EFFECT OF INDUCED CELLULAR REACTION ON THE FATE OF CANCER GRAFTS.*

IV. Studies on Lymphoid Activity.

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Plates 21 to 23.

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It has been shown that the induction of a general lymphocytosis is accompanied by a more or less marked immunity to cancer,[1] and that a local reaction of lymphoid cells, induced in the skin by means of x-rays, renders this tissue an unsuitable locality for the growth of cancer.[2] The reaction about a cancer graft inoculated into a mouse previously injected with homologous living tissue has a striking likeness to a local anaphylactic reaction, and is followed by a more or less complete destruction of the tumor graft. Yet in spite of this constant association of immunity and the cellular reaction, cancer investigators have been inclined to look for other explanations of the immunity phenomena.[3] If this cellular reaction is an important factor and appears to explain more or less the immunity phenomena, it should be possible to bring about a local immunity to cancer, by inducing around a graft a reaction similar to that which occurs in a generally immune animal. As the local anaphylactic reaction has

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[1] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800. Murphy, Jas. B., and Sturm, E., *J. Exp. Med.*, 1919, xxix, 25, 31. Nakahara, W., and Murphy, Jas. B., *J. Exp. Med.*, 1920, xxxi, 13.

[2] Murphy, Jas. B., Hussey, R. G., Nakahara, W., and Sturm, E., *J. Exp. Med.*, 1921, xxxiii, 299.

[3] For a review of the literature see Woglom, W. H., Studies in cancer and allied subjects. The study of experimental cancer. A review, New York, 1913.

similarities with the local effect observed about a graft in cancer-immune animals, we have tested out the influence of the former reaction on cancer grafts.

Foreign Protein Reaction in Mice.

The material most generally used to produce homologous tissue immunity in mice is defibrinated mouse blood; the amount necessary to induce a satisfactory immunity is about 0.2 cc. In order to parallel closely this procedure the same amount of defibrinated rat blood has been used as the foreign protein in the following experiments.

0.2 cc. of defibrinated rat blood was injected into the loose connective tissues of the backs of mice, the first series of which was killed 24 hours later and the tissues prepared for and subjected to histological examination. A considerable degree of lymphoid infiltration in the region of the injected blood (Fig. 1) was present, similar to that which occurs about an injection of defibrinated mouse blood.[4]

10 days later another series of the sensitized mice was given a second small injection of rat blood into the groin, and after another 24 hours the animals were killed and the groin tissue was prepared for histological examination. This tissue showed a marked infiltration of lymphocytes about the injected blood, a reaction far more extensive than that occurring after a single injection and equal to that found about a cancer graft in an immune animal (Fig. 2).

Hence it is readily possible to induce a reaction to foreign protein similar to that accompanying the immunity reaction to cancer. The following experiments were planned to test the effect of this reaction on cancer grafts.

Experiment 1.—A large normal rat was bled from the heart under aseptic precautions and the blood was defibrinated. Of sixteen normal mice from the same strain and of about the same age, eight were injected subcutaneously with 0.2 cc. of the rat blood. 10 days later a 2½ weeks old Bashford adenocarcinoma was removed from a mouse and cut into pieces of approximately equal size. The fragments were placed in a container and thoroughly mixed with a quantity of freshly defibrinated rat blood. The tumor particles were then loaded into a trocar, care being taken to include a drop of the blood with each graft, and inoculated into eight mice formerly injected with rat blood and, in the same manner, into

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

eight normal control mice. Measurements of the grafts were made at weekly intervals. At the end of 3 weeks, of the eight animals which had been sensitized with rat blood and afterwards inoculated with a mixture of rat blood and the cancer, three only showed tumors, while all the control mice inoculated with a mixture of rat blood and the cancer showed tumors.

Experiment 2.—The preceding experiment was repeated with ten mice in the sensitized series and ten in the control. Among the former four tumors developed, or 40 per cent of takes, while among the latter nine tumors developed, or 90 per cent of takes.

Experiment 3.—Twenty mice were given an injection of 0.2 cc. of defibrinated rat blood subcutaneously. 10 days later ten of these were inoculated with a mixture of rat blood and mouse cancer, and the other ten with mouse cancer alone. A control series of ten non-sensitized mice was also inoculated with a mixture of cancer and rat blood. The mice sensitized and inoculated with a mixture of rat blood and cancer showed four tumors, or 40 per cent of takes, the mice sensitized and injected with cancer grafts alone showed ten tumors, or 100 per cent of takes, and the control, non-sensitized mice given cancer plus blood showed nine tumors, or 90 per cent of takes.

Experiment 4.—This experiment was a repetition of Experiment 3, with ten mice in each series. The mice sensitized with rat blood and then inoculated with a mixture of rat blood and mouse tumor showed five tumors, a susceptibility of 50 per cent, the mice sensitized with rat blood and inoculated with tumor alone showed ten tumors, a susceptibility of 100 per cent, and the normal mice inoculated with a mixture of rat blood and mouse tumor showed ten tumors, a susceptibility of 100 per cent.

Experiment 5.—The experiment was again repeated with ten mice in each series. The mice sensitized with rat blood and then inoculated with a mixture of rat blood and mouse cancer showed a susceptibility of 60 per cent, while the animals sensitized and inoculated with cancer material alone were 100 per cent susceptible, as was also the normal control series inoculated with a mixture of rat blood and cancer.

Experiment 6.—This was a repetition of the preceding experiments. The results showed 50 per cent susceptibility in ten animals sensitized with rat blood and later inoculated with a mixture of rat blood and mouse cancer, 77 per cent susceptibility in a series of nine mice sensitized and inoculated with the tumor tissue alone, and 80 per cent susceptibility in the control series of ten normal control mice inoculated with the mixture of rat blood and mouse cancer.

The results obtained in the foregoing experiments are presented in Table I and Text-fig. 1.

TABLE I.

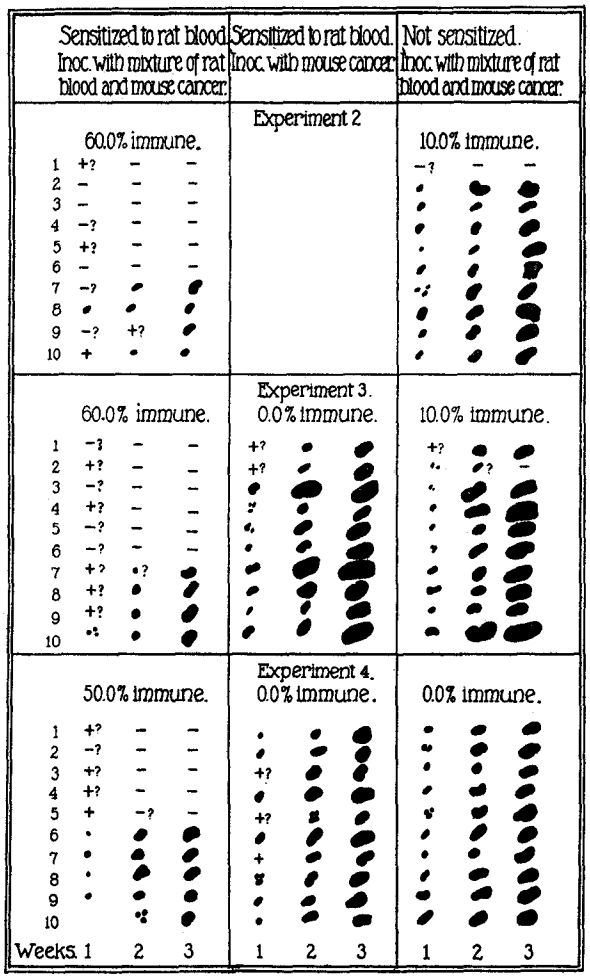
Experiment No.	Group A.*	Group B.	Group C.
1	62.5 per cent immunity (8 mice).		0.0 per cent immunity (8 mice).
2	60.0 per cent immunity (10 mice).		10.0 per cent immunity (10 mice).
3	60.0 per cent immunity (10 mice).	0.0 per cent immunity (10 mice).	10.0 per cent immunity (10 mice).
4	50.0 per cent immunity (10 mice).	0.0 per cent immunity (10 mice).	0.0 per cent immunity (10 mice).
5	40.0 per cent immunity (10 mice).	0.0 per cent immunity (10 mice).	0.0 per cent immunity (10 mice).
6	50.0 per cent immunity (10 mice).	23.0 per cent immunity (9 mice).	20.0 per cent immunity (10 mice).

* Group A was composed of animals sensitized with 0.2 cc. of rat blood and 10 days later inoculated with a mixture of rat blood and mouse cancer. Group B was made up of mice sensitized with 0.2 cc. of rat blood and 10 days later inoculated with mouse cancer alone. Group C was made up of mice not sensitized but inoculated with a mixture of rat blood and mouse cancer.

Histological Study of the Fate of the Cancer-Rat Blood Mixture Inoculated into a Sensitized Animal.

It has been shown that the reaction which takes place about the immunizing injection of mouse blood into a mouse is similar to that about an injection of rat blood in a mouse. The reaction which takes place around a cancer graft in an immunized mouse has been shown to be similar to that which occurs around injected rat blood in a mouse previously sensitized to rat blood. The following experiment was undertaken to supply material for the histological study of the fate of a cancer graft mixed with rat blood when inoculated into a mouse previously sensitized to rat blood.

A series of mice was injected with 0.2 cc. of rat blood and 10 days later inoculated with a mixture of rat blood and mouse tumor. These mice were killed in groups at 24 hour intervals up to the 7th day and histological studies made of the grafts. 24 hours after inoculation there was a massive lymphoid and a mild polymorphonuclear reaction about the graft (Figs. 3 and 4). The reaction was still marked on the



TEXT-FIG. 1. The rate of growth of mouse cancer inoculated in a mixture with rat blood into mice previously sensitized to rat blood, compared to the rate of growth when the cancer alone is inoculated into sensitized mice and the rate of growth when the cancer and rat blood are inoculated into non-sensitized mice.

2nd day, but by the 3rd day it had begun to diminish and the graft was more or less completely destroyed. After this period there was a rapid subsidence of the reaction. The reaction described above is similar to that seen around the cancer graft in an immunized animal.

Desensitizing Effect of Generalized Doses of X-Rays.

In a previously reported experiment it was shown that mice rendered potentially immune by the injection of mouse blood could be reduced to a susceptible state by properly regulated exposure to x-rays, administered between the time of the immunizing dose and that of the cancer inoculation.[5] It has likewise been shown that x-rays administered at certain periods after a foreign protein injection desensitize an animal to such an extent that no anaphylactic shock results after a second injection of the protein.[6] These facts have led us to test the effect of x-rays on the immunity resulting from sensitization of mice with rat blood and the subsequent inoculation of mouse cancer mixed with rat blood.

Normal white mice were injected subcutaneously with 0.2 cc. of defibrinated rat blood. These mice and another group of mice which had not been sensitized were then given daily doses of x-rays for 8 days. The dose was governed by the following factors: spark-gap $2\frac{1}{4}$ inches, milliamperes 10, time 2 minutes, and distance from target 12 inches. 10 days after the sensitizing injection these animals and several normal controls were inoculated with a Bashford adenocarcinoma mixed with defibrinated rat blood. The results from these experiments are given in Table II and Text-fig. 2.

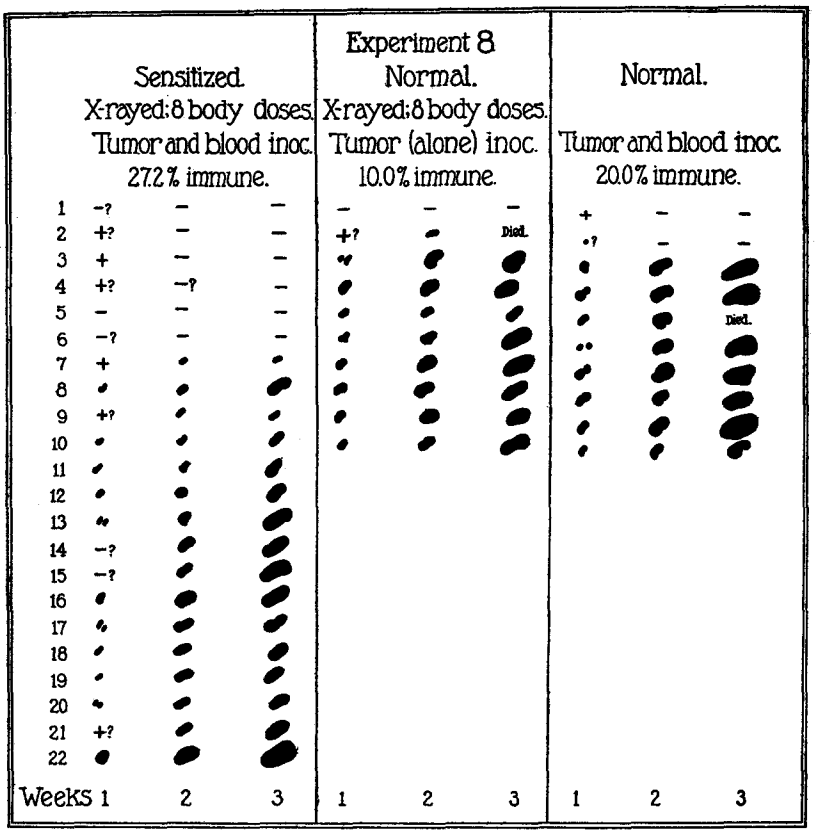
TABLE II.

Experiment No.	Group A.*	Group B.	Group C.
7	10.0 per cent immunity.		20.0 per cent immunity.
8	27.2 " " "	10.0 per cent immunity.	20.0 " " "

* Group A comprises thirty-two mice sensitized to rat blood, which were given eight exposures to x-rays and were then inoculated with a mixture of rat blood and mouse tumor. Group B was made up of ten mice not sensitized but given the same amount of x-rays as Group A and then inoculated with cancer alone. Group C was composed of twenty normal animals inoculated with a mixture of rat blood and mouse cancer, which had received no previous injection of blood and no x-rays. The total number of mice used in these experiments was 62.

[5] Murphy, Jas. B., and Taylor, H. D., *J. Exp. Med.*, 1918, xxviii, 1.

[6] Hussey, R. G., and Murphy, Jas. B., unpublished observation.



TEXT-FIG. 2. The effect of generalized x-rays on the immunity to transplanted cancer resulting from a local anaphylactic reaction.

Effect on the Foreign Protein Reaction of X-Rays Administered Locally.

The foregoing experiments show that mice develop an enhanced refractory state to the growth of transplanted tumor if they are first sensitized with rat blood and inoculated 10 days later with a mixture of rat blood and mouse tumor. Histological examination made at intervals after the tumor inoculation shows that there is a definite local reaction, made up principally of cells of the lymphoid series, which takes place around the tumor cells. This reaction reaches its maximum about 24 hours after inoculation and then gradually sub-

sides. It seems reasonable in the light of previous observations that the cellular elements constituting the reaction play some active part in the mechanism bringing about the refractory state.

On the assumption that the latter statement is true, it would seem to follow that if it were possible to destroy these cells and at the same time not to injure the tumor cells, the refractory state potentially present might be inhibited to some measure. Since it has been shown that the x-rays in moderate amounts have little if any direct effect on the viability of the cells of transplanted tumor when exposures are made directly to the tumor,[7] and since it is well known that the x-rays when properly regulated have a selective destructive action on the lymphoid elements of the body,[8] it was thought possible through these means to effect the purpose stated above.

Normal mice were inoculated subcutaneously with 0.2 cc. of defibrinated rat blood and 10 days later inoculated in the groin with a bit of Bashford adenocarcinoma mixed with defibrinated rat blood. 15 to 20 hours after the inoculation the mice were covered with sheet lead in which an aperture had been made large enough to allow the rays to affect the area around the graft. This area was then exposed to the x-rays in the following dose: spark-gap 8 inches, milliamperes 5, time 4 minutes and 38 seconds, distance from target 8 inches, filtered through 3 mm. of aluminum. For controls to the above experiment, normal mice were inoculated with bits of the same tumor and were exposed to the same amount of x-rays, and other normal mice were inoculated with tumor and rat blood without previous sensitization or after-treatment with x-rays. The results are shown in Table III and Text-fig. 3.

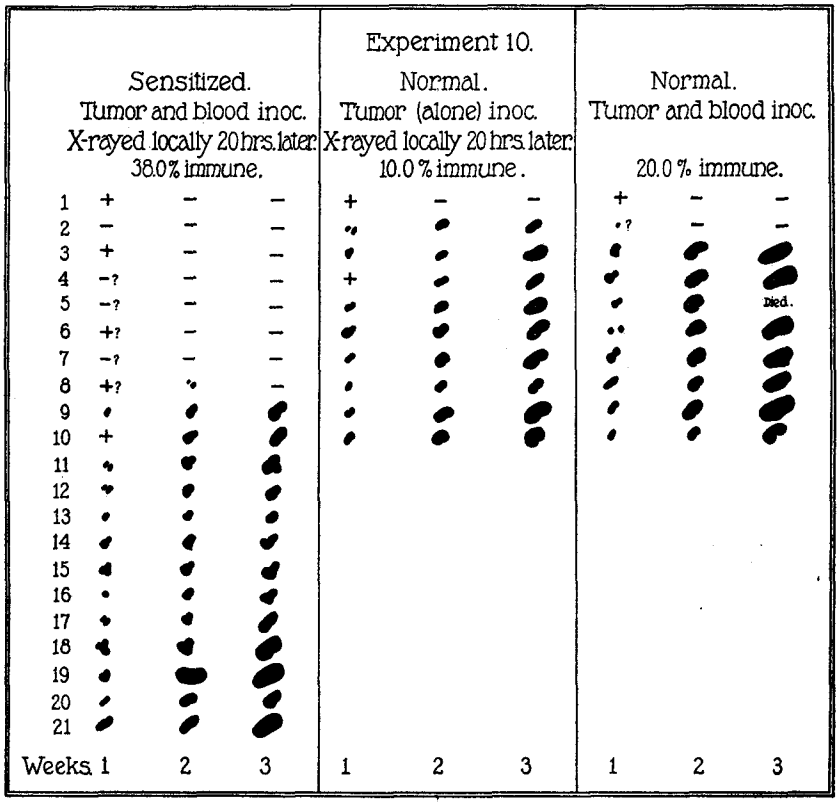
TABLE III.

Experiment No.	Group A.*	Group B.	Group C.
9	30.0 per cent immunity.		20.0 per cent immunity.
10	38.0 " " "	10.0 per cent immunity.	20.0 " " "

* Group A was made up of thirty-one mice, sensitized to rat blood, which were

[7] Hill, E., Morton, J. J., and Witherbee, W. D., *J. Exp. Med.*, 1919, xxix, 89.
[8] Heineke, H., *Mitt. Grenzgeb. Med. u. Chir.*, 1904–05, xiv, 21.

inoculated after 10 days with a mixture of rat blood and mouse cancer; 20 hours later they were given a local dose of x-rays. Group B was composed of normal mice inoculated with a mouse cancer and 20 hours later given a local dose of x-rays. Group C consisted of twenty normal mice inoculated with a mixture of mouse tumor and rat blood.



TEXT-FIG. 3. The effect of x-rays on the immunity to transplanted cancer resulting from a local anaphylactic reaction when the x-rays are administered locally over the cancer graft 20 hours after inoculation.

Histological Study.—Twelve normal white mice were inoculated with 0.2 cc. of rat blood, and 10 days later inoculated with rat blood plus a fragment of a Bashford Adenocarcinoma No. 63. 24 hours after the second inoculation a dose of x-rays was given locally over

the area of the cancer implantation. Two of the mice were killed just before the x-rays were given, and two others immediately afterwards. Eight mice were killed, in groups of two, 24 hours, and 3, 6, and 10 days after x-ray treatment.

24 hours after the second inoculation an extensive cell infiltration was found in the area of the subcutaneous tissue where the mixture of heterologous blood and homologous cancer tissue had been inoculated (Fig. 5). The cells participating in the reaction were chiefly of the lymphoid variety, the polymorphonuclear cells being less numerous.

In the specimens taken immediately after the x-rays were given there was a striking reduction of these cells (Fig. 6) in the area which had been thickly infiltrated immediately before the x-ray treatment. How this reduction of the cells is brought about is a matter of conjecture, but it should be mentioned that no necrotic cells were found in the x-rayed area. 24 hours after the x-ray treatment and later the cell infiltration was gradually restored, although it did not become so extensive as it was before.

The temporary suppression of the lymphoid reaction effected by the local dose of x-rays seems to indicate that the removal of these cells is one of the factors which plays a part in allowing the graft to grow in the sensitized animal.

From the two foregoing groups of experiments the conclusion is drawn that it is possible to overcome, to a certain extent, the immunity to cancer resulting from a local anaphylactic reaction, in two ways. In either case we consider that the effect follows the prevention of the local cellular infiltration from taking place or from becoming effective. In the first of these two experiments animals were desensitized so that the second injection of foreign protein did not call forth the local cellular reaction. In the second experiment the lymphocytes taking part in the local reaction were destroyed by x-rays before they had time materially to affect the cancer graft.

DISCUSSION.

The foregoing experiments offer further evidence of the hypothesis that the so called immunity to the transplanted cancers of mice depends on a local cellular reaction in which cells of the lymphoid

type play the principal part. The usual method of producing this immunity is through the injection of a quantity of living homologous tissue, which leads to a non-specific immunity, which in turn is directed against a great variety of cancers and sarcomas, as well as against transplanted normal tissue. It has been suggested that this immunity phenomenon is analogous to the so called anaphylactic reaction, but the exact nature of the relation had never been demonstrated. The experiments reported here indicate that this relation is quite close. The first injection prepares or sensitizes, and the second injection of the cancer cells calls out a cellular exudate such as is observed in local anaphylactic reactions. Why living cells are necessary for the sensitizing dose is not evident, unless it requires living cells to sensitize to living cells. The fact is unmistakable, from the experiments reported here, that the condition of local anaphylaxis renders the tissues affected unsuitable for the growth of a cancer graft, and the histological changes which arise correspond with those seen about a cancer graft in an animal immunized by a previous injection of homologous tissue. That the cellular exudate is the essential inhibiting agent is indicated by the fact that when this exudate is prevented from arising or is arrested, the protective effect is either annulled or materially reduced. It is still uncertain whether the desensitization induced by x-ray exposure results in such a general destruction of the lymphocytes that the number left is insufficient to yield the local reaction, or whether it depends on some other factor. Reasoning from the observed fact that x-rays are capable of actual desensitization even to the extent of preventing anaphylactic shock from a second injection of foreign protein, one may well consider whether the failure of immunity under these conditions does not arise from the inability of the desensitized animals to call out the usual cellular exudate. Inasmuch as the local destruction of the cellular exudate is sufficient to annul or reduce the immunity, it is unlikely that it is of the nature of a serum-carried resistance, for the amount of x-rays needed for this purpose is so small and its area of application so limited that it could hardly produce a general effect. In brief, there seems to be no other explanation for the results recorded than that cells of the lymphoid type are capable of preventing the growth of a transplanted cancer when present locally in sufficient

numbers. Hence, we conclude that these cells are an active agent in bringing about the so called immunity condition to transplanted cancer.

SUMMARY.

Mice sensitized by an injection of 0.2 cc. of rat blood and 10 days later inoculated with a mixture of rat blood and a transplantable mouse cancer showed a high degree of immunity to the cancer growth, while mice sensitized in the same manner and inoculated with cancer graft with no rat blood showed no immunity. Likewise, non-sensitized mice inoculated with a mixture of rat blood and cancer cells showed no immunity.

Mice sensitized to rat blood and then given a series of doses of x-rays between the time of this injection and the inoculation of the cancer-rat blood mixture showed a suppression of the factors affording protection or immunity, since the cancers grew as well in these animals as in the controls.

Mice were sensitized with rat blood and 10 days later inoculated with a cancer-rat blood mixture. 20 hours after the inoculation when the cellular exudation was at its height, the cells were destroyed by a local dose of x-rays. The degree of immunity was reduced and the cancers grew almost as well as in the controls.

EXPLANATION OF PLATES.

PLATE 21.

FIG. 1. Subcutaneous tissue of a mouse inoculated with rat blood, 24 hours after the inoculation.

FIG. 2. Subcutaneous tissue of a mouse inoculated with rat blood, 24 hours after a second inoculation of rat blood.

PLATE 22.

FIG. 3. Subcutaneous tissue of a mouse sensitized to rat blood, 24 hours after an inoculation with rat blood mixed with mouse tumor.

FIG. 4. The same as Fig. 3, but in higher magnification.

PLATE 23.

FIG. 5. Subcutaneous tissue of a sensitized mouse, 24 hours after an inoculation of a cancer-rat blood mixture, showing an extensive cell infiltration.

FIG. 6. Subcutaneous tissue of a mouse which was sensitized with rat blood, then inoculated with a mouse cancer-rat blood mixture, and 24 hours later given a local dose of x-rays. The tissue was removed immediately after the treatment.

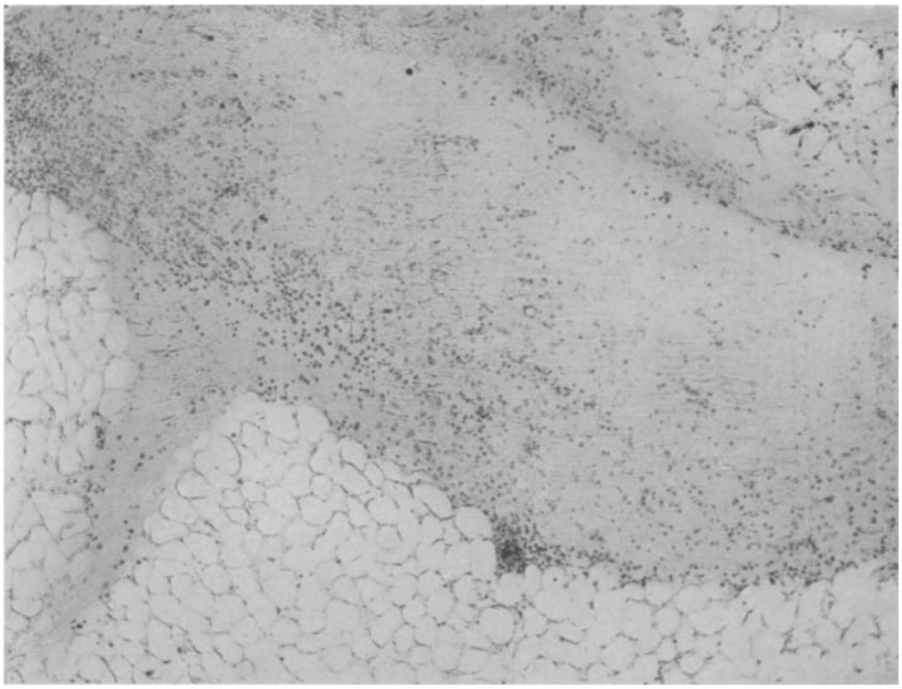


FIG. 1.

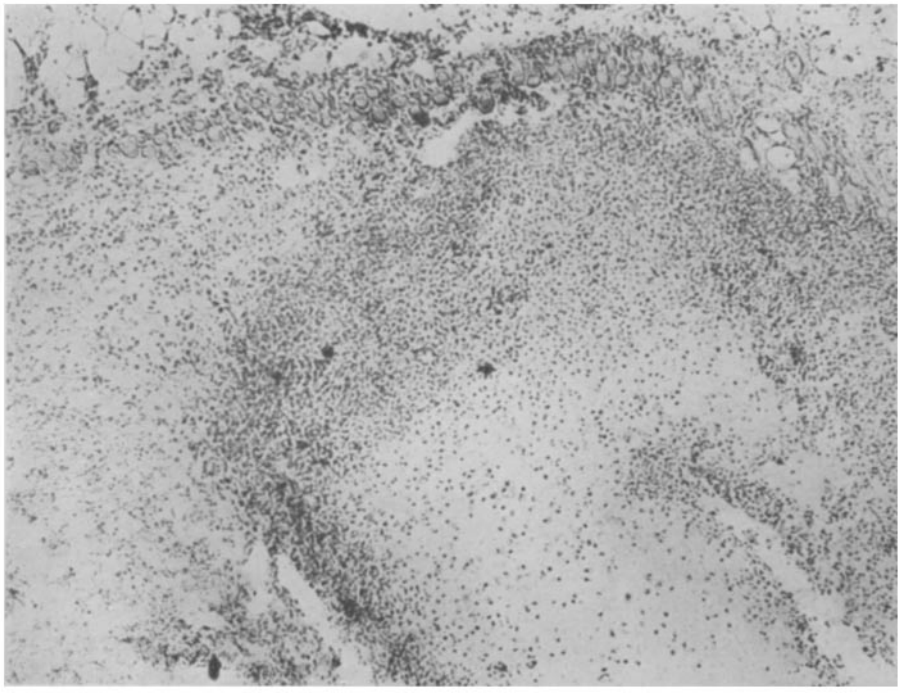


FIG. 2.

(Murphy, Hussey, Sturm, and Nakahara: Cancer grafts. IV.)

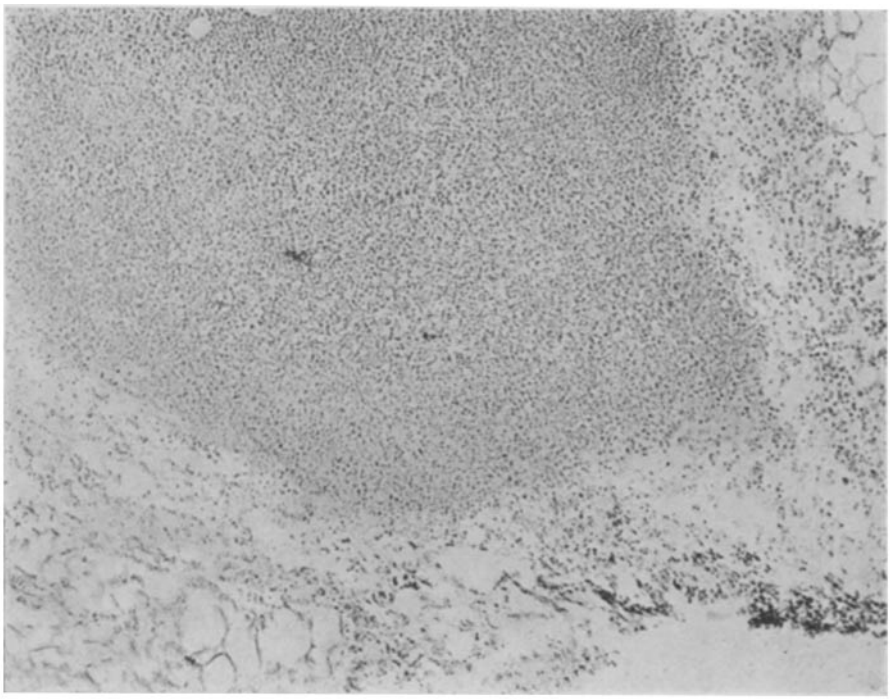


FIG. 3.

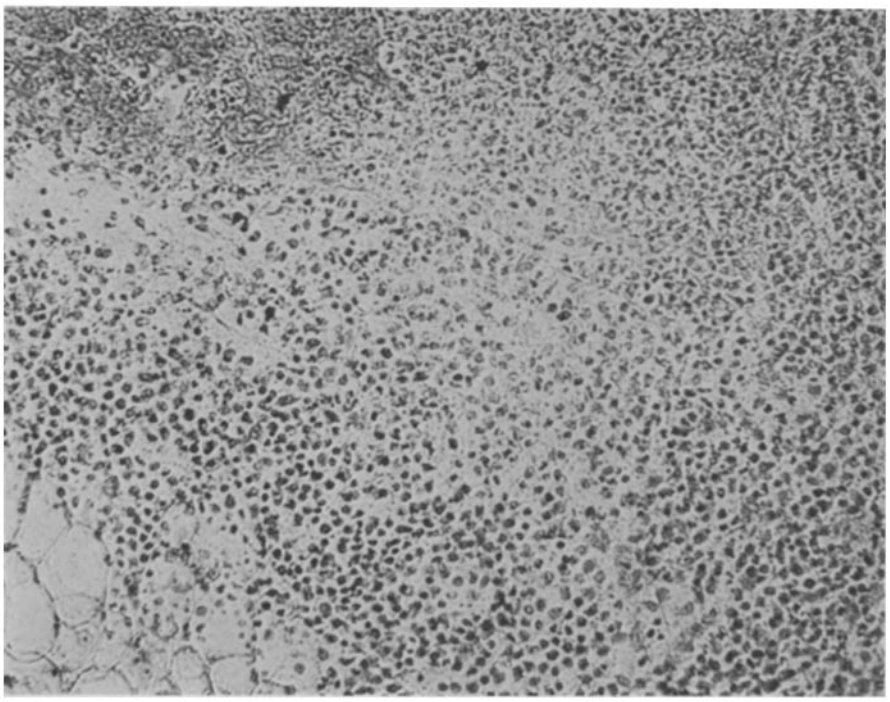


FIG. 4.

(Murphy, Hussey, Sturm, and Nakahara: Cancer grafts. IV.)

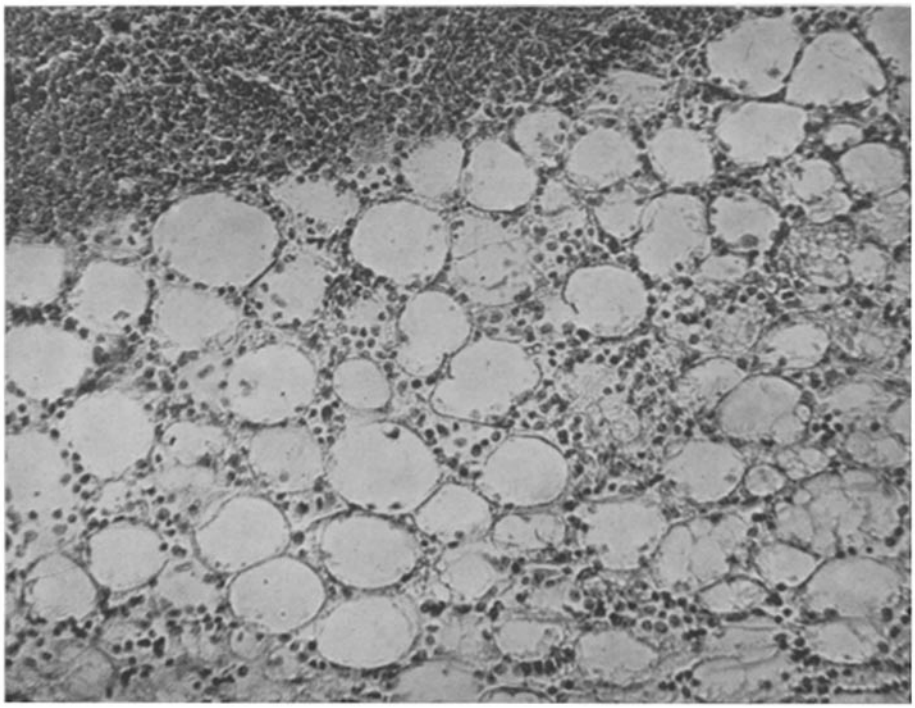


FIG. 5.

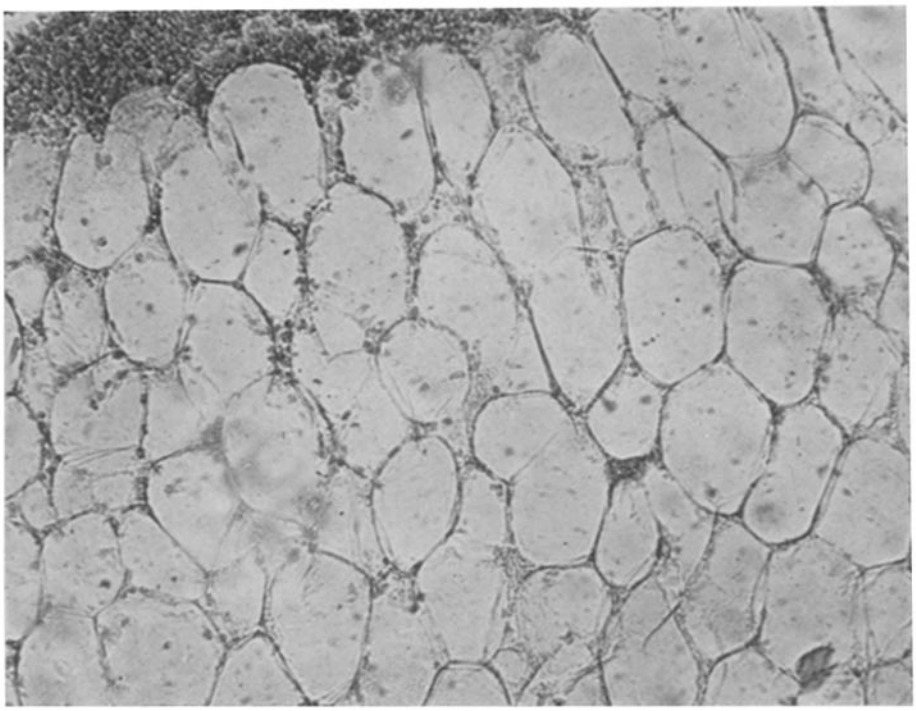


FIG. 6.

(Murphy Hussey, Sturm, and Nakahara: Cancer grafts. IV.)