunized.	29.4
II	" and x-rayed.	50.0
III	Controls.	40.0

Experiment 6.—Twenty mice were immunized and proved immune in the manner described in the preceding experiments, divided into two groups, and one group was given x-ray treatments with the Coolidge tube as follows: $2\frac{1}{4}$ inch spark-gap, 10 milliamperes, 12 inch distance. Five of the daily exposures were of 2 minutes each and one for 5 minutes, the exposures being made during the 3rd week following the initial tumor inoculation. Of the seven animals so treated, 85.7 per cent developed tumors when given a second inoculation in the right groin with the Bashford tumor. A control group of thirteen untreated immune animals from the same lot of mice, inoculated at the same time, showed tumor growth in only 38.8 per cent. 60 per cent of the ten inoculated normal mice developed tumors. The results of the experiment are given in Table VI.

TABLE VI.

Group.	Treatment.	Per cent of takes.
I	Immunized.	38.8
II	" and x-rayed.	85.7
III	Controls.	60.0

Experiment 7.—Seven mice inoculated after being immunized in the usual way gave 28.2 per cent of takes. Inoculated a second time, only 14.2 per cent grew tumors. Of seven other mice, from the same group, 14.2 per cent developed tumors, after an inoculation with the Bashford tumor before treatment. After five daily exposures to the x-rays generated by a Coolidge tube there were 71.3 per cent of takes in this second group. The x-ray factors were the same as those used in Experiment 1. Control mice inoculated at the same time, with the same tumor, showed 71.3 per cent of tumors. The results of the experiment are given in Table VII.

TABLE VII.

Group.	Treatment.	First inoculation. Per cent of takes.	Second inoculation. Per cent of takes.
I	Immunized.	28.2	14.2
II	" and x-rayed.*	14.2*	71.3*
III	Controls.	71.3	—

* X-ray exposures after first and before second inoculation.

Experiment 8.—Twenty-eight mice were immunized, each with 0.2 cc. of defibrinated mouse blood, and divided into two groups. The first group of nine gave 11.1 per cent tumors after the first inoculation into the left groin and 37.5 per cent after the second inoculation into the right groin. They had no x-ray treatment. Of the second group of nine mice, x-rayed before the first inoculation, 55.5 per cent developed tumors. This group was again x-rayed and reinoculated into the right groin. 80 per cent developed tumors. A third group of immune animals was inoculated first in the left groin with the tumor, of which 20 per cent developed tumors. They were x-rayed, and upon reinoculation in the right groin 57.1 per cent of tumors resulted. The x-ray dosage was the same in both series of treatments given the second group of mice, as well as in the series of treatments which the third group received. The x-ray factors were identical with those of Experiment 1. The control mice gave 80 per cent of tumors. The results are given in Table VIII.

TABLE VIII.

Group.	Treatment.	First inoculation. Per cent of takes.	Second inoculation. Per cent of takes.
I	Immunized. No x-ray treatment.	11.1	37.5*
II	Immunized. X-rayed before first and second inoculations.	55.5	80.0†
III	Immunized. X-rayed before second inoculation.	20.0	57.1‡
IV	Controls.	80.0	—

* Only eight living at time of second inoculation.
† Only five living at time of second inoculation.
‡ Only seven living at time of second inoculation.

DISCUSSION.

The experiments described in this paper indicate anew that the lymphocytes are a potent factor in the immunity to cancer which has been studied in the mouse. Taken with other indications the evidence is growing in importance and conclusiveness of the part played by the lymphocyte in bringing about and in maintaining that condition. The main points of evidence now adducible are: (1) the accumulation of lymphocytes about a transplanted cancer graft in an immunized animal; (2) the rise in number in the circulating lymphocytes during the development of the immune state, irrespective of whether the type of immunity induced is artificial or natural; (3) the setting aside of the potential immunity by the x-rays where the dosage employed is sufficient to destroy a large part of the circulating lymphocytes; and finally, (4) as shown by the present experiments, the abolition of the potential immunity for a special tumor strain by means of the lymphocyte-destroying power of the x-rays. These specific points are further supported by the observations of Leo Loeb[9] on the part played by the lymphocyte in respect to homoplastic grafts of normal tissue, and by those of Murphy[3] on heteroplastic tissue grafts.

[9] Loeb, L., *J. Med. Research*, 1917, xxxvii, 229.

SUMMARY.

Mice artificially immunized against a transplantable carcinoma, inoculated, and proved immune, may be again rendered susceptible to the same tumor by exposure to the x-rays.

The immune animals which have not been treated with the x-rays preserve, to a large degree, their resistance to a second inoculation of the tumor in question.