THE LYMPHOCYTE IN NATURAL AND INDUCED RESISTANCE TO TRANSPLANTED CANCER.

II. Studies in Lymphoid Activity.

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In former communications, we have shown that the resistance to heteroplastic tissue grafts apparently depends on the activity of the lymphocyte. The chick embryo, which we found[1] to be devoid of any resisting power to the growth of grafted tissues from a foreign species, could be rendered as refractory as the adult animal, by the introduction of a bit of adult homologous tissue rich in lymphoid elements, such as spleen or bone marrow.[2] Furthermore, an adult animal deprived of the major portion of its lymphoid system by means of repeated small doses of x-ray, no longer resists the growth of heterologous tissues. The tissue cells from a foreign species will grow actively till such a time as the depleted lymphoid system of the animal is well advanced in regeneration.[3]

Histologically, there is a striking resemblance between the appearances presented by the failing heteroplastic graft in the foreign host and the cancer graft in the so called immune animal. The phenomenon is the same, whether the state of resistance to the cancer is one possessed naturally by the animal, a condition termed natural immunity, or whether it is of the nature of induced immunity developed by previous treatment of the animal. The details of the various stages of the processes as observed under the microscope need not be gone into here.[4] It is only necessary to say that the

[1] Murphy, Jas. B., *Jour. Exper. Med.*, 1913, xvii, 482.

[2] Murphy, Jas. B., *ibid.*, 1914, xix, 513.

[3] Murphy, Jas. B., *Jour. Am. Med. Assn.*, 1914, lxii, 1459.

[4] For the literature see Da Fano, C., *Ztschr. f. Immunitätsforsch., Orig.*, 1910, v, 1.

constant and undisputed fact is that in both the heteroplastic tissue graft and the homologous cancer graft in an immune animal, there is a pronounced collection of small round cells at the edges of the grafts and densely infiltrating the tissues about.[5] The lymphoid elements appear quite early in the process and last till it is completed. It is notable that these elements are absent about the growing cancer graft in the highly susceptible animal.

With the above fact at hand it would seem, arguing from analogy, that the lymphocytes might play an as equally important part in immunity to transplanted cancer as they appear to play in the resistance phenomenon to heteroplastic tissue grafts. With this problem in mind the following experiments were planned.

Resistance to Transplanted Cancer.

It is unnecessary to go into the details and theories of the various types of resistance to the transplanted cancer. This has been done at length in numerous communications on the subject.[6] The essential facts are that mice may be rendered relatively immune for a period by giving a subcutaneous or intraperitoneal injection of a certain amount of homologous living tissues at least ten days before inoculating the cancer graft. Various tissues have served, such as hashed embryo, embryo skin, spleen, and red blood cells, for this purpose. The efficiency of the protection varies considerably, depending on the type and amount of tissue used, as well as on the growth power of the cancer inoculated. A certain proportion of mice inoculated with cancer show a natural refractory state which varies in different families of mice and to an extent according to the virulence of the tumor. Sometimes this natural resistance is so effective that the cancer graft does not become established, while in the less resistant animal the introduced tumor may grow for a time and only later be overcome and absorbed. These are the types of immunity which we have elected to study from the point of view of their general lymphoid reaction.

[5] For general review and literature on the subject see Woglom, W. H., The Study of Experimental Cancer, A Review, Studies in Cancer and Allied Subjects, New York, 1913, i, 128.

[6] Woglom, *loc. cit.*

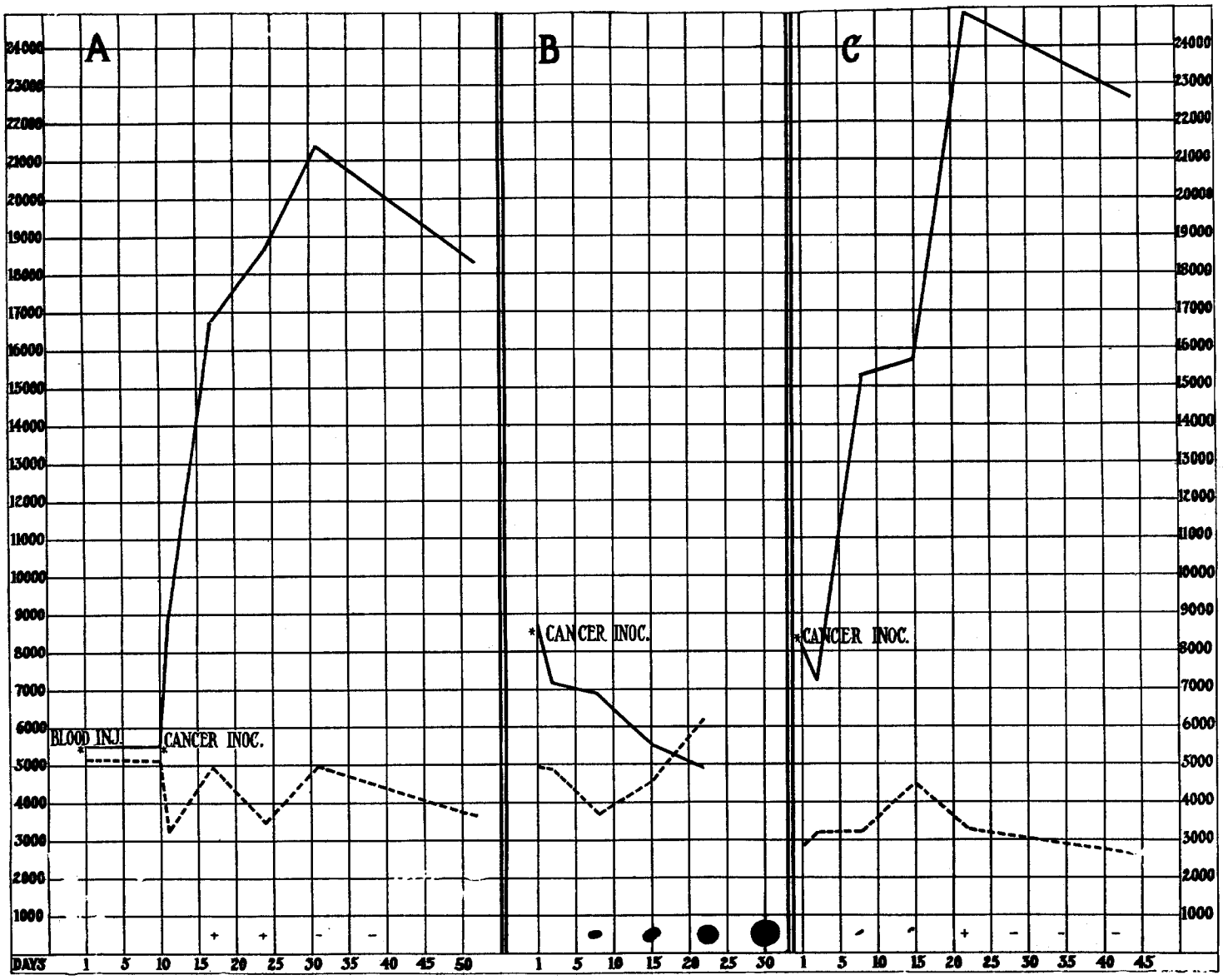
In all we have made detailed studies of the blood in over eighty mice before and at intervals after inoculation with cancer in both immune and susceptible animals. Only one of these experiments will be reported as the others gave practically identical results.

Experiment I.—20 white mice of about the same age and size were selected. A white blood count and a differential count were made to establish the normal in each animal. 10 of the mice were given a subcutaneous injection of 0.3 cc. of defibrinated mouse blood. 10 days later all 20 animals were inoculated with equal sized grafts of a transplantable mammary carcinoma of the mouse.[7] Blood examinations were made after 24 hours and at intervals for something over 50 days. The grafts were measured and recorded weekly. The protection in the immunized animals of this series was 100 per cent. Among the controls, 60 per cent grew their grafts and 40 per cent were naturally immune. From the white count and differential, we have estimated the actual numbers of the various types of white cells present.

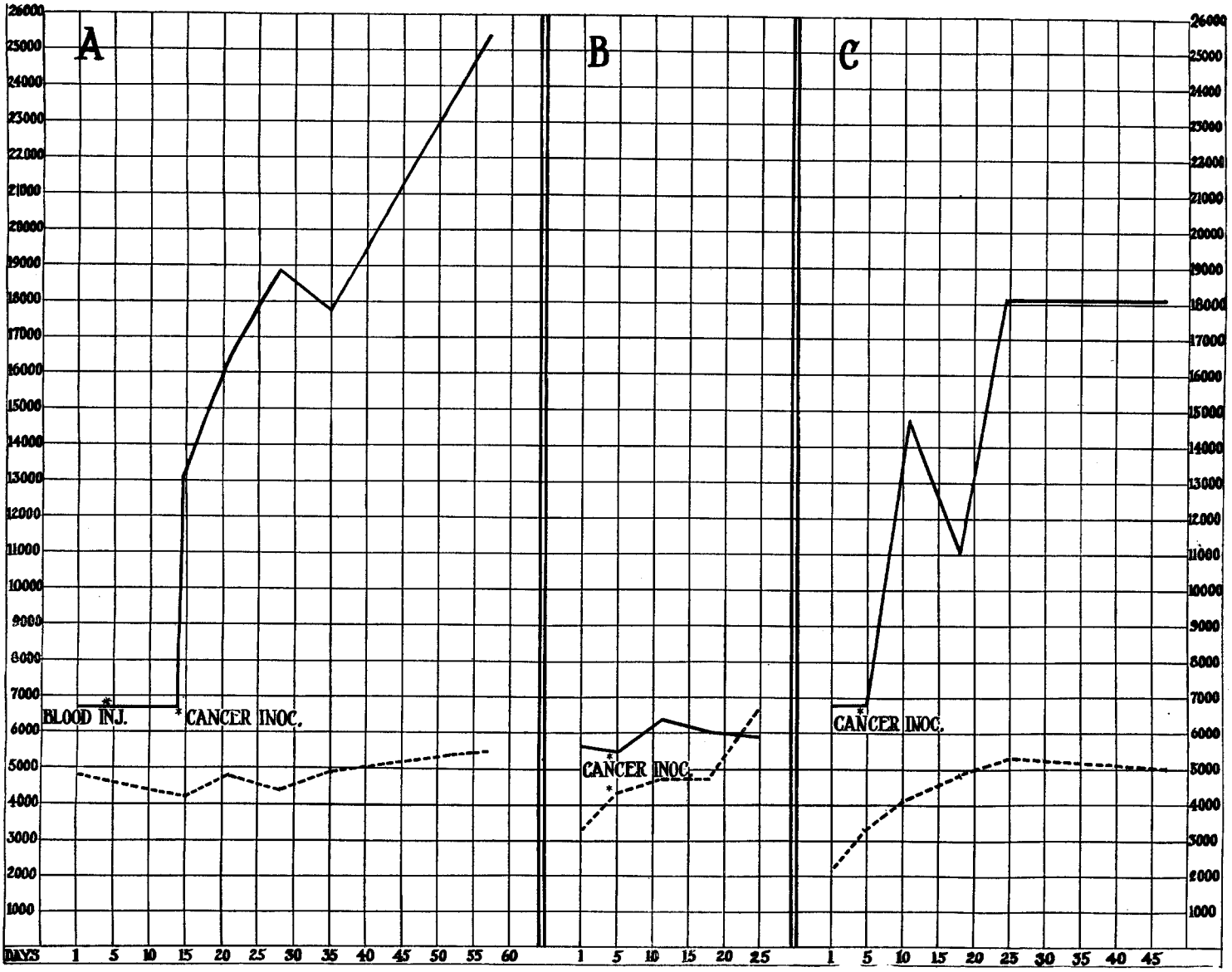
Typical examples of each group are shown in Text-fig. 1, A being an immunized animal, B a control animal with a growing tumor, and C a naturally immune animal. In these counts we have grouped together the large and small lymphocytes; and have not differentiated the types of polynuclear cells. The changes that have taken place have been, however, almost entirely in the small lymphocyte group. In Curve A, here given, no blood examinations were made between the primary count and one twenty-four hours after the cancer inoculation, but a study of a large series of animals has shown no variation in the count before the introduction of the cancer graft. It is assumed, therefore, in this case that the normal level is maintained.

The immediate increase in the circulating lymphocytes present in the immunized animal is a constant finding and in a large proportion of the cases has risen to 100 per cent or more above the normal level during the first twenty-four hours. This is well shown by the composite curve made from the average of all of this series (Text-fig. 2 A). In Curve C of Text-fig. 1 we show a typical example of a naturally immune animal. Here the lymphoid increase is delayed, and is preceded by a slight fall. The composite curve (Text-fig. 2 C), however, shows that as an average there is slight if any change during the first twenty-four hours, but the reaction develops

[7] This tumor was propagated by Dr. Woglom and kindly given us by him.



TEXT-FIG. 1. This shows the blood changes in typical examples of A, an animal with induced immunity; B, one with a growing cancer (shown in silhouette at bottom); and C, a naturally immune animal. The solid line represents the actual number of lymphocytes, and the dotted line the polymorphonuclear leucocytes. The first count in Curve A was made before the immunizing injection, and in the others before the cancer inoculation.



TEXT-FIG. 2. Composite curves formed by averaging the counts for all animals in each group. A, composite curve from a number of immunized animals. B, composite curve from a number of susceptible animals with growing cancers. C, composite curve from a number of animals, naturally immune to transplanted cancer. The solid line represents the actual number of lymphocytes, and the dotted line the polymorphonuclear leucocytes.

sometime during the first week and continues to rise with some variation to a level between 100 and 200 per cent above normal. These animals show, as a rule, some growth of the cancer during the first week but after this, retrogression sets in, continuing to complete absorption. In the susceptible animal (Text-fig. 1 B) there is a decrease in the lymphocytes and a tendency for the polynuclear cells to increase slightly. This particular animal offered little resistance apparently, as the rate of growth of the tumor, shown at the bottom of the text-figure in silhouette, would indicate. This is not uniformly so, however, as is demonstrated in the composite curve (Text-fig. 2 B) taken from a number of animals with growing tumors. Here is shown a slight reaction at the end of the first week which, however, subsides as the tumor increases in size. In one animal in the series, there was a fairly marked reaction of the lymphocytes in the second week, with a marked retardation in the growth of the tumor. The cancer later broke through the resistance which was accompanied by a corresponding drop in the mononuclear elements of the blood.

This series of experiments shows that the resistant state to transplanted cancer, whether of the natural or induced variety, is accompanied by a marked lymphocytosis. In the highly susceptible animals this phenomenon is absent, while in the less resistant ones, where the tumor is retarded but not checked absolutely, there is a slight but apparently inadequate reaction of these cells. Whether the immunity is dependent on the lymphoid increase or not is a different matter. If the reaction is a necessary part of the immunity process, the destruction of the lymphocytes in the immunized animals should be accompanied by a loss of the immunity.

The Effect of Lymphoid Destruction on the Induced Immunities to Transplanted Cancer.

Heineke has shown that x-ray effect is manifest first in its destruction of the lymphocyte.[8] We have found that repeated small doses of x-ray[9] destroy the major portion of the lymphoid system of the mouse without causing apparent injury to other tissues or pro-

[8] Heineke, H., *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1905, xiv, 21.

[9] Murphy, Jas. B., and Ellis, A. W. M., *Jour. Exper. Med.*, 1914, xx, 397.

ducing detrimental effect to the general health of the animal. This method offers a means of testing out the importance of the lymphocytic reaction in the immune states to cancer. We have found it necessary to regulate the dose very carefully, as too large or too frequent doses will cause such a general disturbance in the metabolism that tumors after they develop will grow slowly. Likewise, too small an amount of x-ray will only partially bring about the desired effect. The following experiment is one of four which have given uniform results.

Experiment II.—30 adult mice of about the same age and size were selected. 20 of these were given 0.3 cc. of defibrinated mouse blood, and 10 were put aside for controls. 10 of the immunized animals were subjected to small doses of x-ray for 7 consecutive days by means of the Coolidge tube, 10 milliamperes, 3 inch spark gap, and exposure of 1 to 2 minutes. Blood examination showed this to be sufficient to reduce markedly the circulating lymphocyte, leaving the general health of the animal unaffected. 10 days after the blood injection, all 30 animals were inoculated with a fragment of a transplantable mouse cancer. Text-fig. 3 gives the rate of growth of cancer in the various groups. The x-rayed immunized group shows the same number of takes as does the normal series, but the tumors in the x-rayed animals grew more rapidly.

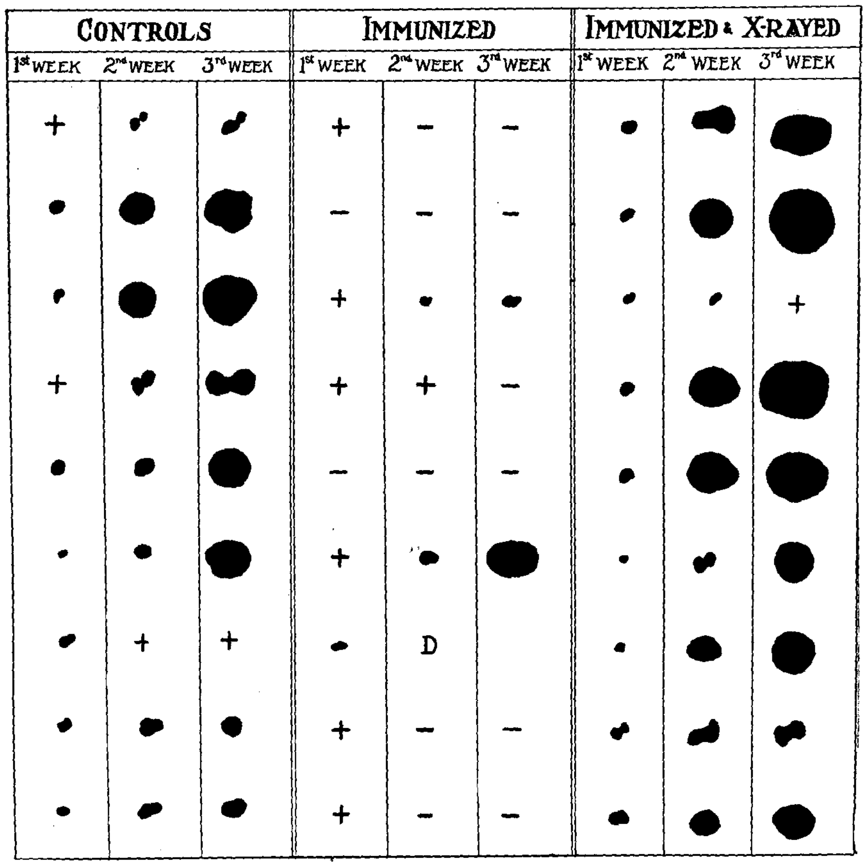
The experiment would seem to indicate that the destruction of the lymphocytes between the period of immunizing injection and cancer inoculation by preventing the usual lymphoid reaction, suffices to abolish the immunity which would otherwise be present.

The Effect of Lymphoid Destruction on Natural Immunity to Transplanted Cancer.

The above experiments have shown that the lymphoid reaction is the same in the naturally immune animals and in the animals with induced immunity. We would expect, therefore, that treatment of normal animals with x-ray before inoculation with cancer should greatly increase the number of takes.

Experiment III. Series A.—20 rats of the same age and size were selected. 10 of them were given 12 doses of x-ray on consecutive days in amounts sufficient to reduce greatly the lymphoid elements. All the 20 animals were then given grafts of the Jensen rat sarcoma. Of the untreated series, only 2 developed tumors and 1 of these later retrogressed, while in the 8 surviving animals in the x-rayed lot, all developed large tumors which eventually caused death.

Result.—Normal series 22 per cent takes; x-rayed animals 100 per cent takes.



TEXT-FIG. 3. This chart shows the effect of x-ray on induced immunity. The silhouettes show the rate of growth of the inoculated cancer for 3 consecutive weeks after inoculation. The first group are the normal animals; the second, the immunized animals; and the third, animals that were immunized and given a series of small exposures to x-ray between the immunizing dose and the cancer inoculation.

Series B.—20 rats of the same lot were selected, and 10 of these were given the same amount of exposures to x-ray as in Series A. All were inoculated with equal sized grafts of the Jensen rat sarcoma. 8 of the normal animals survived, 3 of which number developed tumors, 1 retrogressing, however, after a period of growth. Among the x-rayed animals 6 lived and all of these developed large tumors, but 1 retrogressed after a period.

Result.—Normal animals 37 per cent takes; x-rayed animals 100 per cent takes.

Series C.—20 rats were selected and 10 of these were given x-ray exposures of 4 minutes' duration on 5 consecutive days. A white blood count and a differ-

ential were done before and 3 days after the x-ray treatment was discontinued. The circulating lymphocytes fell during this time on an average to 26 per cent of their former number, while the polymorphonuclear cells remained practically at the same level in actual numbers. All the 20 animals were inoculated with grafts of the Flexner-Jobling carcinoma of the rat. In the normal series only 2 animals developed tumors and 1 of these was of very slow growth. Among the x-rayed animals, all developed tumors which continued to grow till the death of the animals.

Result.—Normal animals 25 per cent takes; x-rayed animals 100 per cent takes.

Series D.—20 mice were used in this experiment, 10 being subjected to 1 minute exposures of x-ray on 10 consecutive days. All the animals were then inoculated with grafts of a transplantable mouse carcinoma. Among the normal animals, 4 out of the 9 surviving developed tumors which progressed till the death of the animal, while the other 5 recovered completely. In the x-rayed series 7 out of 9 surviving mice developed tumors.

Result.—Normal animals 44 per cent takes; x-rayed animals 77 per cent takes.

It will be noted that tumors have been used in this experiment at periods when they were giving a low percentage of takes, so as to make the test more rigid. We have also by this same method successfully transplanted spontaneous tumors to x-rayed animals when like grafts in normal animals failed to grow. They act, however, much as the heteroplastic tissues,[10] retrogressing as the lymphoid tissue regenerates. With more carefully regulated dosage of x-ray it seems likely that uniform takes could be obtained in grafts from the spontaneous as well as from the transplantable tumors.

DISCUSSION.

The facts as they stand from these experiments are (1) that a marked lymphocytosis arises after inoculation with cancer in both the naturally immune animals and in the animals with induced immunity; and (2) when the lymphocytosis is prevented by a previous exposure of the animal to x-ray the resistant state is abolished. All the evidence points, therefore, towards the lymphocyte as a necessary factor in the immunity processes, and the finding offers a probable explanation of the results of Apolant who found that it was more difficult to render splenectomized animals immune to cancer than intact ones.[11]

[10] Murphy, Jas. B., *loc. cit.*

[11] Apolant, H., *Ztschr. f. Immunitätsforsch., Orig.*, 1913, xvii, 219.

Moreover, it has been noted that splenectomized animals exhibit less resistance to the growth of inoculated cancer than do intact ones giving both a higher percentage of takes and more rapidly growing tumors. This fact had been interpreted as the result of removal of one of the chief organs for antibody formation. But as no circulating antibodies have ever been demonstrated for cancer, it would seem more probable in the light of our work that the lymphocytic reaction has been interfered with by the removal of one of the principal lymphoid organs. We are not prepared, however, at the present time to discuss the mechanism of the lymphoid action in cancer immunity, or to do more than state that among the several possibilities that present themselves the data at hand are insufficient to establish one agency above all others.

SUMMARY.

The refractory state to transplanted cancer, induced by the subcutaneous injection of defibrinated blood, is accompanied in every case by a definite lymphoid crisis in the blood. The rise of lymphocytes is not present during the interval between the immunizing injection and the cancer inoculation but comes on sharply within twenty-four hours of the introduction of the cancer graft. In control animals where the graft leads to a definite take there is no such lymphoid response, but in instances of natural immunity the phenomenon is similar to that seen in artificially induced immunity, though the period of rise is often delayed for several days or a week.

The lymphoid crisis is not merely an accompanying factor in the immune period; it is essential to the process. This is demonstrated by the fact that destruction of the lymphocytes by x-ray is accompanied by the loss of natural or induced resistance to the growth of inoculated cancer.

The polymorphonuclear cells show a tendency to increase in the animals with growing tumors, but further study will be necessary before any conclusions regarding them can be drawn.