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THE LYMPHOCYTE IN RESISTANCE TO TISSUE GRAFTING, MALIGNANT DISEASE, AND TUBERCULOUS INFECTION

AN EXPERIMENTAL STUDY

By

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THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

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(*From The Rockefeller Institute for Medical Research.*)

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INTRODUCTION.

The original reports of the investigations reviewed in this monograph have appeared during the past fourteen years for the most part in *The Journal of Experimental Medicine.* As the papers are scattered through something more than sixty publications,[1] covering however a more or less continuous account of the development of a particular line of experimental study it has been deemed worth while to bring them together in a logical fashion in the form of a general summary for the convenience of the reader.

This series of investigations is the product of a group rather than an individual. Dr. John J. Morton and the late Dr. Herbert D. Taylor participated largely in the earlier studies of the manifestations of resistance to cancer, the studies of the biological action of x-ray, and the effect of stimulation of the lymphoid tissue on resistance to the transplanted and spontaneous cancer. Dr. Waro Nakahara collaborated in the cytological study of general tissue changes associated with resistance and susceptibility to cancer and was responsible for the observations on the effect of olive oil and unsaturated fatty acids on resistance to malignant disease. Dr. Raymond G. Hussey cooperated in several of the later investigations and in addition studied the morphological blood changes in animals showing induced resistance to tuberculous infection, the effect of x-ray on protein sensitiveness, and the chemical changes in the blood following x-ray exposure. Mr. Ernest Sturm besides affording material technical assistance, is largely responsible for the observations on the effect of heat on the lymphoid tissue. Acknowledgment is also due for valuable assistance to two volunteers, Professor Joseph Maisin of Louvain University, and Dr. J. Heng Liu, Medical Superintendent of the Hospital of the Pekin Union Medical College. It is a pleasure to express my indebtedness to this earnest group of investigators.

[1] A list of these articles will be found on page 165.

No attempt has been made to cover the enormous literature on the subjects dealt with in this monograph for adequate reviews may be found elsewhere. Nor has the attempt been made to discuss the differences of opinion which have arisen as to the interpretation of the phenomena described. New methods and further elucidation of the problems involved can alone settle these moot points.

I. HETEROPLASTIC TISSUE GRAFTING AND THE MECHANISM OF DEFENSE.

PLATES 1 TO 9.

Until recent years no authoritative examples of transplantation of tissues between different species of warm blooded animals have been recorded, although grafting has long been known as a possibility in some of the lower forms. Joest (1) succeeded in bringing about a permanent union between grafts of *Lumbricus rubellus* and *Allolobophora terrestris*, and Born (2), Harrison (3), and Morgan (4) extended this observation to different varieties of tadpoles. There seems little doubt that the engrafted tissues under these conditions retain their essential character although the engrafted portion may be small and absolutely dependent on the major component for its nervous system, circulation, and general nutrition. In these respects the tissues of the lower order of animals resemble plants where grafting between species has been carried on for centuries and where it is known that the essentials of both the graft and the host are retained unimpaired. These observations are at variance with the strict specificity found to obtain among warm blooded animals, in which it is rarely possible to transplant tissues even between individuals of the same species. If one be permitted to accept the mass of important evidence on this subject acquired by the study of the transplantation of tumors of animals, and there is good evidence to show that the general laws governing this form of grafting is essentially the same as those governing the transplantabiliy of normal tissue (5), then sufficient and dependable evidence is at hand for a discussion of the subject.

Surgeons from earliest times have considered the possibility of utilizing animal tissues for replacement of diseased or destroyed tissues in man. Many ingenious methods have been devised and not a few successes claimed but on final analysis it is generally conceded that these claims are without foundation. Even in recent years extensive reports have been published on grafting of glands of internal secretions, where the claim for success is based on the improvement in

deficiency symptoms in the host. It is difficult to eliminate the psychic factor in many of these experiments on man. These influences must be taken into consideration, as also the temporary effects due to the active principle of the gland carried over from another individual with the engrafted tissue.

From time to time theories have been brought forward in attempted explanation of this remarkable phenomenon of tissue specificity but none of these so far have met with general acceptance. The earlier observers were inclined to attribute the failure of grafts in warm blooded animals to lack of nourishment (Ribbert) but the fact that a temporary vigorous growth was observed seems to eliminate this explanation. As further evidence opposing this idea, Carrel (6) showed that even homoplastic organ transplantation failed in spite of an adequate circulation insured by anastomosis of the blood vessels. The soundness of the technique was demonstrated by the almost invariable success of autotransplantations. Another theory followed the discovery of the development of immune substances in the blood after infection or resulting from vaccination. It was then generally considered that resistance to transplanted tissue could be explained as due to an analogous mechanism. The promptness with which engrafted tissues are destroyed, however, would preclude this explanation, on the ground that insufficient time elapses for antibody formation, and furthermore all attempts to demonstrate antibodies under such conditions have failed.

The most discussed hypothesis of recent years is that of Ehrlich (7) in which he attempted to extend his famous side-chain theory to include an explanation of the factors governing tissue transplantation. According to the athrepsia theory, living cells can utilize only such food substance as their receptors can bind, and these receptors vary in the different species. Therefore when a tissue of one species is grafted into another it is gradually exhausted by the lack of a specific food which is not supplied by the foreign host. The temporary period of survival and growth is accounted for by the small amount of nutriment carried along with the grafted material. The basis for this theory was an experiment which has become familiarly known as the zig-zag experiment in which the tissue of a mouse was grafted alternately between rats and mice. After growing for a time in the

rat, *i.e.* till it had exhausted the specific food, it was returned to the mouse where, after a period of growth it accumulated a sufficient amount of the specific food to be capable of again surviving for a period in the foreign species. The bearing of subsequent work on this explanation will be discussed later.

The writer first became interested in questions of tissue specificity in the course of some experiments with a chicken sarcoma (8), a tumor which showed a high degree of tissue individuality. It failed to grow in any other than blood-related animals during a number of months of transplantation, then for a period it could be propagated only in pure blooded animals of the same variety. Not until after the malignancy had become enhanced, by repeated transplantation, would it grow in chickens of other varieties. The growth vigor of this tumor had never been equalled by other transplanted tumors, yet the cells were capable of only a short survival in other species. It was found, however, that these limitations did not apply to grafts in embryos of a foreign species, for this tumor grew just as well in the embryo of the duck or pigeon as in the embryo of the chicken. This observation suggested the possibility of studying the factors involved in tissue specificity in the embryo; for here we had an organism offering no resistance yet the newly born animal possessed a complete mechanism of defense.

HETEROPLASTIC TISSUE GRAFTS IN THE CHICK EMBRYO.

The avian embryo offered obvious advantages over that of the mammal and was therefore selected for the following experiment. The method developed by Peebles (9) for experimental embryological studies was modified to meet the requirements of our particular problem. A brief description of this follows.

Technique.—A small rectangular piece was carefully cut from the shell with a sharp instrument, without cracking the egg or injuring the underlying shell membrane. With fine sterile forceps the shell membrane was then torn through and pulled back, care being taken that the hole in the membrane was smaller than that in the shell so as to provide a support for the piece of shell when returned. Through such an opening, with proper illumination, one may obtain a good view of the chick and its membrane. In inoculation, it was found most convenient to reduce the tissue to a pulp by forcing it through a fine mesh sieve

and to inject a small amount into the membrane or embryo by means of a syringe. After the inoculation the shell fragment was replaced and sealed into position with paraffin.

Time and Location of Inoculation.—The earliest period at which inoculation into embryos can be made safely has been found to be between the 5th and 7th days of incubation although it is possible to inoculate at a much earlier stage (24 to 48 hours).

For an inoculation from the 5th to 7th day, the position of the embryo is first determined by holding the egg in front of a bright light. The opening is then made directly over the embryo or to one side. The allantois membrane is most suitable as inoculations can be made with a minimum trauma. Furthermore this membrane is the respiratory organ of the chick at this period and is rich in lymphatics and blood vessels.

MAMMALIAN TISSUE IN THE CHICK EMBRYO.

As noted above, a chicken tumor, which was very strictly limited in transplantability to the species in which it arose, grew readily in embryos of a foreign species. This suggested the possibility that the chick embryo might serve as a suitable host for mammalian tissues. Inoculations of rat, mouse, and human tissues were therefore made into the developing chick embryo and with success. A typical experiment of this kind follows.

Experiment 1.—A large rapidly growing Jensen sarcoma of the rat was finely hashed and inoculated by means of a syringe and fine hypodermic needle into the outer membrane (fused chorion and allantois) of 12 chick embryos on the 6th day of incubation. On the 18th day of incubation the eggs were opened. Of the 8 remaining alive, 1 showed no evidence of tumor; all the others showed spherical masses projecting from the membrane at the point of inoculation which varied in size from 0.1 to 1.6 cm. in their greatest diameter (Fig. 1). Histological examination showed these tumors to be made up of cells like those of the original rat tumor (Fig. 2).

Bits of this tissue were inoculated into rats and tumors of the Jensen type developed in them as a result. Many experiments of this kind have been carried out. The Jensen rat sarcoma was used mostly for the reason that its cells are characteristic and easily differentiated from the embryo cells. The strain employed gave a rapidly growing tumor in a high percentage of inoculated rats, and was found to grow readily in the various membranes and in the body

of the chick embryo. The grafts were generally inoculated between the 5th to the 7th days of incubation and allowed to grow till the 18th day. Resulting tumors were found in the membrane as large globular masses sometimes measuring as much as 2.1 cm. in diameter, lying in or suspended from the inner surface of the thin membrane by a broad pedicle. The chick membrane extended over the protruding masses giving them a smooth and glistening surface. Coursing through the membrane and penetrating the semitranslucent greyish tissue of the tumor were numerous dilated vessels. The cut surface of the nodules showed this semitranslucent tissue uniformly, rarely with a small central area of necrosis but more frequently with small scattered hemorrhagic spots.

Microscopically the cells making up these tumors retain the characteristic appearance of those of the same tumor in the rat; fairly large spindle cells with scant, deeply staining protoplasm, and a large, clear vesicular nucleus with a nucleolus. There is some variation in the pattern of growth. In the rat the cells form compact bundles coursing in various directions through the section, while in the chick the arrangement of the cells is less compact, sometimes forming a loose network with clear spaces between, though in some cases the picture resembles that seen in the native host. Whereas in the rat-grown tumors, mitotic figures are only occasionally seen, in those grown in the chick embryo they are found abundantly, as many as five mitotic figures having been seen in a single field of an oil immersion lens (Fig. 3). This fact is all the more striking when it is noted that the number of cells per field in the chick-grown tumors is much less than in the rat-grown. The vessels are much more numerous in the tumors of the embryo occurring either as ingrowths of clusters from the chick membrane or as individuals scattered throughout the tumor.

The most significant observation in the chick-grown tumor is the absence of the usual cellular reaction. The thin continuation of the chick membrane covers the tumor and there is the ingrowth of vessels with their scant accompanying stroma, but no histological evidence of a reaction to the invasion of foreign tissue can be found. This fact is in striking contrast to the occurrence of a defensive reaction under similar conditions in the adult host.

The tumors arising in the rat from inoculations with the chick-grown rat tumor are microscopically identical with those propagated in the usual way from rat to rat.

Prolonged Growth of Rat Tissue in Chick Embryo.

The results of the experiments so far reported suggest the utilization of the chick food material in the nourishment and growth of the rat cell. Partly in order to establish this point beyond dispute, but more particularly to determine if any changes could be brought about in the rat cells by the prolonged growth in the embryos of a foreign species, the embryo-grown tumors were reinoculated into a second series of chick embryo. Not only did the tumors continue to grow rapidly, but they grew in turn when transplanted to a third or fourth series, indicating that growth can be prolonged indefinitely by repeating transplantation. Such an experiment with its controls is given below.

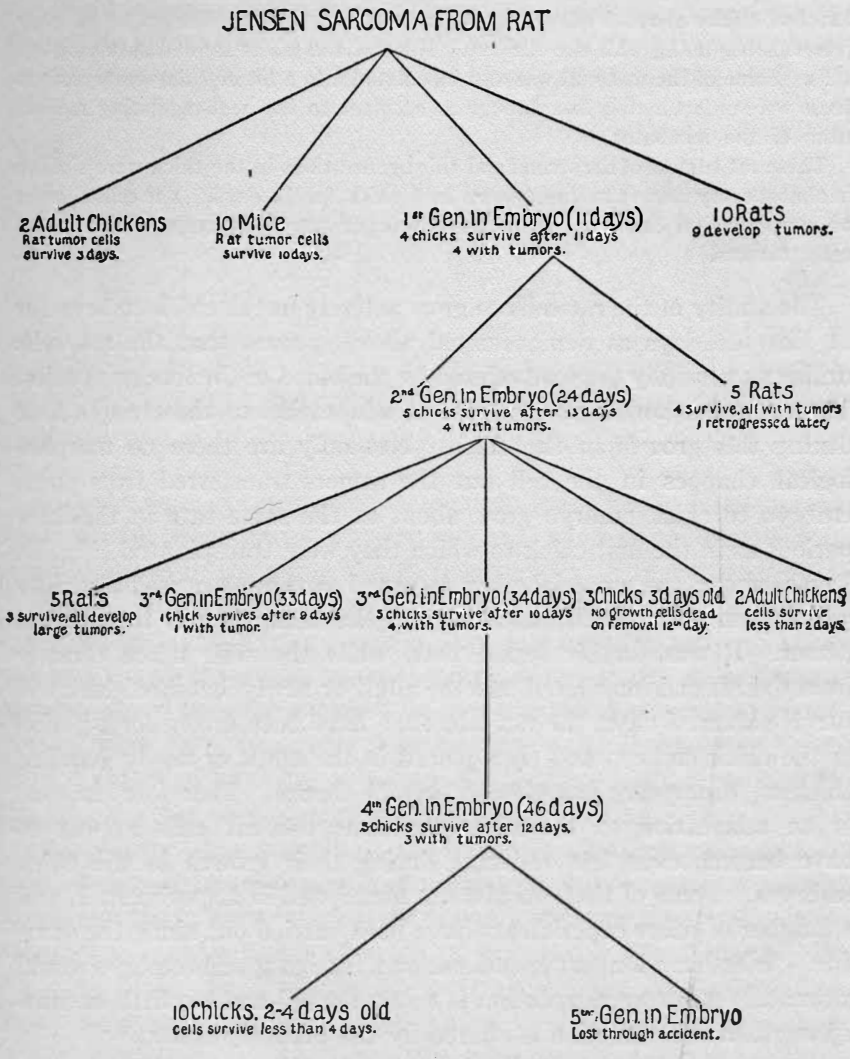
Experiment 2.—(Text-fig. 1.) First generation. A rapidly growing Jensen rat sarcoma was finely hashed and injected into a series of 7 day chick embryos. Some of the same material was inoculated into the following animals as control: (1) 10 rats, 9 of which subsequently developed tumors; (2) 10 mice, of which every 48 hours 1 was killed and the tissues on microscopical examination showed survival of the rat cells till about the 10th day after which they disappeared; (3) 2 adult chickens, each having received 10 grafts removed at 24 hour intervals, which showed survival of rat cells till the 3rd day.[1] The eggs inoculated were opened on the 18th day of incubation, 11 days after the injection; 4 remained alive, all with large tumors.

Second generation. The tumors from the above series of embryo were hashed and inoculated as follows: (1) into 5 rats, 4 of which survived, all having developed tumors; and (2) into a second lot of embryos on the 6th day of incubation. These were opened on the 19th day (13 days later), 5 remaining alive, 4 with tumors.

Third generation. The material from the last series was hashed and inoculated into the following animals: (1) 5 rats, 3 of which survived, all developing tumors; (2) 3 young chickens, hatched less than 3 days before, all developing small nodules which on removal 12 days later were found to be made up of reactive tissue with no rat cells present; (3) 2 adult chickens, 10 grafts each, which

[1] The criteria used here for survival were preservation of cell outline and the retention of the basic staining properties of the nucleus.

on removal at 48 hour intervals showed no rat cell surviving even after the first 48 hours; and (4) into a lot of 8 day embryos, 5 of which survived till the 18th



TEXT-FIG. 1. This represents Experiment 2 in outline.

day, 4 with tumors. The rat tumor at this period had been grown continuously for 34 days in the chick embryo.

Fourth generation. All the material from the above series was used to inocu-

late another lot of 6 day embryos. On the 18th day 5 chicks remained alive, 3 with tumors. Total number of days in embryo, 46.

Fifth generation. The material from the above was inoculated into 10 newly hatched chicks about 3 days old, grafts from which were removed at 48 hour intervals beginning with the 4th day. None of the grafts showed surviving rat cells. Some of the material was also inoculated into a lot of 7 day embryos but these were unfortunately lost through an accident to the heat-regulating mechanism of the incubator.

These rat tumors of the second and third generations in the chick were similar in almost every respect to those grown in 1 chick for 12 days. The cells showed no morphological changes, but the tissue was perhaps more compact than in the early tumors.

The ability of the rat cells to grow actively in the chick embryo for at least 46 days as demonstrated above, proves that the rat cells utilize successfully the food offered by the blood of the foreign species. The rat cells showed no evidence of adaptation to the strange host during this growth in the chick. Not only are there no morphological changes in the cell but the tumors transferred from chick embryo to chick embryo grow about at the same rate in the later periods as in the first chick to which they were transplanted (Fig. 4). Furthermore, the material when returned to the rat caused a rapidly growing sarcoma which was always of the same type as the original tumor. It was further found that, while the cells taken directly from the rat and inoculated into the adult or newly hatched chick will survive about 3 days, the rat cells that have been grown for a period in the chick embryo and then placed in the adult or newly hatched chicken disappear completely in 24 hours. Therefore instead of an adaptation to the new conditions the rat cells appear to have become even less resistant during their growth in the chick embryos. Some of these results are mentioned in Experiment 2, and a number of other experiments have been carried out along the same line. Whether a longer dependence on a foreign species embryo would ultimately effect an adaptation is not indicated and but little encouragement in this direction is offered by the present results.

Growth of Other Tissues in the Chick Embryo.

A variety of tissues besides the rat sarcoma have been grown in the chick embryo for longer or shorter periods. Tissues of the

chick itself, which fail to grow or grow poorly on transplantation to the adult, such as kidney (Fig. 5), testicle, ovary, bone, cartilage, etc., have been grown for a period of from 7 to 10 days. Embryomata formed by inoculation of hashed chick embryo can be carried through several generations, but during this process the epithelial elements become less evident, the resulting mass being predominantly bone, cartilage, and connective tissue elements.

Other foreign tissues grown with success are the Ehrlich sarcoma and chondroma of the mouse, embryomata of the rat and mouse, a mammary carcinoma of the mouse, and the Flexner-Jobling adenocarcinoma of the rat. Attempts with human tumors were less successful, possibly on account of the time that elapsed after the removal from the body. However, in some cases the tissue showed mitotic figures and a copious blood supply was furnished by the embryo.

HISTOLOGICAL MANIFESTATIONS OF RESISTANCE.

With the hope of throwing some light on the factor of resistance to heteroplastic tissue grafts, a study has been made of the histological manifestations in the adult and as they develop in the embryo.

Reaction to Foreign Tissue in the Adult Chicken.—The histologic changes about grafts from a foreign species introduced into the adult chicken closely resemble those observed about grafts of a chicken sarcoma in an immune animal. In fact the histologic process which goes on when a living tissue is grafted into an animal unsuitable for its growth seems to have the same general characteristics regardless of the source of the host's resistance.

Briefly stated, one finds about the strange graft an edema and reaction incidental to trauma. This is followed by an increase in the fibroblasts in the surrounding host tissue, a vascular proliferation and small mononuclear cell infiltration of the tissues round about. In birds, as Loeb and Addison (10) have shown, the accumulation of small round cells may be so marked as to form a considerable nodule of lymphoid tissue. The cells of the graft die more or less quickly, and in the meantime the proliferating connective tissue surrounds and invades the graft remains, and separates the mononuclear cells into islands. Finally the mononuclear cells disappear and the process subsides into a scar.

Reaction to Foreign Tissue in the Newly Hatched Chick.—When grafts from a foreign species are introduced into the newly hatched chick the series of reactions which follow are the same as those observed in the adult, and the foreign cells survive about the same length of time. On the whole the round cell infiltration may be less extensive, the connective tissue reaction more abundant, and the reaction seems to subside more rapidly than in the adult animal.

Reaction to Established Grafts of Foreign Tissue Left in the Embryo during the Development of the Resistant Condition.—In order to determine the time of onset and histological manifestations of the refractory condition, grafts of rat sarcoma were inoculated into a number of chick embryos, long enough in advance of the refractory period to allow them to become established. The time of inoculation most suitable for this purpose was found to be from the 12th to the 15th day of incubation. Beginning with the 17th day the inoculated embryos were killed in groups at 24 hour intervals and the grafts and their surroundings were examined histologically.

As late as the 18th day of incubation the grafts were in active growth, the embryo showing no evidence of a defensive reaction. On the 19th day most of the rat cells appeared to be still in good condition but at the edge of the graft the connective tissue elements had begun to increase. By the 20th day the nuclei of most of the rat cells became pycnotic, mitotic figures became rare, and beginning necrosis appeared in the center of the graft. In a few cases there was a moderate amount of round cell infiltration and in some others a local polymorphonuclear reaction. The uniform and striking change was in the connective tissue which increased markedly forming a capsule and beginning to invade the graft. Specimens taken on the 21st and 22nd days of incubation, showed a continuation of this invasion and replacement. On the 21st day only a few scattered rat cells could be identified in the mass of reactive tissue, and by the 22nd day all of these were reduced to occasional fragments of necrotic tissue staining pink with eosin. At this late period small clumps of round cells were numerous.

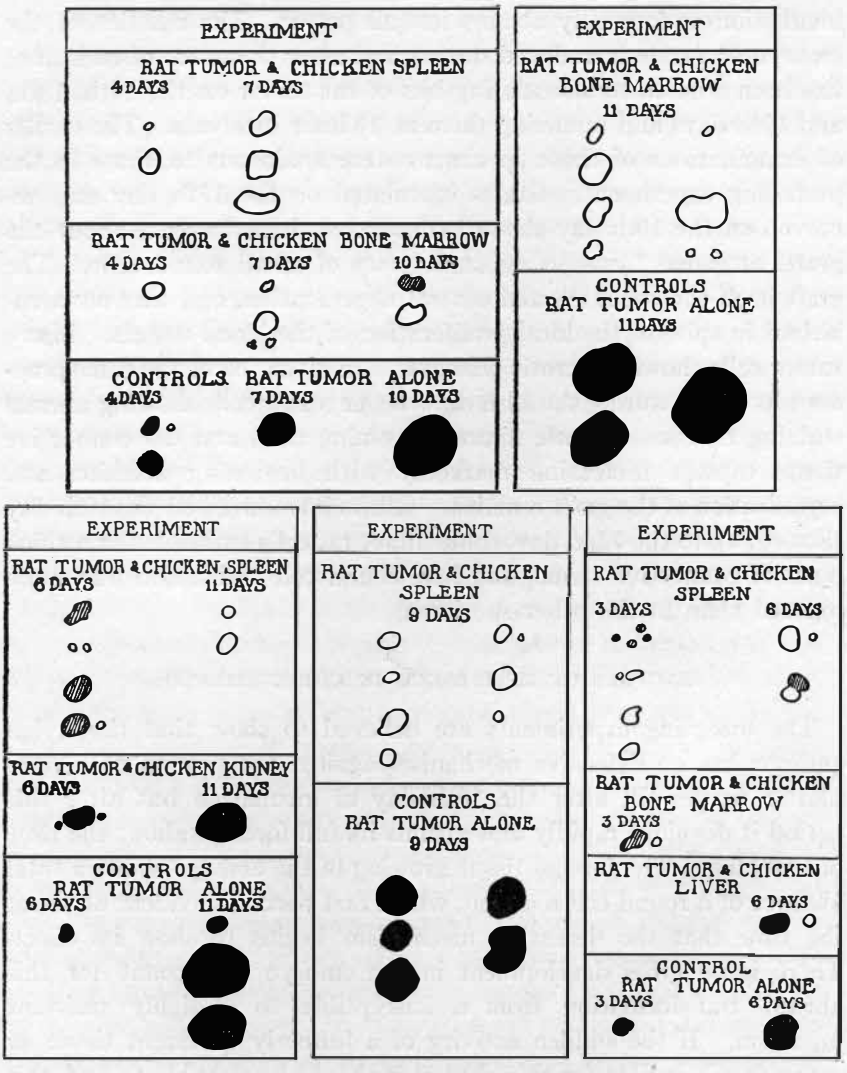
Reaction to Foreign Tissue Implanted during and after the Onset of the Refractory Period in the Embryo.—The foregoing findings show that the refractory period comes promptly after the 18th day of

incubation and quickly attains its full power. The reaction of the embryo to grafts introduced during and after the onset of resistance has been studied by inoculating bits of rat tumor on the 17th, 18th, and 19th days and removing them at 24 hour intervals. The results of examinations of these specimens were analogous to those in the preceding experiment. Grafts inoculated on the 17th day and removed on the 19th day showed connective tissue increase about the graft, attended by an occasional group of small round cells. The graft itself showed a marked central degeneration, and was unvascularized in spite of the local proliferation of the blood vessels. Many tumor cells showed necrotic changes. An extension of the same process was noted during the 20th day: fewer tumor cells showing normal staining reaction, mitotic figures becoming rare, and the connective tissue capsule increasing markedly with beginning invasion and organization of the graft remains. Grafts introduced on the 19th day showed, up to the 22nd day, some intact rat cells embedded in a thick mass of connective tissue, and here round cell infiltration was more marked than in the other specimens.

FACTORS OF RESISTANCE IN CHICK EMBRYO.

The foregoing experiments are believed to show that the avian embryo has no defensive mechanism against the growth of heteroplastic tissues till after the 18th day of incubation but after this period it develops rapidly and attains its full force at about the time of hatching. The foreign tissue growing in the embryo shows a total absence of a round cell reaction, which first becomes evident at about the time that the defensive mechanism begins to show its effect. There is no gross development in the embryo to account for this abrupt transformation from a susceptible to a highly resistant organism. If the sudden activity of a formerly quiescent tissue or organ is responsible for this change it should be possible to find this necessary tissue or organ by testing the effect of various adult tissue grafts in the embryo.

Effect of Adult Chicken Tissue Grafts on Heteroplastic Grafting in the Embryo.—In a series of 20 experiments comprising more than 150 embryos, grafts of rat sarcoma and bits of adult chicken tissues,



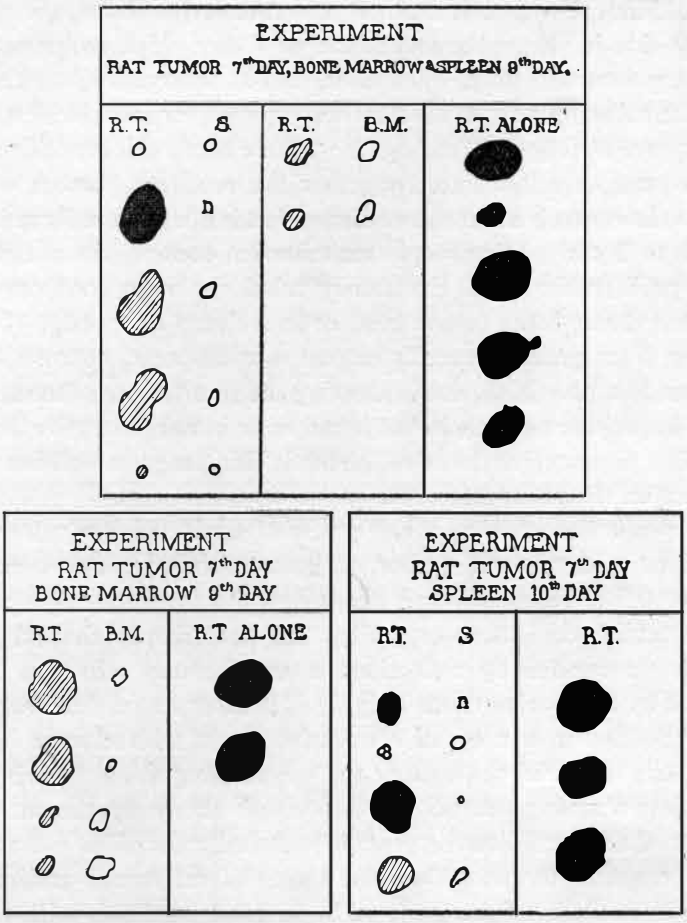
TEXT-FIG. 2. This chart shows in silhouette the results of simultaneous inoculation of rat sarcoma and a graft of adult chicken tissue into the outer membrane of chick embryos. The unshaded nodules were found on microscopic examination to be made up of the adult chicken tissue and reactive tissue, but showed no surviving rat cells. The shaded nodules were found to have a few rat cells embedded in a mass of reactive tissue (figures 2, 3, and 6). The black represents tumors in active growth, with no sign of defensive reaction.

spleen, kidney, liver, bone marrow, and connective tissue, were placed side by side in the outer membrane of 7 day chick embryos. The eggs were returned to the incubator, and at intervals up to the 18th day of incubation part of each lot was opened for examination. The results were as follows (Text-fig. 2): Where adult chicken kidney and rat sarcoma were inoculated together the resultant tumors were as large as the controls of rat tumor alone; that is, they generally measured from 1 to 2 cm. Microscopic examination showed the rat cells in active proliferation, with the kidney tubules, also in good condition, scattered through the tumor mass or in a clump at its edge (Fig. 6). Chicken liver grafts generally caused a widespread necrosis of the membranes of the chick, thus obscuring the result, but, if the rat tissue graft escaped the necrosis it was found to be in as active growth as the controls. Connective tissue of the adult chicken grew well but it had no effect on the rat tumor cells.

The result was striking when grafts of adult chicken spleen were inoculated with the rat tumor. The resulting tumors were flat, often mottled, yellowish, and opaque, while the spleen grafts were well established. Microscopically the rat tissue showed much necrosis surrounded by collections of small round cells and largely replaced by connective tissue (Fig. 7). In later stages dead rat tissue was embedded in a mass of connective tissue with clumps of small round cells scattered throughout the surrounding tissue. This condition offers a strong contrast to the picture shown by the rat tumor inoculated alone. Here the graft is practically devoid of stroma and also of reactive tissue. The final stages of rat tumor and chicken spleen showed the spleen graft to be in good condition with no evidence of the rat tissue remaining (Fig. 8).

The effect of bone marrow resembled that of the spleen but was less complete. The bone marrow grafts as they survived were composed for the most part of fat cells and collections of the lymphoid elements (Fig. 9).

Effect of Chicken Spleen and Bone Marrow on Established Grafts.— The tissues in the foregoing experiments were growing side by side, often intermingling. The next series of experiments was planned to avoid the contact and to give the spleen and bone marrow a more



TEXT-FIG. 3. This chart shows in silhouette the effect of adult chicken spleen and bone marrow on established and growing rat tumor in the embryo, when the adult tissues were inoculated at a distance. In the column with double rows of silhouettes the one on the left is the rat tumor (R.T.) and that on the right the bone marrow (B.M.) or spleen (S.) in the same embryo. The last column gives the controls of rat tumor alone. The day of incubation at which the inoculation was made is given in the caption. All tumors were removed at the 18th day of incubation. Black indicates that the tumors are composed of rat cells in active proliferation; the shaded outlines, that the rat tissue is much degenerated, with pronounced infiltration with round cells (figures 9 and 10). The unshaded outlines indicate that none of the rat cells survived. N indicates that graft did not take.

severe test. The kidney and other tissues mentioned above have no evident effect even in contact and they were therefore disregarded.

Series of eggs were inoculated with rat sarcoma on the 7th day of incubation. 2 or more days later an opening was made on the opposite side of the eggs, and adult chicken spleen or bone marrow graft was placed in the outer membrane. Some of these results are shown in Text-fig. 3. On examination, 11 days later, the controls inoculated with rat tumor alone showed, almost without exception, large, well established tumors. In the embryos carrying a graft of adult spleen or bone marrow the tumors were flat, yellowish, and opaque. In some of these only a flake of tumor survived. Microscopic examination of the tumors showed massive collections of lymphocytes around the edges and in clumps associated with the blood vessels throughout the tumor (Fig. 10), and a great increase in the connective tissue elements. The rat cells themselves showed many degenerated forms and mitotic figures were rare.

From this result spleen and bone marrow would seem capable of inhibiting the growth of rat tumor even though the graft of spleen or bone marrow be some distance from the foreign tissue and introduced after the latter has become established (Figs. 11 and 12).

HETEROPLASTIC TISSUE GRAFTS IN THE ADULT BRAIN.

In addition to the avian embryo, there is another subject in which heteroplastic tissue grafting can be successfully carried out. This is the adult mammalian or avian brain—a locus first utilized by Shirai (11). The original observation, as reported by Shirai, gives little more than the fact that rat tumors had been grown successfully in the brains of chickens, pigeons, and other animals. The important bearing of this finding on the question of the mechanism of resistance led the writer to attempt an analysis of the factors responsible for this unsuspected absence of control in the central nervous system. The method of tissue implantation in the brain as worked out by us follows.

Method.—After etherization of the animal, the head was shaved and a midline incision made, enabling the skin and fascia to be retracted. A small hole of sufficient size to admit a No. 18 gauge trocar was made in the skull. The trocar was provided with a shoulder of metal about 3 mm. from the point, in order to limit the depth of penetration. The point was beveled slightly so as not to damage the brain tissue unnecessarily. The material for inoculation was loaded into the trocar and pushed into the brain by means of a plunger, after which the trocar was withdrawn slowly so as not to dislocate the graft. Bleeding was

stopped by pulling the fascia over the opening or by applying a small piece of muscle.

Mouse Tumors in the Brains of Rats.—The cerebellum proved to be unsatisfactory as the location for inoculations for reasons not altogether clear. Save occasional excellent growths the graft was represented by a mass of reaction tissue similar to the remnant of an heteroplastic graft in the subcutaneous tissue. Implantations into the posterior lateral part of the cerebrum likewise gave irregular results. We found, as a possible explanation of this fact, that a graft lying in the ventricle or even coming into contact with it failed, but grafts entirely in the brain substance grew almost invariably with great rapidity.

On the basis of this observation we selected the anterior part of the frontal lobe on account of the thickness of the cortex and the shallowness of the ventricle at this point. Grafting in this region gave 80 to 90 per cent of tumors in the animals inoculated, and when the implant failed to grow, examination showed that almost invariably it had come into contact with the ventricle.

The growth resulting from implantation of mouse sarcoma into the rat brain was generally a discrete, rounded nodule, pushing the brain tissue away, rather than invading it directly. The tumors had a copious vascular supply and rarely showed any considerable areas of necrosis, which is in contrast with the extensive central necrosis usually found in nodules developing in the subcutaneous tissue of the native host. The number of mitotic figures is remarkable (Fig. 13). No cellular reaction is present about the edges of the typical growth lying entirely within the brain substance, though there is a pronounced perivascular round cell reaction nearby, and also often a blocking of the lumina of small vessels with lymphocytes (Fig. 14). Ordinarily the cellular accumulations remain confined, and do not invade the brain tissue. However, if, in the course of growth, the tumor reaches the ventricle, the choroid plexus becomes enormously swollen and engorged with lymphocytes (Fig. 15) and these may break through and actively invade the tumor with resultant necrosis of the portion lying near the ventricle (Fig. 16).

The rapidity of growth of the mouse sarcoma in the brains of rats may be judged by the fact that pin-head sized grafts produced tumors in 7 to 9 days which replaced the entire frontal lobe. This mouse tumor has also grown with equal rapidity in the brains of guinea pigs and pigeons, as did also a mouse carcinoma. The latter tumor tended to flatten on the surface of the brain and to extend apparently by invasion, sending finger-like processes into the brain. Necrosis was observed more frequently in the carcinoma than in the sarcoma, although less in extent than occurs in the subcutaneous tumor of the native host.

Effect of Autografts of the Spleen on the Growth of Foreign Tissue in the Brain.—As previously noted, an organism which is non-resistant to heteroplastic tissue, like the chick embryo, may be rendered resistant by a graft of adult spleen. This suggests the possible significance of the observation that no cellular reaction occurs about a foreign tissue in the brain. The following experiments were therefore undertaken to throw some light on this question.

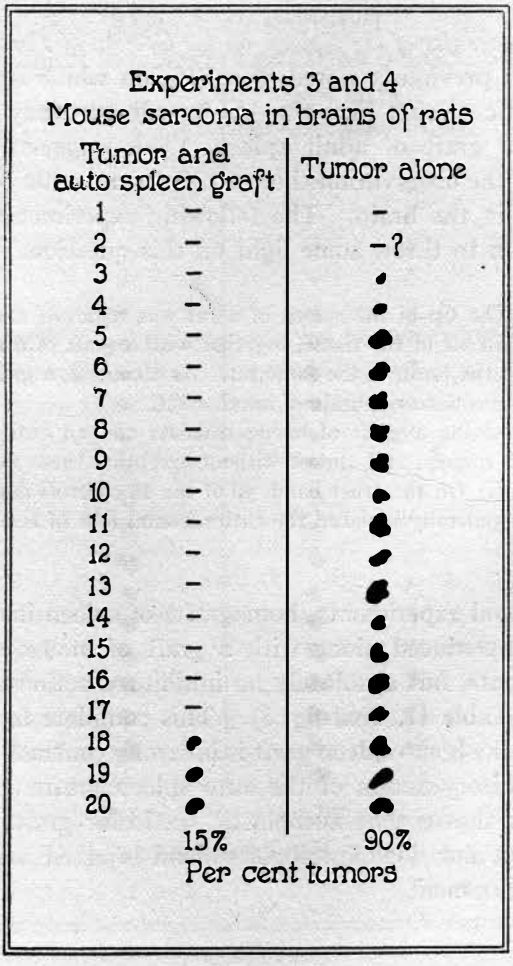
Experiment.—The tip of the spleen of a rat was removed under ether anesthesia, and a small bit of the tissue, together with a graft of a mouse sarcoma, was implanted in the brain of the same rat. As a control, a graft of the tumor alone was introduced into the brain of another rat.

Of 50 rats receiving a graft of mouse sarcoma and an autograft of spleen, only 8 developed tumors, and almost without exception these were small, often only nests of cells. On the other hand, 40 of the 48 controls developed tumors, and the growths generally replaced the entire frontal lobe of the brain (Table I, Text-fig. 4).

In 2 additional experiments, homografts of spleen instead of autografts, were introduced along with a graft of mouse sarcoma into the brains of rats, but absolutely no inhibitory action on the tumor was effected (Table II, Text-fig. 5). This complete lack of activity on the part of the homo spleen graft is in strong contrast to the almost complete inhibitory action of the auto spleen grafts. As a further control it was shown that autoplastic testicular grafts exert no inhibitory action, nor does autologous blood injected with the tumor affect its development.

TABLE I.

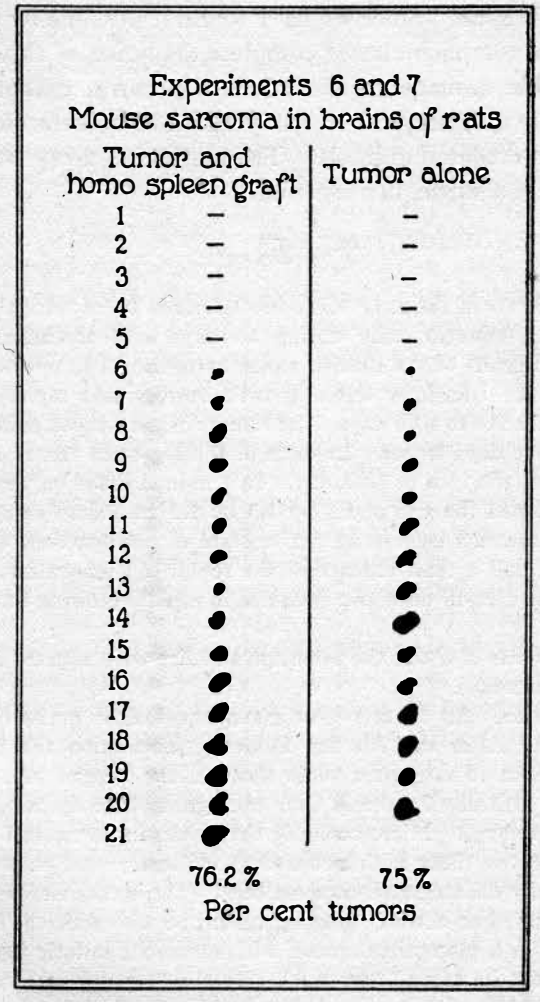
Experiment No.	Tumor and auto spleen graft.		Tumor alone.	
	Takes.	No. of rats.	Takes.	No. of rats.
	per cent		*per cent*	
1	11.1	9	66.6	9
2	33.3	9	80.0	10
3	10.0	10	80.0	10
4	20.0	10	100.0	10
5	8.5	12	88.8	9
Average or total.....	16.0	50	83.3	48



TEXT-FIG. 4.

TABLE II.

Experiment No.	Tumor and homo spleen graft.		Tumor alone.	
	Takes.	No. of rats.	Takes.	No. of rats.
	per cent		*per cent*	
6	72.7	11	80.0	10
7	80.0	10	70.0	10
Average or total.....	76.2	21	75.0	20



TEXT-FIG. 5.

HETEROPLASTIC GRAFTING IN THE ADULT ANIMAL DEPRIVED OF LYMPHOID TISSUE.

The fact that a soil unresistant to heteroplastic tissues can be made resistant by the introduction of lymphoid tissue suggests the importance of this histological element in the resistance mechanism. If this supposition is correct, it should be possible to effect heterotransplantation in the normally resistant host by lessening the activity of the lymphoid tissue. Since we have found it possible by proper doses of x-rays to accomplish almost complete depletion of this tissue without perceptible damage to the other structures, excepting the sex glands, we have attempted to study the fate of heterologous tumor grafts in such depleted animals. The method of x-ray treatment will be described in a subsequent section.

Experiments.

Series 1.—20 rats of the same size were selected, 10 of which were exposed to x-rays for 5 minutes daily during 10 days. All the animals were then inoculated with grafts of the Ehrlich mouse sarcoma. The mouse tumor in the normal rats, after transitory initial growth, retrogressed rapidly to complete absorption by the 9th to 10th day. The tumors in the x-rayed animals continued to grow for some days longer. In some of these growth ceased after the 14th day, in others at the 15th or 16th day. In 1 animal killed on the 18th day the tumor had attained the size of 1.8 by 1.3 by 1.2 cm., showing in sections the typical mouse sarcoma cells in an active state of proliferation, with numerous mitotic figures, and a total absence of the round cell infiltration. This tumor was reinoculated into 10 mice and gave rise to rapidly growing tumors in all the 10.

Series 2.—10 rats of about the same age and size were selected and 5 of these were exposed to x-rays.

First generation. All 10 rats were given equal sized grafts of the Ehrlich mouse sarcoma. After the 7th day following inoculation the tumors in the normal rats began to retrogress while those in the x-rayed rats continued to grow. On the 11th day 2 animals from each group were operated on and part of the tumors removed. Microscopically the masses in the normal rats consisted mainly of connective tissue with a round cell infiltration and an occasional small group of partially disintegrated sarcoma cells. The sections of tumor from the x-rayed rats showed an actively growing tumor, no whit different from the same tumor growing in a susceptible mouse, with numerous mitotic figures and with no reaction about its edges (Tumor A). By the 14th day after inoculation all of the tumors in the normal series had retrogressed till only very small nodules

could be felt. In the x-rayed animals 1 tumor had retrogressed, 2 had become stationary and 2 were increasing in size. 1 each of the x-rayed and normal rats were operated on and part of the tumors excised. The nodule from the normal animal was made up entirely of reaction tissue with no surviving mouse tissue, while that in the x-rayed animal was a healthy, vigorously growing tumor with active mitosis and with no reaction about its edges (Tumor B). By the 23rd day after inoculation no trace of the mouse sarcoma grafts was found in the normal animals. Among the x-rayed rats the tumors had become stationary, one having attained considerable size. Microscopically this latter tumor consisted of healthy looking sarcoma cells, with occasional mitotic figures and a beginning round cell reaction at the edges.

Second generation A. 6 irradiated rats were inoculated with a mouse sarcoma which had grown for 11 days in the first generation of rats (Tumor A). For control this tumor was also inoculated into 3 mice, all of which developed progressively growing tumors. By the 12th day all of the x-rayed rats had developed tumors ranging in size from 0.5 to 0.7 cm. in diameter. Some of the tumors then retrogressed while others continued to grow 3 to 5 more days. Microscopic examination showed them to be typical Ehrlich mouse sarcomata.

Second generation B. A mouse tumor removed after 14 days' growth in the first of x-rayed rats (Tumor B) was inoculated into 3 mice and 6 x-rayed rats. 2 of the 3 mice developed typical tumors. In the rats all of the grafts produced tumors by the 11th day. Sections proved them to be the vigorously growing typical mouse sarcoma with no reaction in the surrounding tissues (Fig. 18). After this period, as in the preceding series, some variation in the length of survival and growth occurred. 1 tumor was removed on the 13th day and reinoculated into 6 x-rayed rats (third generation). At this period the mouse tumor had grown continuously in x-rayed rats for 27 days.

Third generation. A tumor from Second Generation B was inoculated into 6 x-rayed rats. The resulting tumors grew less rapidly than in the previous generation, none attaining a large size, and they became more or less stationary after 8 days. 2 tumors removed on the 13th day were typical sarcomas with some mitosis and a beginning local reaction. Thus the mouse cells had been growing continuously in x-rayed rats for the period of 40 days.

Series 3.—A mammary carcinoma of the mouse was inoculated into 5 x-rayed and 5 normal rats. Small tumors developed in the normal animals, but after 4 or 5 days they rapidly retrogressed. In the x-rayed animals, however, the masses continued to grow from 11 to 15 days before they became stationary. On the 12th day no surviving tumor cells were found in grafts from the normal rats while those from the x-rayed animals were composed of actively growing cancer cells with little or no reaction in the surrounding tissue (Fig. 17).

Second generation. Grafts from the latter tumor after 12 days' growth in x-rayed rats were transplanted into 2 other x-rayed rats. After 9 days they were removed for histological examination, which revealed the tumor cells to be

still multiplying and retaining their usual structure. This mouse tumor had grown continuously in x-rayed rats for 21 days.

Series 4.—10 rats, after proper x-ray doses, were implanted with grafts from a chicken sarcoma (Chicken Tumor 18), along with a like number of normal rats. The tumors in the normal rats disappeared by the 5th or 6th day showing only a mass of reaction tissue. In the x-rayed animals, however, the tumor continued to grow, in some cases as long as 16 to 18 days, and in 1 the tumor was still active when the animal was killed 25 days after inoculation.

The difference in the time of survival of foreign tissue in x-rayed animals might be accounted for by the individual variation in the rate of lymphoid regeneration after depleting doses of x-ray. We found that a rat which supported a graft of mouse tumor for 23 days had only 30 per cent lymphocytes, while in another animal of the same series in which the foreign tumor had retrogressed earlier the lymphocytes formed 90 per cent of the circulating white cells. Judging from the condition of the blood and the amount of local reaction the foreign tissue will grow actively in an x-rayed animal till such time as the lymphoid system is well started in the regenerative process. Furthermore such foreign tissue may be repeatedly transferred to other x-rayed animals.

SUMMARY AND DISCUSSION.

Athrepsia Theory.—The fact that such tissues as rat and mouse tumors may be transplanted successfully to the chick embryo, the forebrain of a different species, and into x-rayed animals of a different species, and furthermore may be repeatedly transplanted in the foreign hosts over long periods, leaves no doubt that the failure of heteroplastic grafts under usual conditions is not due to a lack of suitable nutrient material. Therefore the athrepsia theory of Ehrlich seems untenable in the light of the above results.

Stroma and Vascular Supply Theory.—The explanation offered for the failure of heteroplastic grafts on the ground that the foreign host does not furnish the engrafted tissue with a suitable blood supply or connective tissue stroma has been subjected to an extensive experimental examination. The details of these studies will not be gone into as the majority of investigators are inclined to consider this explanation inadequate. One observation recorded above has a direct bearing

on this point, namely that a rat tissue graft in the chick embryo with an adequate blood supply may be eliminated in the course of 48 hours after the resistance mechanism develops at the time of hatching.

Antibody Theory.—The promptness with which foreign tissues are eliminated is strong evidence that the development of antibodies is not responsible for the failure of heteroplastic grafts. Furthermore all attempts to demonstrate antibodies under the conditions of these experiments have failed. It is true that cytotoxins to foreign cells may be developed but only after massive immunization. From the experimental material presented here, the fact that heteroplastic tissues will grow readily in the embryo and in x-rayed animals could not be considered as evidence against the antibody theory, for embryos are deficient in antibody formation and x-rayed animals develop these protective substances very slowly. On the other hand the fact that foreign tissues will grow in the brain where they have every opportunity of being affected by circulating antibodies speaks rather strongly for the absence of any such system of protection in the case of heteroplastic tissue grafts.

Cellular Reaction.—There is considerable evidence that the cellular reaction is of first importance in determining the fate of a heteroplastic graft. The accumulation of the small round cell about foreign tissue introduced into the higher animals is a constant finding and has been noted and commented on by many investigators. Where this reaction is absent as in the embryo, the x-rayed animal, or in the brain, the heteroplastic graft meets with no resistance and its rate of growth is at least as great as it would be in the native host. The absence of resistance in the chick embryo and the adult brain may be successfully counteracted by the introduction of native lymphoid tissue with the foreign graft. Under these conditions the degree of resistance and its manifestation are similar to that which normally takes place about a heteroplastic graft in the subcutaneous tissue of an adult animal.

Certainly all of this evidence points without exception to the importance of the lymphoid type of cell in the resistance to foreign tissue grafts. In the absence of these cells the foreign tissue meets with no check in its growth and with them even non-resisting organisms or organs, such as the chick embryo and the adult brain, resistance is

practically complete. It has been suggested that the cellular reaction is in the nature of an associated phenomenon and not the primary resisting force, but until some other efficient factor is shown to operate this opinion does not seem justified in view of the experimental facts presented above.

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For a general review of tissue grafting up to 1913 see *Proceedings of Seventeenth International Congress of Medicine*, London, Section III.

EXPLANATION OF PLATES.

PLATE 1.

FIG. 1. The Jensen rat sarcoma in the outer membrane of an 18 day embryo, 11 days after inoculation. The tumor measured 1.7 by 1.5 cm.

FIG. 2. A section of rat sarcoma after 12 days' growth in a chick embryo. A and B = mitotic figures.

PLATE 2.

FIG. 3. A rat sarcoma after 11 days in a chick embryo, showing five mitotic figures.

FIG. 4. A section of rat sarcoma after four generations in chick embryos (46 days). A = mitotic figures.

PLATE 3.

FIG. 5. Growing kidney tubules in the outer membrane of an 18 day chick, resulting from the inoculation of embryonic kidney.

FIG. 6. The edge of a tumor resulting from a 10 days' growth of a Jensen rat sarcoma and adult chicken kidney inoculated into the outer membrane of a 7 day embryo. The kidney tubules are seen scattered around the edge of the tumor mass, which is made up of the rapidly grown rat cells.

PLATE 4.

FIG. 7. A section of a tumor resulting from a simultaneous inoculation of adult chicken spleen and a rat sarcoma, after 5 days' growth in the outer membrane of a chick embryo. A = spleen graft. B = the sarcoma cells surrounded and largely replaced by small round cells and connective tissue.

FIG. 8. The resulting tumor from a simultaneous inoculation of a 7 day embryo with a rat sarcoma and a graft of adult chicken spleen, after 11 days. The spleen graft is seen on the left and the location of the sarcoma graft is on the right. There are no evidences of the rat cells remaining.

PLATE 5.

FIG. 9. This section shows the effect of a chicken bone marrow graft on a rat sarcoma after 6 days in the embryo. The rat cells (A) are embedded in a mass of small round cells (B).

FIG. 10. Section of rat tumor in a chick embryo which had at some distance away a graft of adult bone marrow. A = round cell infiltration. B = degenerated rat cells.

PLATE 6.

FIG. 11. A drawing, somewhat enlarged, showing a tumor in the outer membrane of an 18 day old embryo, resulting from a simultaneous inoculation 11 days previously of grafts of rat sarcoma and adult chicken kidney. The kidney is shown as the bluish nodule in the concavity of the tumor. The controls of rat tumor alone ranged about the same size.

FIG. 12. A drawing, somewhat enlarged, showing the effect of adult chicken spleen on an established graft of rat tumor in a chick embryo. The lower figure is the control of rat tumor alone after 11 days of growth in the outer membrane of chick embryo. The upper figure is the outer membrane of an embryo inoculated at the same time as the above with rat tumor (yellowish area), but 2 days later a graft of adult chicken spleen was added (pink nodule).

PLATE 7.

FIG. 13. A mouse sarcoma growing in the brain of a rat. M = mitotic figures.

FIG. 14. Perivascular infiltration in the brain of a rat, which occurs near a foreign tumor graft.

PLATE 8.

FIG. 15. Choroid plexus engorgement with round cells resulting from the encroachment of a foreign tumor.

FIG. 16. Result of encroachment of a foreign tumor on the ventricle.

PLATE 9.

FIG. 17. Low and high power photomicrograph of a mouse carcinoma grown in x-rayed rats. M = mitotic figures.

FIG. 18. A mouse sarcoma grown for 24 days in x-rayed rats. M = mitotic figures.

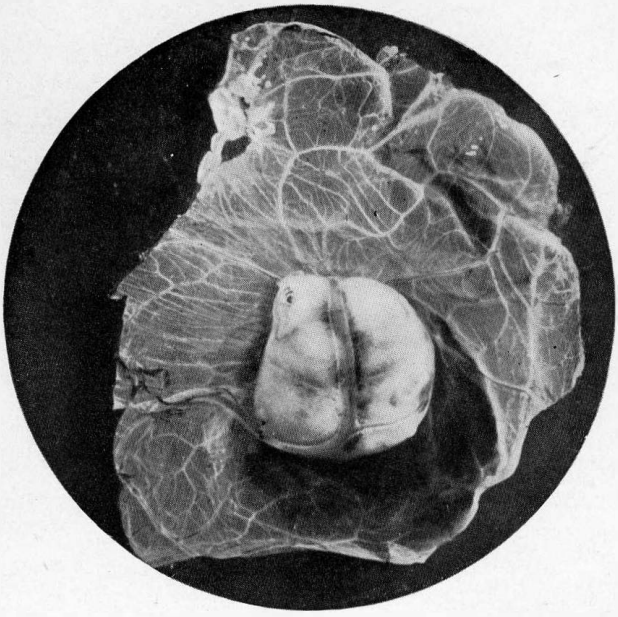


FIG. 1.

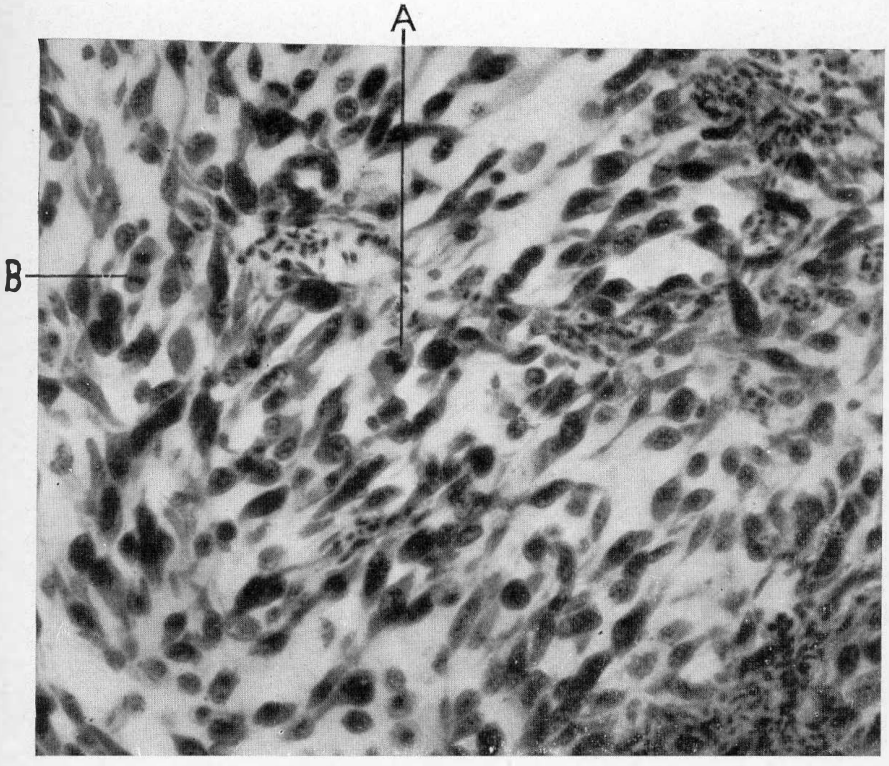


FIG. 2.

(Murphy: Heteroplastic tissue grafting.)

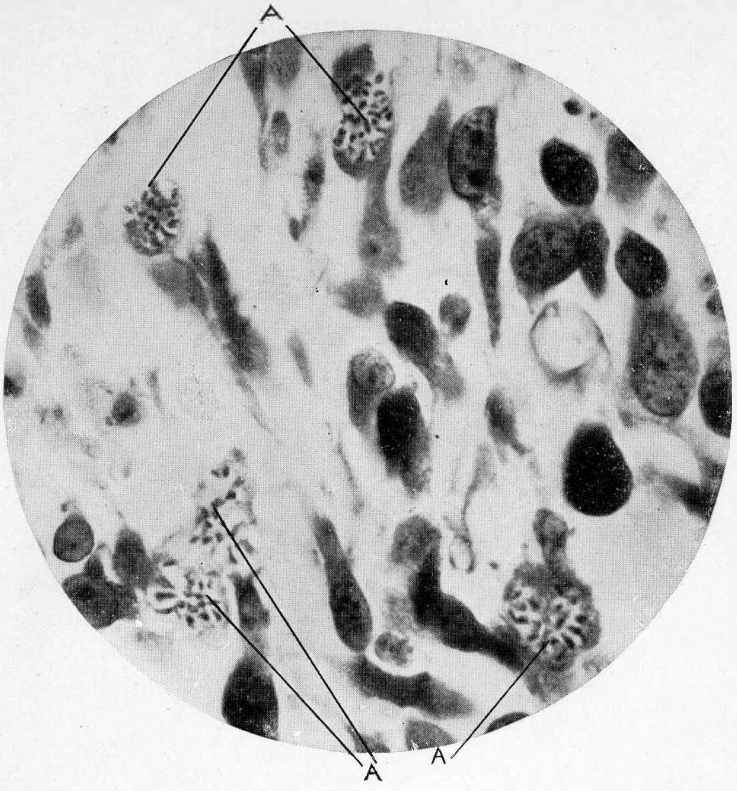


FIG. 3.

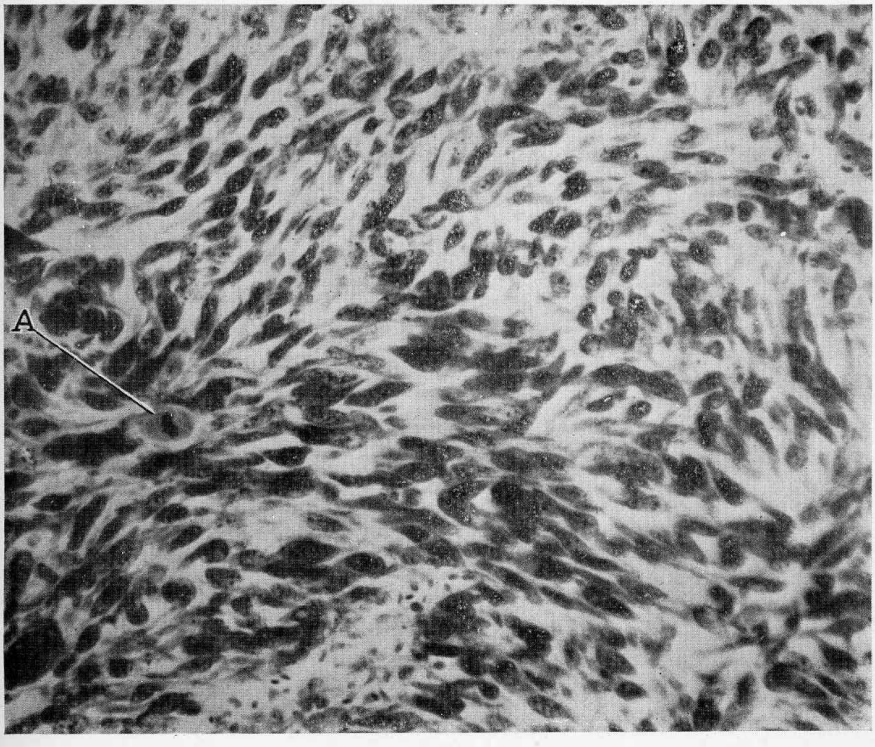


FIG. 4.

(Murphy: Heteroplastic tissue grafting.)

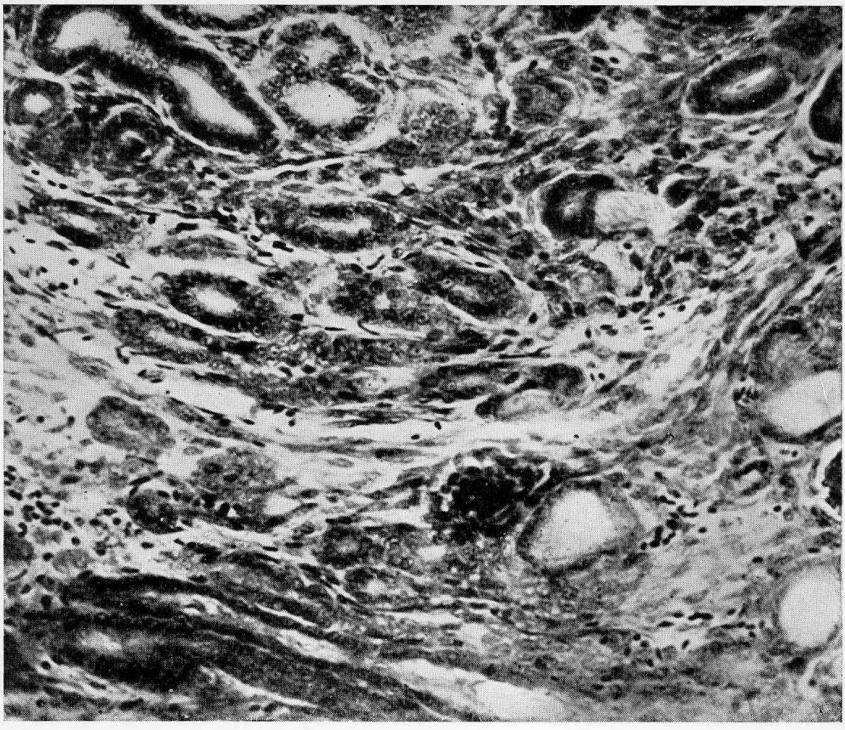


FIG. 5.

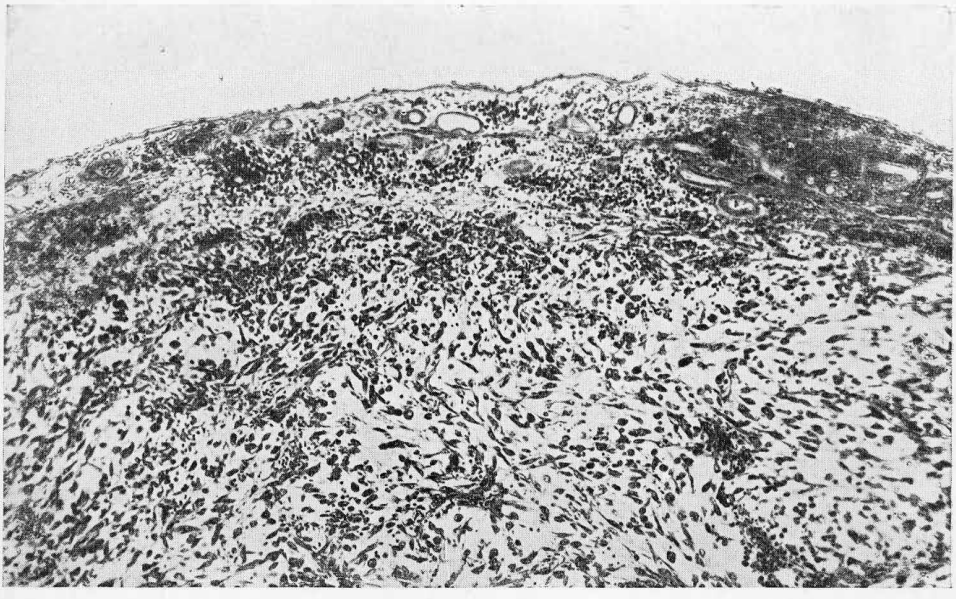


FIG. 6.

(Murphy: Heteroplastic tissue grafting.)

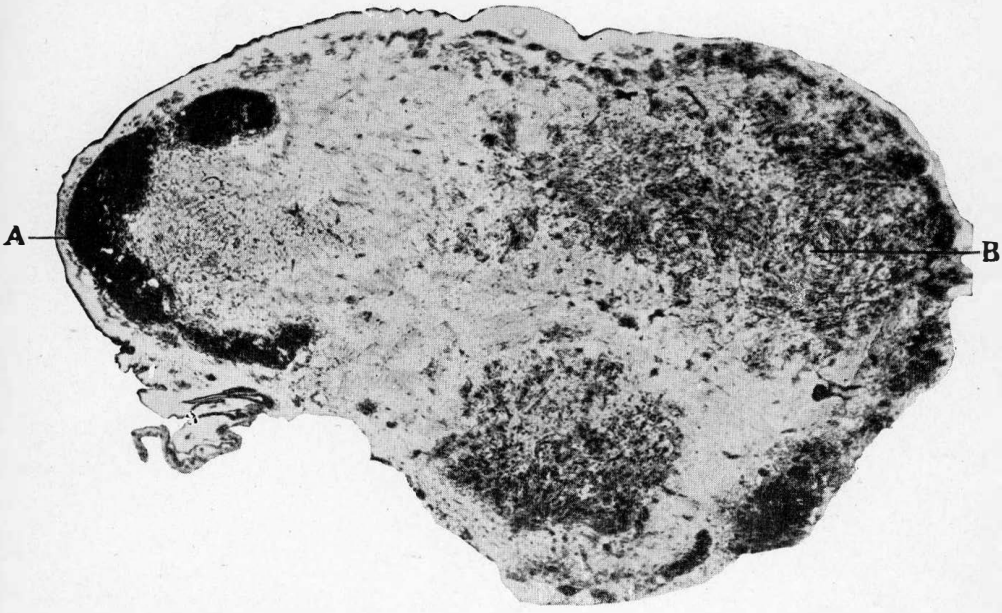


FIG. 7.

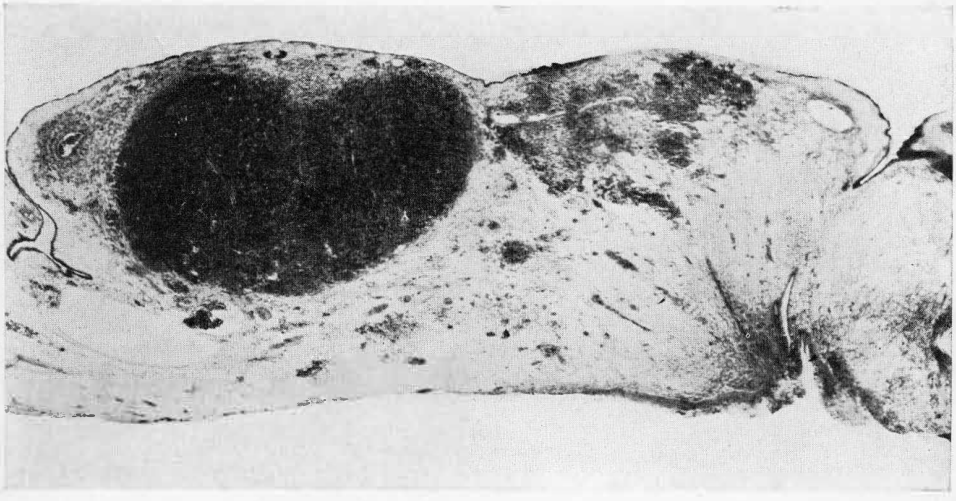


FIG. 8.

(Murphy: Heteroplastic tissue grafting.)

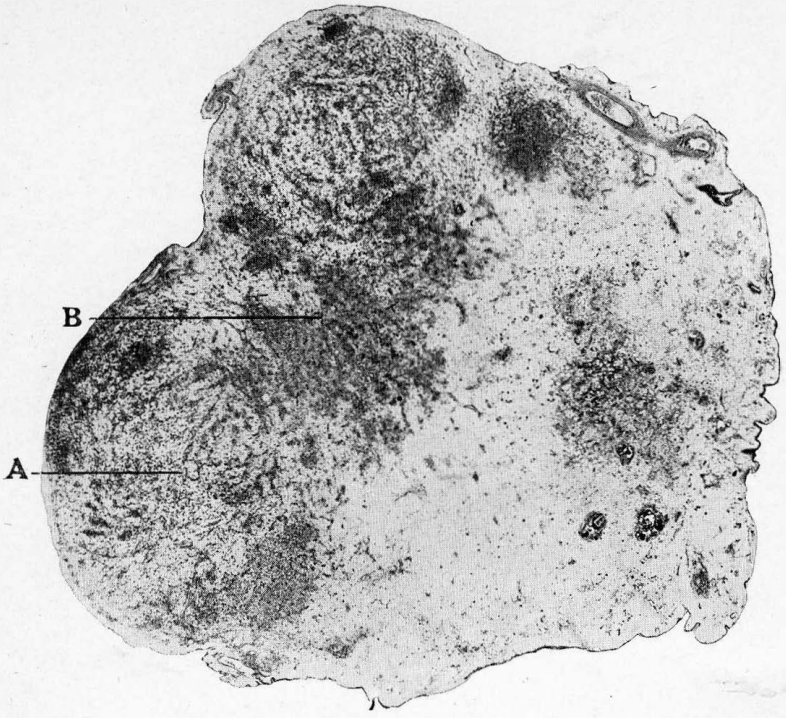
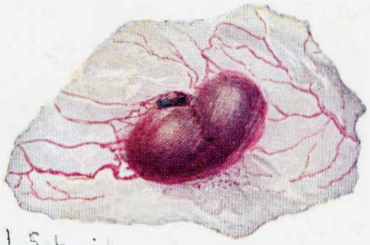


FIG. 9.

FIG. 10.

(Murphy: Heteroplastic tissue grafting.)



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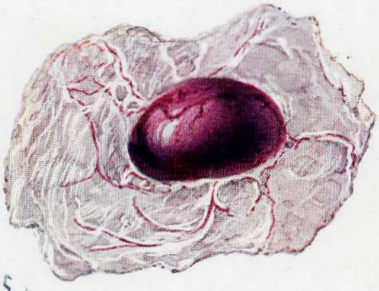


FIG. 12.

(Murphy: Heteroplastic tissue grafting.)

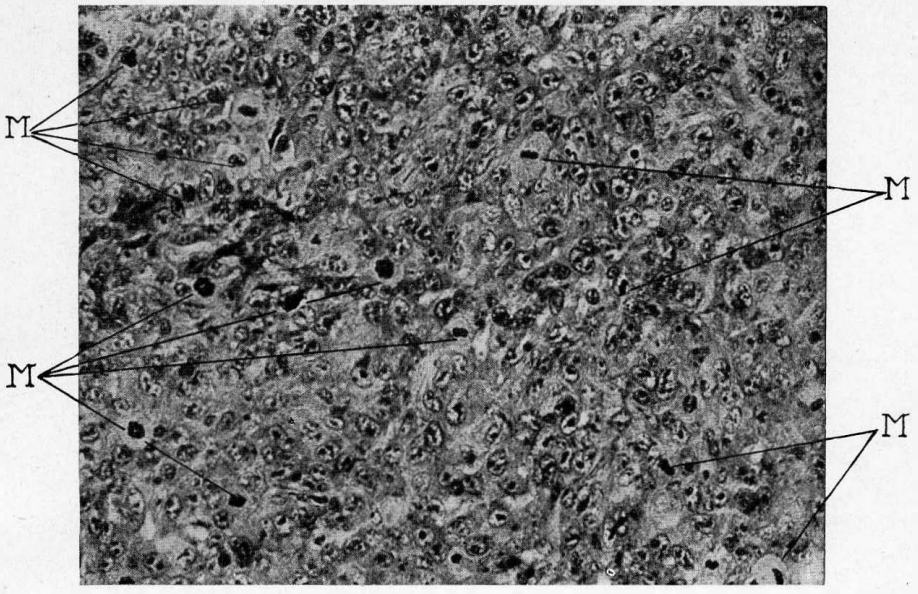


FIG. 13.

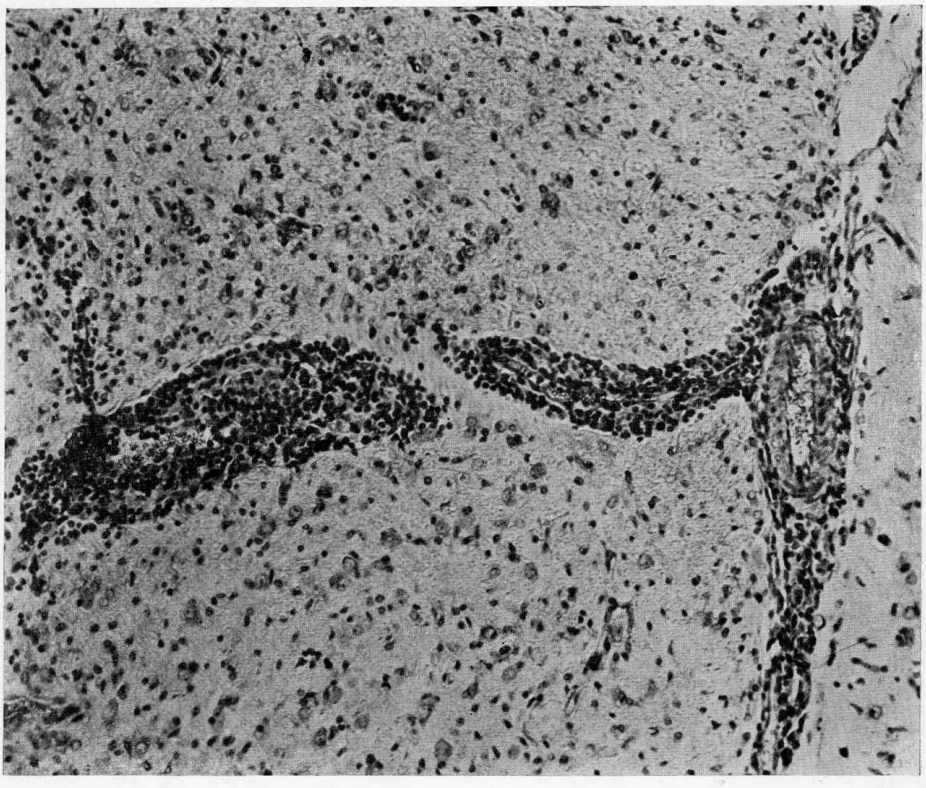
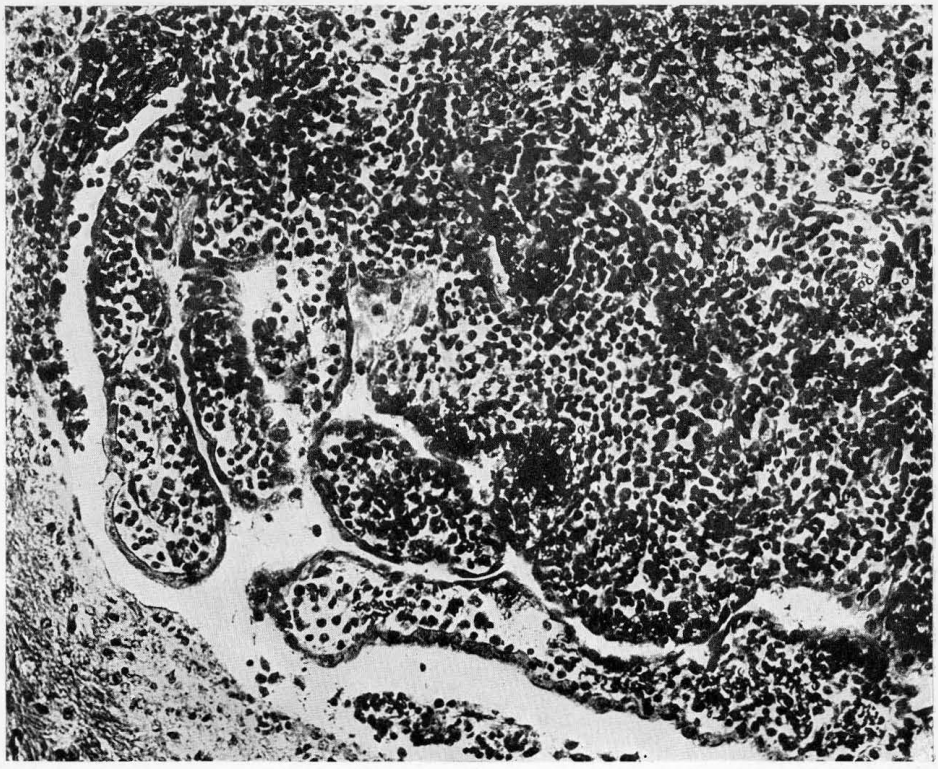


FIG. 14.

(Murphy: Heteroplastic tissue grafting.)



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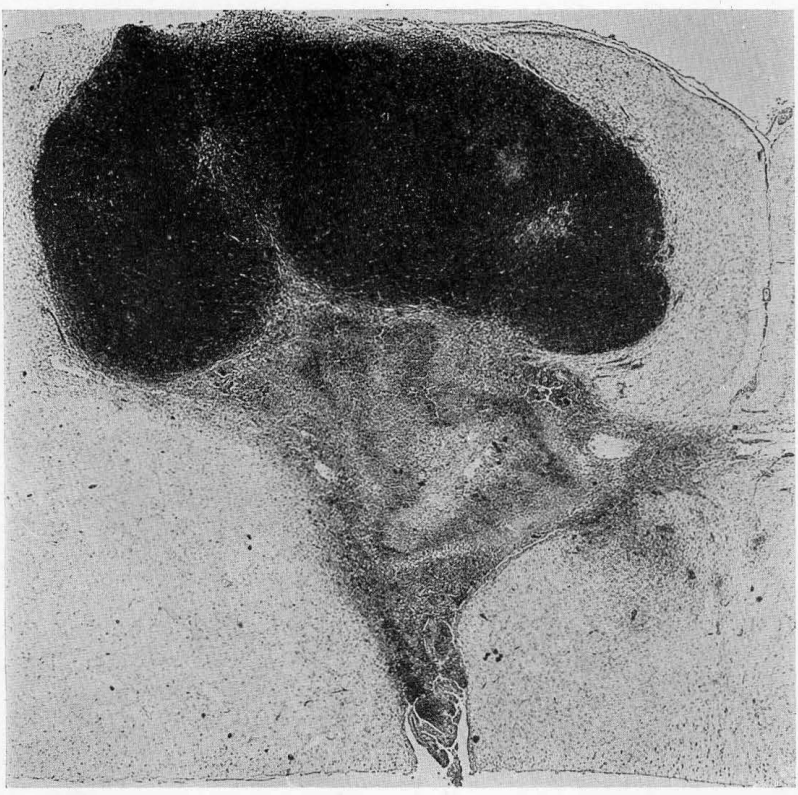
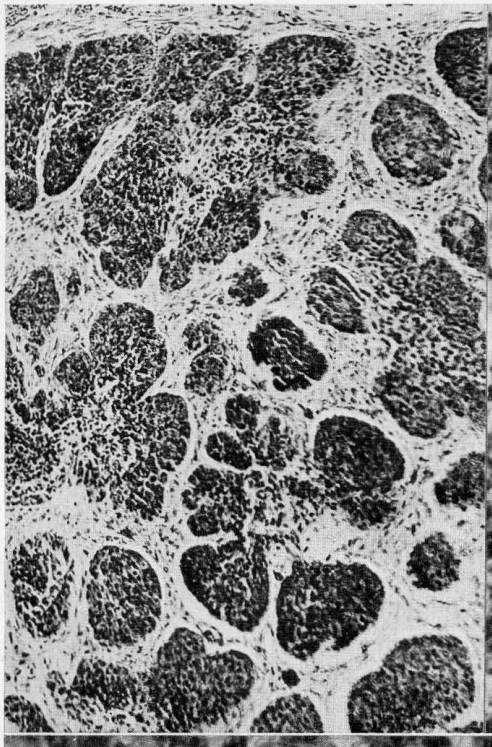
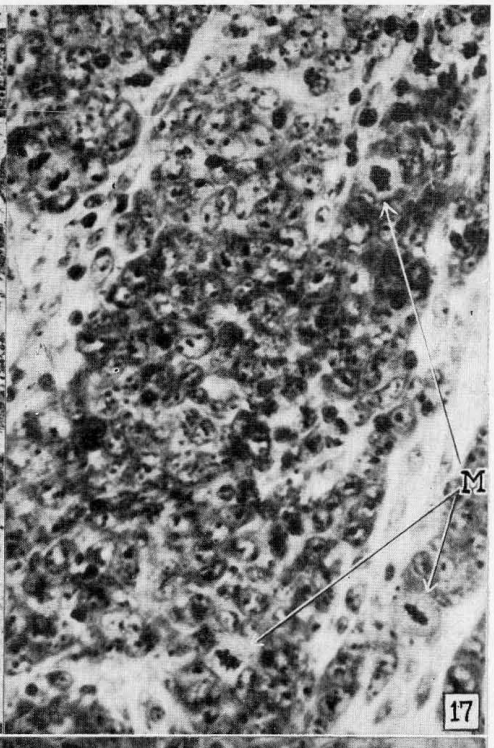
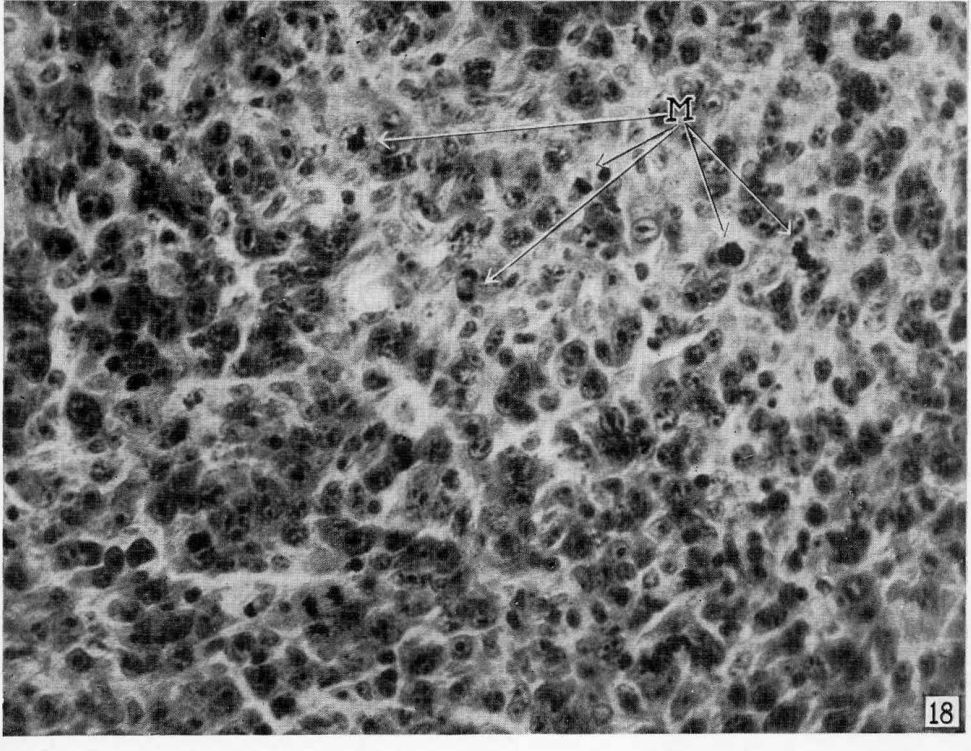


FIG. 16.







(Murphy: Heteroplastic tissue grafting.)

II. HISTOLOGICAL MANIFESTATIONS ACCOMPANYING RESISTANCE TO INOCULATED TUMOR.

PLATES 10 TO 12.

Resistance to transplanted tumors may be either naturally present in the animal, or acquired so that a tumor, after a period of growth, is overcome and absorbed, or it may be induced (1). The underlying process which enables such resistant animals to destroy engrafted tumors is still undetermined and hence matter for speculation, for no adequate theory has thus far been advanced.

LOCAL REACTIONS ABOUT CANCER GRAFTS IN RESISTANT ANIMALS.

Whether the resistance to tumor be natural or induced, the local changes which take place about a cancer graft are the same, the difference, if any, being only in the time at which the various reactions occur (2). In brief, the cells of the cancer graft, in a resistant host, survive for some days and may actually proliferate actively. During the first few hours the reactions in the surrounding tissue are principally those associated with the trauma resulting from the inoculation, and are identical in resistant and susceptible animals. Later, in the resistant animal, a small round cell reaction is found surrounding nearby vessels and appearing as a halo around the tumor, but not in contact with it. The infiltration gradually increases and approaches the tumor, and finally actively invades it. Other cells appear in this reaction, such as macrophages, plasma cells, and fibroblasts, but their numbers are variable and if one may judge by the histological pictures, they play a minor rôle in the process. The fibroblasts are very active in the last stages but they seem to come in only after the cancer cells are well on their way to disintegration. In a retrogressing tumor there is a constant association of lymphoid reaction with the process, and even in growing tumors, local areas of healing are frequently observed associated with local accumulations of round cell reaction.

Da Fano (3) was probably the first to venture an opinion based on experimental work on the part played by the lymphocyte in the proc-

esses of resistance. His observations included only the local phenomena and changes in the subcutaneous tissues, but they tended to point to lymphoid reaction at least as an "histological sign of resistance."

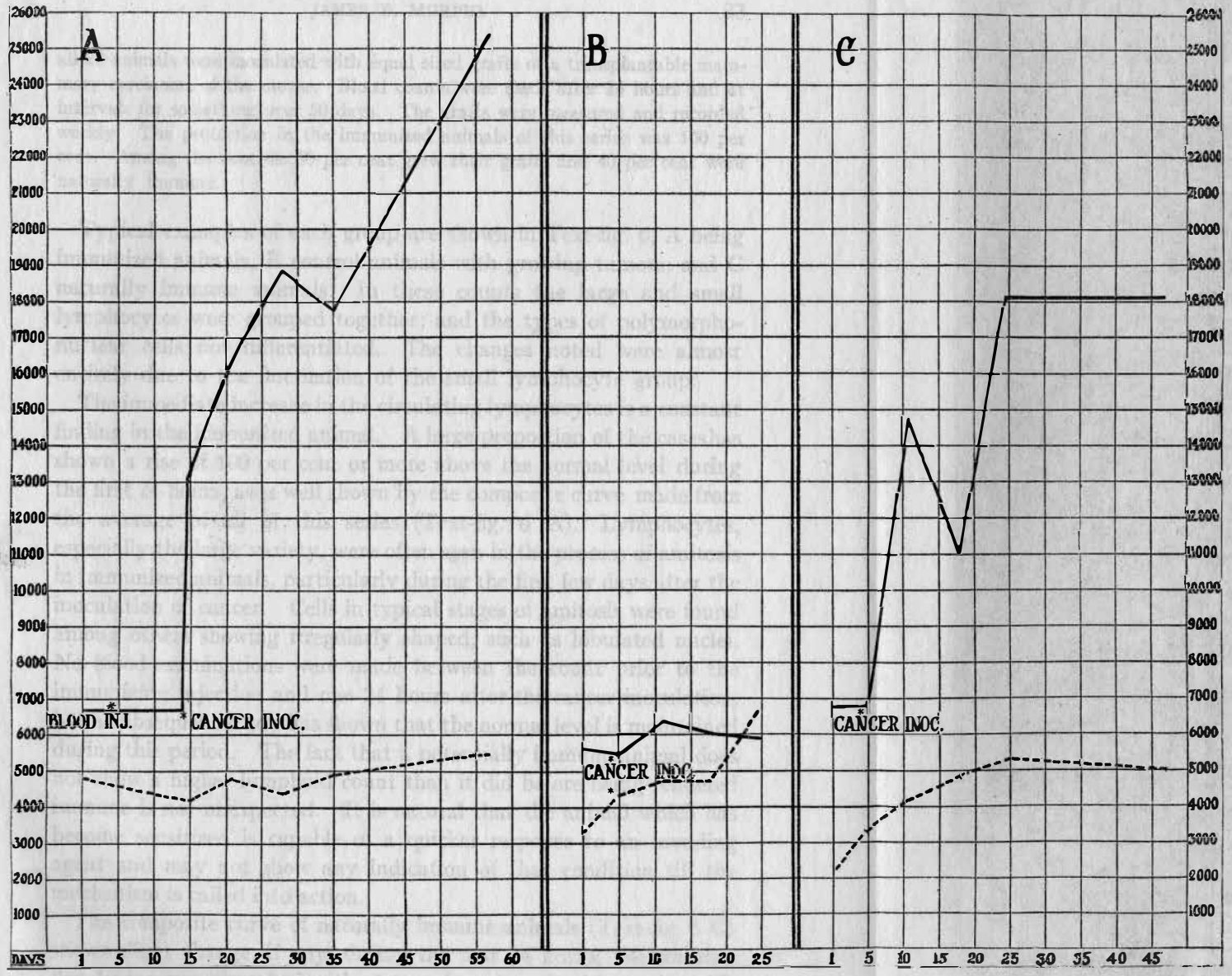
In the light of our studies of the fate of heteroplastic grafts and the probable participation of the lymphoid cell in the defensive mechanism, it was particularly suggestive that the series of events taking place about a tissue graft in a resistant host is the same whether this refractory state be due to species, variety, or individual variation (4). On this evidence we were led to make a study of the importance of the lymphoid type of cell in the resistance to transplanted tumor. The prominence of these cells in the local reaction has already been noted. If this local manifestation of resistance is significant, it seemed to us there should be a general reflection of the reaction in the blood and organs of the animal body.

CHANGES IN THE BLOOD ASSOCIATED WITH RESISTANCE TO TRANSPLANTED CANCER.

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 10 days before inoculating the cancer graft (5). Furthermore, a certain proportion of mice inoculated with cancer are naturally refractory, as indicated above. This natural resistance is sometimes so effective that the cancer graft does not become established but usually the introduced tumor grows in the resistant animal for a time, then retrogresses and is absorbed later. We have chosen these two types of immunity, the induced and the natural, for study from the point of view of the general lymphoid reaction.

In a series of experiments, studies have been made of the blood picture in a large number of mice before and at intervals after inoculation with cancer in both resistant and susceptible animals. As the different experiments gave similar results only one will be given in detail.

Experiment.—A white blood count and a differential count were made on 20 white mice of about the same age and size. 10 of the mice were then given a subcutaneous injection of 0.3 cc. of defibrinated mouse blood. 10 days later



TEXT-FIG. 6. 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.

all 20 animals were inoculated with equal sized grafts of a transplantable mammary carcinoma of the mouse. Blood counts 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.

Typical examples of each group are shown in Text-fig. 6, A being immunized animals, B control animals with growing tumors, and C naturally immune animals. In these counts the large and small lymphocytes were grouped together; and the types of polymorphonuclear cells not differentiated. The changes noted were almost entirely due to the fluctuation of the small lymphocyte group.

The immediate increase in the circulating lymphocytes is a constant finding in the immunized animal. A large proportion of the cases has shown a rise of 100 per cent or more above the normal level during the first 24 hours, as is well shown by the composite curve made from the average of all of this series (Text-fig. 6 A). Lymphocytes, especially the large variety, were often seen in the process of amitosis in immunized animals, particularly during the first few days after the inoculation of cancer. Cells in typical stages of amitosis were found among others showing irregularly shaped, such as lobulated nuclei. No blood examinations were made between the count prior to the immunizing injection and one 24 hours after the cancer inoculation, but a subsequent study has shown that the normal level is maintained during this period. The fact that a potentially immune animal does not show a higher lymphoid count than it did before being rendered immune is not unexpected. It is natural that the animal which has become sensitized is capable of a quicker response to an invading agent and may not show any indication of that condition till the mechanism is called into action.

The composite curve of naturally immune animals (Text-fig. 6 C) shows slight change, if any, during the first 24 hours, the reaction developing sometime during the 1st week and continuing to rise with some variation to a level between 100 and 200 per cent above normal. Some growth of the cancer takes place in these animals during the 1st week, after which the grafts retrogress to complete absorption.

In the susceptible animal there is a slight reaction at the end of the

1st week which, however, subsides as the tumor increases in size (Text-fig. 6 B). In one animal in the series there was a fairly marked reaction in the lymphocytes in the 2nd week, with a marked retardation in the growth of the tumor. The cancer later resumed its rapid growth and this was accompanied by a corresponding drop in the number of lymphocytes.

These experiments show that the natural as well as induced resistant state to transplanted cancer is accompanied by a marked lymphocytosis. This reaction is totally absent in the highly susceptible animals, while in the less resistant ones, in which the tumor growth is merely retarded, there is a slight but apparently inadequate reaction.

HISTOLOGICAL COMPARISON OF THE LYMPHOID TISSUE OF NATURALLY RESISTANT AND SUSCEPTIBLE MICE.

The demonstration of the striking change in the number of circulating lymphocytes during the development of cancer resistance naturally raises the question as to whether there is an accompanying reaction in the lymphoid organs.

Experiment.—In order to study histological changes in lymphoid organs, after cancer inoculation, young adult white mice were implanted with Bashford Adenocarcinoma No. 63 and were killed for tissues 3 weeks later. The tissues were fixed in Carnoy's 6-3-1 and stained with eosin methylene blue, Ehrlich's hematoxylin, and eosin, or Heidenhain's iron-hematoxylin. In all we have brought together for study the spleen and lymph nodes from 105 mice, of which 29 came from mice immune to the inoculated cancer, and the remaining 76 from animals with tumors.

The results of histological examination of the lymphoid organs of the naturally immune and susceptible mice showed no clear-cut distinction between these two groups, but there exists a complete series of intergradations in the changes. However, the two groups differ decidedly in the general tendency of the changes, which may be described as follows:

Spleen.—A spleen from a typical resistant animal shows a characteristic general histology. The hypertrophy of the Malpighian bodies is prominent (Fig. 19). The germ centers are also enlarged and contain a striking number of mitotic figures (Fig. 20), which may be taken as indicative of a hyperactivity in tissue proliferation. An

increased number of lymphoid cells are noted in the spleen pulp especially around the vessels, and even in these cells a considerable number of mitotic figures occur. There are comparatively few necrotic cells in the lymphoid tissue. The pulp spaces contain a very small amount of blood and the deposits of pigment are inconspicuous.

These findings in general agree with the observations of Mottram and Russ (6), who showed that the spleens of rats resistant to the Jensen sarcoma tended to show a higher lymphocytic content than the spleens of normal animals.

A different histological picture occurs in a typically susceptible animal (Fig. 21). The Malpighian bodies are small, mitotic figures rare, the large macrophages with ingested cell fragments are abundant in the germ centers, and some necrosis may be found in the periphery of the Malpighian body. The splenic pulp is sparingly supplied with the lymphoid tissue while hemosiderosis and hyperemia are pronounced (Fig. 22).

Lymph Nodes.—The changes in the several lymph nodes are entirely parallel to those found in the spleen, although not so striking. In typical examples of immune mice the lymphoid tissue, even in the medulla, contains many mitotic figures and scarcely any necrotic cells. A large number of lymphocytes filled up the pulp spaces (Fig. 23) suppressing the proliferation of the endothelial elements.

In contrast to these conditions typically susceptible animals show a striking inhibition of the lymphoid tissue proliferation, as may be judged from the scarcity of mitotic figures. The pulp spaces are occupied largely by endothelial cells mixed with only a few lymphocytes (Fig. 24). In several instances a large number of plasma cells appear in the lymph cords, portions of which being almost entirely made up of these cells. Also a few susceptible animals show a considerable number of polymorphonuclear leucocytes in the lymph cords and even in the cortex, and mast cells in the peripheral sinuses.

The descriptions above refer to the extreme cases. As might be expected, almost every gradation is encountered between the extremes, for it is well known that various degrees of resistance exist. In the highly resistant animals the tumor grafts are quickly destroyed, while in others only after a period of growth is the cancer overcome. The highly susceptible animals offer little or no inhibitory effect, and the

grafted tumor grows at a rate limited only by its growth energy and the ability of the host to supply stroma, while the less susceptible animals show evidences of a definite retarding effect on the rate of growth of the graft.

In order to show the degrees and variations in the points of difference between the histological appearance of the lymphoid tissue of the susceptible and the resistant animals, we have presented in Tables III and IV the results of the study of the individual mice. For the spleen we have included the following points: (a) the relative size of the nodules, (b) the amount of lymphoid tissue in the pulp, (c) the number of mitotic figures present, (d) the amount of necrosis of the cells, and (e) the amount of blood and pigment present. For the lymph nodes we have indicated (a) the number of mitotic figures, (b) the degree of necrosis, and (c) the number of lymphocytes and endothelial cells present in the pulp. The material from the individual animals was studied and the records were made without any knowledge of whether the tissues came from a susceptible or immune mouse. (Tables III, IV, and V follow.)

In Table V a summary is given of the percentage of the animals in the susceptible and resistant groups showing the various histological changes indicated above.

In the above study the difference in the nature of the changes in the lymphoid organs of immune and of susceptible mice deserves special consideration. While immune mice tend to show more or less marked indications of lymphoid hyperplasia, varying degrees of lymphoid depletion seem to be the general rule in susceptible mice. The extreme cases of the susceptible type are suggestive of the lymphoid destruction induced by a large dose of x-ray. These findings seem significant in connection with the results of the blood studies previously described.

LYMPHOID TISSUE IN ANIMALS WITH INDUCED RESISTANCE TO TRANSPLANTED CANCER.

The lymphoid organs in animals with induced immunity to cancer have been next studied. The material for the study has been collected from 96 mice which were immunized by a subcutaneous injection of 0.3 cc. of defibrinated mouse blood.

TABLE III.

Immune Mice.

Immune Mouse No.	Spleen.						Lymph nodes.			
	Size of nodules.	Lymphoid tissue in pulp.	Mitosis in lymphoid tissue.	Necrosis in lymphoid tissue.	Blood.	Pigment.	Mitosis in lymphoid tissue.	Necrosis in lymphoid tissue.	Lymphocytes in pulp spaces.	Endothelial cells in pulp spaces.
1	±*	+	±	−	±	±	−	−	±	±
2	−	±	−	−	±	+	±	−	±	−
3	±	−	±	−	−	±	−	±	+	±
4	+	+	+	−	−	±	−	−	±	±
5	−	±	−	±	+	−	−	±	+	±
6	+	+	+	±	−	−	±	±	±	+
7	±	+	−	±	±	±	±	−	+	±
8	+	+	+	±	−	−	−	−	±	±
9	+	+	±	−	±	±	+	±	±	±
10	+	±	±	−	±	−	±	−	±	−
11	+	+	+	−	−	±	±	−	±	−
12	+	+	±	±	−	±	±	−	±	−
13	+	+	−	−	±	−	+	−	+	−
14	+	+	±	+	±	−	+	−	+	±
15	+	+	±	±	±	±	+	−	±	−
16	+	±	+	±	±	−	+	±	+	−
17	±	±	±	+	±	±	±	−	±	+
18	±	±	±	±	+	±	−	±	−	±
19	+	±	±	+	±	±	±	−	±	±
20	+	±	±	−	−	±	±	±	+	±
21	+	+	+	±	±	−	±	−	±	±
22	+	±	±	±	±	±	±	±	±	+
23	+	±	+	±	±	±	+	−	+	±
24	±	±	±	±	±	±	±	±	+	±
25	+	±	±	−	−	±	±	±	+	±
26	+	±	+	±	±	−	±	±	+	±
27	+	±	±	±	−	−	±	−	+	−
28	±	±	±	±	±	−	±	±	+	±
29	+	±	−	±	−	−	±	−	±	±

*In the tables + indicates an increase above the normal; ±, approximately normal; −, a decrease below the normal.

TABLE IV.

Susceptible Mice.

Susceptible Mouse No.	Spleen.						Lymph nodes.			
	Size of nodules.	Lymphoid tissue in pulp.	Mitosis in lymphoid tissue.	Necrosis in lymphoid tissue.	Blood.	Pigment.	Mitosis in lymphoid tissue.	Necrosis in lymphoid tissue.	Lymphocytes in pulp spaces.	Endothelial cells in pulp spaces.
1	±	±	−	−	±	+	−	−	±	+
2	−	±	±	−	−	±	−	−	±	±
3	−	−	−	±	+	+	−	−	±	+
4	−	±	−	±	±	±	−	−	±	±
5	±	−	−	±	+	+	−	±	−	±
6	−	−	−	−	±	+	−	±	±	−
7	−	±	−	−	±	±	−	−	−	±
8	−	−	±	−	−	−	−	−	+	±
9	−	±	±	±	−	±	−	+	±	±
10	−	−	±	±	−	±	−	+	−	−
11	−	±	−	±	±	±	−	+	±	−
12	±	+	±	−	±	−	±	+	±	±
13	+	+	+	±	±	−	±	−	±	±
14	−	−	−	±	+	−	−	+	±	+
15	±	−	−	±	+	±	−	±	−	±
16	−	±	−	±	−	+	−	±	+	±
17	±	±	±	±	−	−	−	+	±	+
18	±	±	±	±	−	−	−	+	+	±
19	−	−	−	+	+	±	−	+	±	+
20	−	−	−	+	+	−	−	±	−	+
21	−	±	−	+	±	−	±	±	−	+
22	±	±	−	±	±	±	−	−	±	±
23	±	±	±	±	−	±	−	±	−	±
24	±	−	−	±	+	±	±	±	±	±
25	−	±	−	±	±	±	−	±	±	±
26	−	−	−	+	+	−	−	−	±	+
27	−	±	±	+	±	−	±	−	±	±
28	±	+	±	±	±	±	±	+	±	+
29	−	±	−	+	±	±	−	±	−	+
30	−	−	−	+	+	±	±	+	±	+
31	−	−	−	±	±	±	−	±	−	+
32	−	−	−	−	±	±	−	±	±	±
33	±	−	−	±	+	−	±	+	−	±
34	±	−	±	+	+	±	±	−	−	+
35	−	±	−	+	+	±	−	+	±	+
36	−	±	−	+	+	±	±	±	−	+
37	±	−	−	+	+	±	±	±	±	+

TABLE IV—*Concluded.*

Susceptible Mouse No.	Spleen.						Lymph nodes.			
	Size of nodules.	Lymphoid tissue in pulp.	Mitosis in lymphoid tissue.	Necrosis in lymphoid tissue.	Blood.	Pigment.	Mitosis in lymphoid tissue.	Necrosis in lymphoid tissue.	Lymphocytes in pulp spaces.	Endothelial cells in pulp spaces.
38	+	−	+	±	+	±	±	+	−	+
39	−	−	−	+	+	±	±	−	±	±
40	±	−	−	±	+	±	−	+	−	±
41	+	±	±	±	−	±	−	±	+	±
42	+	±	±	+	+	±	±	±	−	+
43	±	±	±	+	±	−	−	+	−	+
44	±	±	±	+	+	±	−	±	−	+
45	−	±	−	±	+	±	±	±	±	+
46	±	±	−	+	±	±	−	+	±	+
47	±	±	±	+	−	±	−	±	+	±
48	±	−	−	+	±	±	−	±	±	+
49	−	−	−	+	+	±	−	±	+	+
50	−	−	−	±	+	+	−	−	±	±
51	±	−	−	+	+	±	±	±	±	+
52	±	±	−	+	±	±	−	±	+	±
53	±	−	±	±	±	±	−	±	−	±
54	±	−	−	±	+	+	−	±	±	+
55	±	−	−	+	+	±	−	−	−	±
56	±	±	−	±	+	±	−	±	±	±
57	−	+	−	+	−	−	−	±	±	±
58	±	−	±	±	+	±	±	±	−	±
59	±	−	−	+	+	±	−	±	−	+
60	−	−	−	+	+	+	±	±	±	±
61	±	−	−	+	±	±	±	±	±	+
62	−	±	−	+	±	±	±	±	−	+
63	±	−	±	−	±	±	±	−	−	+
64	−	±	−	+	±	+	±	±	−	+
65	+	−	+	±	±	±	±	±	±	+
66	±	−	−	+	±	±	−	+	−	±
67	±	−	±	±	±	±	±	+	+	±
68	±	±	±	±	−	±	−	±	±	+
69	−	−	−	±	±	±	±	±	−	+
70	±	−	−	±	−	−	±	−	±	+
71	±	−	−	±	−	±	−	±	±	±
72	±	−	±	±	±	±	−	±	−	±
73	−	−	−	+	+	±	−	+	−	±
74	−	−	−	±	+	±	−	±	−	+
75	±	−	−	±	+	+	−	+	−	±
76	−	±	±	±	−	±	±	−	±	±

TABLE V.

Percentage of Immune Mice and Susceptible Mice.

Organ.	Points of difference.		Immune mice.	Susceptible mice.
			per cent	*per cent*
Spleen.	Size of nodules.	+	69	7
		±	24	47
		−	7	46
	Lymphoid tissue in pulp.	+	41	5
		±	55	39
		−	4	56
	Mitosis in lymphoid tissue.	+	28	4
		±	55	30
		−	17	66
	Necrosis in lymphoid tissue.	+	10	39
		±	55	50
		−	35	11
	Blood.	+	7	42
		±	58	38
		−	35	20
	Pigment.	+	4	13
		±	55	69
		−	41	18
Lymph nodes.	Mitosis in lymphoid tissue.	+	21	0
		±	58	36
		−	21	64
	Necrosis in lymphoid tissue.	+	0	26
		±	42	51
		−	58	23
	Lymphocytes in pulp spaces.	+	45	11
		±	52	50
		−	3	39
	Endothelial cells in pulp spaces.	+	10	47
		±	62	49
		−	28	4

Material.

38 mice were killed in groups, 24, 48 hours, 4, 6, 8, and 10 days after the immunizing injection. The remaining 58 mice were inoculated, 10 days after the blood injection, with bits of Bashford Adenocarcinoma No. 63, and were killed in groups, 24 hours, 4, 5, 15, 25, and 35 days after the inoculation. All the mice were of about the same size and were from the same stock. The virulence of the tumors used in each experiment was tested by inoculation into a number of normal mice. The tissues were fixed in Carnoy's 6–3–1, and sections stained with Heidenhain's iron-hematoxylin or eosin methylene blue.

Much variation has been noted in the extent of the reaction in different animals, due largely to the fact that a certain percentage of the immunized mice will grow tumors actively. What appear to be typical changes were as follows:

Spleen.—The stimulation of germinal centers, evidenced by a large number of mitotic figures, was manifest 48 hours after the blood injection. A few large cells with pycnotic and fragmented nuclei were among the cells of the germ center and the usual number of pigmented cells and megalocaryocytes were found in the pulp spaces. Mitosis in the germ center after 4 days was apparently more active than before, while after 5 days the number of mitotic figures was found to be somewhat decreased. By the 10th day the proliferative activity of the germ centers had almost returned to the normal level but even at this period a few mitotic figures were to be seen.

At 24 to 48 hours after the cancer inoculation numerous mitotic figures appeared in germ centers accompanied by a general enlargement of the splenic nodules. This stimulation was far more extensive than that occurring in the previous period. The enhanced cell division continued for about 1 week following the cancer inoculation, the normal rate being gradually resumed after this period. Toward the later period pycnotic cells increased in the nodules. About 25 days after the inoculation the general condition of the organ was approximately normal.

Lymph Glands.—Similar changes were observed in the mesenteric and inguinal lymph glands. A distinct acceleration of mitosis was observed 24 hours after the immunizing injection, dividing cells being numerous in the germ centers of the nodules and not infrequent even in the lymph cord. At 48 hours the number of mitotic figures

was more or less decreased, though considerable variation among individual mice was noted. Slight stimulation was still present as late as 10 days after the treatment. No other appreciable change was observed.

The activity of the germ center became decidedly increased soon after the cancer inoculation, and numerous mitotic figures appeared. This condition, in a less marked degree, lasted for a considerable length of time. Cell division subsided to the normal rate about 35 days after the inoculation.

Other Organs.—The thymus and thyroid glands, the liver, kidney, and bone marrow showed no significant changes. In a few instances, however, there was an increase in the number of mitotic figures in the cells of the thymus, and mitosis was often observed among the perivascular lymphoid cells of the liver.

Subcutaneous Connective Tissue.—With the hope of checking and possibly extending the previous work of Da Fano, changes in the subcutaneous connective tissue have also been investigated. For this purpose, loose connective tissue from the subcutaneous layer was carefully spread over the slide, fixed with absolute alcohol, and stained with methylene blue and eosin. 24 hours after the injection of the blood a well marked cellular reaction appeared about the groups of red cells. Cells participating in this reaction were mostly small lymphocytes, with some polymorphonuclear leucocytes and macrophages. After 4 days the injected blood became more generally distributed in the subcutaneous tissue and correspondingly the local reaction became more diffuse. At this period while the lymphocyte persisted as the dominant cell, many plasma cells and macrophages appeared in the reaction. The injected red blood cells were found scattered through a very extensive area of the subcutaneous tissue and were everywhere associated with an infiltration of lymphocytes, plasma cells, and macrophages. No cellular reactions were found in the loose connective tissue at a distance from the area infiltrated with red blood cells. Furthermore, mice immunized by an intraperitoneal injection showed no change in the subcutaneous tissue.

Da Fano states that the plasma cells, which are absent in normal connective tissue of the mouse, appear 48 hours after the immunizing injection of the blood, becoming more numerous in the succeeding

days until the 4th day, when small groups of the cells are seen in every section. It is highly probable that Da Fano has misinterpreted the local reaction about the mass of injected blood cells as a general reaction in immunity.

SUMMARY AND DISCUSSION.

The conclusions suggested by the results just reported, regarding the rôle of the lymphocytes in resistance to transplanted cancer are not only in harmony with the previous observations on the factor of resistance to heteroplastic tissue grafting and on cellular reactions about the cancer graft in animals with natural or induced resistance but have also sustained subsequent experimental tests as well. Not only do the circulating lymphocytes increase markedly in a resistant animal after inoculation with cancer, but there is a corresponding enhancement of the rate of cell division in the lymphoid centers of spleen and lymph nodes. While animals with induced immunity show no change in the lymphocyte count they yet show a hyperactivity of the lymphogenic centers. The mechanism of the lymphoid reaction has become, as it were, sensitized so that a very small amount of tumor is sufficient to induce a relatively large blood lymphoid response.

From the study of the response of the animal to the immunizing injection of homologous tissue, it seems probable that the lymphoid cells of an untreated animal are sufficient in quantity and quality to be an efficient defense mechanism against normal cells with no or little proliferative ability but as a result of this injection the capacity of the lymphoid tissue to react has become so enhanced that it is capable of a massive reaction when the cancer is inoculated.

The irregularity in the results of the study of the lymphoid organs of animals with natural and induced resistance were to be expected. It is well known that a proportion of mice immunized to cancer show no more resistance than normal mice, while in still others the resistance to cancer growth is at first not evident, the mechanism asserting itself sufficiently to overcome the cancer only after a period of growth has occurred. Unfortunately at the time of the greatest changes in the spleen and lymph nodes it is impossible to predict which way the animals would have arranged themselves according to this grouping. To give an example, 10 mice are inoculated with cancer 10 days after

receiving an immunizing injection of homologous tissue. If permitted to live, judging by the average experiment with our strain of tumor, the following would result: about 2 would develop progressive tumors, 2 more would show a temporary growth followed by retrogression, and the remaining 6 would have been immune from the beginning. If all of these animals are killed during the first few days after inoculation we have no way of comparing the extent of the reaction in the spleen with the degree of resistance the animal might have shown if allowed to live. Therefore, we should expect, if the changes in the spleen and lymph nodes are an index to the resistance, only a proportion of the mice to show a marked reaction and from 10 to 20 per cent delayed reaction or none at all. These figures correspond approximately with the reported findings.

In the natural resistance another type of limitation was encountered. The resistant animals could not be determined with any degree of certainty till about the 3rd week after inoculation. It seems probable from our other studies that the more resistant animals react promptly, and with the tumor material eliminated the reaction subsides at an earlier date than with the less resistant ones. Even with these limitations the study has yielded unmistakable evidence of a grouping according to the condition of the lymphoid tissue which agrees with the susceptibility or resistance of the animals. From the blood study where the fate of the tumor could be determined the degree of resistance followed fairly closely the extent of the lymphocytosis.

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EXPLANATION OF PLATES.

PLATE 10.

FIG. 19. General histological appearance of the spleen of a mouse naturally immune to transplanted tumor. A low power view.

FIG. 20. Germ center of the spleen of an immune mouse, showing numerous mitotic figures (M).

PLATE 11.

FIG. 21. General histological appearance of the spleen of a mouse susceptible to transplanted tumor. A low power view.

FIG. 22. Deposits of pigment in the spleen of a susceptible mouse.

PLATE 12.

FIG. 23. Lymph node of a naturally immune mouse, showing abundant lymphocytes in the pulp spaces and a few mitotic figures in the lymph cords (M).

FIG. 24. Lymph node of a susceptible mouse, showing the proliferation of endothelial cells in the pulp spaces and pycnotic cells in the lymph cords.

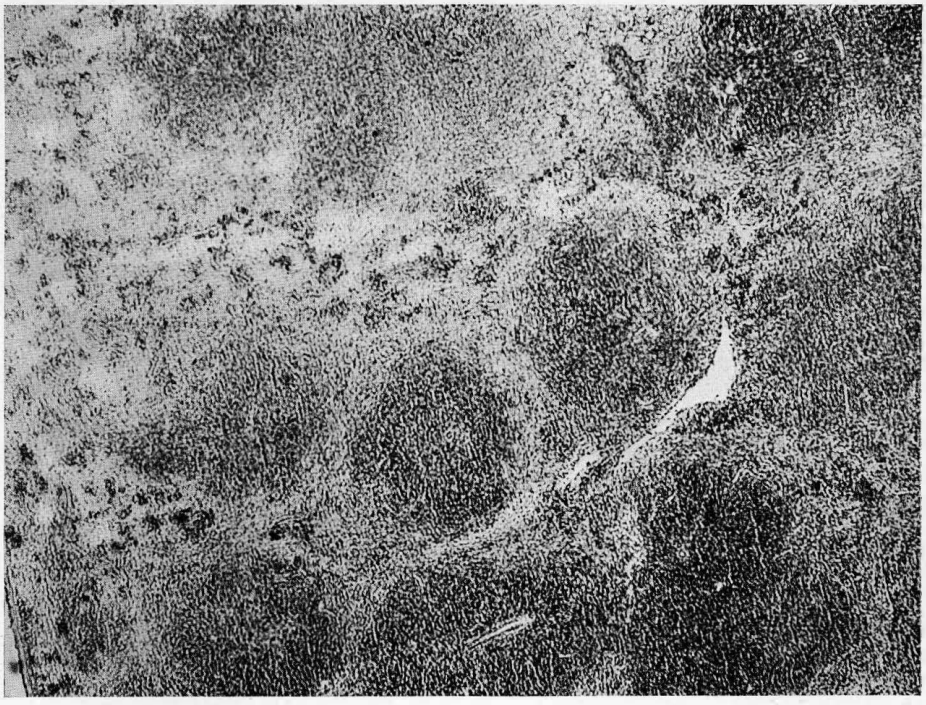


FIG. 19.

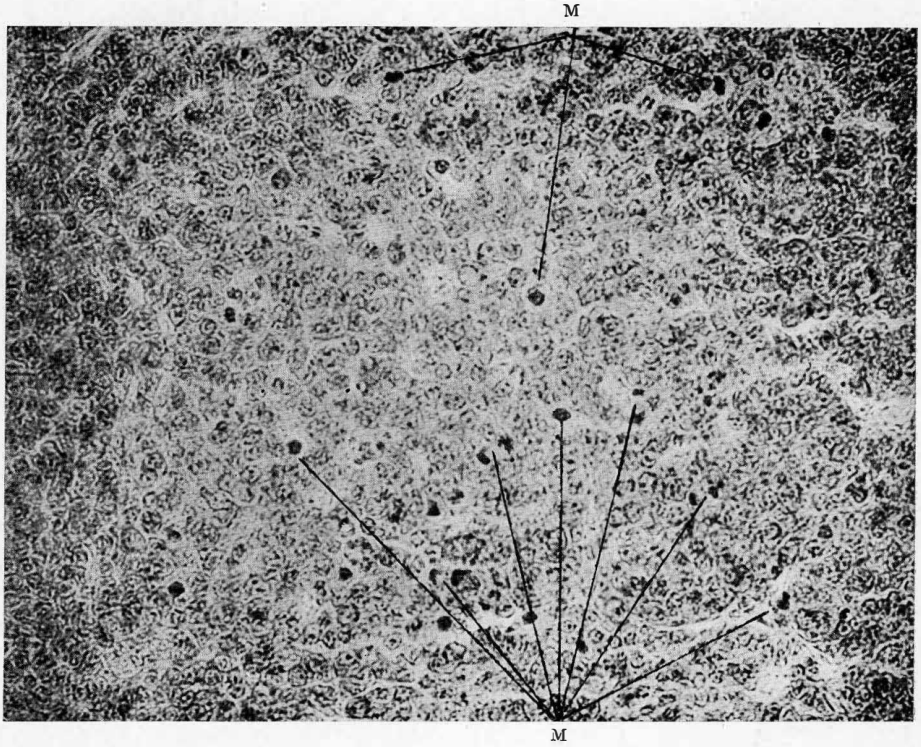


FIG. 20.

(Murphy: Resistance to inoculated tumor.)

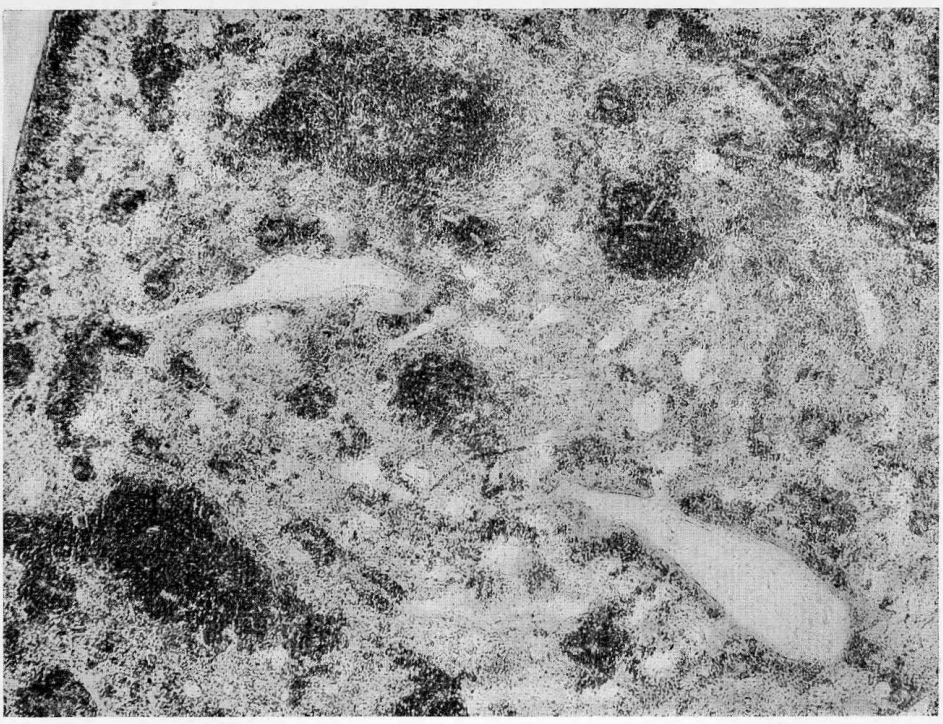


FIG. 21.

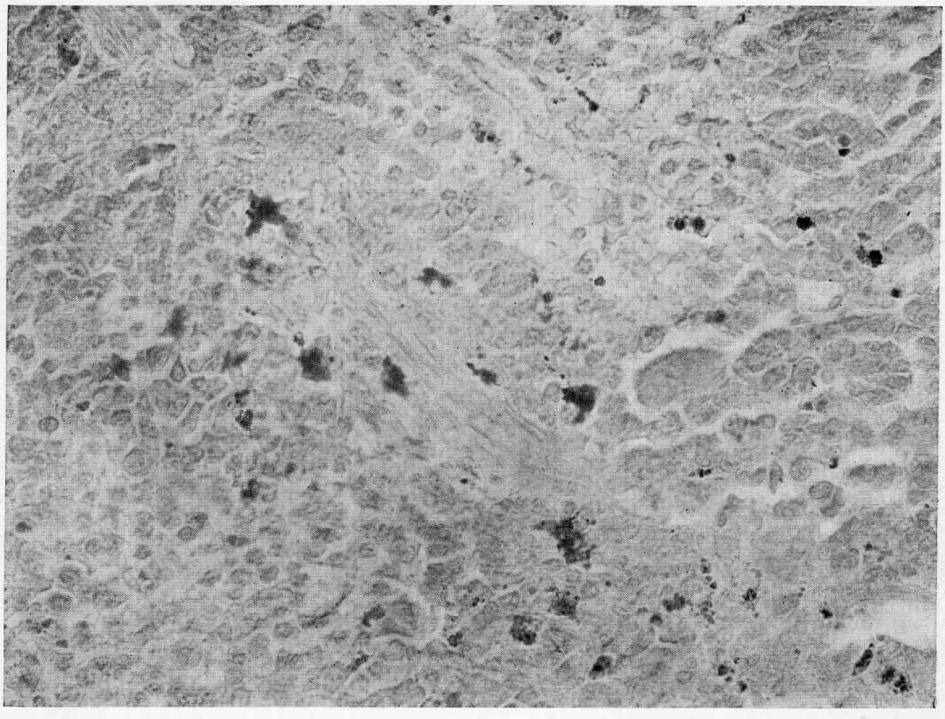


FIG. 22.

(Murphy: Resistance to inoculated tumor.)

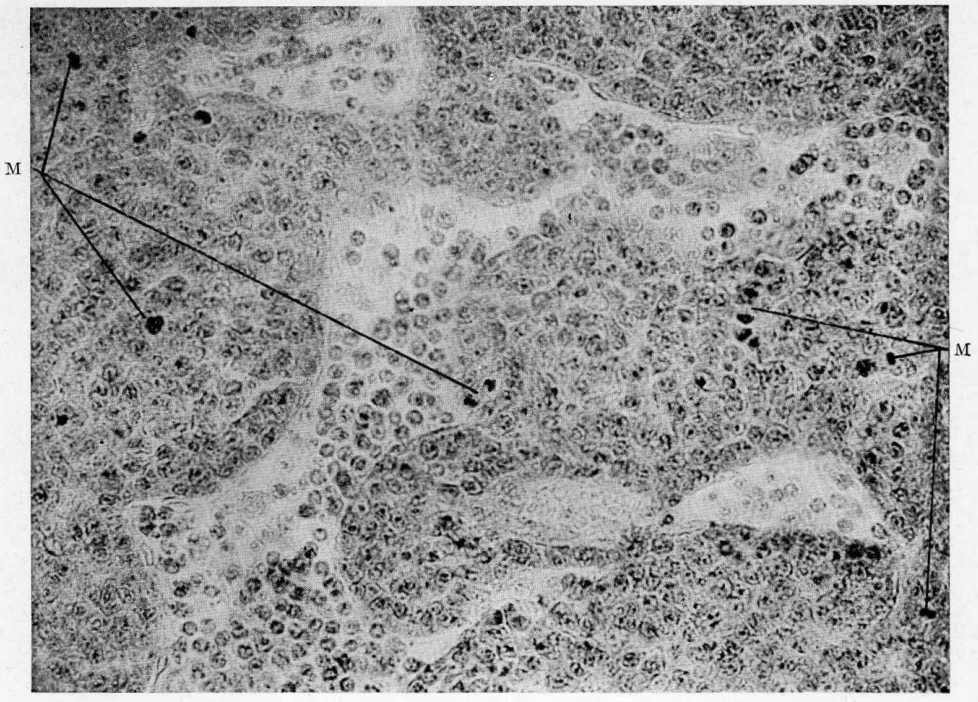


FIG. 23.

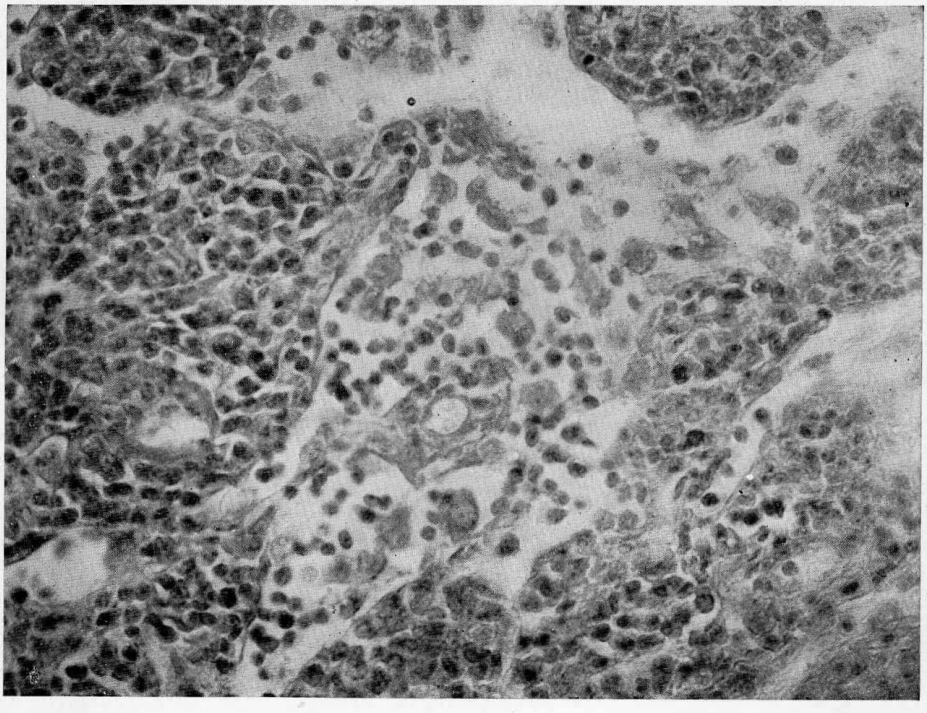


FIG. 24.

(Murphy: Resistance to inoculated tumor.)

III. EFFECT OF DESTRUCTION OR SUPPRESSION OF THE LYMPHOID TISSUE ACTIVITY ON RESISTANCE TO TRANSPLANTED CANCER.

So far as the histological evidence is concerned there are strong indications that the lymphoid cell is implicated in the mechanism of resistance to transplanted cancer. Would this mechanism of defense be effective in the absence of the lymphocytes? In other words, is the round cell reaction an accompanying phenomenon and the result of the resistance or is it an essential factor in the process? In answer to this question methods were sought whereby the lymphoid cell may be prevented from taking part in the general response. Two such methods and their effect on the resistance mechanism are described in the following pages.

DESTRUCTIVE ACTION OF X-RAY ON THE LYMPHOID TISSUE.

Heineke (1) first called attention to the fact that large doses of x-ray administered to an animal affected primarily the lymphoid system. Degeneration of the follicles of the spleen and lymph nodes and a diminution in the number of circulating lymphocytes were the prominent early changes observed. Warthin (2) in an extension of Heineke's work noted that the Malpighian bodies were first affected and later the lymph nodes and bone marrow were involved in the degenerative process. Helber and Linser (3) made a more exhaustive study of the action on the blood and demonstrated that the blood stream could be almost depleted of the lymphoid type of cell by a long continued exposure of an animal to x-ray.

The dosage used by all of these investigators was of such magnitude as to cause extensive changes in skin, kidneys, bone marrow, and other organs and almost invariably resulted in the death of the animals. These results showed the extreme sensitiveness of the lymphoid tissue to x-ray and suggested the possibility of utilizing this agent for our purpose.

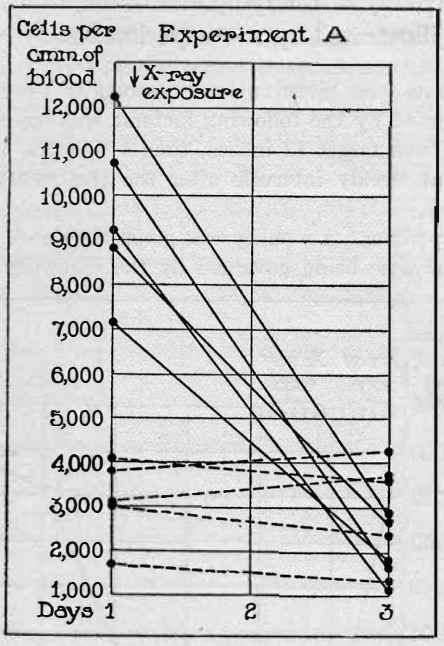
The nature of our problem required that a marked depletion of the

lymphoid elements be secured without any considerable disturbance of any important organs, particularly of the bone marrow. Furthermore, it was of prime importance that the general nutrition and well being of the animals should not be interfered with, for it is well known that the transplantable tumors grow slowly or not at all in sickly animals. It was soon found that this result could not be induced with a single exposure to x-ray, for if the dose was so small as not to injure the other tissues, the depression of the lymphoid tissue was not of sufficient duration to answer the requirements of the experiment. The system finally evolved was to administer small daily exposures till the circulating lymphocytes were reduced to a low level without any very marked reduction in the numbers of polymorphonuclear cells. Large groups of animals treated in this manner were kept under close observation over long periods in order to detect any signs of late deleterious effects. A careful histological study was made of the tissues beginning immediately after the treatments and at intervals over an extended period. The observations were made on rabbits, guinea pigs, cats, monkeys, rats, mice, and a small horse. As the general results were much the same, only the data from a few typical experiments will be reported.

Differential Action on Lymphoid Cells.—The following experiments selected from a large number are quoted to show how markedly the circulating lymphocytes may be depressed with little or no effect on the polymorphonuclear leucocytes.

Experiment A.—5 normal adult rats were simultaneously exposed for the same length of time to a single unfiltered dose of x-ray. Total and differential white blood counts were made on each animal immediately before and 48 hours after exposure. The average for the 5 animals showed that the lymphocytes decreased from 9,660 cells per c.mm. of blood before x-ray, to 2,020 cells 48 hours afterward, while polymorphonuclears remained practically stationary (3,150 before x-ray and 3,080 after). The results in the individual animals are shown in Text-fig. 7.

Experiment B.—3 adult white rats were exposed to unfiltered x-rays generated by a Coolidge tube, the dose being governed by the following factors: spark-gap 3 inches, milliamperes 10, distance from target to skin 12 inches, and time 4 minutes. This dose was repeated daily for 5 days, giving a total of 30 Holzknecht units. The total and differential white cell counts before and 48 hours after the last x-ray exposure showed that the lymphocytes decreased on an



TEXT-FIG. 7.

average from 8,870 cells before x-ray, to 2,055 cells per c.mm. afterward. The polymorphonuclear cells increased from 2,627 to 3,879 during the same period.

The results of several experiments have been brought together in the following table.

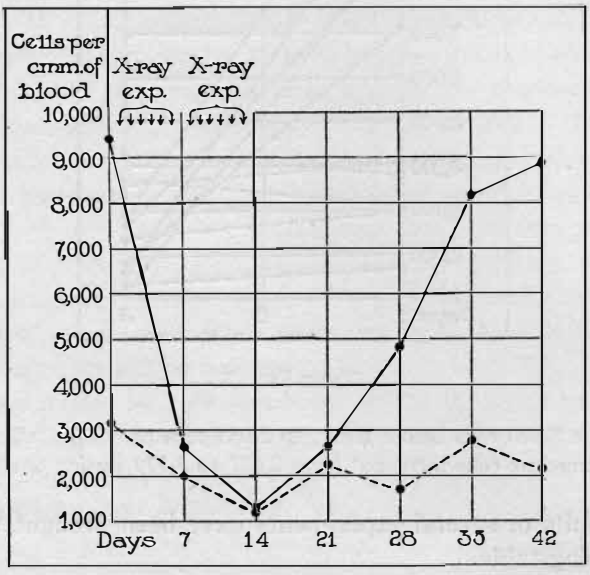
TABLE VI.

Animals.	Average No. of lymphocytes.		Average No. of polynuclears.	
	Before x-ray.	After x-ray.	Before x-ray.	After x-ray.
	per c. mm.	*per c. mm.*	*per c. mm.*	*per c. mm.*
5 rats.	9,660	2,020	3,150	3,080
3 "	8,870	2,055	2,627	3,879
10 "	9,495	2,803	3,180	2,003
5 mice.	10,200	2,840		
4 "	7,428	2,749	4,454	4,695
4 monkeys.	13,290	1,879	9,379	14,882

The rate of recovery of the lymphoid tissue after destructive doses of x-ray may be illustrated by two experiments.

In the first, 10 rats were given twelve exposures to x-ray at daily intervals. The dose was governed by the following factors: spark-gap 3 inches, milliamperes 10, distance from target 12 inches, time 4 minutes. Blood counts were made before and at weekly intervals after the treatments. The results are shown in Text-fig. 8.

In the second experiment, a monkey was given a series of seven exposures to unfiltered x-ray, the dose being governed by the following factors: spark-gap

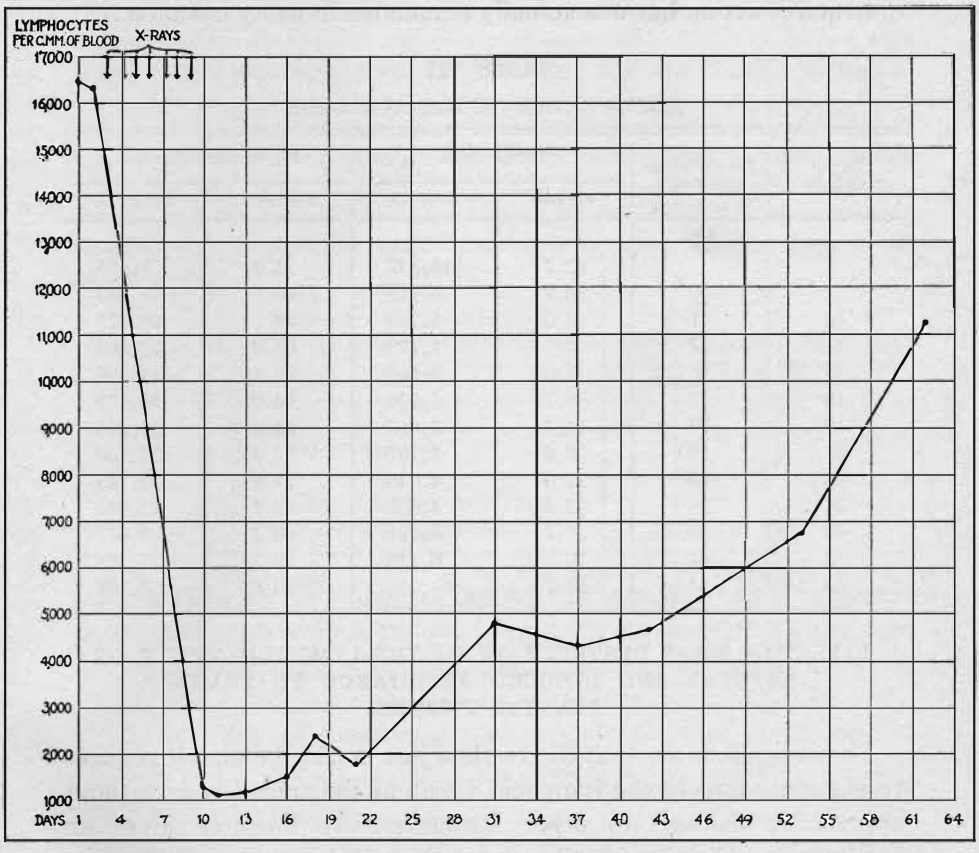


TEXT-FIG. 8.

3 inches, milliamperes 10, distance from target 12 inches, time 4 minutes. The doses at daily intervals were given alternately to the dorsal and ventral surface of the body. The behavior of the circulating lymphocytes is shown in Text-fig. 9 and Table VII. The polymorphonuclear leucocytes increased from 9,495 cells per c.mm. before exposure to 30,375 after, and did not return to the normal level till some 30 days later.

In general, the reduction in the number of circulating lymphocytes reaches its lowest level about 48 hours after the last x-ray treatment. Following this fall there is a more or less slow recovery which requires a considerable period before it regains the normal level. In some

instances the lymphocytes had not regained their original numbers even 54 days after the x-ray treatment. The foregoing experiments further demonstrate that this extraordinary depletion may be brought about without any material reduction of the polymorphonuclear cells. As a matter of fact these cells were more often increased than decreased



TEXT-FIG. 9. The effect of x-ray treatment on the circulating lymphocytes of a *Macacus rhesus*.

by the x-ray treatment. This would suggest that no damage had been done to the bone marrow.

Histological Changes.—The more minute histological changes in the lymphoid organs following x-ray need not be gone into here for they have been adequately described by other workers. The changes

induced by the broken dose system are identical in this respect to those produced by a single long exposure. A careful study of animals treated by our method has failed to show any early or late deleterious effects in any of the organs with the exception of some damage to the sex glands. The bone marrow not only showed no signs of the destructive action but was actually stimulated in many instances.

TABLE VII.

Monkey Receiving 42 Holzknecht Units.

Day of experiment.	Length of time after x-rays.	Lymphocytes.		Polymorphonuclear cells.	
		Per cent.	Total No.	Per cent.	Total No.
	days				
1		62.7	16,537	36.0	9,495
2		56.7	16,415	40.7	11,783
10	1	4.0	1,283	94.7	30,375
11	2	4.7	1,128	95.0	22,800
13	4	7.0	1,155	92.7	15,296
16	7	5.7	1,526	94.0	25,169
18	9	11.7	2,463	88.0	18,524
21	12	7.0	1,787	92.3	23,560
28	19	27.0	4,846	71.0	12,745
37	28	23.3	4,351	74.7	13,950
42	33	31.7	4,771	64.3	9,677
53	44	29.0	6,779	66.7	15,598
63	54	55.7	11,224	40.7	8,201

EFFECT OF X-RAY DESTRUCTION OF THE LYMPHOID TISSUE ON NATURAL AND INDUCED RESISTANCE TO TRANSPLANTED TUMORS.

The indications are that the method just outlined makes it possible to eliminate largely the lymphoid tissue of the animal body without appreciable damage to other structures. It therefore gives an opportunity of testing whether or not the lymphocyte is as important in the resistance mechanism as the histological studies indicated.

Natural Resistance.—For these and all subsequent experiments it has been found necessary to regulate the dose of x-ray with extreme care as too large or too frequent doses will cause such a general disturbance in the metabolism that the inoculated tumors will grow slowly or not at all. Likewise, too small an amount of x-ray will only

partially bring about the desired result. The factors controlling the dose when published are of little value except as indicating in general the approximate dosage and the degree of hardness of the rays. With the change of the type of x-ray generator it has invariably been necessary to restandardize the dosage system.

Experiments.—In order to make the test more rigid, strains of tumors were selected which were giving a very low percentage of successful grafts on inoculation. The animals were prepared by a series of daily exposures to small doses of x-ray. As a rule rats were given twelve treatments and mice from seven to ten. Following the last exposure the animals together with an equal number of normals were inoculated with tumor grafts. Rats were grafted with the Jensen sarcoma and the Flexner-Jobling carcinoma and the mice with a transplantable mammary carcinoma. The results of some of the experiments are given in the following table.

TABLE VIII.

Animals.	Tumor.	Takes in x-rayed animals.	Takes in normal animals.
		per cent	*per cent*
Rats.	Jensen sarcoma.	100	22
"	" "	100	37
"	Flexner-Jobling carcinoma.	100	25
Mice.	Mammary carcinoma.	77	44

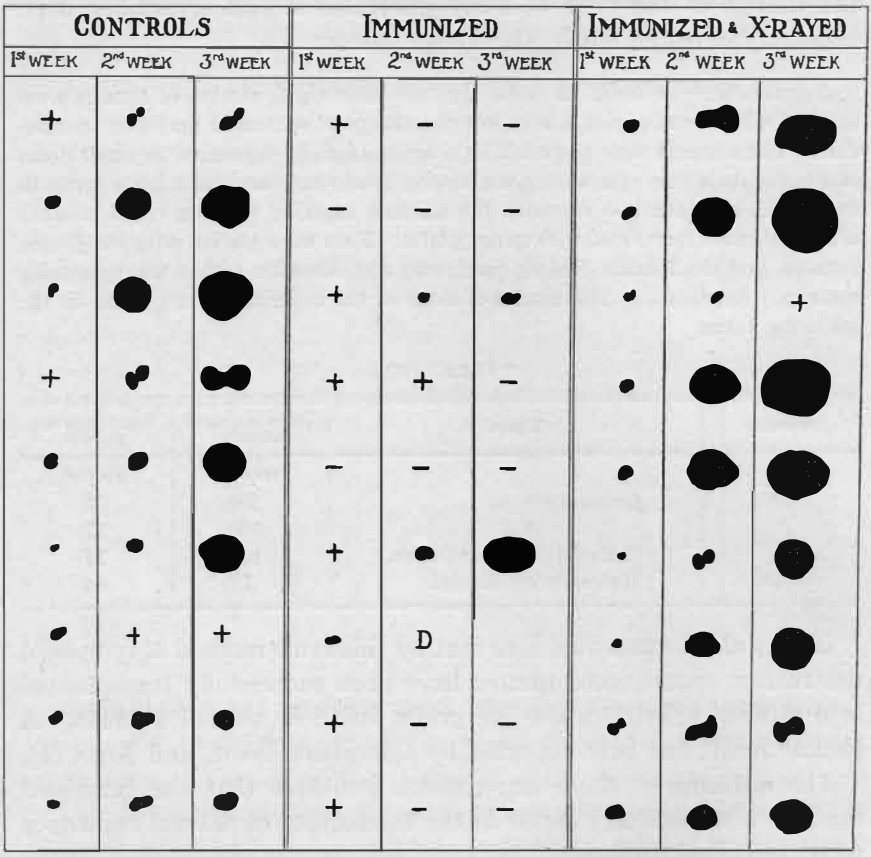
It may also be recorded here that by this same method of lymphoid destruction spontaneous tumors have been successfully transplanted into x-rayed animals when like grafts failed in normal animals. A similar result has been reported by Chambers, Scott, and Russ (4).

The outcome of these experiments indicates that the lymphoid reaction is a necessary factor in the mechanism of natural resistance to transplanted tumors.

ACTION OF X-RAY ON INDUCED POTENTIAL RESISTANCE TO TRANSPLANTED TUMORS.

Mice injected subcutaneously with homologous living tissue cells, as has already been pointed out, become highly resistant to inoculated tumors. The maximum effectiveness of this reaction is about 10 days after the injection. While the circulating lymphocytes show no increase during the interval between the immunizing injection and the

cancer inoculation, histological study shows a distinct increased activity on the part of the lymphogenic centers. Furthermore, this system has gained in mobility as shown by the fact that it responds



TEXT-FIG. 10. This chart shows the effect of x-ray on induced resistance. 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.

to a cancer inoculation by the outpouring of enormous numbers of cells into the circulation. This process may be looked on as a more active form of resistance than that encountered in the natural re-

fractiveness where the reaction occurs more slowly and at a later period.

Prevention of the Development of Resistant State.

The next point to be investigated was the action of x-ray lymphoid destruction on the development of immunity following homologous tissue injections. For this purpose the broken doses of x-ray were applied during the period between the blood injection and the inoculation with cancer. A typical experiment, one of four giving uniform results, will be quoted here.

Experiment.—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. 10 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.

EFFECT OF DESTRUCTION OF THE LYMPHOID TISSUE ON ESTABLISHED RESISTANCE TO TRANSPLANTED CANCER.

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. There is a possibility that while the lymphocyte might be a potent factor in bringing about the potential immunity, after the resistance becomes established by implantation of one of the transplantable tumors, this cell might no longer play a part in the maintenance of the immunity. This point was next investigated.

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 had shown to be adequate to destroy the major portion of the lymphoid tissue without appearing to affect the general health of the animal.

Eight experiments have been carried out with consistent results. The reinoculation of 147 mice immune to one inoculation of tumor

TABLE IX.

Experiment No.	Takes in.		
	Reinoculated immunized mice.	Reinoculated x-rayed immunized mice.	Control mice.
	per cent	*per cent*	*per cent*
1	21.1	52.4	90.9
2	12.0	64.0	94.4
3	10.5	73.3	90.0
4	33.3	50.0	66.6
5	29.4	50.0	40.0
6	38.8	85.7	60.0
7	14.2	71.3	71.3
8	37.5 20.0	80.0 57.1	80.0

resulted in takes in from 12 to 38 per cent. Of the 108 mice of tested resistance, tumors developed in from 50 to 80 per cent when x-rayed before the second inoculation. The takes in the 84 controls ranged from 40 to 94 per cent. The results of the individual experiments are given in Table IX.

The above experiments answer definitely the question as towhether or not the mechanism of defense against the transplantable tumor would be effective in the absence of the lymphocyte. In the light of the reported results, apparently the lymphocyte is a necessary factor in this phenomenon.

SUPPRESSIVE EFFECT OF OLIVE OIL ON THE LYMPHOID TISSUE AND ON INDUCED RESISTANCE TO INOCULATED CANCER (NAKAHARA).

In the course of some studies on the biological action of certain oils and fatty acids (see page 85), it was observed that when as much as 0.7 cc. of olive oil was injected intraperitoneally in mice a distinct suppression of the proliferative activity of the lymphoid organs resulted. Animals receiving this injection failed to show the usual cellular increase in the peritoneal fluid which follows a smaller injection. The germ centers of the spleen and lymph nodes were very small and mitotic figures in the lymphogenic tissue were practically absent. In the following experiments we have tested the influence of this suppression on the development of resistance to transplanted cancer.

TABLE X.

Experiment No.	Takes in.		
	Immunized mice.	Immunized and oil-injected mice.	Control mice.
	per cent	*per cent*	*per cent*
1	22.2	71.5	100.0
2	10.0	50.0	70.0
3	0.0	35.3	44.4
4	25.0	65.0	
Average.....	13.9 (36 mice.)	53.2 (62 mice.)	71.4 (28 mice.)

Experiments.—A group of mice were immunized by the subcutaneous injection of 0.2 cc. of defibrinated mouse blood. 10 days later half of these received an intraperitoneal injection of 0.7 cc. of olive oil and immediately afterwards were inoculated with the Bashford adenocarcinoma. The other half of the immunized mice which had received no olive oil and a suitable number of normal mice were inoculated with the same tumor. The results of four such experiments are shown in Table X.

In a second group of experiments the duration of the suppressive effect was tested. The details of the experiments were the same as those above except that the tumor was not inoculated till the 17th day after the blood injection, which was 7 days after the adminis-

tration of the olive oil. The outcome of the individual experiments is shown in Table XI.

In a third series of experiments, the details were the same as the first except that the olive oil was injected 24 hours after the cancer inoculation. Table XII shows the results of two such experiments.

It may be concluded from these three groups of experiments that olive oil, in the dosage used, modifies the potential resistant state

TABLE XI.

Experiment No.	Takes in.		
	Immunized mice.	Immunized and oil-injected mice.	Control mice.
	per cent	*per cent*	*per cent*
5	33.3	54.5	66.6
6	40.0	70.0	75.0
7	30.0	55.5	70.0
Average.....	34.5 (29 mice.)	60.0 (30 mice.)	70.3 (27 mice.)

TABLE XII.

Experiment No.	Takes in.		
	Immunized mice.	Immunized and oil-injected mice.	Control mice.
	per cent	*per cent*	*per cent*
8	10.0	10.0	70.0
9	30.0	20.0	70.0
Average.....	20.0	15.0	70.0

which develops after an homologous tissue injection but is incapable of influencing the resistance mechanism after it has been activated. In other words, the oil is only effective in interfering with the immune state if given before the cancer inoculation and is without activity when injected after the cancer inoculation.

RESULTS OF OLIVE OIL INJECTION ON THE CELLULAR REACTIONS ACCOMPANYING RESISTANCE.

The three characteristic cellular manifestations accompanying the state of resistance to transplanted cancer as shown in the previous pages are: (1) local lymphocytic infiltration around the cancer graft; (2) hyperactivity of the lymphogenic centers; and (3) blood lymphocytosis. The influence of the olive oil injection on these cellular reactions was next studied.

TABLE XIII.

Lymphocyte Counts on Series A (Immunized but Not Oil-Injected).

Mouse No.	1 day before blood injection.	3 days after cancer inoculation.	10 days after cancer inoculation.	Outcome of cancer inoculation.
1	9,534	10,455	7,613	+
2	12,364	11,181	9,858	+
Average for susceptible mice.....	10,949	10,813	8,735	
3	9,106	16,862	14,703	—
4	6,528	19,759	12,483	—
5	8,827	9,445	14,468	—
6	11,496	16,855	12,695	—
7	10,024	13,344	10,719	—
8	6,287	12,854	15,411	—
Average for immune mice.....	8,711	14,853	13,413	
Average for all mice...	9,273	13,843	12,368	75.0 per cent immune.

Material.—Mice were immunized by a subcutaneous injection of mouse blood, and 10 days later were first given 0.7 cc. of olive oil intraperitoneally and then inoculated with a transplantable tumor. For controls, immunized mice were inoculated with tumor but no olive oil was given. These animals were killed in groups at 24, 48 hours, 3, 4, and 5 days after the inoculation, and a histological examination was made of the graft and lymphoid organs.

Local Reaction.—After the initial reaction to the trauma of inoculation the tissue surrounding the cancer grafts, in the oil-injected series, showed slight evidence of cellular reaction. With few exceptions

little lymphoid reaction appeared surrounding the cancer graft and connective tissue proliferation was slight. In the later stages the stroma became more abundant, the graft received a copious vascular supply but no more than a slight lymphocytic infiltration appeared.

TABLE XIV.

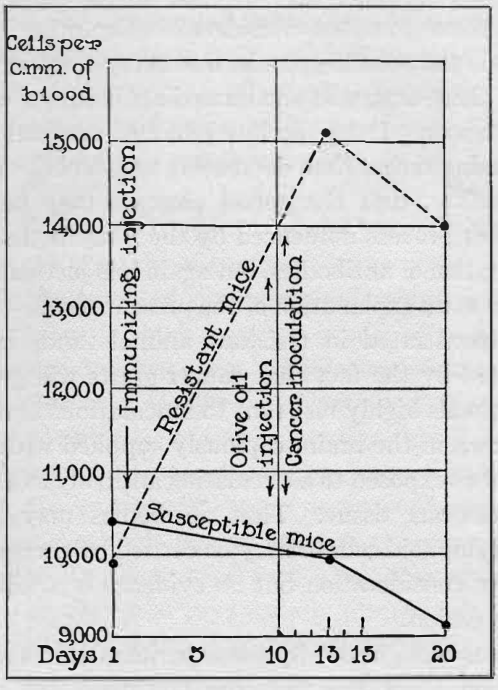
Lymphocyte Counts on Series B (Immunized and Oil-Injected).

Mouse No.	1 day before blood injection.	3 days after cancer inoculation.	10 days after cancer inoculation.	Outcome of cancer inoculation.
1	10,473	8,894	8,675	+
2	6,578	7,249	6,555	+
3	10,381	10,903	10,087	+
4	12,276	8,711	8,648	+
5	7,741	7,613	7,076	+
6	10,332	10,451	8,627	+
7	9,778	11,198	9,936	+
8	11,838	13,239	12,282	+
9	10,331	6,986	9,104	+
10	6,980	9,615	9,679	+
11	8,659	6,673	6,128	+
12	13,848	10,539	11,833	+
13	17,042	17,153	10,685	+
Average for susceptible mice.....	10,481	9,940	9,178	
14	12,211	21,386	18,195	—
15	13,868	15,733	18,316	—
16	11,026	15,377	16,243	—
17	5,660	13,210	12,506	—
18	7,694	14,358	11,334	—
19	13,442	16,896	12,921	—
20	5,752	8,890	8,812	—
Average for immune mice.....	9,951	15,121	14,046	
Average for all mice...	10,296	11,753	10,882	35 per cent immune.

The cancer grafts from the series which received no olive oil showed the typical local reaction in cancer immunity, namely a dense infiltration of the tissues surrounding the graft, made up primarily of lymphocytes with plasma cells and fibroblasts.

Lymphoid Organs.—The difference between the two groups here was not so pronounced. The germ centers were less active in the animals which had received oil, than in those which had not.

Blood Lymphocytosis.—The mice were immunized and 10 days later injected with oil and then inoculated with cancer. White blood cell counts were made before the immunizing injection, and 3 and 10 days



TEXT-FIG. 11.

after cancer inoculation. The same procedure was carried out on a group of mice with the oil injection omitted for control. The analysis of the blood counts in the resistant and susceptible mice is shown in Tables XIII and XIV and in Text-fig. 11.

SUMMARY AND DISCUSSION.

The repeated exposure to small doses of x-ray as far as can be determined has practically a selective destructive action on the lymphoid

tissue. The regeneration following such a depletion is sufficiently slow to answer the experimental requirements. The fact that animals treated by this system will support a growing tumor at least as well as an untreated animal is assurance that the general metabolism has not been seriously interfered with, for it is known that sickly or poorly nourished animals are poor hosts for the transplantable tumors.

That other factors may vary with the amount and reactibility of the lymphoid tissue must be considered. The other tissue affected most by x-ray is that of the gonads, but many tests have shown that disturbance of these organs has no immediate influence on the animal's resistance to cancer. Even the late effect of removal of the gonads is one of increasing rather than decreasing resistance. It is more than probable, therefore, that the gonad changes may be ignored as a factor. Another process influenced by the x-ray is that having to do with the elaboration of antibodies, but again it is justifiable to eliminate this occurrence as an explanation of the present result. No antibodies have been demonstrated in resistant animals and their absence is further indicated by the fact that cancer grafts will grow actively in the brain of animals highly resistant to inoculations in other locations. The tumor grown in the brain, copiously supplied with blood vessels, is certainly just as exposed to a circulating antibody as a tumor growing in the subcutaneous tissue. That antibodies may be elaborated locally by the lymphoid cells or may be carried by these cells is a possibility worthy of consideration but no evidence is available either for or against this notion.

From the facts brought out by the experiments, it appears that the only explanation tenable is that the lymphoid cell is a necessary factor in the resistance mechanism. Destruction of these cells by x-ray or suppression of their reactibility by olive oil results in the practical annulment of resistance to the transplanted tumors.

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IV. EFFECT OF STIMULATION OF THE LYMPHOID TISSUE ON RESISTANCE TO TRANSPLANTED CANCER.

PLATES 13 TO 17.

That resistance to transplanted cancer can be overcome by agents which destroy or depress the activity of the lymphoid tissue seems to be established by the experiments reported in the preceding pages. Our next investigation had to do with the effects of stimulation of the lymphoid tissue on the resistance phenomena.

STIMULATIVE ACTION OF X-RAY ON THE LYMPHOID TISSUE.

In the course of the study of the destructive action of x-ray it was noted that while the repeated small dose did extensive damage to the lymphoid tissue, a single small exposure produced a slight amount of destruction followed quickly by a stimulative effect on this tissue. This observation led to a careful investigation of the effect of small doses of x-ray on the blood count and the principal organs of the body. As the earlier observations indicated that soft x-ray was more effective than hard rays the subsequent study was confined to the former. In order to obtain these soft rays in greatest concentration we used a special tube of the Coolidge[1] type with a window of very thin glass which allowed the passage of rays usually filtered out by the thicker glass of the standard tube.

Experiment.—The special tube described was operated with a spark-gap ½ inch, 11 milliamperes, and 6 inches distance from the animals. In preliminary observations mice were exposed in groups for ½, 1, 2½, 4, 5, 10, 20, 30, or 60 minutes to this dose, filtered through a thin sheet of cardboard to protect the animals from the heat of the tube. Blood counts made on all of these animals before treatment and a week after showed that the 1 minute treatment gave a definite lymphocytosis.

This dose was given a more extensive test by the exposure of 35 mice. Lymphocyte counts were made before and at intervals for 18 days after the x-ray ex-

[1] We wish to express our indebtedness to Dr. Coolidge and to the General Electric Company for designing and constructing this tube.

TABLE XV.

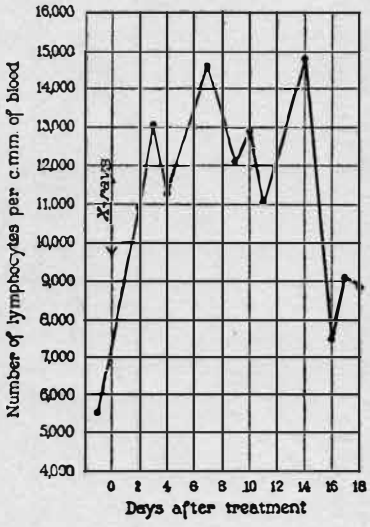
Mouse No.	Count before treatment.	Count after treatment.										
		No. of days after x-ray treatment.										
		2	3	4	7	9	10	11	14	16	17	18
1	6,018				15,908				13,250			
2	6,541				11,105				17,713			8,430
3	5,897				14,455							
4	5,899				16,210				14,446			
5	5,526				15,463				14,744			
6	5,823	11,041				12,252				7,091		
7	4,617	7,658										
8	4,336	12,070				16,532				11,645		
9	6,585	17,070				10,603				8,455		
10	4,239	10,972				13,650				9,423		
11	4,288	7,604				11,766				5,471		
12	4,998	14,734				10,567				5,816		
13	7,104	7,070				12,786				7,005		
14	5,477	7,406				10,472				6,808		
15	5,185	12,878				10,332				6,520		
16	6,110		16,122				12,800				8,888	
17	6,113		12,775				16,861				7,183	
18	4,215		9,435				12,890				6,008	
19	4,909		12,002				7,338				5,360	
20	4,790		7,007				9,495				9,231	
21	3,714		12,064				9,304				8,232	
22	4,145		10,989				12,543				9,382	
23	4,425		16,261				15,814				13,209	

24	6,203		18,252				15,681				12,124	
25	7,155		15,352				17,055				12,836	
26	3,594			7,527				9,303				
27	9,313			7,027				8,975				9,897
28	4,428			8,815				7,196				6,675
29	5,264			3,767				7,986				6,878
30	4,404			12,452				12,870				10,178
31	3,974			15,300				15,082				
32	8,330			13,167				10,277				8,571
33	7,329			16,480				17,274				14,796
34	6,175			16,787				12,894				9,893
35	5,635			11,851				8,518				5,092
Average.....	5,507	10,850	13,026	11,317	14,628	12,105	12,978	11,037	15,038	7,581	9,245	8,934

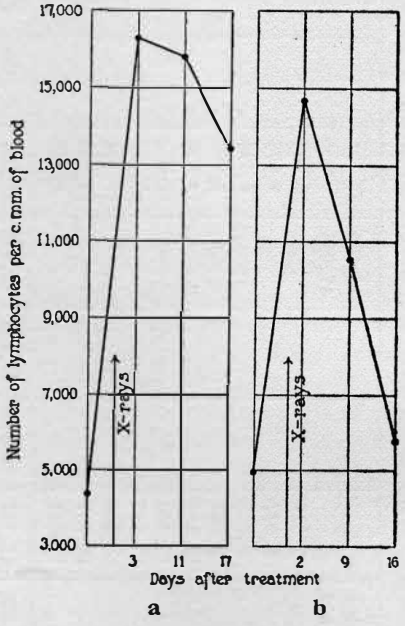
The counts before treatment were made a number of days in advance so that no animal had more than one count in a week. For example, on the group of mice counted 2 days after treatment, the pretreatment count was made 5 days before, thus allowing a week between counts.

posure. A composite curve of all of the 35 animals is shown in Text-fig. 12 and the curves of 2 typical individual mice in Text-fig. 13. The counts on the individual animals are recorded in Table XV.

The counts showed that no definite alteration in the absolute number of polymorphonuclear leucocytes resulted but the lymphocytes increased considerably above the initial count. Generally speaking



TEXT-FIG. 12.



TEXT-FIG. 13.

TEXT-FIG. 12. Composite curve of lymphocyte counts on 35 mice before and after an exposure to low frequency x-rays.

TEXT-FIG. 13, *a* and *b*. (*a*) Curve of lymphocyte counts on Mouse 23 in Table XV. (*b*) Curve of lymphocyte counts on Mouse 12 in the same table.

these latter cells had almost doubled their number by the 2nd day and continued to increase with some variations until the 14th day, after which they subsided but were still above their initial level on the 18th day. Considerable variation in the extent and duration of the reaction in individual mice was observed but in only two animals did a drop in the lymphocytes occur at the time of the first count after the x-ray treatment. One of these, however, showed a reaction later

with an increase of over 30 per cent, but in the other no stimulation was observed. The particular animals used to illustrate this experiment do not show the initial depression preceding the stimulation as the counts were not made at an early enough period but this point has been definitely established by other experiments.

HISTOLOGICAL CHANGES IN THE LYMPHOID ORGANS AFTER SMALL X-RAY EXPOSURE.

The duration of the lymphocytosis and the time at which it occurs following the x-ray treatment suggest that it represents a true stimulation in the actual production of these cells. The following study was designed to substantiate this deduction.

Material.—The mice used in the experiments were carried as a parallel series to the one used for the blood studies. The groups of mice were given a stimulative dose of x-ray as determined by the preceding experiment (special tube, spark-gap ½ inch, milliamperes 11, distance 6 inches, time 1 minute). The mice for material were killed at 24 hours, 4, 7, 10, and 14 days after the treatment. The spleen, lymph nodes, bone marrow, thymus, thyroid, liver, kidneys, suprarenal, ovary, and testes were fixed in Carnoy's 6–3–1 solution and the sections stained with Heidenhain's iron-hematoxylin for mitotic figures or with Ehrlich's acid hematoxylin and eosin. The material was analyzed mainly from the standpoint of the presence of mitotic figures.

Lymphoid Organs.—The changes in the spleen and lymph nodes were practically identical and will be described together. At 24 hours after treatment there was no demonstrable change, but by the 4th day very numerous mitotic figures were to be found in all of the lymphoid organs. This condition was equally as pronounced in the animals killed on the 7th day. By the 10th to the 14th day the activity had diminished to about the normal rate.

Suprarenal Glands.—At autopsy of the treated mice the suprarenals were found to be reddish in color and somewhat enlarged. Histological examination developed the fact that the sinus-like spaces between the cortex and medulla were much distended with blood, this dilatation extending to the capillaries between the cortical cell columns, which were separated by a wide margin. This vascular change was observed in varying degrees in all of the mice examined except those killed 24 hours after the treatment. The sinusoids of

the medulla showed little if any modification. There was no sign of hemorrhage or of necrosis of the suprarenal tissue in any of the specimens examined (Figs. 25 and 26).

Other Organs.—There was no histological evidence of changes in the other organs examined after the x-ray treatment. Even the germ cells of the ovaries and testes, well known to be extremely sensitive, showed no detectable damage. In a few instances the interstitial tissue of the testis was found to be hypertrophied. Distinct perivascular lymphoid infiltration was observed in a number of the livers and kidneys examined, but these findings were not uniform enough to be of great importance. This condition is also found occasionally in normal animals.

Experiments with Standard X-Ray Tubes.—As in a number of the tests to be described later, the standard Coolidge x-ray tube was used, and a report will be given showing that stimulation may be produced as well by this tube as with the special one.

Experiments.—A group of mice were given an exposure to x-ray generated by the Coolidge tube, the factors governing the dose being as follows: spark-gap $\frac{7}{8}$ inch between points, milliamperes 25, distance from target to back of animal 8 inches. The time of exposure was 20, 10, and 5 minutes respectively for the three groups studied. These will be designated as A, B, and C and described separately.

Group A (20 minute exposure). At 24 hours after the x-ray treatment, the lymphoid tissue was found to contain numerous degenerated cells with pycnotic or fragmented nuclei. The Malpighian bodies of the spleen were small and inconspicuous. A moderate number of mitotic figures were distributed irregularly in the spleen but were almost totally absent in the lymph nodes.

Three days after treatment there was less evidence of the injured cells, the Malpighian bodies were more evident and showed a few mitotic figures. From this period on the lymphoid organs regained their normal appearance and showed no evidence of an increase in the mitotic figures.

Group B. For these mice the same factors for controlling the dose of x-ray were used except that the duration of exposure was 10 minutes.

A definite increase in the number of mitotic figures in follicles of the spleen was seen at 24 hours and at 4 days after the treatment (Fig. 27). The estimated number of these figures in each nodule of the spleen was from five to six, a number which is in striking contrast to the normal condition of the organ. The 24 hour specimens showed an appreciable number of pycnotic cells in the pulp, but this manifestation was less than in the first series.

The lymph nodes showed an equally pronounced increase in mitotic figures during the corresponding period with these figures occurring frequently in the medulla of the gland (Fig. 28).

At 8 days after the x-ray treatment the spleen and lymph nodes as regards the number of division figures were normal in appearance but the sections gave the impression that the lymphoid elements were more abundant than normally seen.

Group C. Here the dosage used was the same as that of the two previous groups except the time of exposure was 5 minutes.

The histological examination of the animals after this treatment showed no definite increase in the number of mitotic figures in the lymphoid tissue. The evidences of cell damage which were so marked in the first series, and much less so in the second one, were not present at all in this series of animals.

It may be concluded from this study that stimulation of the lymphoid tissue may be induced by a small dose of x-ray generated by the standard tube. Furthermore, there seems to exist a definite relationship between the extent of cellular destruction and the degree of stimulation following x-radiation. A longer exposure with fairly pronounced destruction results in slight if any stimulation, a less exposure causing no destruction results in no stimulation, but with a slight destruction, marked stimulation occurs.

Extensive studies with other animals, principally rats and rabbits, have shown that a similar stimulation may be induced but as these animals were not used in the subsequent experiments, the details of these observations will not be given. The only variation worthy of note is the fact that in rabbits the stimulation occurs with no evidence of cell destruction which suggests that if our study of mice had been continued to include intermediate doses to those used, a dose might have been determined which would give a primary stimulation.

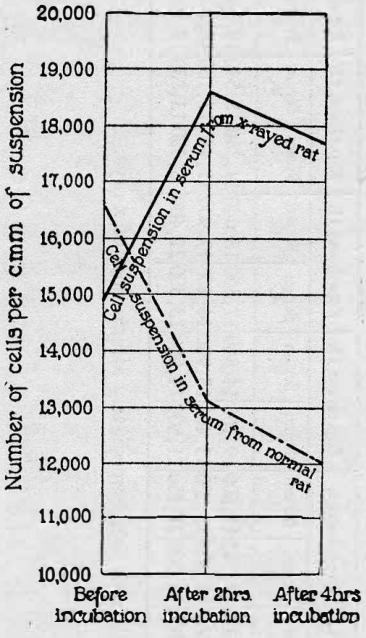
TABLE XVI.

Time.	No. of cells in suspension.													
	Experiment 1.		Experiment 2.		Experiment 3.		Experiment 4.		Experiment 5.		Experiment 6.		Experiment 7.	
	Serum A.	Serum B.	Serum A.	Serum B.	Serum A.	Serum B.	Serum A.	Serum B.	Serum A.	Serum B.	Serum A.	Serum B.	Serum A.	Serum B.
Before incubation.....	18,840	18,250	15,280	17,260	13,900	15,000	23,700	14,500	20,200	12,160	8,180	9,925	16,920	15,500
After 2 hrs. incubation.....	17,340	24,880	15,400	23,550	13,460	20,460	13,080	17,250	17,500	16,000	6,500	11,890	12,200	17,500
" 4 " "	16,960	27,600	13,190	21,100	13,920	18,750	10,080	15,500	16,900	12,500	5,700	9,590	13,000	19,150
" 24 " "	10,050	24,670												

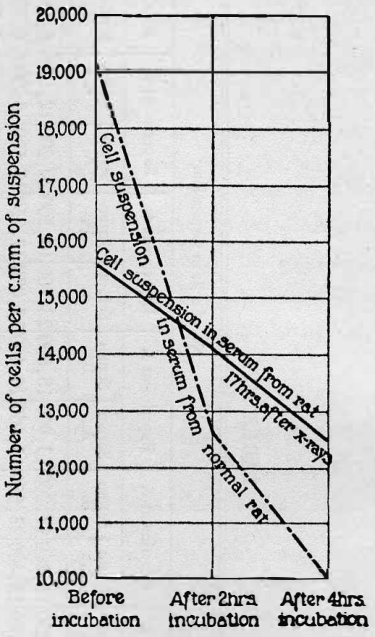
Time.	No. of cells in suspension.													
	Experiment 8.		Experiment 9.		Experiment 10.		Experiment 11.		Experiment 12.		Experiment 13.		Experiment 14.	
	Serum A.	Serum B.	Serum A.	Serum B.	Serum A.	Serum B.	Serum A.	Serum B.	Serum A.	Serum B.	Serum A.	Serum B.	Serum A.	Serum B.
Before incubation.....	19,500	18,500	17,240	17,600	19,100	14,800	17,500	15,040	14,880	15,540	19,040	11,820	9,850	13,800
After 2 hrs. incubation.....	17,100	21,300	13,000	23,200	13,400	15,900	14,450	19,900	11,440	17,160	13,500	15,640	6,300	15,760
" 4 " "	13,240	24,700	15,000	21,550	11,000	14,000	13,300	21,400	8,350	13,830	12,200	13,520	5,300	14,850

Serum A is from normal rats, Serum B from rats immediately after a dose of x-rays.

The average of fourteen experiments (Text-fig. 14 and Table XVI) shows that the cells suspended in normal serum decreased by over 3,000 during the first 2 hours and by another thousand by the end of the 4 hour period. The cells in the serum from x-rayed animals increased by over 3,000 cells in the first 2 hours and showed only a slight drop between the 2 and 4 hour periods. At the end of the period of ob-



TEXT-FIG. 14.



TEXT-FIG. 15.

TEXT-FIG. 14. Graphic representation of the average of Experiments 1 to 14.
TEXT-FIG. 15. Graphic representation of the average of Experiments 15 to 17.

servation the counts showed the suspensions still had some 3,000 cells more per c.mm. than the original suspension.

Examination of a large number of stained films made from these suspensions at the 2 hour period showed among the cells suspended in serum from x-rayed animals a fairly large number of mitotic figures. The average was a little less than one mitosis to a thin film, and occasionally three or more were found in a film (Fig. 29). In only one instance was a dividing cell found in the normal serum suspension.

The amount of disintegration of the cells, judged by the number of degenerated forms found in the smears, is just as rapid in the serum from x-rayed animals as in that from normal animals. Apparently, therefore, the proliferation of the cells in contact with serum from x-rayed animals is sufficient to replace not only the disintegrated cells but also actually to increase the total number. A large number of films prepared from the suspensions before incubation failed to show any mitotic figures, thus ruling out the question of the dividing cells being carried over from the glands in any appreciable numbers.

Further points developed were that the stimulative quality of the serum of x-rayed rats was lost by 17 hours after the treatment (Text-fig. 15, Table XVII), but even here the rate of disintegration was

TABLE XVII.

Time.	No. of cells in suspension.					
	Experiment 15.		Experiment 16.		Experiment 17.	
	Serum A.	Serum B.	Serum A.	Serum B.	Serum A.	Serum B.
Before incubation.....	23,700	15,500	14,880	17,480	19,040	14,100
After 2 hrs. incubation.....	14,080	14,350	11,440	15,240	13,500	12,700
" 4 " "	10,080	14,750	8,350	13,250	12,200	12,500

Serum A is from normal rats, Serum B from rats 17 hours after a dose of x-rays.

retarded as compared with the cells in normal serum. Blood or serum x-rayed *in vitro* proved to be devoid of stimulative effect, as was also the serum from rats given a very large dose of x-ray. This study was confined to rats for the reason that the lymphoid cells were resistant while those of rabbits and guinea pigs were too fragile to withstand the necessary manipulation.

EFFECT OF X-RAY STIMULATION OF THE LYMPHOID SYSTEM ON RESISTANCE TO TRANSPLANTED CANCER.

We have shown that the small dose of soft x-ray induced a definite stimulation of the lymphoid system, this stimulation manifesting itself both by an increased number of lymphocytes in the circulation and an increased activity of the lymphogenic centers. The only other

detectable change resulting from the treatment was a hyperemia of the suprarenals. The association of this condition and the relation if any to the following results must remain for the present an open question.

Experiment.—Young adult white mice were exposed to the following dose of x-rays (the standard Coolidge tube): spark-gap ⅛ inch, milliamperes 25, distance from target to back of mouse in the ordinary attitude 8 inches, and time of exposure 10 minutes. From 3 to 7 days afterwards the mice, together with a suitable number of controls, were inoculated subcutaneously in the left groin with a bit of a transplantable cancer (Bashford Adenocarcinoma No. 63). The result of seven such experiments are given in Table XVIII.

TABLE XVIII.

Experiment No.	Interval between exposure to x-rays and tumor inoculation.	Immunity in x-rayed animals.	Immunity in controls.
	days		
1	3	40.0 per cent (10 mice).	11.1 per cent (9 mice).
2	5	50.0 " " (16 ").	10.0 " " (20 ").
3	5	20.0 " " (10 ").	0.0 " " (10 ").
4	7	75.0 " " (8 ").	23.3 " " (9 ").
5	7	10.0 " " (10 ").	0.0 " " (10 ").
6	7	30.0 " " (10 ").	0.0 " " (10 ").
7	7	71.4 " " (21 ").	40.0 " " (10 ").
Total or average.....		45.9 " " (85 ").	11.5 " " (78 ").

During the course of these experiments the virulence of the tumor used varied considerably, and when the controls showed no resistance the immunity was correspondingly low among the x-rayed animals. With the exception of one group the resistant mice were at least 30 per cent higher in the x-rayed groups than in the controls. In the one exception noted the difference was only 10 per cent but even here the tumors developed much later and grew at a slower rate in the x-rayed group than in the controls. This variation in the results is not surprising when the variability of output of the average x-ray generator is considered.

The above tests have been repeated using the special tube described

above, which emits a larger proportion of the soft rays than the standard tubes. The dose used was governed by the following factors: spark-gap $\frac{1}{2}$ inch, milliamperes 11, distance 6 inches, and time 1 minute. The results of cancer inoculation in mice treated in this manner, contrasted with the fate of the tumor in normal mice, is shown in Table XIX.

The evidence seems conclusive that the small dose of x-ray greatly increases the resistance of mice to inoculated tumors. From these two series of experiments, of the 157 mice x-rayed, 42.6 per cent proved to be resistant to the tumor inoculation, while of the 128 untreated mice inoculated with the same tumor, only 9.3 per cent were resistant. While the proportion of resistant animals is not as high following the

TABLE XIX.

Experiment No.	Days between x-ray and tumor inoculation.	Resistance in x-rayed mice.	Resistance in normal mice.
1	7	30.0 per cent (10 mice).	11.1 per cent (9 mice).
2	7	29.4 " " (17 ").	9.9 " " (11 ").
3	10	37.5 " " (8 ").	0.0 " " (10 ").
4	10	33.3 " " (18 ").	0.0 " " (10 ").
5	10	57.8 " " (19 ").	10.0 " " (10 ").
Total or average.....		38.8 " " (72 ").	6.0 " " (50 ").

x-ray treatment as it is after the injection of homologous living tissue, yet in the twelve experiments listed above, and more might be added, the percentage of refractory animals was consistently higher in the treated series.

The resistance of mice inoculated with cancer immediately after a stimulating dose of x-ray is no higher than that of normal mice and on the average is somewhat lower. On the other hand as in the preceding experiments the mice inoculated with cancer a week after the stimulating exposure show a considerably higher degree of resistance, which reveals itself both in the number of takes and in the rate of growth of the tumor.

Influence of Early Cancer Inoculation on the X-Ray Lymphoid Stimu-

lation.—The fact that early inoculation after a stimulative dose of x-ray has no effect brings up an interesting question. Does the cancer inoculation interfere with the action of the stimulus, or does this result depend on the fact that the cancer graft is established and actively growing before the increase in lymphocytes takes place? The question was answered by the following experiment.

Experiment.—A group of mice were exposed to a stimulative dose of x-ray and immediately afterwards were inoculated with a transplantable tumor. These were killed in groups at 48 hours, 4 and 6 days after inoculation and an histological examination was made of the lymphoid organs.

On an average the spleen and lymph nodes were smaller than in normal mice. Microscopically, a considerable number of pycnotic cells were noted in all of the lymphoid organs but the most striking feature was the almost complete suppression of the proliferative activity of the lymphoid cells in half of the animals. In the others mitotic figures were found, but in no instance were they more numerous than would be expected in a normal animal.

Other mice subjected to the same treatment but not inoculated with cancer showed the usual hyperactivity of the lymphoid tissue in about 90 per cent of the animals. Furthermore mice given the stimulating exposure and inoculated a week later presented evidence of very marked lymphoid stimulation in the majority of animals.

Hence it appears that cancer inoculation made immediately after a stimulative dose of x-ray interferes with the lymphoid reaction and little or no stimulation results, while cancer inoculation made at the height of the process augments the lymphoid reaction and the proliferative activity of the cells continues longer than in animals receiving the same stimulative exposure but without the cancer inoculation.

Accompanying Lymphocytosis.—A proportion of the animals given the stimulating dose of x-ray fail to react and a proportion also fail to show any increased resistance to cancer. In order to determine if these two fractions coincide a study of the blood has been made.

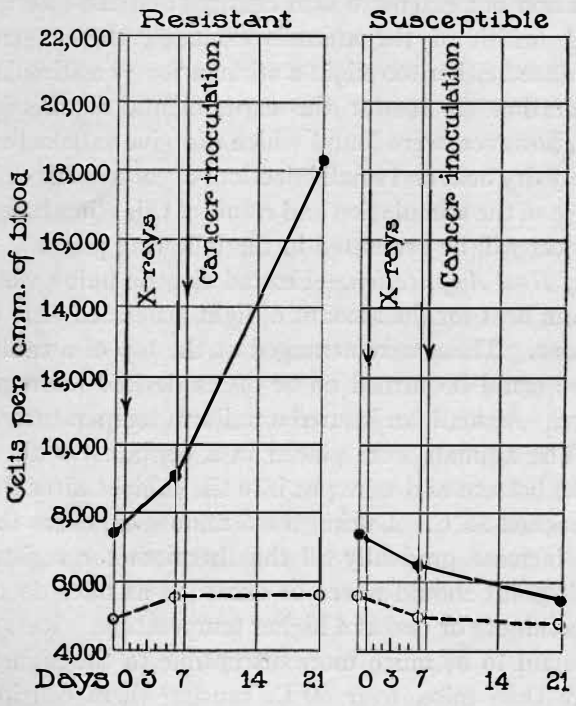
Experiment.—Blood counts were made on a group of mice and then they were given a stimulating dose of x-ray. A week later a second count was made followed by the inoculation with cancer. A third count was made 14 days later.

Of the 50 animals, 46 per cent proved to be resistant to the inoculation. In

these animals the lymphoid count was found to be increased on an average of about 2,000 cells per c.mm. 1 week after the x-ray treatment and increased to 18,000 or over, doubling the initial number by the 14th day after inoculation. In the

TABLE XX.

Time.	Resistant mice.		Susceptible mice.	
	Lymphocytes.	Polymorpho-nuclears.	Lymphocytes.	Polymorpho-nuclears.
	per c. mm.	*per c. mm.*	*per c. mm.*	*per c. mm.*
Before x-ray.....	7,590	5,040	7,460	5,300
7 days after x-ray.....	9,290	5,590	6,340	4,900
21 " " "	18,100	5,600	5,360	4,700



TEXT-FIG. 16.

susceptible group the lymphocytes had fallen slightly on the average 1 week after x-ray and still more by the 14th day after inoculation. The composite figures for this experiment are illustrated by Table XX and Text-fig. 16.

STIMULATION OF THE LYMPHOID TISSUE BY DRY HEAT.

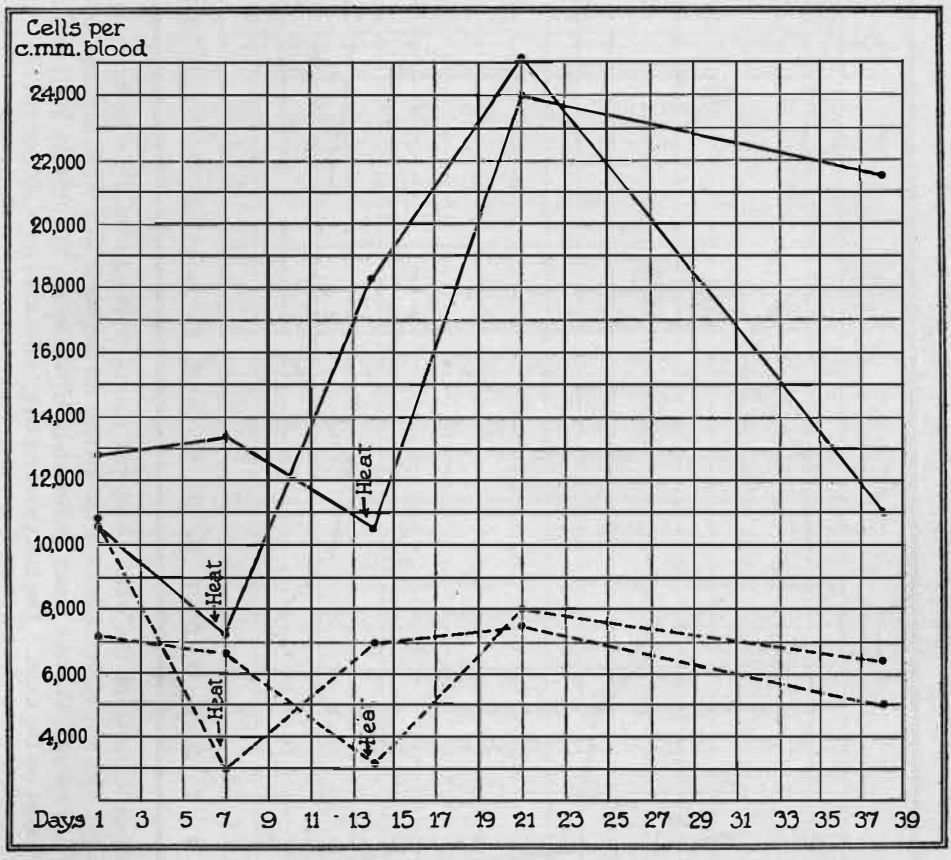
The effect of stimulation of the lymphoid tissues by x-ray on the resistance phenomenon seemed of such importance that further investigation was undertaken with the idea of finding other methods which shared with x-ray the stimulating factor, yet lacked some of the other x-ray effects on other tissues. Certain classes of drugs are known to increase the number of circulating lymphocytes, but an investigation showed this to be of short duration and not an expression of an actual stimulation. The temporary increase of these cells in the circulation was due to a forcing out of the lymphoid cells from the pulp of the spleen and was without an increased production. Ultraviolet and sunlight properly administered gave what appeared to be a real stimulation but extensive skin changes and the interference with the general health of the animals excluded these agents. Other methods induced either too slight a stimulation or a stimulation of too short a duration to answer the experimental requirements. Two procedures, however, were found which did give satisfactory stimulations, namely dry heat and small injections of certain oils or fatty acids. The evidence of the stimulation and result of this stimulation on transplanted cancer will be presented in the following pages.

Method of Heat Application.—Frosted electric bulbs which give off the maximum heat for the amount of light generated were used as the source of heat. These were arranged at the top of a cabinet so that one or more could be turned on or off as desired for regulating the temperature. A small fan insured a uniform temperature throughout the box. The animals were placed in a container with sawdust or paper on the bottom and were put into the cabinet after the temperature had reached 55°C. During the 5 minute exposure the heat was allowed to increase gradually till the thermometer registered about 65°C. This point should never be exceeded as mice do not survive more than a minute or two at a higher temperature. Rats and guinea pigs were found to be much more susceptible to the higher ranges of temperature than mice, over 60°C. causing them considerable discomfort. For this reason such animals were given a longer exposure at a lower temperature.

Effect of Dry Heat on the Leucocytes of Mice.—Total and differential white cell counts were made on the mice a week in advance of the heat

exposure. Counts were again made immediately and at intervals for 21 days after the heating.

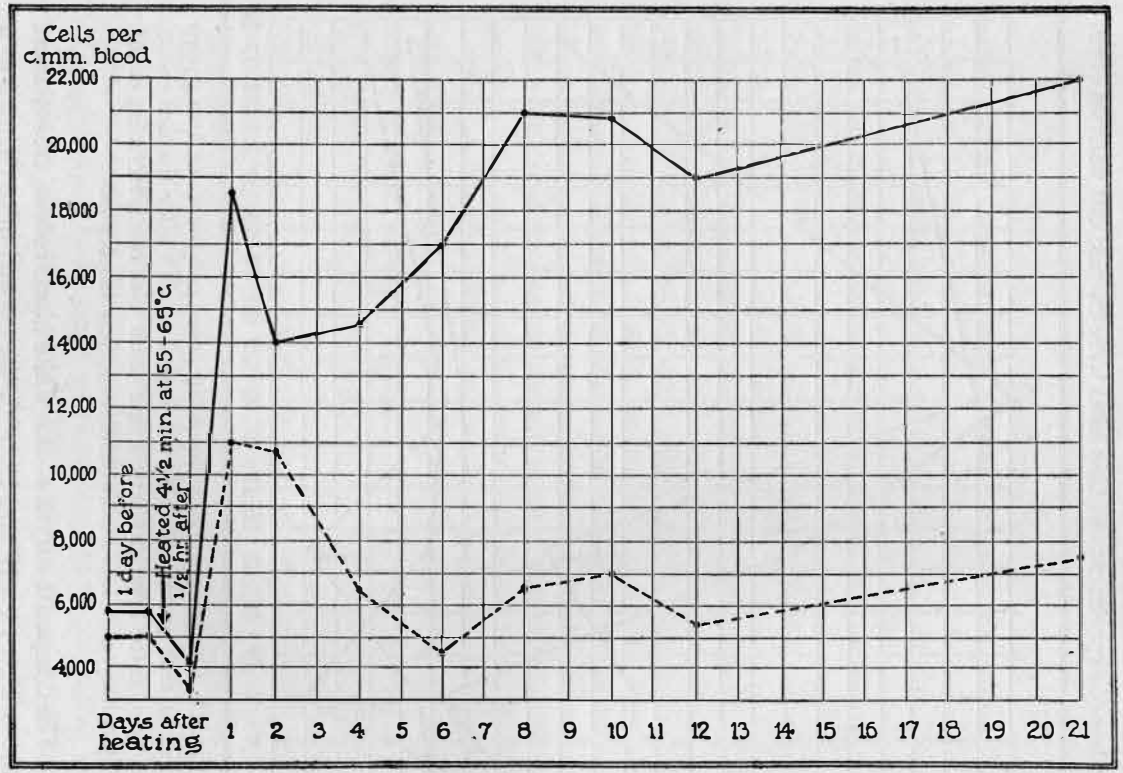
A number of experiments of this kind were carried out with uniform



TEXT-FIG. 17. Composite curve of the white blood cell counts on two groups of mice, five in each group. One lot was heated a week after the first count, the others were heated 2 weeks after the first count with one intervening count. ———Lymphocytes. .....Polymorphonuclear leucocytes.

results. The composite curves of the white blood cells of two of these are shown in Text-figs. 17 and 18, with the normal mice counted as a control.

The lymphocytes as a rule were found to be somewhat decreased in



TEXT-FIG. 18. Composite curve of the white blood cell counts on twenty healthy mice before and at intervals after heating. The first count represents the average for the whole lot. After heating they were counted in groups of five animals, the first group immediately after heating, the next 24 hours later, and then at intervals indicated on the chart. The last count represents the average of all twenty mice.

number immediately after the exposure to heat, but in 24 hours they had increased on an average to 200 per cent above their initial number. With some fluctuation this high level was maintained throughout the duration of the observation. The polymorphonuclear leucocytes also showed a slight fall immediately after heat and in the majority of the experiments the normal level was regained after which there was no further variation from the normal. In some instances, however, these cells increased somewhat during the first 2 or 3 days but later fluctuated only within the normal limits.

Rats, guinea pigs, and rabbits were found to respond to dry heat exposures in much the same manner as mice.

Source of the Lymphocytosis Induced by Heat.—In order to determine whether or not the increased number of lymphocytes in the circulating blood following exposures to dry heat was accompanied by an actual stimulation in production, the lymphoid organs were subjected to histological examination.

Material.—The spleen and lymph nodes were collected from a number of mice carried as a parallel series to the experiments reported above. The duration of the exposure was 5 minutes at a temperature ranging from 55 to 65°C. Specimens were taken at intervals for 14 days.

Spleen.—A large number of degenerating cells were present not only in the nodules but also in the pulp of the spleens removed immediately after the heat exposure. The germ centers, however, remained unaffected although there was no evidence of proliferative activity; only rarely was a mitotic figure to be seen.

48 hours after treatment the general appearance of the spleen was normal with perhaps still an unusual number of necrotic cells. However, the germinal centers contained a great number of mitotic figures, a significant fact when it is recalled that in the normal spleen of the adult, the cells of the germinal centers are not usually active.

The specimens examined at later periods showed a gradual elimination of the necrotic cells with the increased number of mitotic figures in the germ centers persisting, but to a less extent than in the 48 hour specimens.

It was noted that the spleen apparently underwent a marked enlargement at about the 6th to the 8th day after treatment, some being

approximately four times the normal size at this period. The size of the spleen varies greatly even among apparently normal mice but that the average size in these heated animals is increased seems beyond doubt.

Lymph Nodes.—The cortex lymph cords of the mesenteric lymph node were found to be full of necrotic cells immediately after the heat treatment. As in the spleen, the germ centers were unaffected and mitotic figures were rare. By 48 hours the necrotic cells had been largely eliminated. At this period the cells of the germ centers were in a state of active proliferation as evidenced by the large number of mitotic figures. A few division figures were to be seen even in the lymph cords. The condition of the nodes from the 4th to the 14th day seemed normal except for some evidence of increased numbers of mitotic figures in the germinal centers.

Grossly, as in the spleen, a general enlargement of the nodes was apparent about the 8th day.

Mechanism of Heat Stimulation.—The first histological change noted following heat exposure was a destructive process, a condition similar to that observed after x-ray exposures. The general character of the stimulation was of the same nature as that following x-ray except it was of greater intensity. To complete this comparison a study was made on the effect of serum from heated animals on lymphocytes *in vitro*.

The details of the experiment were the same as those reported under the x-ray effects. The individual tests need not be given in detail as the results were practically identical with those in which the serums from x-rayed animals were used. The only exception of note was that the serum taken immediately after the heat exposure had no stimulating power on the lymphocytes but the serum taken from 2 to 17 hours after the exposure was quite active in its stimulating power.

EFFECT OF DRY HEAT ON RESISTANCE TO TRANSPLANTED CANCER.

We may conclude from the observation reported in the preceding pages that dry heat has an even more profound effect as a stimulus to the lymphoid tissue than x-ray. The stimulation is both extensive and durable enough to answer the experimental requirements and it was therefore tested for its effect on resistance to transplanted cancer.

Experiment.—Mice were exposed to dry heat for 5 minutes at a temperature ranging from 55 to 65°C. A week later these animals together with a suitable number of untreated mice were inoculated with a transplantable cancer (Bashford Adenocarcinoma No. 63). Three experiments of this kind were carried out with consistent results which are shown in Table XXI.

Relationship between Time of Inoculation and Degree of Resistance.—It will be observed that the animals in the above experiments were inoculated a week after the exposure to heat. This period was selected because the blood study had shown that at about this time the stimulation was at its height. With x-ray the degree of resistance depended on the time at which the inoculation was made in relationship to the x-ray exposure. Unless the stimulation phase had actually set in at the time the cancer grafts were introduced little evidence of enhanced

TABLE XXI.

Experiment No.	Resistant heated mice.	Resistant normal mice.
1	70.0 per cent (10 mice).	22.2 per cent (9 mice).
2	70.0 " " (17 ").	31.3 " " (16 ").
3	50.0 " " (36 ").	5.5 " " (18 ").
Total or average...	58.7 " " (63 ").	18.6 " " (43 ").

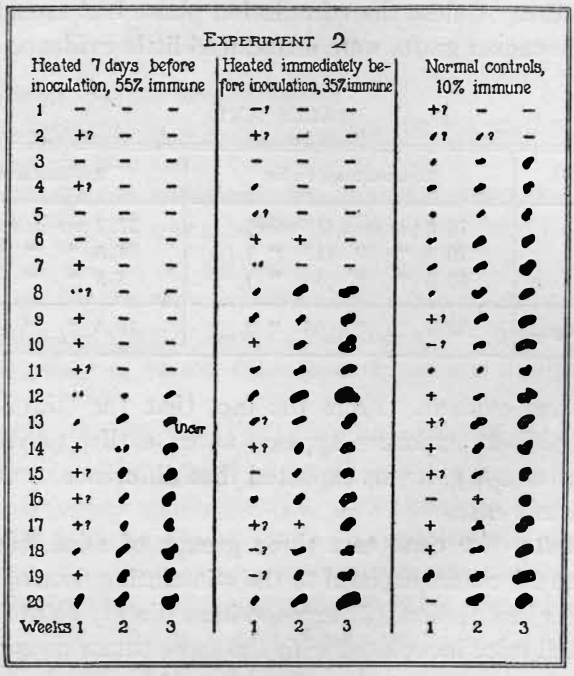
resistance was evident. From the fact that the stimulation phase following the heat exposure appears at an earlier period than that occurring after x-ray, it was expected that difference would not be so marked in this case.

Experiment.—For each test three groups of mice were used, (1) in which the mice were exposed to the stimulating dose of heat a week before cancer inoculation; (2) exposed immediately before inoculation; (3) untreated mice inoculated with the same tumor as controls. The results of three experiments comprising 118 animals are shown in Table XXII (Text-fig. 19).

Here as with the preceding experiments with x-ray, the degree of resistance depends on the phase of the stimulation at which the inoculation was made.

TABLE XXII.

Experiment No.	Resistant mice inoculated.		
	1 week after heat.	Immediately after heat.	Controls.
	per cent	*per cent*	*per cent*
1	66.6	40.0	20.0
2	55.0	35.0	10.0
3	50.0	30.0	10.0
Average.....	57.2	35.0	13.3



TEXT-FIG. 19. The effect of dry heat on the immunity to transplanted cancer when exposure to heat was done 7 days and immediately before inoculation.

STIMULATION OF THE LYMPHOID TISSUE BY OLIVE OIL (NAKAHARA).

Bergel (2), among others, has shown that a local lymphoid cell reaction follows the injection of fatty oils. An investigation was undertaken to determine whether or not this local reaction was accompanied by a general lymphoid stimulation.

General Response to Injections of Olive Oil.—A preliminary study was made to determine the dosage of olive oil which would have the maximum effect. For this purpose, commercial olive oil, described as the first expression, was injected intraperitoneally, in varying doses into a number of mice. Histological examinations were made of the lymphoid organs at intervals afterwards, with special attention to the number of mitotic figures present, as this has been shown to be a fair index of the degree of stimulation.

Experiment.—Group A received each 0.1 cc. of the oil intraperitoneally, and the condition of the lymphoid system was determined by killing groups of the animals at 24, 48 hours, 3, 4, and 5 days afterwards. No unusual features were found in the lymphoid organs of any of these mice except one killed on the 4th day, and here the germ centers of the spleen and nodes showed a marked increase of mitotic figures.

Group B. These animals received 0.2 cc. of olive oil and were examined as in the preceding group. Here there was a definite increase in mitotic figures, particularly in the germ centers, beginning at 48 hours and continuing for 5 days (Fig. 30).

Group C. The mice received 0.3 cc. of oil and were followed as in the above tests. The increase in mitotic figures appeared at 48 hours and persisted for 5 days but was less marked than the stimulation following 0.2 cc.

Group D. The dose of oil for this group was 0.5 cc. Judged by the number of mitotic figures, proliferative activity was retarded in the 24 and 48 hour specimens but returned to about the normal rate at the later periods.

Group E. The mice of this group were given 0.7 cc. of olive oil. The spleens and lymph nodes removed at 24 and 48 hour periods were much reduced in size. There was almost complete suppression of mitosis, a reduction of the amount of lymphoid tissue, and in the splenic pulp vacuoles of various sizes. At later periods, while the organs were still small, the rate of mitosis was approximately normal.

From this study 0.2 cc. of olive oil apparently gives the most definite evidence of stimulation. A more extensive study was made of the changes following this dose. The results confirmed the preliminary observation. The increase in mitosis usually appeared at 48 hours

after the injection, reached the maximum intensity between 4 and 7 days, and persisted for 10 days.

Other Organs.—The livers of certain of the mice injected with olive oil showed many intracellular vacuoles suggestive of fatty inclusions. Occasionally the capillaries and sinus-like spaces of the suprarenal gland were found to be much dilated. Another occasional finding was an increase in the number of mitotic figures in the cortical cells of the thymus. These were all of irregular occurrence and, therefore, are not to be classed as typical changes induced by the oil injection. No special alterations were noted in the thyroid, kidneys, or bone marrow.

The peritoneal fluid showed numerous lymphoid cells, including large and small lymphocytes, and many cells resembling macrophages. There was no evidence of a blood lymphocytosis.

EFFECT OF OLIVE OIL AND FATTY ACIDS ON RESISTANCE TO TRANSPLANTED CANCER.

An olive oil injection induces a condition as far as the lymphoid tissue is concerned essentially similar to that following an immunizing injection of homologous living tissue. The time at which the showers of mitotic figures appear and the duration of the effect are the same as is also the absence of a blood lymphocytosis. With these facts it seemed worth while to investigate whether or not the olive oil will induce an increased resistance to tumor grafts.

The first experiment was designed to determine the relationship of dosage to the degree of resistance.

Experiment.—A group of mice were each given a single injection of 0.1 cc. of olive oil and 10 days later inoculated with Bashford Adenocarcinoma No. 63. In a like manner other groups were given respectively 0.2, 0.3, 0.5, and 0.7 cc. of oil and inoculated. For controls to each group a number of normal mice were inoculated with the same tumor. The results are summarized in Table XXIII.

It is significant that the group showing the highest percentage of resistant mice should be the one which received 0.2 cc. of oil for this was the amount which gave the most pronounced lymphoid stimulation. A summary of five experiments in which the mice were inoculated with cancer 10 days after receiving 0.2 cc. of olive oil is given in Table XXIV.

Additional experiments which need not be given in detail have established that no increased resistance is evident if the cancers are inoculated immediately after the oil injection. It is not very evident at 5 days but reaches its maximum effectiveness when the grafts are introduced 10 days after the oil. After this period there is a gradual diminution (Table XXV).

TABLE XXIII.

Amount of olive oil.	Treated mice.		Controls.	
	Resistance.	No. of mice.	Resistance.	No. of mice.
cc.	*per cent*		*per cent*	
0.1	20.5	19	0.0	10
0.2	40.0	20	0.0	19
0.3	25.0	20	5.5	21
0.5	6.1	23	11.1	9
0.7	0.0	9	0.0	9

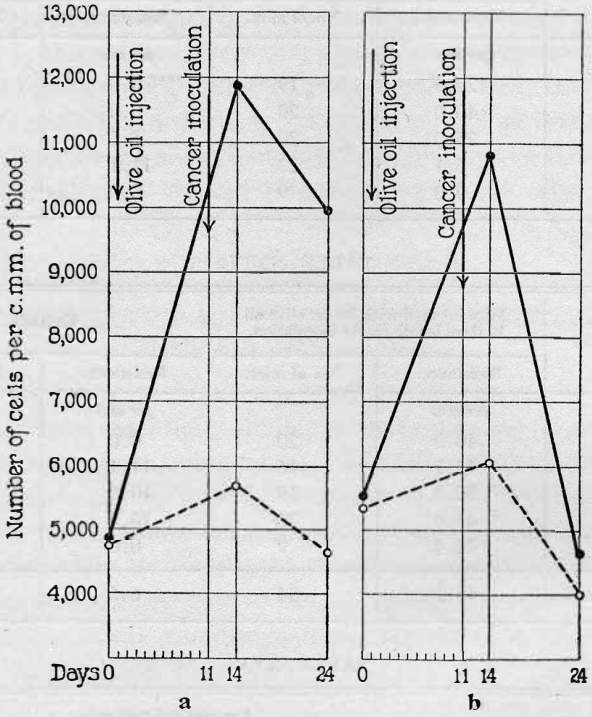
TABLE XXIV.

Experiment No.	Mice injected with 0.2 cc. olive oil 10 days before cancer inoculation.		Controls.	
	Resistance.	No. of mice.	Resistance.	No. of mice.
	per cent		*per cent*	
1	40.0	20	0.0	10
2	43.7	16	11.1	9
3	52.6	19	10.0	10
4	45.0	20	10.5	19
5	44.4	9	0.0	12
Average or total..	45.2	84	6.6	60

TABLE XXV.

Interval between oil and tumor inoculation.	Per cent resistant mice.	
	Oil-injected.	Normals.
1 to 2 hours.	6.9 (29 mice).	3.3 (30 mice).
5 days.	20.0 (10 ").	0.0 (12 ").
10 "	41.6 (36 ").	2.3 (42 ").
15 "	30.0 (10 ").	0.0 (12 ").
25 "	10.0 (10 ").	0.0 (12 ").

Histological Changes Accompanying the Resistance Induced by Olive Oil.—Associated with resistance to tumors definite local and general reactions occur whether the resistance is natural, or whether it is induced by homologous tissue injection, a small dose of x-ray, or dry heat. An examination has been made of animals made resistant by



TEXT-FIG. 20. Composite curves of white cell counts on mice injected with 0.2 cc. of olive oil and inoculated with Bashford Adenocarcinoma No. 63, 10 days later. (*a*) Composite curves from ten mice proved to be immune; (*b*) composite curves from nine mice proved to be susceptible. ——— Lymphocytes..... Polymorphonuclears.

olive oil to determine if the same manifestations accompany this immune state.

Local Changes about Cancer Grafts.—The reaction about the cancer grafts removed from mice made resistant by the injection of olive oil, during the first 48 hours is essentially a reaction to trauma and is

similar to that which takes place about a graft in a susceptible animal. By the 3rd day, however, this polymorphonuclear reaction has subsided and there is a marked infiltration of lymphocytes, plasma cells, and fibroblasts, a reaction similar to that which occurs in mice made resistant by other means. This cell infiltration was found in all the grafts removed from 4 to 5 days after inoculation but there was a variation in the intensity of the reaction.

Lymphoid Organs.—Spleens and lymph nodes taken as early as 24 hours after cancer inoculation in the oil-injected animals, showed that the number of mitotic figures was in excess of the normal. From 48 hours to 3 days the reaction seemed to reach its height and during this time mitotic figures were found in great numbers in the germ centers of the spleen (Fig. 31) and lymph nodes and often in considerable numbers even in the lymph cord of the node. An occasional animal failed to show any increase of mitotic figures in the lymphoid tissue, an irregularity without significance.

Blood Lymphocytes.—The resistance to cancer induced by other means invariably was accompanied by an increase in circulating lymphocytes. This point has been investigated for oil-induced immunity.

Mice were injected with olive oil and cancer was inoculated 10 days later. Blood counts were made before the oil injection, 3 and 13 days after the cancer inoculation. The results are presented in composite curves of the variation of the principal white cell groups of the resistant and susceptible animals (Text-fig. 20). While the lymphocytes in both groups increased immediately after cancer inoculation, in the susceptible group these cells fell rapidly to below their initial level. On the other hand in the resistant animals the lymphocytes were still 100 per cent above their normal number 2 weeks after the cancer inoculation.

It may be said from the foregoing experiments that olive oil induces a considerable resistance to transplanted cancer. The manifestations of this resistance are similar to those accompanying resistance induced by the several other methods reported. In common with the immunity induced by homologous tissue injections, no blood lymphocytosis occurs prior to the cancer inoculation but there is evidence of a very active stimulation of the proliferative activity in the lymphogenic tissues.

RESISTANCE INDUCED BY FATTY ACIDS AND SOAPS.

In an effort to determine the active factor in olive oil which is responsible for its immunizing power, the effects of several soaps and fatty acids have been investigated. The details of the experiment which determines the optimum dose need not be gone into. The soaps and fatty acids were injected intraperitoneally in the form of 1 per cent solutions or emulsions made up with distilled water. Among the tested substances which exerted no effect on resistance were sodium palmitate and sodium stearate. On the other hand, sodium oleate was found to be just as active as olive oil. This finding led to the

TABLE XXVI.

Substance.	Dose.	Resistance in.	
		Treated mice.	Normal mice.
Sodium oleate.	6 mg.	50.0 per cent (36 mice).	10.3 per cent (29 mice).
Oleic acid.	0.004 to 0.008 cc.	60.6 " " (61 ").	18.2 " " (55 ").
Linoleic acid.	0.004 to 0.006 cc.	54.0 " " (50 ").	19.2 " " (52 ").
Linolenic acid.	0.004 to 0.005 cc.	66.6 " " (30 ").	25.0 " " (32 ").

testing of the unsaturated fatty acids, three of which were selected representing three different chemical series. The large number of experiments involved in these tests has been summarized in Table XXVI.

It is evident that an unsaturated soap and the whole group of unsaturated fatty acids tested induce a material increase in the resistance against transplants of cancers.

QUANTITATIVE RELATIONSHIP BETWEEN THE EXTENT OF LYMPHOID STIMULATION AND THE DEGREE OF INDUCED CANCER RESISTANCE.

Three methods have been described by which a definite stimulation of the lymphoid tissue may be brought about; namely, small doses of

x-ray, dry heat, and olive oil or certain unsaturated fatty acids. The type of reaction produced by these methods varies somewhat both in the time at which the stimulation occurs and the extensiveness of the response. For example, the stimulation induced by x-ray is preceded by a period during which evidences of the destructive action of this agent on the lymphoid centers are the prominent features. This is followed by a period during which the stimulation alone is evident, reaching its height by the 4th day and then quickly subsiding. This represents the least pronounced and the least durable of the reactions produced. The stimulation induced by dry heat is much greater in extent and of longer duration. Here too the stimulation is preceded by evidences of cell destruction in the lymphoid centers but this period is of shorter duration than that following x-ray and the

TABLE XXVII.

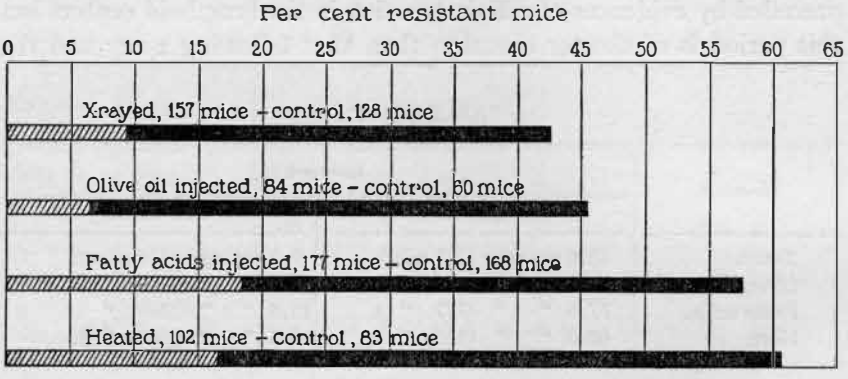
Treatment.	Resistance in.	
	Treated mice.	Control mice.
X-ray.	42.6 per cent (157 mice).	9.3 per cent (128 mice).
Olive oil.	45.2 " " (84 ").	6.6 " " (60 ").
Fatty acids.	57.6 " " (177 ").	18.5 " " (168 ").
Heat.	60.3 " " (102 ").	16.5 " " (83 ").

stimulation phase follows more promptly and is of greater volume. The effect of olive oil is a stimulation which in volume is about between the two others; it appears less promptly than the heat stimulation and is of longer duration than the x-ray stimulation.

An analysis of the percentages of animals resistant to tumor inoculation, resulting from these three methods of treatment indicates quantitative relationship between the extent and time of the stimulation phase and the degree of resistance. The greatest proportion of immune mice occurred among those heated, 60.3 per cent in 102 animals; while the x-rayed ones had the lowest proportion, 42.6 per cent among 157 mice. Those treated with olive oil gave a somewhat higher rate than the x-rayed mice (45.2 per cent, 84 mice), and the unsaturated fatty acid induced a still higher proportion of immunes (57.6 per cent, 177 mice). For comparison these results have been summarized in Table XXVII (Text-fig. 21).

These differences between the groups are not so pronounced if the figures are considered in relationship to the percentage of resistant animals among the controls, but even taking this fact into account the groups still retain their relative positions in the scale.

Another point not without significance is the time at which the resistance appears after treatment. Animals inoculated immediately after the x-ray exposure or the oil injection yield no higher proportion of immunes than the untreated controls. In both these instances the histological studies indicated that the stimulation was very slow in appearing. On the other hand, animals inoculated immediately after heat showed a distinctly higher percentage of resistant animals than



TEXT-FIG. 21. The shaded area represents the percentage of immune animals among the controls and the total length of the column, the percentage of resistant mice in the treated series.

occurred in the controls, but less than occurred when the inoculation was made a week after heat. Here the stimulation sets in almost immediately after the treatment.

From these facts it follows that not only the amount of stimulation plays a part but that the fate of the cancer graft is determined by the condition of the lymphoid tissue at the time of inoculation. Why a stimulation occurring after the inoculation is without effect except for a slowing down of growth rate probably depends on the absence of the initial local reaction. Another explanation is suggested by study of the x-rayed animals inoculated immediately after treatment. In this group there was a complete failure of the stimulation. Al-

though this finding was not tested by the other methods, yet it suggests that early inoculation annuls the effect of the treatment so that no increase in the lymphoid tissue takes place.

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EXPLANATION OF PLATES.

PLATE 13.

FIG. 25. Suprarenal gland of mouse 7 days after an exposure to low frequency x-rays, showing dilatation and engorgement of capillaries.

FIG. 26. The same as Fig. 25; higher power view.

PLATE 14.

FIG. 27. A splenic nodule, 24 hours after a 10 minute exposure to the small dose of x-ray. M = mitotic figure.

PLATE 15.

FIG. 28. A nodule of the mesenteric lymph gland, 4 days after a 10 minute exposure to the small dose of x-ray. M = mitotic figure.

PLATE 16.

FIG. 29. Mitotic figures found among the cells suspended in serum from x-rayed animals.

PLATE 17.

FIG. 30. Germ center of spleen, 4 days after an intraperitoneal injection of 0.2 cc. of olive oil. M = mitotic figure.

FIG. 31. Germ center of spleen of a mouse injected with 0.2 cc. of olive oil and inoculated with cancer 10 days later, 3 days after cancer inoculation. M = mitotic figure.

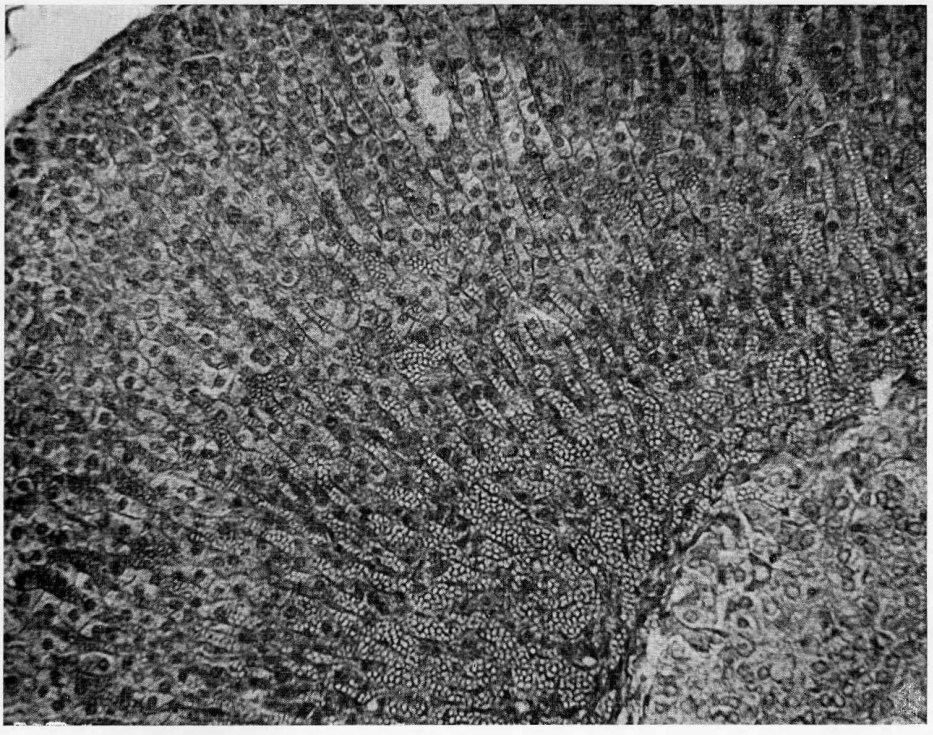


FIG. 25.

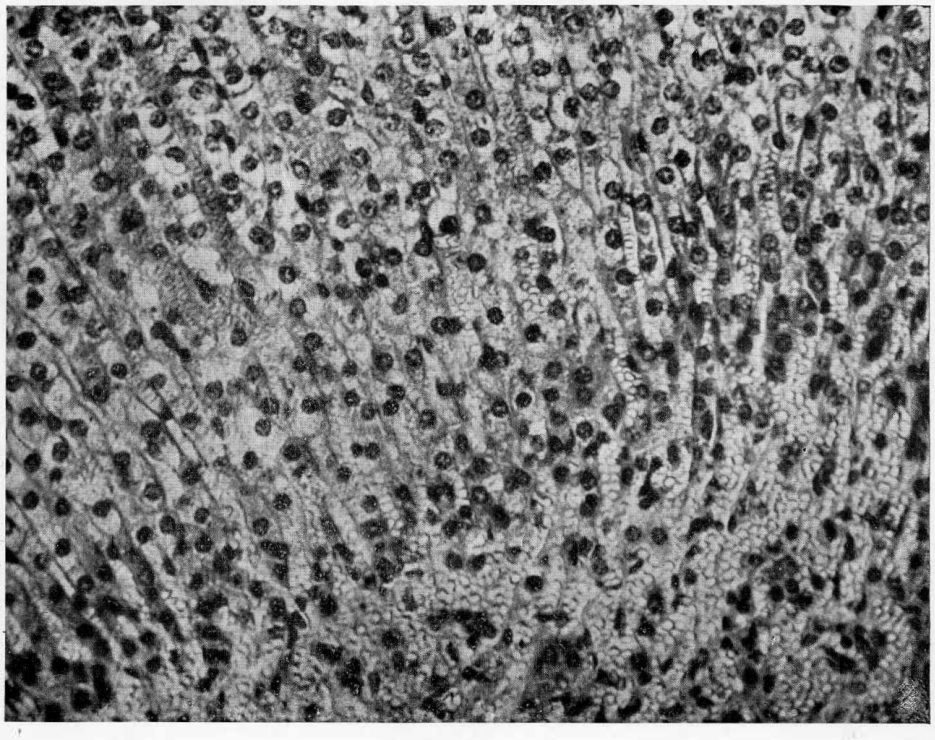


FIG. 26.

(Murphy: Lymphoid stimulation and cancer resistance.)

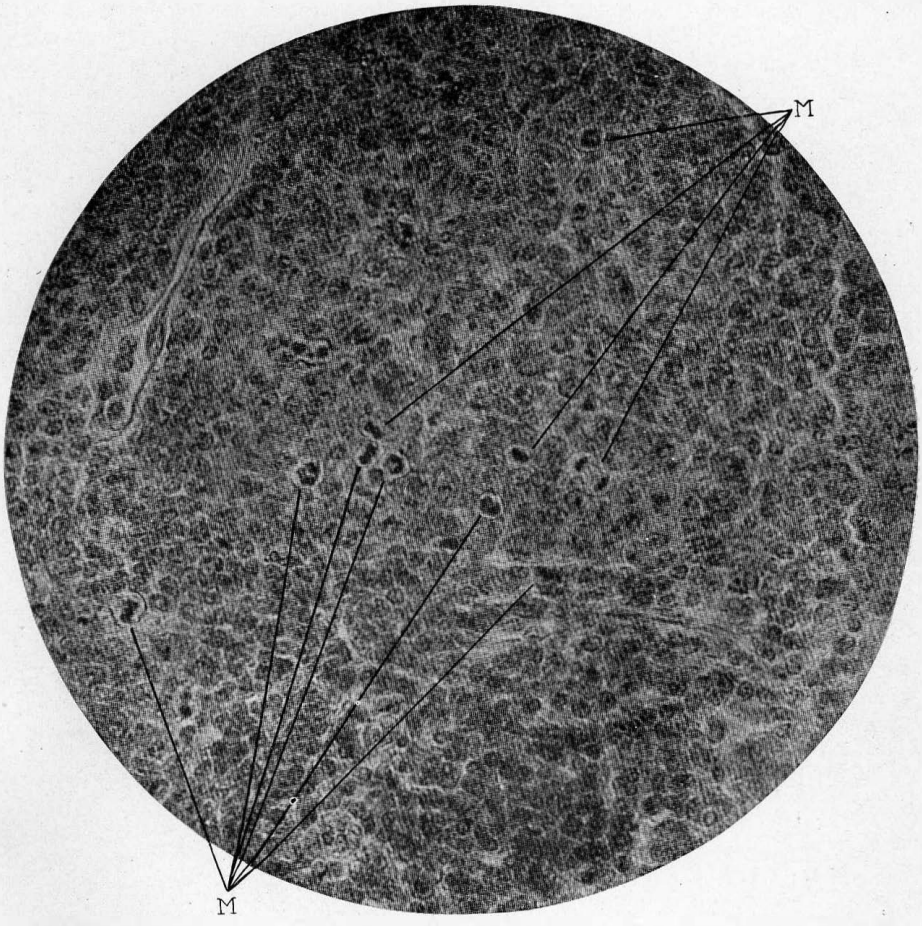


FIG. 27.

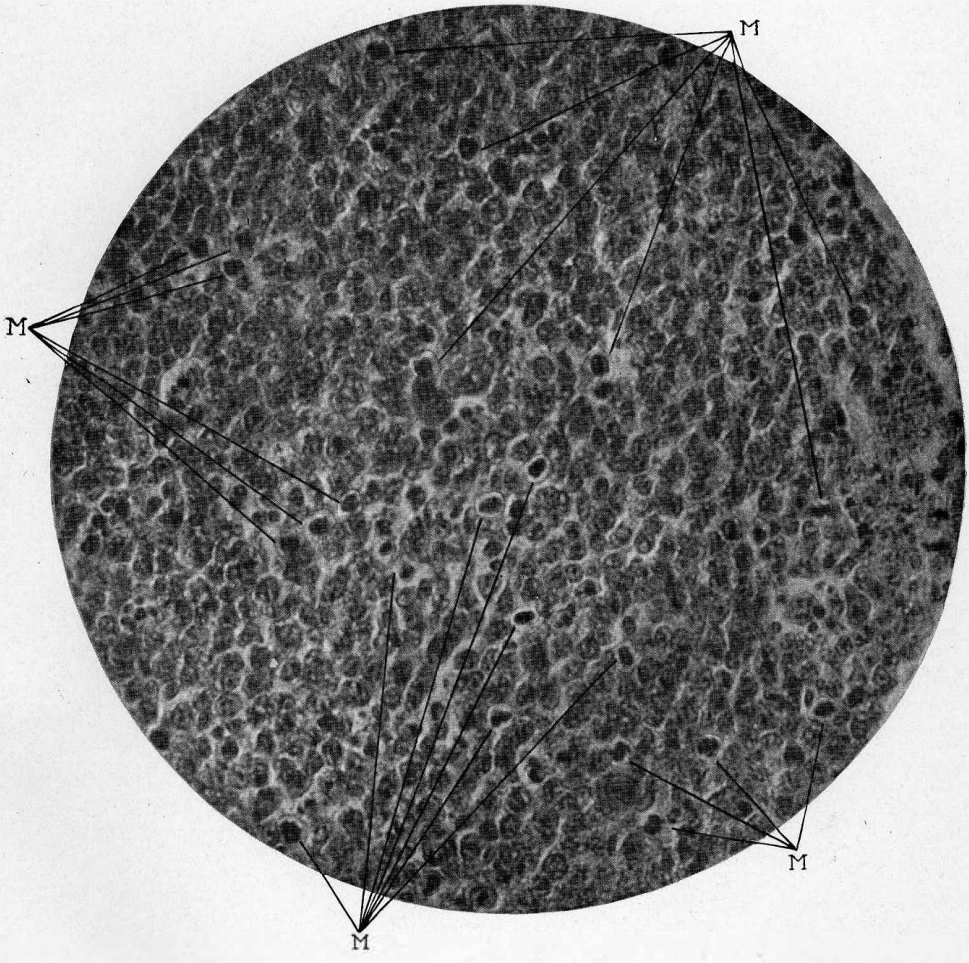


FIG. 28.

(Murphy: Lymphoid stimulation and cancer resistance.)

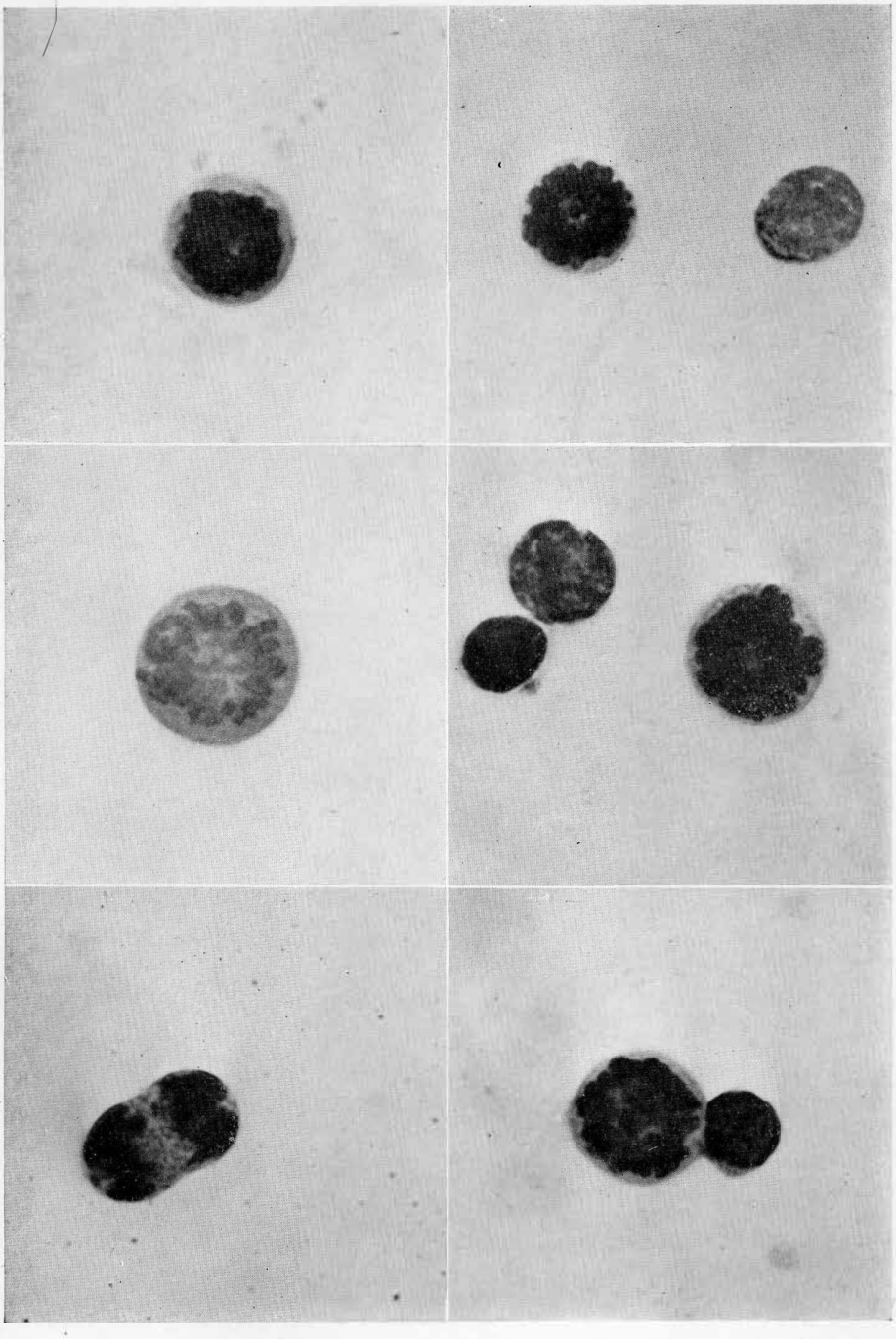


FIG. 29.

(Murphy: Lymphoid stimulation and cancer resistance.)

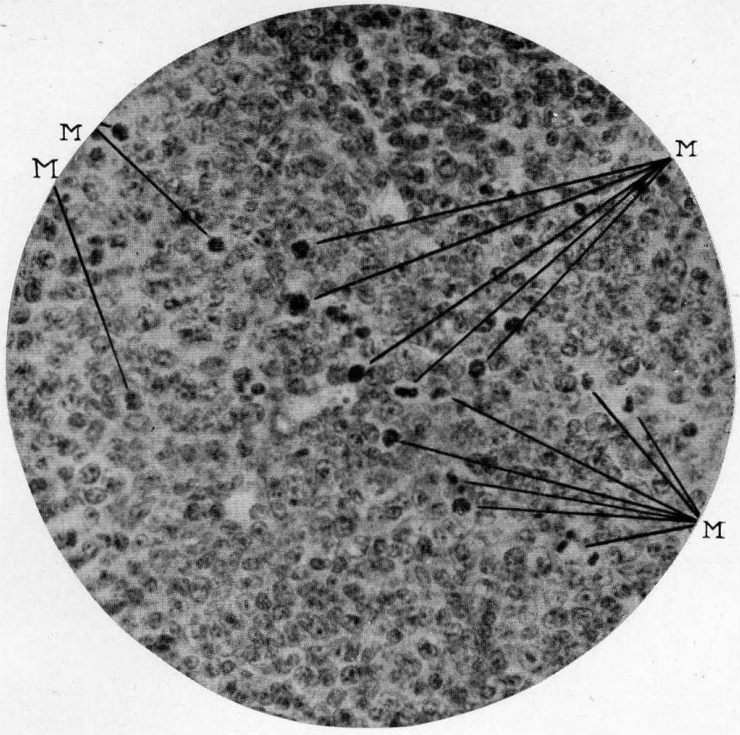


FIG. 30.

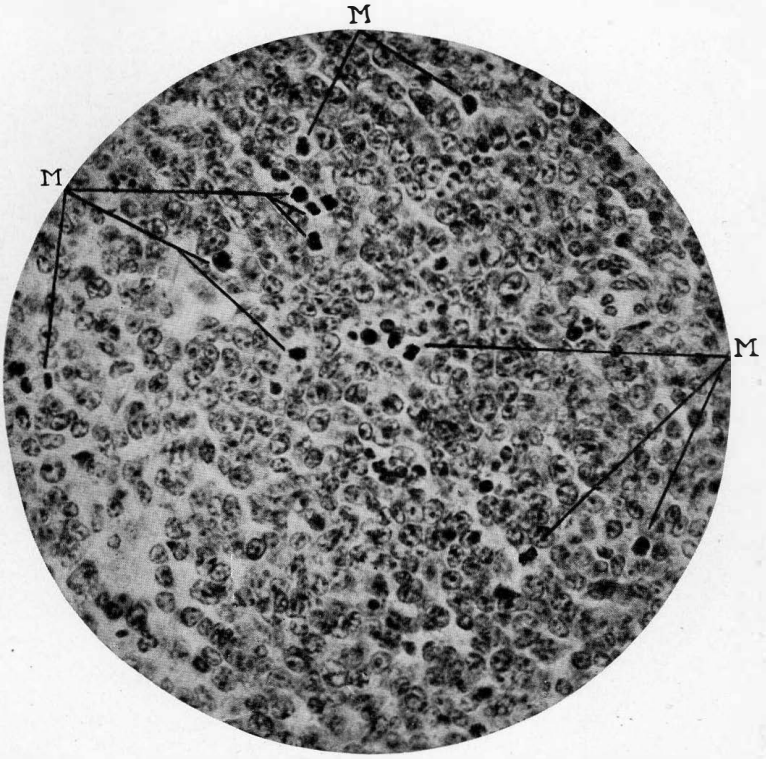


FIG. 31.

(Murphy: Lymphoid stimulation and cancer resistance.)

V. LOCAL CELLULAR REACTION AND RESISTANCE TO TRANSPLANTED CANCER.

PLATES 18 TO 20.

As noted above the resistance to transplanted cancer manifests itself by a local cellular reaction about the introduced cancer graft. This resistant state may be induced by several procedures which apparently involve primarily a general stimulation of the lymphoid tissues. What part does the local reaction play in the process? Is it only part of the mechanism or does it represent the mobilized strength of the resisting force? To answer these suggestions we have searched for methods whereby a local round cell reaction may be produced without inducing at the same time a general reaction; the reverse, in which local reaction may be suppressed in a resistant host.

LOCAL REACTION OF SENSITIZED MICE TO FOREIGN BLOOD, AND ITS EFFECT ON CANCER GRAFTS.

On the basis of the similarities existing between the local anaphylactic reaction and the local manifestations in cancer immunity, we have tested the influence of the foreign protein reaction on cancer grafts. Since defibrinated mouse blood is most generally used for producing homologous tissue immunity in mice, in order to parallel closely this procedure, defibrinated rat blood has been used as the foreign protein in the following experiments.

Experiment.—0.2 cc. of defibrinated rat blood was injected subcutaneously into the backs of a number of mice. Some of these were killed 24 hours later for histological examination. There was a considerable lymphoid infiltration in the region of the injected blood, simulating the process which occurs at the site of homologous blood injection.

10 days later the remaining sensitized mice were given a second small subcutaneous injection of rat blood into the groin, and these were killed after another 24 hours. In these animals the infiltration of lymphocytes about the injected blood was far more extensive than that occurring after a single injection and equal to that found about a cancer graft in an immune animal.

It is apparent that a reaction to foreign protein is very similar to that which accompanies the immunity reaction to transplanted cancer. If this similarity is more than a coincidence it should be possible to influence the growth of cancer grafts by means of this foreign protein reaction.

Experiment.—Rat blood was collected aseptically and defibrinated. A number of mice were injected subcutaneously with 0.2 cc. of this blood and 10 days later part of a transplantable adenocarcinoma was minced finely and thoroughly mixed with fresh defibrinated rat blood. The particles of tumor were then loaded into a

TABLE XXVIII.

Experiment No.	Sensitized rat blood. Inoculated mouse tumor and rat blood.	Sensitized rat blood. Inoculated mouse tumor alone.	Non-sensitized. Inoculated mouse tumor and rat blood.
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).
Average....	53.5 per cent immunity (58 mice).	5.2 per cent immunity (39 mice).	7.0 per cent immunity (58 mice).

trocar, care being taken to include a drop of blood with each graft. This mixture was then inoculated into half of the mice which had been sensitized 10 days before, and also into an equal number of untreated mice. The remaining part of the tumor not mixed with rat blood was inoculated into the other half of the sensitized animals. We thus had sensitized mice inoculated with a mixture of rat blood and tumor cells, sensitized mice inoculated with tumor cells alone, and normal mice inoculated with a mixture of tumor cells and rat blood.

This experiment was repeated six times with the result that of 58 mice sensitized with rat blood and later inoculated with a mixture of rat blood and mouse cancer, 53.5 per cent were resistant, while the 39

animals sensitized with rat blood and inoculated with tumor alone yielded only 5.2 per cent of immunes. The last group composed of 58 normal mice inoculated with the mixture of tumor and rat blood had 7.0 per cent of resistant animals (Table XXVIII).

HISTOLOGICAL STUDY OF THE FATE OF THE CANCER-RAT BLOOD MIXTURE INOCULATED INTO A SENSITIZED ANIMAL.

In order to study histologically the fate of a cancer graft mixed with rat blood and inoculated into a previously sensitized mouse 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. At first a massive lymphoid and a mild polymorphonuclear reaction appeared about the graft, and this reaction lasted until the 3rd day after which it began to diminish. The graft was by this time more or less completely destroyed, and the reaction rapidly subsided after this period. It may be noted that the reaction around the cancer graft in an immunized animal is essentially similar to that just described.

DESENSITIZING EFFECT OF GENERALIZED DOSES OF X-RAYS.

We have previously shown that the state of potential immunity in mice could be reduced to a state of susceptibility by a proper exposure to x-rays, given between the immunizing injection and the cancer inoculation. X-rays administered at certain periods after a foreign protein injection likewise bring about a desensitization, so that anaphylactic shock which should result after a second injection of the protein can be prevented. In view of these facts 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 seems worthy of study.

Experiment.—Normal white mice were injected subcutaneously with 0.2 cc. of defibrinated rat blood. These mice and a group of mice which had not been sensitized were then given daily doses of x-rays (spark-gap 2¼ inches, milliamperes 10, time 2 minutes, and distance from target 12 inches) for 8 days. 10 days after the sensitizing injection these animals and several normal controls were inoculated with a Bashford adenocarcinoma mixed with defibrinated rat blood.

Thirty-two mice sensitized to rat blood, exposed to the dose of x-rays, and inoculated with a mixture of rat blood and mouse tumor, showed from 10 to 27.2 per cent immunity. Ten mice not sensitized but given the same dose of x-rays as the first group and then inoculated with cancer alone showed 10 per cent immunity. Twenty normal mice inoculated with a mixture of rat blood and mouse cancer, without the previous blood injection and x-rays, showed 20 per cent immunity (Table XXIX).

TABLE XXIX.

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.

EFFECT OF X-RAYS ADMINISTERED LOCALLY ON LOCAL RESISTANCE TO CANCER.

The foregoing experiments show that mice develop an enhanced refractory state to the growth of transplanted cancer coincident with a definite local lymphoid reaction which takes place as a result of the reinjection of a foreign protein in a previously sensitized animal. In the light of the accumulated evidence it seems probable that the cellular elements in the reaction play an active part in the refractory mechanism. If so, the destruction of these cells alone might be sufficient to inhibit the potential resistance. Since x-ray in moderate amounts is known to have no detectable effect on the viability of the transplantable tumor cells, it may be used to bring about the destruction of the local lymphoid reaction.

Experiment.—Normal mice were injected subcutaneously with 0.2 cc. of de-

fibrinated rat blood and 10 days later inoculated with a bit of Bashford adenocarcinoma mixed with rat blood. From 15 to 24 hours after the inoculation the mice were covered with sheet lead in which an aperture had been made large enough to expose the area around the graft. Over this area the following dose of x-rays was then given: 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 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 the mixture of tumor and rat blood without previous sensitization or after-treatment with x-rays.

The results of two experiments of this type were as follows: Among thirty-one mice, sensitized to rat blood, inoculated after 10 days with a mixture of rat blood and mouse tumor, and given a local dose of x-rays 20 hours later, the per cent of immunity was from 30 to 38. Ten normal mice inoculated with a mouse tumor and given a local dose of x-rays 20 hours later showed 10 per cent immunity. Twenty normal mice inoculated with a mixture of mouse tumor and rat blood showed 20 per cent immunity.

The above process has been examined histologically. It was found that 24 hours after the inoculation of rat blood plus mouse tumor in previously sensitized mice an extensive cell infiltration was found about the inoculated material. The great majority of the cells participating in the reaction was of the lymphoid series, the polymorphonuclear cells being less numerous.

Immediately after the local dose of x-rays a striking reduction of these cells took place in the area which had been thickly infiltrated previous to the x-ray treatment. How this reduction of the cells is brought about is not clear, but these cells were not destroyed *in situ* for no evidence of them was found in this area. The cell infiltration was gradually restored later on but it never reached the preirradiation extent. These findings support the idea that the temporary removal of the lymphoid reaction effected by the local dose of x-rays permits the graft to grow in the sensitized animal.

The conclusion may be drawn that there are two ways in which it is possible to overcome partially the immunity to cancer resulting from a local anaphylactic reaction. In either case the effect follows the prevention or suppression of the local cellular infiltration; *i.e.*, either the animals are desensitized so that the second injection of foreign

protein does not call forth the local cellular reaction, or the local lymphocytic reaction is suppressed by x-ray.

EFFECT OF LOCAL CELLULAR REACTION INDUCED BY X-RAY ON THE FATE OF CANCER GRAFTS.

The erythema in the skin produced by x-ray, besides the dilatation of the vessels, is characterized mainly by a lymphoid cell infiltration. Little change is noted histologically till about 7 days after treatment and then examination shows a marked accumulation of the lymphoid cells in the skin layers, particularly in the stratum papillare of the corium. The reaction is confined to the skin layers, the underlying subcutaneous tissue being entirely free from cellular infiltration. This observation offers an opportunity for further testing the effect of local cellular reaction on the fate of cancer grafts for we have in close proximity two regions, one with and one without a cellular reaction.

Experiment.—Healthy young mice were shaved over the region extending from the upper abdomen down to and including both groins. After being anesthetized the animals were secured on a small board and the entire body covered with sheet lead in which an opening 15 × 20 mm. was cut, exposing a region in the left groin extending to the midline. This area was then exposed to the following dose of x-rays: 3 inch spark-gap, 10 milliamperes, 6 inches distance from target, and 2½ minutes exposure. 7 days later when a mild erythema with some scaliness was appearing in the irradiated area of the skin, small grafts of Bashford Adenocarcinoma No. 63, were inoculated intracutaneously in the center of the x-rayed area and also in the corresponding position of the unexposed right groin. Great care was taken to avoid thrusting the grafts through into the subcutaneous tissue, though this accident occasionally occurred and unquestionably accounts for some of the positive tumor grafts in the x-rayed areas.

The fate of cancer grafts inoculated into x-rayed and normal areas of skin was studied in four experiments of this kind. In the 57 mice the cancer grafts failed in the x-rayed area in 61.4 per cent, while in the protected areas of the same animals they failed in only 3.5 per cent (Table XXX and Fig. 32).

In a second series of experiments, the details of which were the same as the above except that the tumor grafts were inoculated intracutaneously 2 hours after the x-ray exposure, while the number of failures of the graft in the x-rayed area was higher (76.2 per cent) than in the

first experiment, so were the failures in the normal areas. On a comparative basis the result was about as striking as in the first group of animals x-rayed 7 days before inoculation (Table XXXI, Text-fig. 22).

In a third series of experiments the details of which were the same as above, the x-ray dose was given 20 hours after the intracutaneous inoculation of the tumor. The result here was just as pronounced as in the two preceding experiments (Table XXXII).

TABLE XXX.

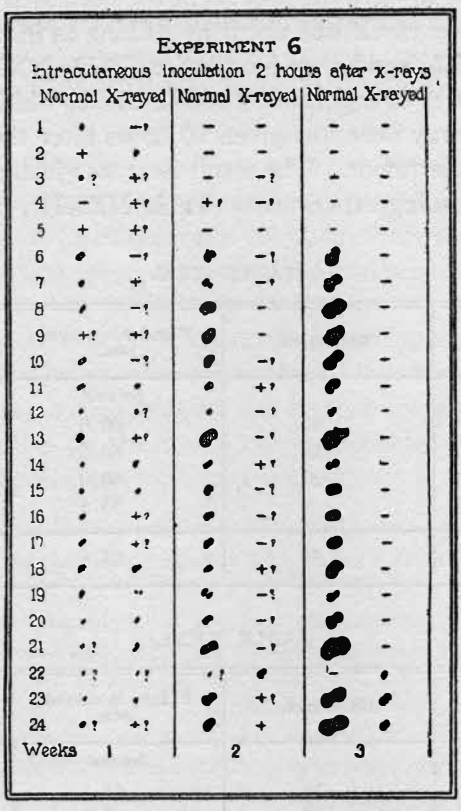
Experiment No.	No. of mice.	Negative in x-rayed area.	Negative in protected area.
		per cent	*per cent*
1	10	60.0	10.0
2	18	66.6	5.6
3	20	60.0	0.0
4	9	55.5	0.0
Total or average....	57	61.4	3.5

TABLE XXXI.

Experiment No.	No. of mice.	Failures in x-rayed area.	Failures in protected area.
		per cent	*per cent*
5	18	61.1	11.1
6	24	87.5	25.0
Total or average...	42	76.2	19.0

Histological Examination.—The tumor grafts were usually found to be in the tela subcutanea. In the animals x-rayed 7 days previous to the inoculation, the cancer tissue was found 3 days later to be surrounded by a dense lymphoid cell reaction (Fig. 33). This reaction persisted as long as any living tumor cells remained; with the disappearance of the tumor cells it subsided. There was practically no reaction about the cancer graft in the normal skin, and the cancer cells were proliferating actively (Fig. 34).

The examination of the grafts inoculated immediately after the



TEXT-FIG. 22. The growth of cancer grafts inoculated intracutaneously in an area of skin 2 hours after the skin had been exposed to x-ray treatment compared to the fate of similarly inoculated grafts in an untreated area in the same animals.

TABLE XXXII.

Experiment No.	No. of mice.	Failures in x-rayed area.	Failures in protected area.
		per cent	*per cent*
7	7	85.7	14.3
8	11	90.9	36.3
9	20	85.0	15.0
10	18	50.0	16.6
Total or average...	56	75.0	19.6

x-ray exposure or x-rayed after inoculation showed the same picture. Extensive lymphoid infiltration appeared in the x-rayed area about the tumor graft by the 4th day. The tumor tissue had disappeared by the 7th day, but there remained an intense lymphoid infiltration of the skin. In the untreated side well established tumors were present accompanied by only a slight cellular reaction.

BLOOD SUPPLY TO TUMOR GRAFTS IN X-RAYED AREA.

Thus it appears that cancer grafts fail to grow when inoculated into an area of skin previously exposed to x-ray. There is a possible explanation of this fact which deserves consideration, namely an interference with the vascularization of the graft. There is no evidence that so mild a dose of x-ray damages the vessels but this possibility has been examined.

A number of mice which had received a local exposure to x-ray and had been inoculated intracutaneously with a cancer graft in this area and also in a protected area were killed with ether and the skins removed. When these were held before a light a clear definition of the blood vessels was obtained. The grafts in both areas were surrounded by dilated vessels and little difference between the sides could be distinguished, but when a difference was noted it was in favor of the x-rayed side.

This point was further tested by injecting India ink into the heart of a number of these animals. The patent vessels were just as numerous around and in the graft in the x-rayed area as in the normal skin.

FATE OF SUBCUTANEOUS CANCER GRAFTS IN X-RAYED AREAS.

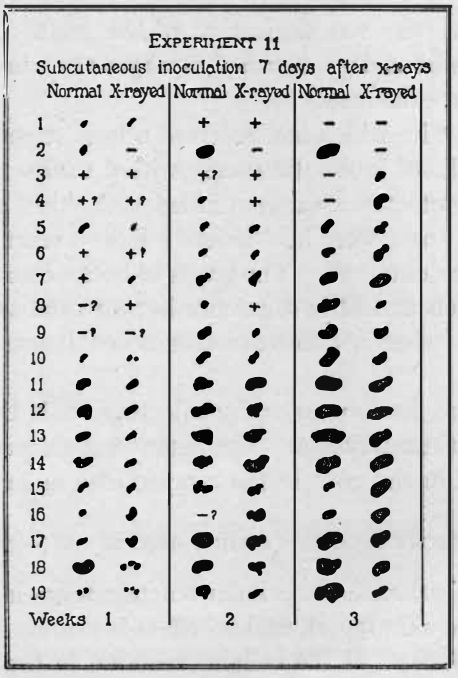
As already mentioned, the cellular reaction following x-ray exposure is confined to the skin layers, and no effect is evident in the underlying subcutaneous tissue. If the cellular reaction in the skin determines the local resistance we should expect the cancer grafts to grow at the normal rate if inoculated under the skin instead of into the skin of an x-rayed area.

Experiment.—The mice were prepared in the same fashion as in the preceding experiments and given the same local dose of x-ray. A week later a cancer graft was inoculated into the x-rayed area just under the skin. At the same time an-

other graft was introduced in the opposite side of the animals, an area which had been protected from x-ray.

TABLE XXXIII.

Experiment No.	No. of mice.	Failures in x-rayed area.	Failures in protected area.
		per cent	*per cent*
11	19	10.5	15.8
12	9	11.1	11.1
Total or average.....	28	10.7	14.35



TEXT-FIG. 23. A graphic representation of the results of subcutaneous inoculations of cancer grafts into x-rayed areas as compared with the subcutaneous inoculations into untreated areas.

Two experiments of this nature were carried out with the result that the cancers grew just as well in the subcutaneous tissue of the x-rayed area as in the protected area (Table XXXIII, Text-fig. 23).

The experimental results offer an interesting point in relation to the

cellular infiltration as determining the fate of cancer grafts. The cellular reaction induced by x-ray was confined to the skin layers and was absent in the loose subcutaneous tissue, and the skin proved an unsuitable soil for the growth of cancer, while the subcutaneous tissue was not affected in this respect by the treatment. While the difference in amount of x-ray received by these two tissues must have been very slight, yet it was considered worth while to determine if direct exposure of the subcutaneous tissue would affect the fate of a subsequently implanted cancer graft in this location.

TABLE XXXIV.

Experiment No.	Resistant x-rayed mice.	Resistant control mice.
1	66.6 per cent (15 mice).	23.0 per cent (13 mice).
2	68.4 " " (19 ").	0.0 " " (6 ").
3	73.3 " " (15 ").	29.4 " " (17 ").
4	50.0 " " (10 ").	0.0 " " (10 ").
Average.....	66.1 " " (59 ").	17.4 " " (46 ").

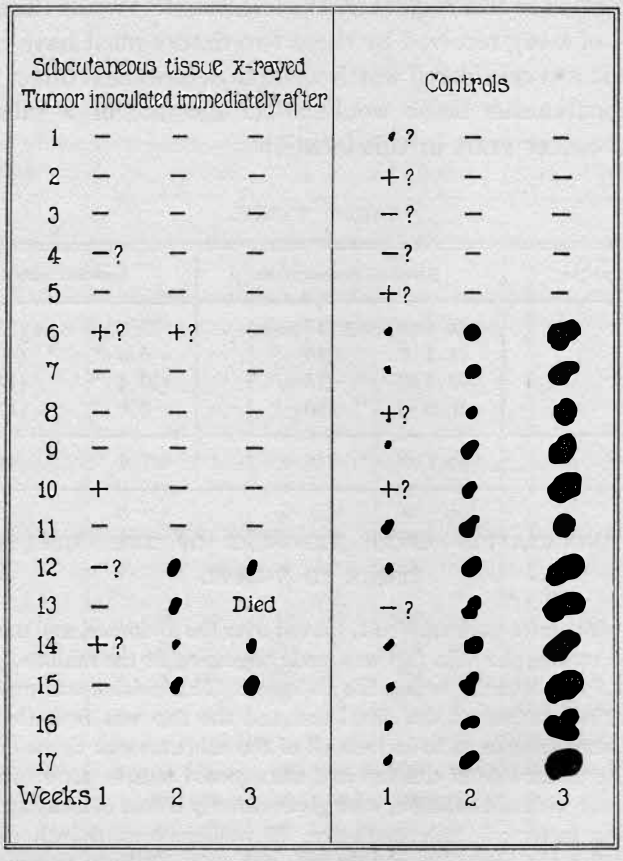
TUMOR INOCULATION AFTER EXPOSURE OF THE SUBCUTANEOUS TISSUE TO X-RAYS.

Normal white mice were etherized, shaved over the abdomen, and under aseptic conditions a rectangular skin flap was made beginning at the midline and extending about 1.5 cm. laterally across the abdomen. The incision was made so as to leave the skin attached at the distal end and the flap was separated from the underlying structures so as to include all of the subcutaneous tissue down to the muscle. The under side of the flap and the exposed muscle, after being covered with gauze wet with salt solution, were given directly a dose of x-rays governed by the following factors: 3 inch spark-gap, 10 milliamperes, 6 inch distance, $2\frac{1}{2}$ minutes. With the exception of this area the animal's body was protected by sheet lead. Immediately after the treatment a cancer graft was introduced into the loose connective tissue of the under side of the flap and the skin sutured back into place. As a control, another series of animals was treated in precisely the same fashion except that no x-rays were given.

In practically all of the animals of both series the wounds healed within 5 or 6 days by primary intention, with no detectable difference between the x-rayed and control animals. Weekly examinations were made to determine the fate of the cancer grafts and later verified by autopsy.

The results of four such experiments are given in Table XXXIV.

It is apparent from these experiments that an erythema dose of x-rays applied directly to the subcutaneous tissue brings about some change which renders this tissue decidedly less suitable as a soil for the growth of implanted cancer (Text-fig. 24). Another point of



TEXT-FIG. 24. Results of inoculation of tumor into subcutaneous tissue previously exposed directly to x-rays, compared with a like inoculation in normal mice.

interest is that the cancer grafts which took in the x-rayed animals showed a tendency to grow inward toward the abdominal cavity with a flat inactive base on the side near the skin. Some of these did not produce even a slight elevation of the overlying skin and were only detected at autopsy.

To serve as a control for the above experiments and in order to confirm the finding that the effect of a local erythema dose of x-rays applied to the skin does not extend to the underlying subcutaneous tissue, the following experiment was carried out.

SUBCUTANEOUS INOCULATION OF TUMOR AFTER EXPOSURE OF THE SKIN TO X-RAYS.

Ten normal white mice were given a dose of x-rays over the left lower quadrant of the abdomen, the dose being governed by the same factors as those used in the preceding experiment. Immediately following the exposure, a skin flap was made in the x-rayed area and a cancer graft (Bashford No. 63) was introduced into the loose areolar tissue of the under side of the flap and the wound closed with sutures. The tumor grew in all of these animals, from which it may be concluded that an erythema dose of x-rays given to the intact skin does not increase the resistance of the underlying subcutaneous tissue.

TUMOR INOCULATION IN A PROTECTED AREA AFTER A LOCAL EXPOSURE OF THE SUBCUTANEOUS TISSUE TO X-RAYS.

In order to determine whether the exposure to x-rays of a small area of subcutaneous tissue affects the general resistance to cancer grafts, a series of thirteen mice was operated upon and after the skin flap was made on the left side of the abdomen they were x-rayed over the open wound and then the flap was sutured back into place. Cancer grafts inoculated immediately afterwards in the right side resulted in tumors in 76.9 per cent of the animals, or in about the proportion observed in normal control mice.

HISTOLOGICAL CHANGES AFTER DIRECT EXPOSURE OF THE SUBCUTANEOUS TISSUE TO X-RAYS.

Two series of twelve mice each were shaved, and, under ether, flaps of skin and subcutaneous tissue were made over the left lower abdominal region. One series was x-rayed with an erythema dose directly on the under side of the skin flap and on the denuded surface of the abdominal muscle, the remaining parts of the animal being protected by sheet lead. The other series was operated on in the same manner but not x-rayed. The wounds in both were sutured with great care as to the approximation of the skin edges. The animals were

killed in groups of two, 24 hours, 3, 5, 7, 9, and 14 days after operation for examination.

Up to the 5th day the process of repair formed so prominent a part of the picture that it was impossible to detect any difference in the extent and character of the cellular infiltration from histological study. The 7 and 9 day preparations, however, in which the process of repair was in the last stages, showed distinctly that while in the animals not x-rayed, only a layer of new connective tissue between the subcutaneous and muscle layers was slightly infiltrated with round cells, in the x-rayed animals large numbers of lymphocytes occurred, chiefly in the loose connective tissue, and in about half of the animals examined these cells had infiltrated the thickness of the muscle and formed a heavy layer between the muscle and the parietal peritoneum. At the end of 2 weeks this lymphocytic infiltration, although still evident, had subsided somewhat.

Two other groups of mice were operated on in the same manner as in the preceding experiment, and one of the groups was given a dose of x-rays over the exposed subcutaneous tissue and muscle. Before the skin flap was sutured back into place each animal received a cancer graft into the connective tissue underlying the flap. The microscopic appearances of the sections of tissue taken at intervals from the animals were so complicated, through operation, x-ray treatment, inoculation of tumor, natural differences in susceptibility, and in some cases, mild infections, that it was impossible to draw any conclusions in regard to the cellular reactions.

The observations reported here bring out the fact that x-rays can be made to induce a local change in the subcutaneous tissue similar to that which this agent will induce in the skin. This change, in both instances, renders the locality resistant to the growth of implanted cancer cells, but does not affect the general resistance of the animal. This is an additional point to be taken into consideration in determining the method of treatment and the interpretation of clinical results following the use of x-rays as a therapeutic agent.

Changes in the Cancer Cells Inoculated into an X-Rayed Area.— The familiar phases of cancer cell degeneration following x-ray treatment in man have generally been attributed to the direct injury produced by the irradiation, but there is little experimental proof to

support this notion. On the contrary, recent studies have indicated that the dose of x-rays has little, if any, direct effect on the cancer cells. The above tests suggest that the curative action of this agent depends largely, if not entirely, on the reaction induced in the surrounding tissue. Under the conditions of the experiments just reported the cancer cells inoculated into an x-rayed area themselves received no x-ray, and therefore any changes taking place in them must be secondary to the altered condition induced by x-ray in the surrounding tissue.

Experiment.—An area of skin in the groins of mice was exposed to x-ray in the same manner as that described in the foregoing experiments. 7 days later cancer grafts were inoculated intracutaneously into the x-rayed areas, and like grafts in the corresponding locations of the non-radiated groin. The animals were killed in groups for histological study, 48 hours, 4 days, and 7 days after the cancer inoculation.

The grafts removed 24 hours after inoculation were found to consist of a necrotic mass with islands of healthy cancer cells scattered here and there. In and around the grafts there was an acute polymorphonuclear and a mild lymphoid reaction. In the condition of the inoculated material and the reaction immediately surrounding it, no difference could be discovered between the grafts from the two sides, but in the x-rayed area there was a marked lymphoid reaction in the skin layers, which, however, did not come into direct contact with the graft.

In the x-rayed area the necrotic debris of the original mass of graft tissue had been largely removed at the end of the 48 hour and 3 day periods, and the islands of healthy looking cancer cells showed frequent mitotic figures and seemed larger than at the previous period. These islands often formed a more or less continuous ring around the remains of the necrotic mass. The polymorphonuclear reaction had almost subsided and was replaced by a more extensive reaction of the cells of the lymphoid variety. There was also pronounced activity of the fibroblastic tissue, and the cellular infiltration of the skin layers was still very prominent. At the same period cancer grafts in the protected areas were found to be in a more active stage of growth, islands of cancer cells tending to coalesce and to form irregularly shaped

masses of healthy looking tissue. The polymorphonuclear reaction was much reduced, as in the x-rayed area, while the lymphoid cell infiltration was now present to only a limited extent around the cancer grafts.

At the 4 to 5 day periods the difference between the cancer grafts in x-rayed areas and those in protected areas was that degenerative changes had become even more prominent in the former, while the latter continued to grow actively.

The first event in this degenerative process, as seen under the microscope, was the swelling of both nucleus and cytoplasm. The cytoplasm gradually became more acidophilic, and the nucleus hyperchromatic. The nucleus finally lost its structure, became more deeply stained, and later uniformly pycnotic. The general cell structure also became less and less distinct, and with the fragmentation of the pycnotic nuclei the cells were reduced to debris.

It was observed also that two or more cells often coalesced to form giant cells with highly vacuolated cytoplasm, this type of change being found more frequently in small cell groups which had become imbedded in a fibrous matrix. It was not uncommon to find the nuclei pushed to the periphery of some cells by large masses of inclusion bodies. In later stages of degeneration the nuclei lost their staining capacity and appeared as the so called "ghost cells" (Figs. 35 and 36) and then finally became unrecognizable, leaving the cells as irregularly shaped masses of homogeneous appearance.

This degenerative process was generally complete by the 7th day, when the remains of the graft in the x-rayed area were represented by a small mass of necrotic debris, attended by some polymorphonuclear cells and macrophage reaction.

It may be noted that the changes described are in every respect similar to those that have been reported as the typical changes taking place in cancer cells following radiation and attributed to the direct action of the ray on tumor cells.

ABSENCE OF LOCAL CELLULAR REACTION AND THE GROWTH OF CANCER GRAFTS.

The converse to the above experiments would be the study of the effect of the absence of a local reaction in a resistant animal. As noted

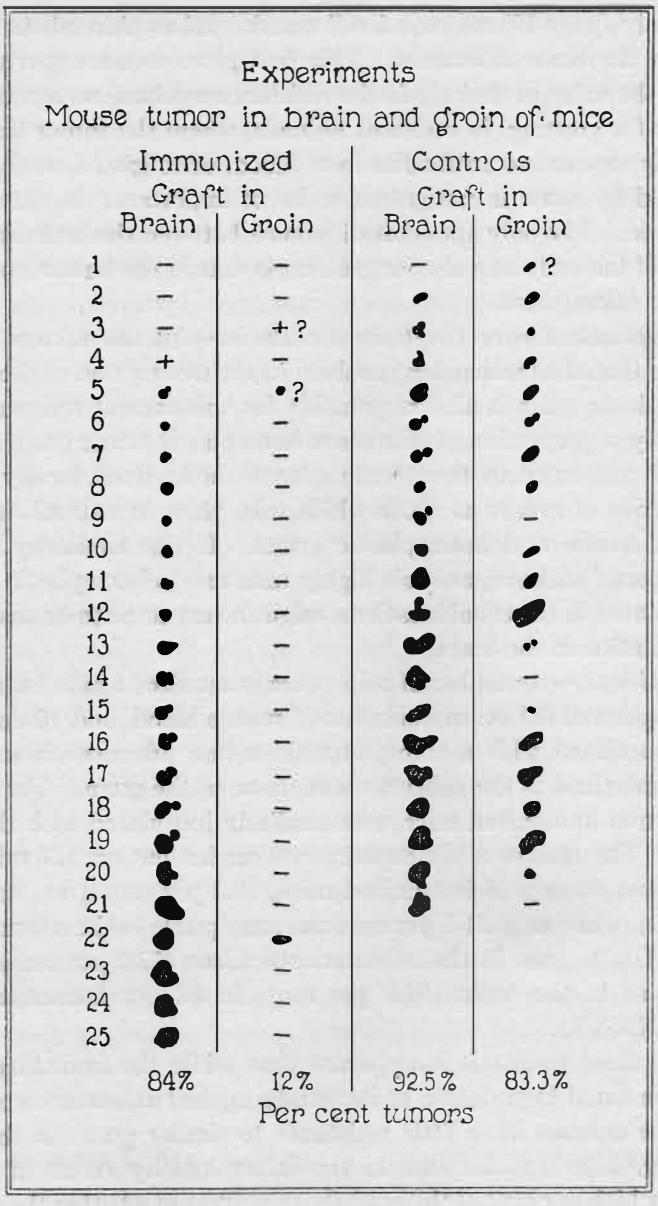
previously (page 19) no round cell reaction takes place about a tissue graft in the brain substance. This fact gives us an opportunity of testing the value of the cells in the resistance mechanism, for we have a locality for grafting, in resistant animals, where the tumor tissue will be freely exposed to antibodies from blood, or to local fibrosis, factors supposed by some investigators to be of importance in determining resistance. The only apparent difference between the brain and other tissues of the body as a site for grafting is that in the former no cellular reaction takes place.

Homoplastic Tumor Grafts in the Brain.—All the factors studied indicate that the mechanism involved in the destruction of the heteroplastic tissue graft is also responsible for the natural resistance possessed by a proportion of animals to homoplastic tumor grafts. Even induced resistance to tumor transplants shows itself locally by the same series of events as those which take place in natural resistance to both homo- and heteroplastic grafts. If this similarity is more than a superficial one, animals highly resistant to homoplastic tumors, transplanted in the usual locations, might be expected to be susceptible to inoculation in the brain.

Experiment.—A number of mice were immunized against tumors by the injection of 0.2 cc. of defibrinated mouse blood, and 10 days later were inoculated with a transplantable mouse adenocarcinoma, both in the brain and in the subcutaneous tissue of the groin. For control, normal non-immunized mice were similarly inoculated with the same tumor. The results of six experiments carried out on 127 mice were as follows: Among 65 immunized mice, 89.2 per cent grew tumors in the brain, while only 21.5 per cent was susceptible to the subcutaneous graft. Grafts grew in the subcutaneous tissue (82.2 per cent) almost as well as in the brain (91.9 per cent) in 62 non-immunized mice (Table XXXV).

From these results it is apparent that while the immunized mice show the usual high degree of immunity against subcutaneous grafts, the same animals have little resistance to similar grafts in the brain (Text-fig. 25). Inoculations in the latter locality result in tumors in a very high per cent of the animals, regardless of whether the animals are immunized or not.

The histological study brought out, as in the case of the hetero-


Experiments
Mouse tumor in brain and groin of mice
Immunized
Graft in
Brain
Groin
Controls
Graft in
Brain
Groin
1 – – – –?
2 – – – –
3 – +? – •
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21 – – – –
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84% 12% 92.5% 83.3%
Per cent tumors

TEXT-FIG. 25.

plastic tissue experiment previously noted, that when a graft came in contact with the ventricle in a resistant mouse, a marked cellular reaction occurred, producing a more or less complete destruction of the tumor graft. On the other hand, grafts in susceptible mice frequently grew into the ventricle and invaded the choroid plexus without inducing an unusual reaction.

With heteroplastic tissue grafts in the brain it was found that a resistance could be supplied by the local introduction of autologous lymphoid tissue. This same point has been investigated with homoplastic tumor grafts in resistant animals with the addition of auto- and homospleen tissue.

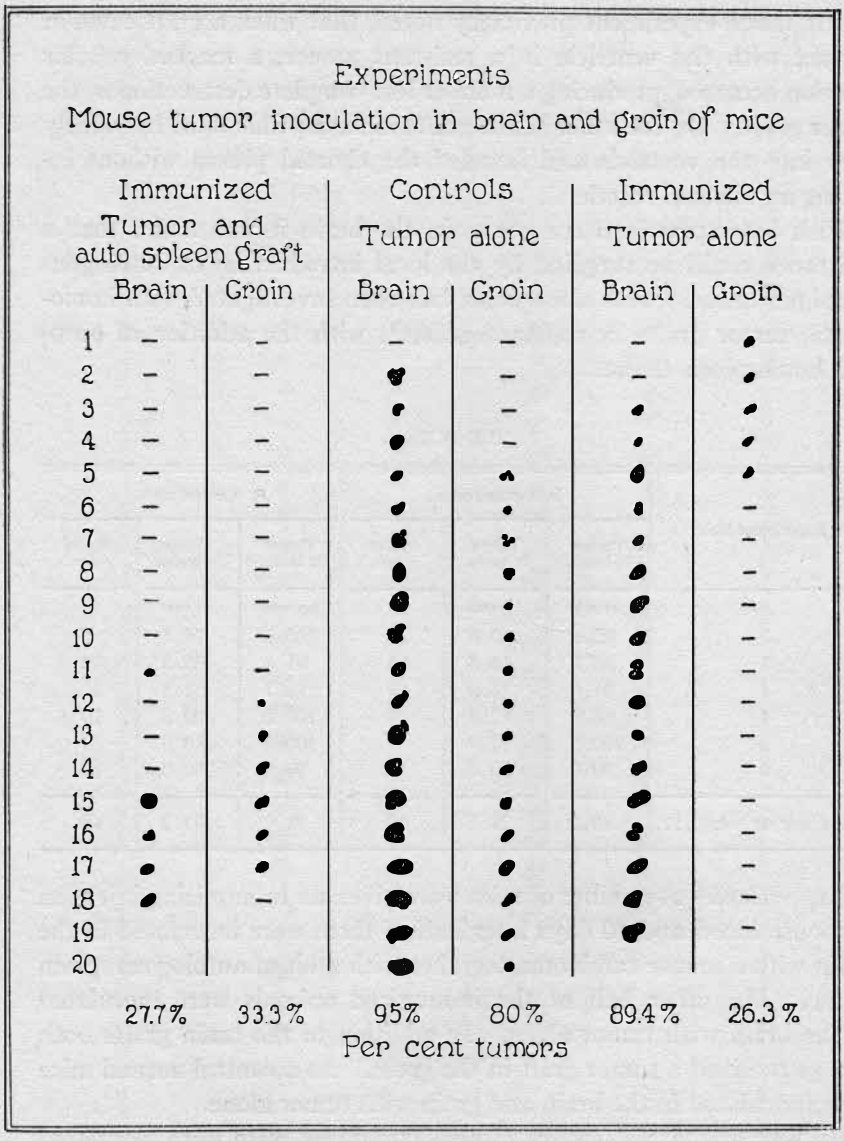
TABLE XXXV.

Experiment No.	Immunized mice.			Control mice.		
	Tumor in brain.	Tumor in groin.	No. of mice.	Tumor in brain.	Tumor in groin.	No. of mice.
	per cent	*per cent*		*per cent*	*per cent*	
1	80.0	10.0	10	100.0	77.7	9
2	86.6	13.3	15	91.6	83.3	12
3	91.6	25.0	12	72.7	81.8	11
4	88.8	12.0	9	100.0	90.0	10
5	100.0	22.0	9	100.0	80.0	10
6	90.0	30.0	10	90.0	80.0	10
Average or total.....	89.2	21.5	65	91.9	82.2	62

Experiment.—A number of mice were given an immunizing injection of mouse blood and 10 days later half of them were inoculated in the brain with a mouse carcinoma together with a bit of autologous spleen tissue. The other half of the immunized animals were inoculated in the brain with tumor alone. In addition to the brain grafts both groups received a tumor graft in the groin. As a control normal mice were inoculated in the brain and groin with tumor alone.

By way of contrast groups of non-immunized mice were inoculated in the brain with auto spleen grafts and tumor, with homo spleen grafts and tumor, and with tumor alone. All of these animals also received tumor grafts in the groin (Table XXXVI).

By comparing the figures in the table it is evident that the inocula-



TEXT-FIG. 26.

tion of autospleen along with the tumor tissue into the brain of immunized mice renders this location as resistant as the subcutaneous

tissue (Text-fig. 26). Even in non-immunized mice this combined inoculation in the brain results in fewer tumors than would be expected if the tumor had been inoculated alone, but this difference is not so great as that seen in the immunized animals. On the other hand, the addition of homosplenic tissue has no influence on the fate of tumor grafts in the brain.

A study of the effect of auto- and homoplastic splenic tissue on the fate of tumor grafts inoculated in the subcutaneous tissue may be

TABLE XXXVI.*

Immunized mice.				Control mice.	
27 mice inoculated with.		28 mice inoculated with.		30 mice inoculated with.	
Auto spleen graft and tumor in brain.	Tumor alone in goin.	Tumor alone in brain.	Tumor alone in groin.	Tumor alone in brain.	Tumor alone in groin.
per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
29.6	25.9	89.3	31.4	96.6	83.3
Non-immunized mice.				Control mice.	
20 mice inoculated with.		19 mice inoculated with.		20 mice inoculated with.	
Auto spleen graft and tumor in brain.	Tumor alone in groin.	Homo spleen graft and tumor in brain.	Tumor alone in groin.	Tumor alone in brain.	Tumor alone in groin.
per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
55.0	75.0	84.2	84.2	95.0	70.0

* The figures in this table are based on the percentage of progressive tumors in each group.

reported in connection with the above results. The splenic tissue in this experiment was mixed with the tumor cells before inoculation. The results based on the inoculation of 114 animals were 54.6 per cent of takes when autosplenic tissue was used, 80 per cent of takes with homosplenic tissue, and 94 per cent when the tumor was inoculated alone. These figures are closely in line with those from the brain inoculation.

SUMMARY AND DISCUSSION.

Two methods were utilized to induce a local cellular reaction of the kind which ordinarily takes place about a cancer graft in a resistant animal and the effect of this reaction in both cases proved deleterious to implanted cancer cells. The local lymphoid reaction induced by injecting foreign blood into a sensitized animal proves almost as effective in controlling the growth of a cancer graft as does the massive general reaction of a resistant animal. The skin reaction which follows a local x-ray exposure results in the appearance of a resistant state but the enhanced resistance does not extend even to the underlying connective tissue. In this instance the refractory state was observed only in the presence of the cellular reaction. Why this reaction can be induced in the subcutaneous tissue when it is exposed directly but does not take place if the rays first pass through the skin is not clear, but under these circumstances the subcutaneous tissue shows a definite increased resistance to tumor growth.

The reverse condition, the absence of a cellular reaction in a resistant animal, results in the failure of the resistance mechanism. No evidence is at hand to explain the absence of lymphoid reaction in the brain tissue but no reaction of this kind does take place and even highly resistant animals prove susceptible if the grafts are placed in the brain. As in the case of heteroplastic grafts, the resistance mechanism is effective if autosplenic tissue is supplied to that organ.

The local reaction, therefore, appears to constitute the resisting force and may be effective in the absence of a general reaction; conversely, the general reaction without local response results in no resistance to tumor growth.

EXPLANATION OF PLATES.

PLATE 18.

FIG. 32. The result of an intracutaneous inoculation of cancer grafts in an area previously exposed to x-ray (left side) compared with the result of a similar inoculation in an untreated area (right side).

PLATE 19.

FIG. 33. Cancer graft in an x-rayed area showing intensity of cellular reaction. Remains of cancer cells seen on left.

FIG. 34. Cancer graft in an untreated area. Actively growing cancer cells are seen on left with an absence of any reaction either at the edge or in the surrounding tissue.

PLATE 20.

FIG. 35. A small group of cancer cells imbedded in fibrous tissue, showing binucleated and highly vacuolated giant cell (A), and giant cells with nuclei pushed to the periphery (B).

FIG. 36. The same as Fig. 35, showing a binucleated giant cell (A), and so called "ghost cells" (B).

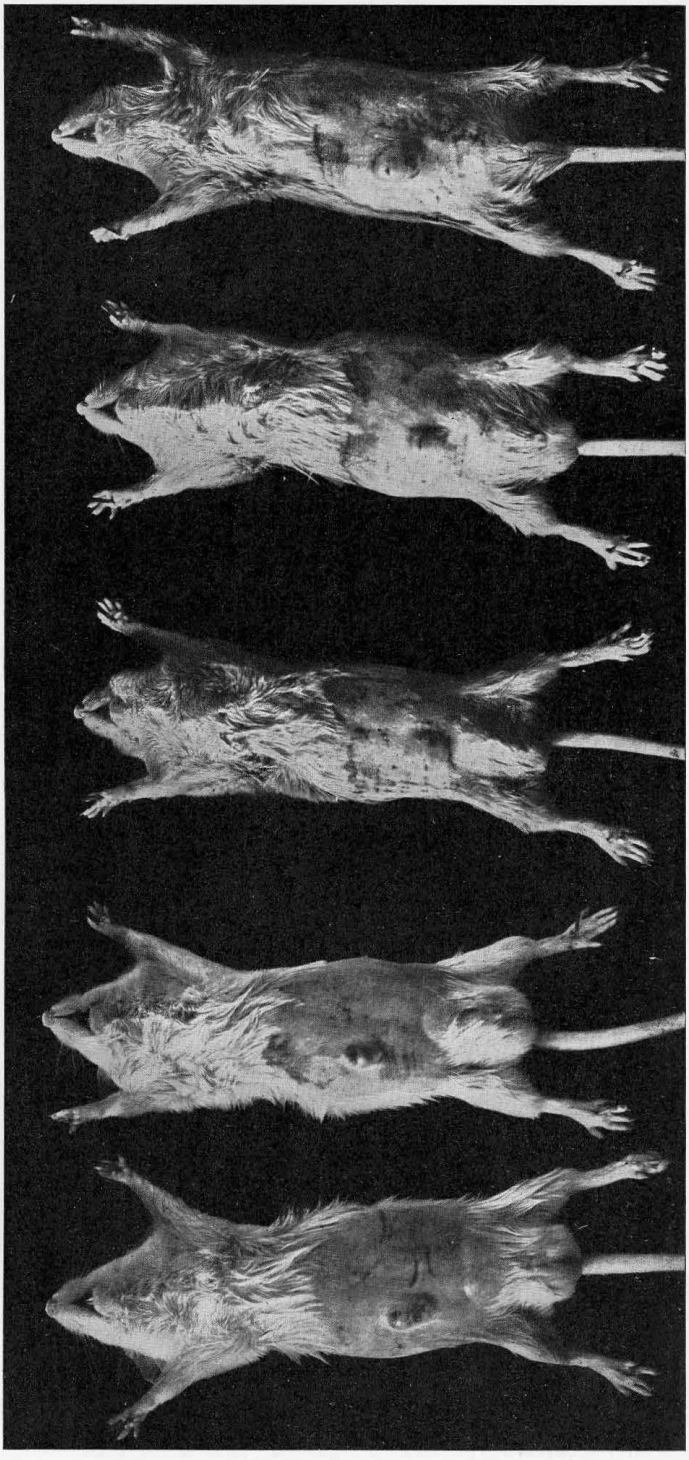


FIG. 32.

(Murphy: Cellular reaction and resistance to cancer.)

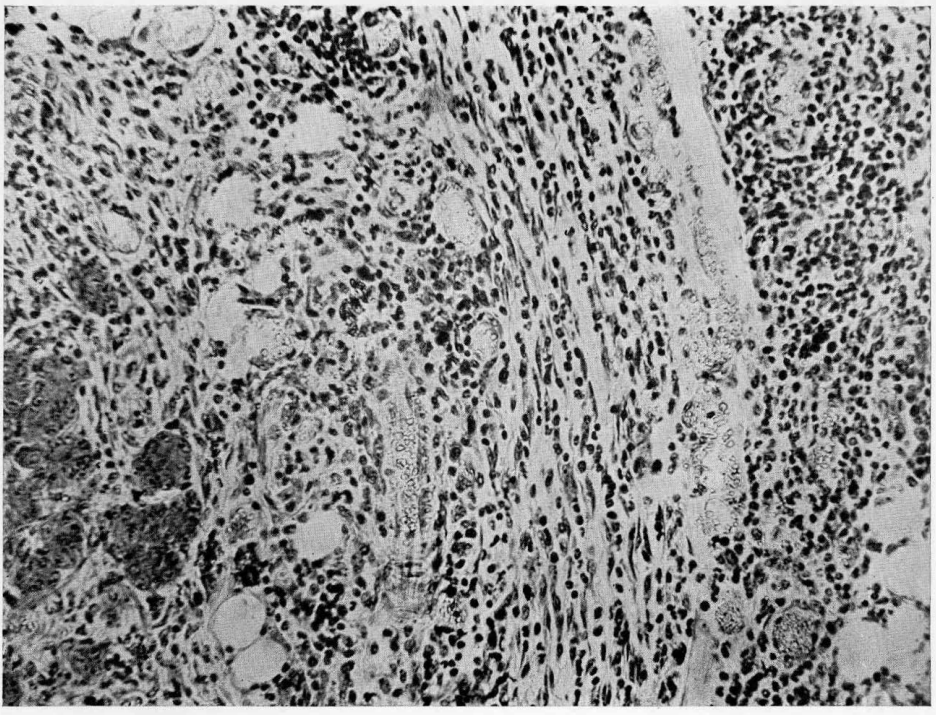


FIG. 33.

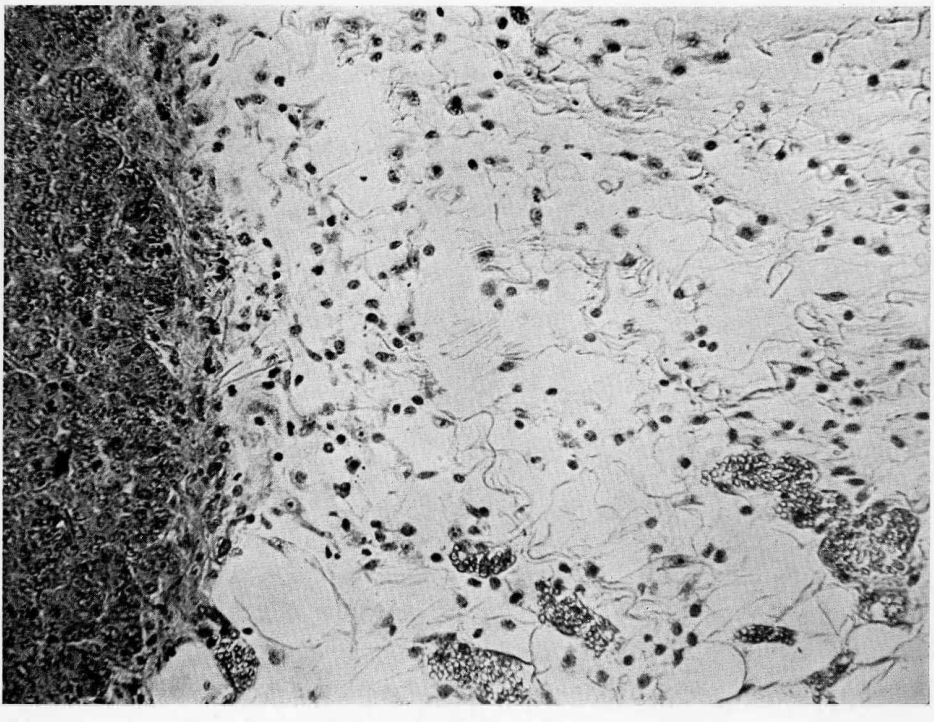


FIG. 34.

(Murphy: Cellular reaction and resistance to cancer.)

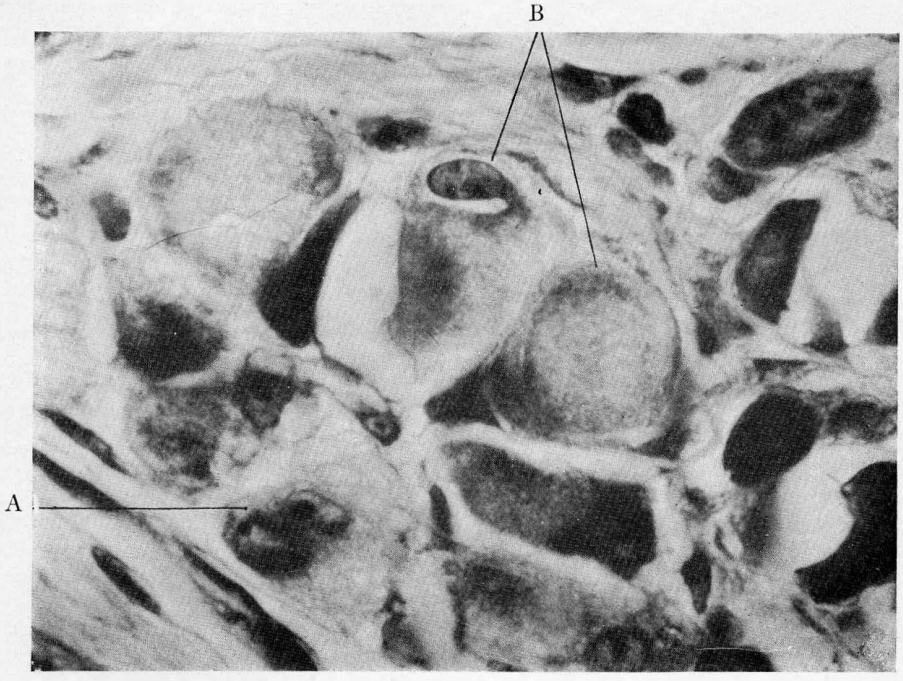


FIG. 35.

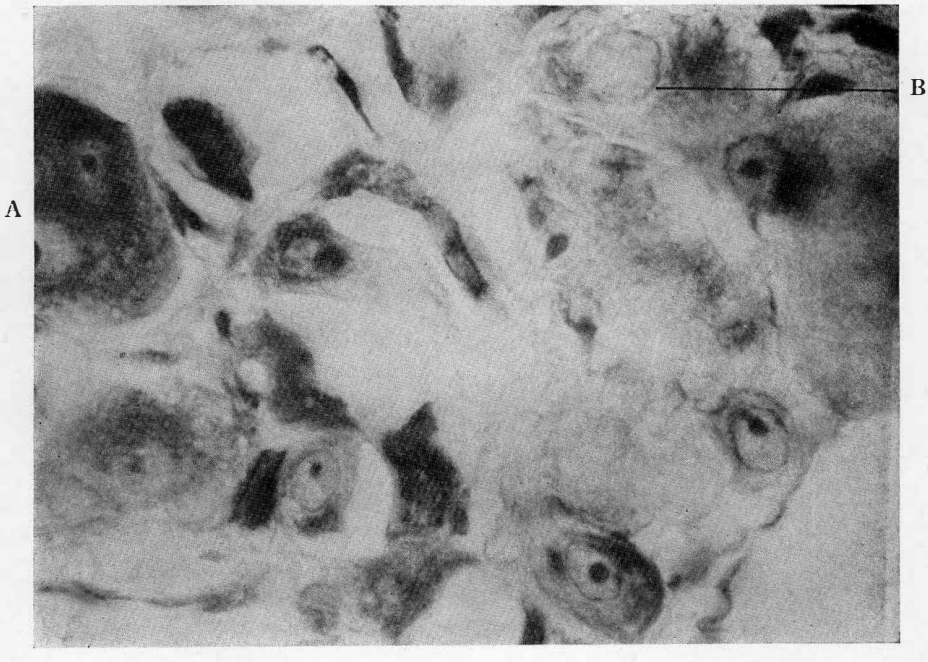


FIG. 36.

(Murphy: Cellular reaction and resistance to cancer.

VI. EFFECT OF GENERAL AND LOCAL LYMPHOID STIMULATION ON RESISTANCE TO SPONTANEOUS TUMORS.

In the preceding section we have shown that a small dose of x-ray, an exposure to dry heat, or an injection of a fatty acid, will increase resistance of mice to transplanted cancer. It is generally conceded that any deduction from experiments with transplanted tumors, when applied to cancer as a disease, must be accepted with definite reservations. The transplanted cancer unquestionably retains the essential tissue character of its original bearer, although it often produces on inoculation into other animals, a general affection with manifestations entirely analogous to those of the spontaneous disease.

Until the present experiments were made no means of influencing the resistance of animals to their own spontaneous tumors had been found. A few instances of spontaneous regression had been reported, but these were so rare as to be of no importance. Even after careful surgical removal, the disease recurs in 50 per cent or more cases (Murray (1) and Haaland (2)), while autografts succeed in practically 100 per cent.

Before we could apply the deductions made from the observations of the influence of x-ray, heat, and fatty acids on resistance to transplanted tumors to cancer as a disease, it was necessary to confirm the results with spontaneous cancer. There were certain obvious difficulties, such as the belief that the cancer cells are more susceptible to x-ray and heat than are normal cells. Therefore it was a requirement that the animal should receive the full benefit of the treatment but that the tumor should be spared. On this account we resorted to the measure of removing the tumor at operation, and then subjected the animal to x-ray or heat exposures, immediately followed by a return of a piece of the tumor. As noted above it cannot be considered that removal and replanting spontaneous tumor material interrupt the progress of the disease, for local recurrences take place

in 50 per cent of the animals and approximately 100 per cent of the returned tumors grow. From these facts we consider that any change brought about in these figures may be interpreted as interference with the natural progress of the disease.

EFFECT OF X-RAY ON THE RATE OF GROWTH OF SPONTANEOUS TUMORS IN MICE.

Series 1.—This group consisted of 52 mice with various types of spontaneous mammary cancers. The tumors were removed as completely as possible by operation, and, with the cancer out, the animal was exposed to a stimulating dose of x-rays (Coolidge tube) previously described. Immediately afterwards a graft of the original cancer was implanted in the groin of the animal. A complete immunity to the recurrence of the disease resulted in 26 of the 52 animals treated in this manner. In these animals there was no evidence of a local recurrence at the site of operation, or of the implanted graft, or of metastasis. Among the remaining 26 animals of the series the average time for the graft to become palpable was 5 weeks and 4 days, a figure which contrasts strongly with the figure for the control animals. The number of recurrences at the original location of the tumor was 11 among the 52 animals, all occurring in the latter 26. Only those animals were included in this number that lived and remained in good physical condition for at least 5 weeks after the treatment. The majority lived from 2 to 4 months, some to 8 months and over.

Total white blood cell counts and differentials were done on all of the mice before operation or treatment and on part of them at intervals afterwards. In the limited number counted systematically the animals rendered resistant showed a definite increase in the lymphocytes. Blood counts on a large number of mice with spontaneous tumors showed a lymphoid cell content in the blood at least equal to that of normal animals.

Series 2.—This control series was made up of 29 mice with spontaneous tumors of various sorts. The tumors were removed by operation and autografts reimplanted in the same manner as in the first series, but without x-ray treatment to either the animal or the cancer. In making autografts tumors were kept outside the body

for the same length of time as in the first series. In 28 of the 29 animals the grafts grew progressively, and these became palpable on the average of 1 week and 5 days after implantation. In 1 the graft grew for a period and then retrogressed to complete absorption. Local recurrences at the site of operation occurred in 14 of the 29 animals.

Series 3.—10 mice were freed from their spontaneous tumors in the same manner as in the first two series. The removed cancers were subjected to the same amount of x-radiation that the animals had received in the first series, and an autograft from the cancer, after this treatment *in vitro*, was returned to the groin of the original host. All ten of the returned grafts grew, becoming palpable on the

TABLE XXXVII.

Series No.*	Immune.	Susceptible.	Local recurrence of tumor.	Average time for appearance of graft.
	per cent	*per cent*	*per cent*	
1	50.0	50.0	21.2	5 weeks, 4 days.
2	3.4	96.6	48.3	1 " 5 "
3	0.0	100.0	40.0	1 " 3 "

* Series 1, animals treated by x-ray, while cancer was out. Later a graft of the tumor was returned. Series 2, control animals in which cancer was removed and a graft returned without treatment to either animal or tumor. Series 3, cancers removed and subjected direct to x-ray treatment and a graft returned to the original host.

average of 1 week and 3 days after implantation. 4 of the 10 animals showed local recurrences.

For the sake of comparison the figures for the three experiments are given in Table XXXVII.

The above experiments show conclusively that a small dose of x-ray administered to mice with spontaneous cancers is capable of rendering 50 per cent of the animals resistant to returned autografts, while the same amount of x-ray given direct to the cancer outside of the body is without effect. The difference in percentages of local recurrences between the treated and untreated groups cannot be due to the direct action of the rays on the tumor cells for it is evident that x-ray in this dosage is without effect. The contrast between the

numbers of positive autografts in the control and x-rayed series is striking as is also the delay in the time of appearance of the graft in the latter series.

EFFECT OF DRY HEAT ON RESISTANCE TO SPONTANEOUS TUMORS.

In the next experiment dry heat was used as the method of producing lymphoid stimulation instead of x-rays. An exposure for 5 minutes to dry heat at a temperature ranging from 55 to 63°C. will engender in a mouse so treated a marked increase in the circulating lymphocytes, preceded by a slight transitory fall, and, as already noted, this lymphocytosis lasts from 2 to 4 weeks. The increase in the circulating lymphocytes is associated with a marked hyperactivity on the part of the lymphoid germ centers as evidenced by the appearance in them of numerous mitotic figures (see Section IV).

Experiment.—61 mice showing various types of mammary carcinomas were used. The tumors were removed as completely as possible by operation, and while the tumor was out the animal was heated for 5 minutes according to the previously described technique. Immediately after heating, a graft of the tumor was reinoculated subcutaneously into the left groin of the original animal.

36 of the 61 animals treated in the above manner proved to be completely resistant to the growth of the autograft. No metastasis was observed in any of these 36 animals at autopsy. Among the remaining 25 animals which were not resistant the average time for the graft to become palpable was 2 weeks and 5 days. Local recurrences occurred in 7. In some instances a recurrence later retrogressed, but these have not been recorded as resistant animals. Only those mice were included in the series which lived for at least 4 weeks and remained in good condition, but the majority lived for a much longer period than this.

For control we used Series 2 of our x-ray experiment just described. The animals in this series were derived from the same stock and were *treated in the same way as the above series except that they were* not heated. Of 29 animals the returned graft grew in 28, and the average time required for the graft to become palpable was 1 week and 5 days. Local recurrences were present in 14 out of the 29 animals. Table XXXVIII gives a comparison of the two series.

Blood counts were made on 38 of the animals of the treated series, one before operation, the next, 1 week after operation and exposure to heat, and subsequent counts at weekly intervals. The average number of lymphocytes per c.mm. of blood before operation was about 12,000. 1 week after operation and heating they had risen to approximately 16,000, and they continued to increase until by the fourth count there was an average of 20,000 lymphocytes per c.mm. of blood. The polymorphonuclear leucocytes were somewhat lower after treatment in 20 of 38 animals. In the other 18, except in a few in which extensive infection occurred, there was only a slight gain. These fluctuations in the white cells correspond with those reported for normal animals after exposure to heat. Animals

TABLE XXXVIII.

Series No.*	Immune.	Susceptible.	Local recurrences.	Grafts alone.	Time for appearance of graft.
	per cent	*per cent*	*per cent*	*per cent*	
1	59.4	40.6	11.3	14.7	2 weeks, 5 days.
2	3.4	96.6	48.3	48.3	1 " 5 "

* Series 1, 61 mice with spontaneous cancers heated after the removal of the tumor with later a return of a graft. Series 2, 29 control mice with cancer removed at operation and later a return of a graft.

subjected to the same surgical procedure without being heated showed no such change.

The result of the heat treatment is even more marked than that of x-ray. The percentage of animals resistant to autograft results from the former treatment was 59 per cent and for the latter 50 per cent. This difference was also evident in the local recurrence, for after heat there were 11.3 per cent and after x-ray 21.2 per cent. This difference in favor of the heat treatment is detectable in the amount of stimulation of the lymphoid tissue and also in the degree of resistance induced to the transplanted tumors.

RESISTANCE TO SPONTANEOUS MOUSE TUMORS INDUCED BY INJECTIONS OF OLEIC ACID (NAKAHARA).

We have shown in a preceding section that resistance to transplanted cancer in mice can be intensified by a previous injection of a

small quantity of olive oil, or such unsaturated fatty acids as oleic, linoleic, and linolenic acids. It is of interest in our study of the question of resistance to spontaneous tumors to test the action of fatty acids in this respect.

Material.

The mice for these experiments were taken from the tumor stock maintained at The Rockefeller Institute, a continuation of the Lathrop strains.

All the tumors included in this study occurred in the mammary region of female mice. Histological examination showed about 55 per cent to be adenocarcinomas and about 40 per cent alveolar carcinomas, both groups including a fair proportion of the cystic and hemorrhagic varieties. There was a single case each of squamous cell carcinoma, lymphoma, and adenocarcinoma sarcomatodes.

The age of mice at the time of operation varied between 10 and 28 months, with the average about 17½ months. The tumors, in the majority of instances, were of recent origin, but it is difficult to be certain of the duration of the disease.

PREVENTION OF POSTOPERATIVE RECURRENCE.

We have first tested the effect of oleic acid injections on the incidence of return of spontaneous mammary cancers in mice after an attempt at their complete removal by operation.

Experiment 1.—50 mice were operated on and their tumors removed as completely as possible. 48 hours later, the mice received an intraperitoneal injection of 0.5 cc. of 1 per cent emulsion of oleic acid. A second injection of the same amount was given 2 weeks later, and repeated thereafter at intervals of 1 to 2 months. The mice were kept under uniform living conditions and were observed as long as they lived. Careful autopsy was performed as soon after their death as possible.

For control, 25 mice were operated on and the tumors excised in the same manner as in the experimental series. These mice were given no injection, but were merely placed under the same living condition as the other group for observation. Autopsy was likewise performed at their death.

Mice that died within 6 weeks after operation were only included in the final consideration of results when they had shown a recurrence. It may be stated that under ordinary circumstances local recurrence usually appears within 6 weeks, if ever.

The oleic acid emulsions were prepared either by the addition of a sufficient amount of hydrochloric acid to a solution of sodiumoleate (Merck) or directly from oleic acid with the addition of sodium hydroxide to stabilize the emulsion.

The results of this experiment are summarized in Table XXXIX and are graphically represented in Text-fig. 27. The points noted are: local recurrence at the site of operation, development of new primary tumors at other locations, and macroscopic metastases encountered at autopsy.

Some of the internal tumors found at autopsy were of such a histological character that they must be regarded as primary tumors, rather than as metastases, but these have not been tabulated separately. With one exception the metastases were in the lungs.

The average postoperative longevity in the treated mice was 147 days, and 49 days longer than that in the control series (98 days). This difference appears significant in view of the similarity of the

TABLE XXXIX.

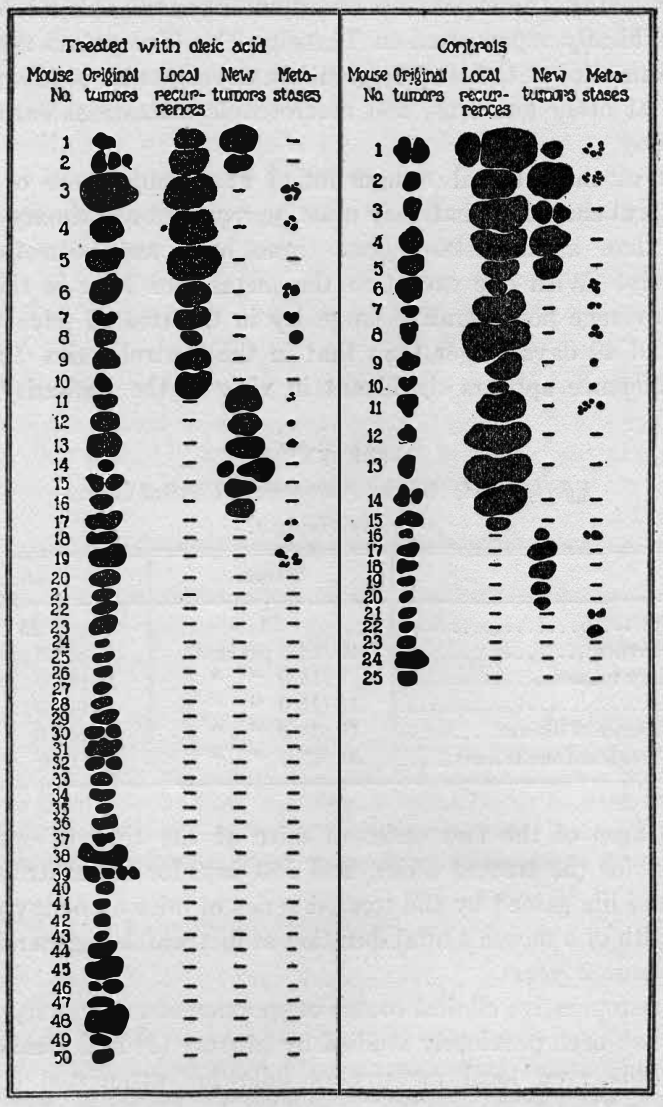
Effect of Oleic Acid on Postoperative Clinical Course.

Experiment 1.

	Treated.	Controls.
No. of mice	50	25
Local recurrence	10 (20.0 per cent).	15 (60.0 per cent).
New primary tumors	8 (16.0 " ").	10 (40.0 " ").
Metastases	11 (22.0 " ").	11 (44.0 " ").
Total recurrence of disease	19 (38.0 " ").	22 (88.0 " ").
Complete freedom from tumors	31 (62.0 " ").	3 (12.0 " ").

average ages of the two series of mice at the time of operation: 581 days for the treated series, and 560 days for the controls. The 49 days of life gained by the treated series of mice amount to nearly one-twelfth of a mouse's total duration of life, which is generally held to be about 2 years.

The postoperative clinical course of spontaneous mammary cancers in mice has been previously studied by Murray (1) and Haaland (2). Murray observed local recurrences following attempted complete removal in 23 of 48 operated mice, and Haaland in 96 of 174. In our own series, reported above, we obtained approximately the same proportion. The postoperative longevity in Murray's series "averaged 3 to 6 weeks in the later operations and in 5 cases was more than 100 days." Haaland reported the average to be about 15 weeks



TEXT-FIG. 27. Relative sizes of tumors in Experiment 1. The blackened masses represent the original tumors at the time of their excision, local recurrences, primary tumors subsequently developed, and the metastases found at autopsy.

(105 days). These figures are in close agreement with our findings in the control series.

SUPPRESSION OF AUTOPLASTIC TUMOR GRAFTS.

In order to confirm and extend the results of the above experiment, we next tested the influence of oleic acid injections on the growth of autoplastic grafts of spontaneous tumors in mice.

Experiment 2.—54 mice with spontaneous mammary carcinomas were freed from their tumors as completely as possible by operation, and grafts of the neoplastic material were at once reimplanted in the subcutaneous tissue of the animals through a hollow needle. 48 hours later, these mice were injected intraperitoneally with 0.5 cc. of 1 per cent emulsion of oleic acid, and subsequent injections

TABLE XL.

Effect of Oleic Acid on Autografts.

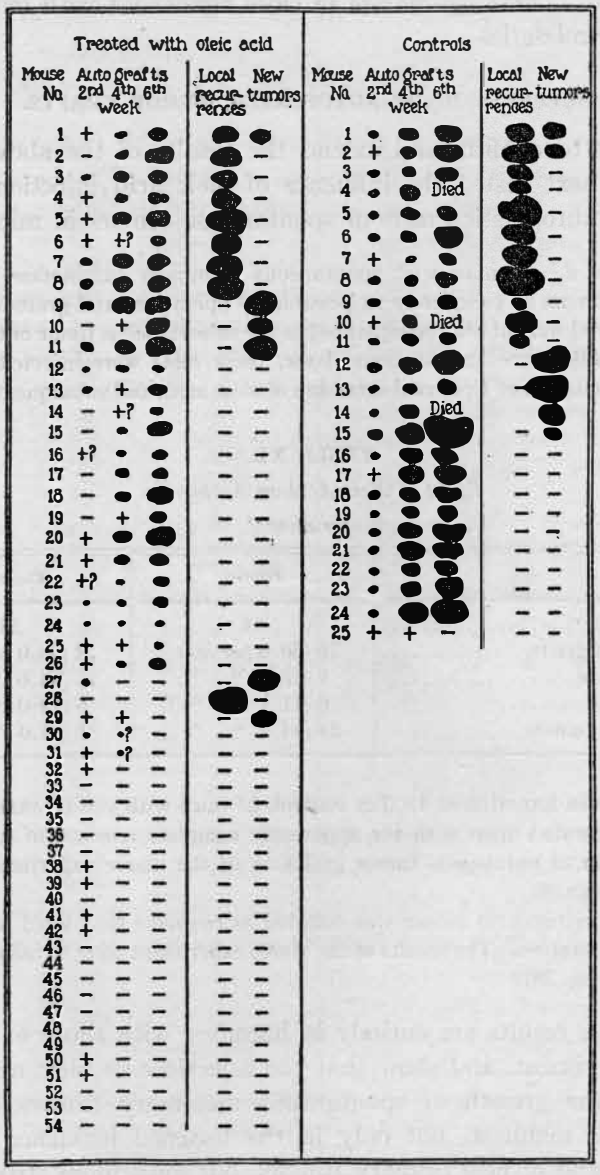
Experiment 2.

	Treated.	Controls.
No. of mice	54	25
Positive tumor grafts	26 (50.0 per cent).	24 (96.0 per cent).
Local recurrence	9 (16.6 " ").	11 (44.0 " ").
New tumors	6 (11.1 " ").	6 (24.0 " ").
Freedom from tumors	24 (44.4 " ").	1 (4.0 " ").

were given as in Experiment 1. For control, 25 mice with similar mammary tumors were operated upon with the apparently complete removal of tumors and reimplantation of autologous tumor grafts as in the above experiment but no oleic acid was given.

In this experiment no mouse was counted as negative that lived less than 6 weeks after operation. The results of the above experiment were as follows (Table XL and Text-fig. 28):

The above results are entirely in harmony with those of the preceding experiment, and show that the injections of oleic acid interfere with the growth of spontaneous mammary tumors in mice. This fact is manifest, not only in the lessened incidence of local recurrence and of new primary tumors, but even more strikingly in the diminished frequency of takes of autoplastic tumor grafts. It may again be pointed out that the results in the control series are in close agreement with those of previous investigators.



TEXT-FIG. 28. Relative sizes of tumors in Experiment 2, showing the rate of growth of autoplastic cancer grafts during the 6 weeks immediately after operation, and the local recurrences and new primary tumors existing at the end of this time.

LOCAL RESISTANCE TO SPONTANEOUS MOUSE CANCER INDUCED BY X-RAY.

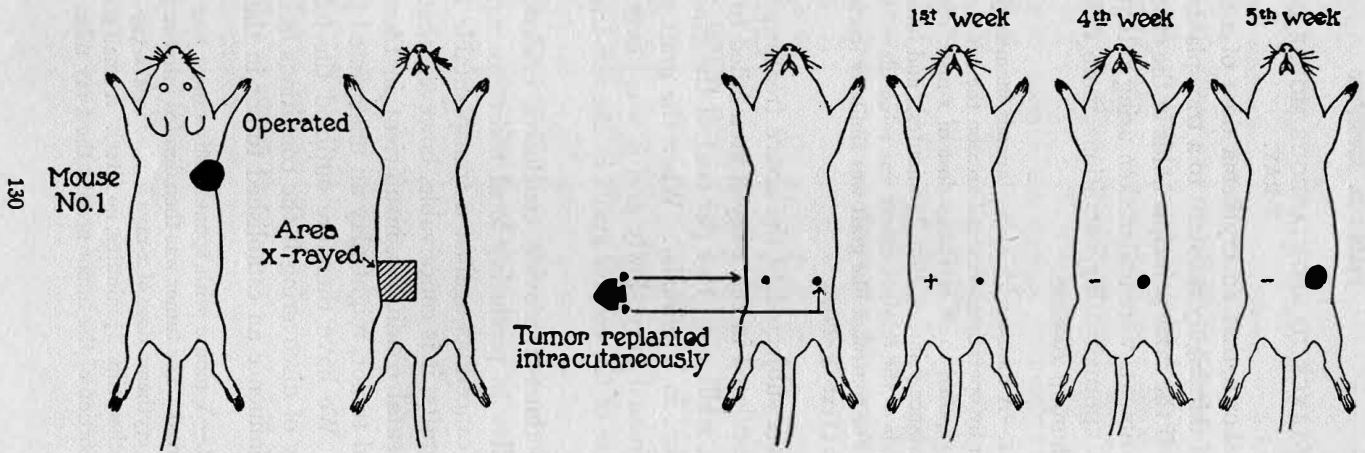
We have shown that an erythema dose of x-rays renders the exposed area of skin highly resistant to a transplanted cancer. In order to determine if the same principle holds in the case of the spontaneous disease, experiments were undertaken using autografts of spontaneous cancer, thus approximating conditions more closely to those existing for the human disease.

Experiment 1.—A mouse with a spontaneous mammary cancer was first freed from the tumor by operation. A small area on the left flank, measuring 12 × 15 mm., was then exposed to an erythema dose of x-rays (spark-gap 3 inches, milliamperes 10, distance 6 inches, time 2½ minutes), and immediately following this treatment a graft of the original cancer was reinoculated intracutaneously in the x-rayed area. For control, a like graft was similarly inoculated in the right non-irradiated flank (Text-fig. 29).

49 mice were subjected to the above treatment. 71.4 per cent of the grafts inoculated in the x-rayed area failed to grow (*i.e.*, in 35 of the animals), while only 16.4 per cent of the grafts in the protected area failed (*i.e.*, in 8 animals). When the graft grew in the x-rayed area, it progressed very slowly and it was never more than a fraction of the size of the control grafts in the non-irradiated skin (Text-fig. 30).

These experiments provide conclusive evidence that x-rays are just as effective in producing local resistance against autografts of spontaneous cancers as against a transplantable tumor. In dealing with tumor grafts, it is conceivable, however, that slightly unfavorable environmental conditions, insufficient in themselves to influence an established tumor, might play an unexpected part in experiments of this sort. We have therefore outlined the following experiment with the view of discovering if the conditions induced by x-rays are sufficient to influence an established tumor in the skin.

Experiment 2.—A mouse with spontaneous cancer was operated on and the tumor was removed. The tumor was then divided into two parts, and one of these subjected to an erythema dose of x-rays *in vitro* (spark-gap 3 inches, milliamperes 10, distance 6 inches, time 2½ minutes, no filter). A small graft was taken from the surface of this portion of the tumor nearest the x-ray tube, and it was inoculated


Spontaneous tumor mice
Autografts grew in x-rayed area in 30.6%, in normal area in 83.7%
50 mice so treated
1st week
4th week
5th week
Mouse No.1
Operated
Area x-rayed
Tumor replanted intracutaneously
+
-
-

TEXT-FIG. 29.

intracutaneously in the right flank of the original animal.[1] A similar graft from the non-irradiated portion of the tumor was inoculated in the left flank, also intracutaneously (Text-fig. 31). 50 mice were included in this experiment.

The cancer grafts x-rayed *in vitro* grew as rapidly as the untreated grafts. When the new tumors had become established, after about 10 days or longer, those originated from the non-radiated graft were given the same erythema dose of x-rays *in situ*, the irradiation including the surrounding normal skin as well as the tumor. A prompt disappearance of the tumor followed in 38 of the 50 animals (76 per cent) so treated. The grafts x-rayed *in vitro*, in the meantime, continued to grow rapidly, failing in only three animals (6 per cent). In instances in which the tumor x-rayed *in situ* did not disappear, there was a marked slowing down of the rate of tumor growth (Text-fig. 32).

These results demonstrate anew that a treatment dose of x-rays has little if any direct effect on the cancer cells, yet the same dose given to the surrounding normal tissue brings about healing in a majority of cases.

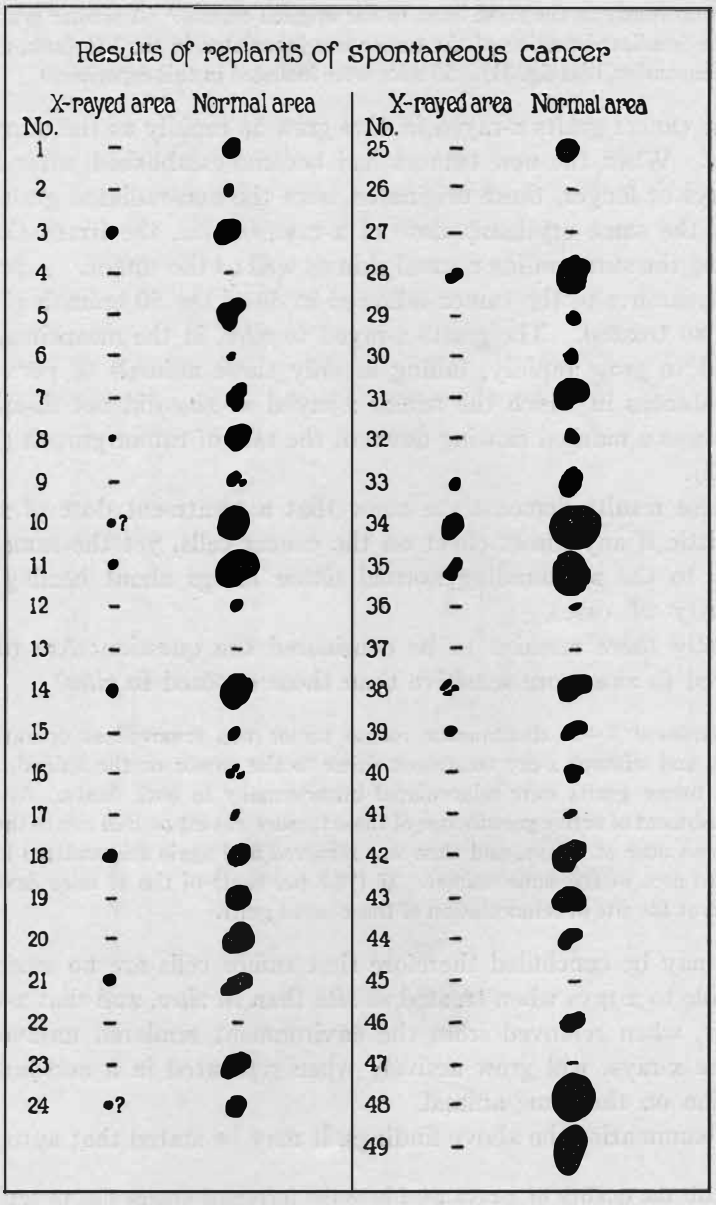
Lastly there remains to be considered the question: Are tumors x-rayed *in situ* more sensitive than those exposed *in vitro*?

Experiment 3.—A spontaneous mouse tumor was removed at operation as before, and without x-ray treatment either to the tumor or the animal, autologous tumor grafts were reinoculated intradermally in both flanks. After the establishment of active growth, one of these tumors was exposed *in situ* to the same erythema dose of x-rays, and then was removed and again reinoculated into an unrayed area of the same animal. 37 (78.8 per cent) of the 47 mice developed tumors at the site of reinoculation of the x-rayed graft.

It may be concluded therefore that tumor cells are no more susceptible to x-rays when treated *in situ* than *in vitro*, and that x-rayed tumor, when removed from the environment rendered unfavorable by the x-rays, will grow actively when replanted in a non-radiated location on the same animal.

To summarize the above findings, it may be stated that autografts

[1] With the quality of x-rays used here the increased dosage due to scattering would be theoretically as great in the locality from which the graft was taken as in a tumor of the surface layers of an animal exposed to the same initial dosage.


Results of replants of spontaneous cancer
No.
X-rayed area
Normal area
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
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28
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31
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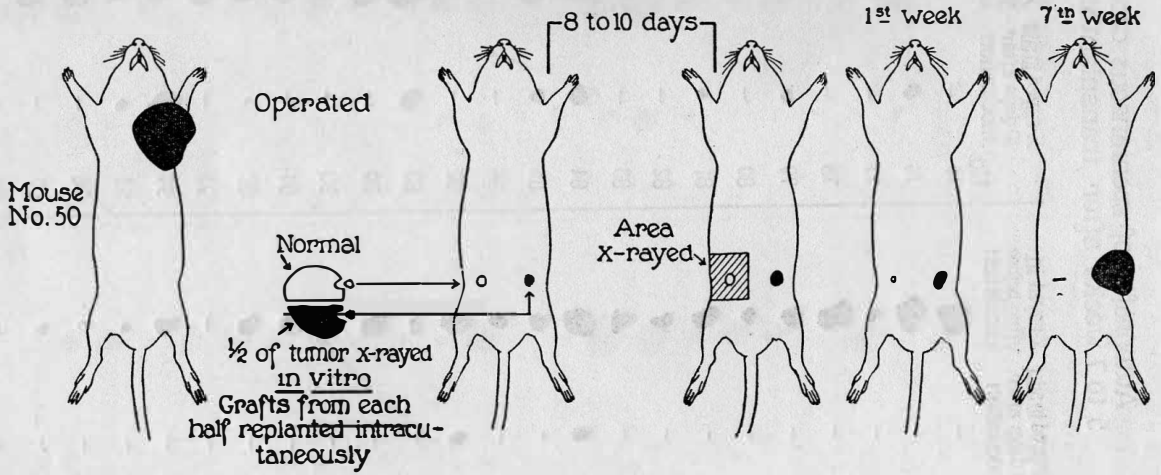
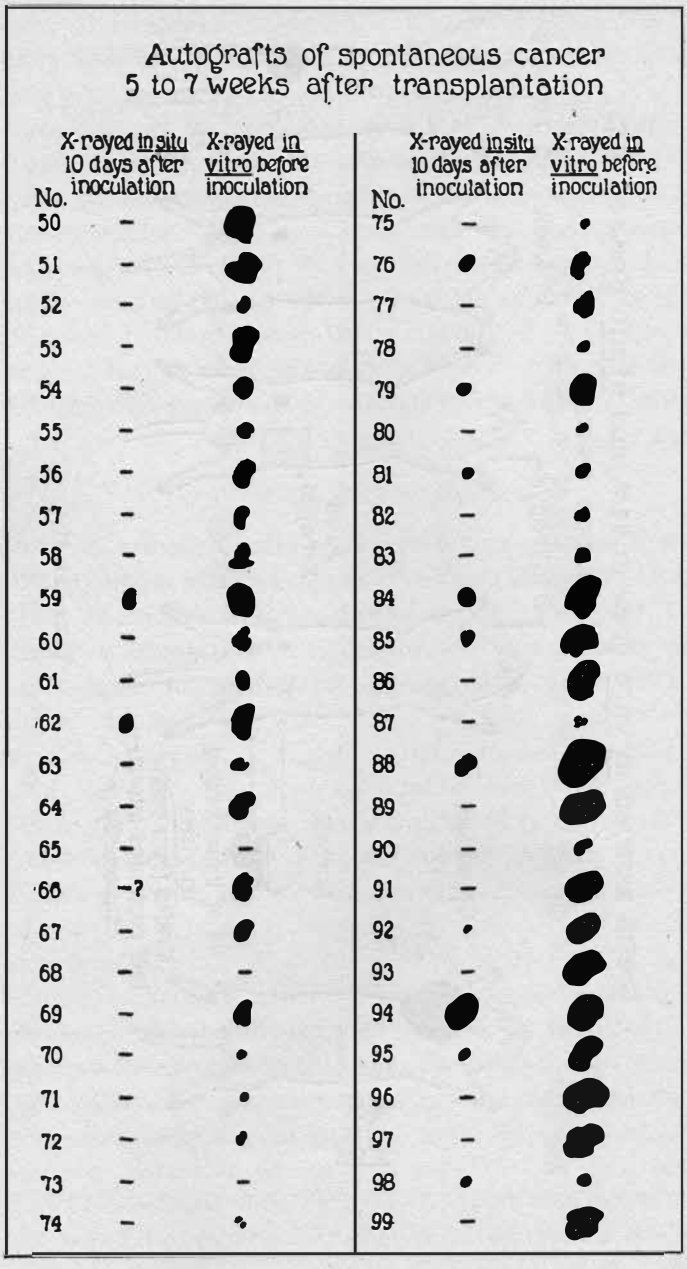
TEXT-FIG. 30.


Grafts x-rayed in vitro grew in 94 % and in situ grew in 24 %
50 mice so treated
8 to 10 days
1st week
7th week
Operated
Mouse
No. 50
Normal
Area
X-rayed
½ of tumor x-rayed
in vitro
Grafts from each
half replanted intracu-
taneously

TEXT-FIG. 31.


Autografts of spontaneous cancer
5 to 7 weeks after transplantation
No.
X-rayed in situ 10 days after inoculation
X-rayed in vitro before inoculation
50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74
75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99

TEXT-FIG. 32.

from spontaneous cancers of mice when replanted into areas previously exposed to an erythema dose of x-rays, failed to grow in the majority of instances. As is well known, autografts inoculated into the normal areas grew in a large proportion of the animals.

Even established intracutaneous autografts of spontaneous cancer usually disappeared when the tumor and surrounding tissues had been exposed to an erythema dose of x-rays. A like dose of x-rays if given directly to tumor cells outside of the body had no influence. That the cancer cells are no more susceptible to x-ray *in situ* than *in vitro* was shown by the fact that tumors irradiated *in situ* and then replanted in a protected location in the same animal grew actively. It is evident that no direct damage to the cancer cells has been done by x-rays.

SUMMARY AND DISCUSSION.

Spontaneous cancer as it occurs in mice is similar in all essential respects to cancer in man. There can be little doubt that they are one and the same disease showing minor differences which can probably be explained on differences in anatomical structure. For example cancer in man usually metastasizes through the lymph channels while in mice as a rule the metastases are blood-borne. This is perhaps understandable when it is considered that the cells of the mouse are at least as large as the human cell while the average dimensions of the lymphatic channels are considerably smaller and probably too small to permit the passage of a cancer cell.

Previous attempts to influence the course of the disease in mice have met with little success. Surgical removal is followed by local recurrence in from 40 to 50 per cent of the animals, and autografts grow in almost a hundred per cent. There is no doubt that the three methods employed in the foregoing experiments do have a decided influence on the expected outcome of surgical removal and implantation of an autograft. Local recurrences are reduced by the treatments to 14.7 and 21.2 per cent in the different treated groups, contrasted with 40 to 60 per cent in the untreated animals. The failure of autografts in the treated animals was from 44 to 60 per cent while in the untreated animals the autografts failed in less than 4 per cent (two instances in 54 mice).

The only detectable biological effect induced in common by these three agents, x-ray, dry heat, and fatty acid, is the stimulative action on the lymphoid system. Again it would seem that the only possible conclusion to be drawn from the facts presented is that an increased activity of the lymphoid system is responsible for the increased resistance of the mice to growth of spontaneous cancer.

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VII. LYMPHOCYTE IN RESISTANCE TO EXPERIMENTAL TUBERCULOSIS.

So far we have dealt exclusively with the apparent rôle of the lymphocytes in response to tissue growth, both in regard to resistance to heteroplastic grafts and to cancer. In infections much attention has been centered around the polymorphonuclear cells as the first line defense. Not only do these cells accumulate in large numbers at the focus of certain types of infection and show an active increase in numbers in the circulating blood, but they are actually capable of engulfing and digesting bacteria. All these points are so easily demonstrated that experimentation was scarcely necessary to establish the importance of this cell. The evidence of alignment of the lymphoid cell as a factor in resistance to certain infections is not so clear from the observation of disease processes as they naturally occur. This may perhaps be attributed to the fact that lymphoid reactions are associated with the types of infections which are relatively slow in their progress. Furthermore the lymphoid cell, in performing its function, does nothing so spectacular as engulfing and digesting bacteria. As for numerical changes in the circulating blood, these have been frequently overlooked due to the fact that the observers were primarily looking for changes in the polymorphonuclear cells or that the numerical fluctuations in the number of lymphoid cells are masked by marked changes in the other cells due to associated secondary infections.

The evidence of involvement of the lymphoid type of cell in the process of defense is sufficiently suggestive to deserve an experimental examination of the subject. This is particularly true in tuberculosis. The lesion is characterized by the surrounding zone of round cells. The extent of this reaction varies somewhat with the degree of resistance, as for example in the more rapidly advancing miliary tuberculosis, the zone of reaction forming the individual tubercle is comparatively small while in the subacute type of the disease, where a higher resistance may be assumed, very large num-

bers of cells participate in the reaction (1). The blood picture, when analyzed, offers an interesting and consistent result. In the more rapidly advancing types the lymphocytes fall often below 10 per cent of the circulating white cells, whereas in patients with early, healed, or healing tuberculous lesions these cells are increased, sometimes forming more than 50 per cent of the total leucocytes (1, 2). The frequency with which such changes are overlooked is well exemplified in a recent edition of a standard text-book where the statement is made that there are no blood cell changes characteristic of tuberculosis. While the normal number of lymphocytes per c.mm. is about 2,000, in the two counts published to substantiate the above statement, one case had 530 lymphocytes per c.mm. and the other only 227.

Until the present work was started very little experimentation had been carried on with the view of determining the importance of these cells in resistance to tuberculosis infection, but many clinicians had come to consider the lymphoid index as of considerable prognostic importance. Webb (3), among others, had collected evidence based both on clinical experience and experimentation which led him to express a strong belief in the importance of the lymphoid reaction. Krause (4) had called attention to the fact that resistant animals responded to inoculation by a massive cellular outpouring, but he attributed no importance to this as part of the defensive mechanism. The work of Bartel (5) and his coworkers which led them to associate the lymphoid tissue with resistance had failed to be confirmed.

The immediate starting point of our investigation was the observation of Lewis and Margot (6), who noting that rats and mice experimentally infected with tuberculosis developed large spleens, tested the effect of splenectomy on the course of the disease in these animals. They expected a lowering of resistance from this removal of the spleen, but on the contrary found that the splenectomized animals lived longer than unoperated animals after experimental infection.

EFFECT OF DESTRUCTION OF THE LYMPHOCYTES ON RESISTANCE TO THE TUBERCLE BACILLUS.

It occurred to us that this unexpected and anomalous result might be due to the effect of splenectomy on the other lymphoid structures, for blood counts made on a number of splenectomized animals had indicated that there was temporary overcompensation of the lymphoid elements. Immediately after splenectomy there was a fall in the total number of circulating lymphoid cells, but by the 19th to 21st day a gain of over 100 per cent above the normal count, or an average increase of 11,000 lymphocytes per c.mm. of blood, took place in three-fourths of the splenectomized mice. The remaining 25 per cent of animals averaged a loss of about 8 per cent. As Lewis and Margot inoculated their animals between the 2nd and 3rd week after splenectomy, or at the time of the lymphoid overcompensation, sufficient grounds existed to submit the phenomenon to a closer analysis from our point of view.

The almost specific destructive action of x-ray on the lymphoid tissue offered an excellent experimental method for testing the validity of our supposition.

Counteraction of the Increased Resistance Following Splenectomy.—For this experiment the compensatory overproduction in the lymphoid system which follows splenectomy was prevented by subjecting the animals to small repeated x-ray exposures for 14 to 17 days following the operation. The resistance of such animals was compared with that of (1) unoperated mice exposed to the same x-ray dosage, (2) mice splenectomized a few hours before inoculation, (3) mice splenectomized 8 to 24 days before inoculation, and (4) normal mice. Each animal was inoculated with an emulsion of a 6 weeks old glycerol veal bouillon culture of bovine tubercle bacilli, the dose amounting to 1 mg. of the dry bacilli suspended in 0.8 cc. of normal salt solution.

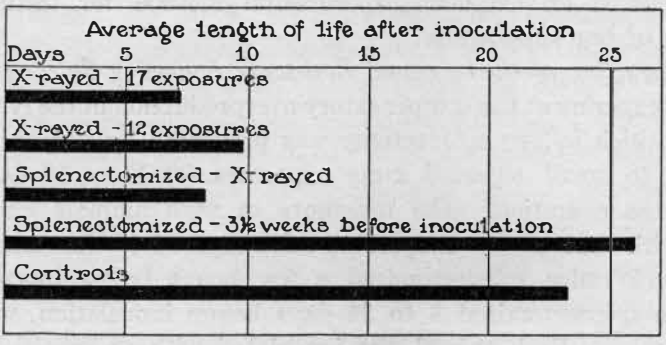
The average length of life of the various groups is shown in Table XLI. Tubercle bacilli were found widely disseminated in the exudate, spleen, liver, kidney, lung, and frequently in the heart's blood. There was no essential difference in this respect between the groups. That the early death of the x-rayed animals was not due to the x-ray effect alone was shown by the fact that large numbers of mice given

the same doses of x-ray and kept under the same laboratory conditions showed no ill effects from the treatment.

As may be seen from the above figures the resistance of a mouse as judged by its length of life after inoculation with a massive dose of tubercle bacilli varies directly with the amount of lymphoid tissue. The x-rayed animals having a depleted system survive the shortest

TABLE XLI.

Groups.	Average time of survival.	No. of mice.
	days	
Splenectomized and x-rayed	7.8	18
X-rayed alone	7.7	35
Splenectomized a few hrs	9.1	10
" 8 to 10 days	19.7	10
" 3½ wks	23.3	10
Normal	20.9	20

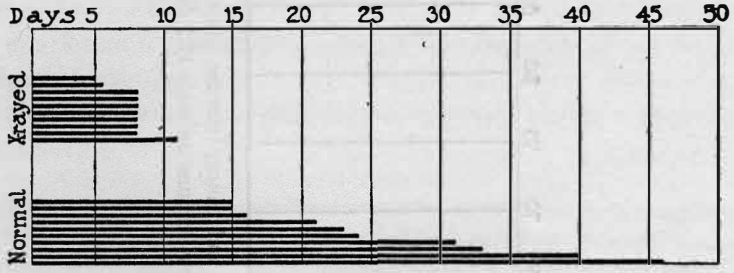


TEXT-FIG. 33.

time; while the splenectomized ones which apparently have a somewhat more active lymphoid system than normal have a somewhat enhanced resistance (Text-fig. 33).

More definite stimulation of the lymphoid tissue can be induced by other means as has already been shown and the influence of such stimulation on the course of tubercular infection will be dealt with in the next section. The principal point to be emphasized in the

above experiment is the fact that the destructive doses of x-ray reduce the resistance to the vanishing point (Text-fig. 34).



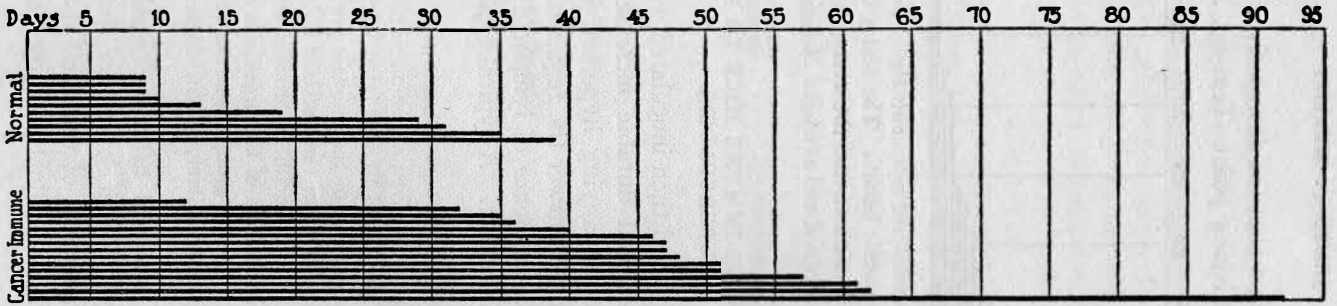
TEXT-FIG. 34. Each horizontal line represents the time of survival of a mouse after infection with bovine tubercle bacilli. The first group received a series of small doses of x-ray before inoculation, and the average duration of life was 7.8 days. The second group was normal and averaged 26.4 days of life after inoculation.

RESISTANCE OF CANCER-IMMUNE MICE TO EXPERIMENTAL TUBERCULOSIS.

Mice immunized against and then inoculated with a transplantable cancer develop a marked and durable increase in the circulating lymphocytes with an accompanying hyperactivity of the lymphogenic centers. Such animals answer the requirement for the experiment designed to test the influence of lymphoid stimulation on the course of a tuberculosis infection. A typical experiment of this kind will be quoted.

Method.—A number of mice were given an immunizing injection consisting of 0.3 cc. of defibrinated mouse blood, and 10 days later inoculated with transplantable cancer. A very large proportion of the animals proved to be resistant. 3 weeks after the tumor inoculation all of the resistant animals were given 3 mg. of a 9 day culture of bovine tubercle bacilli. Autopsies were performed on all of the mice at death and the distribution of the organisms determined. No significant difference was noted between the groups as all showed a wide dissemination including the peritoneal fluid, kidneys, liver, spleen, lungs, and heart's blood.

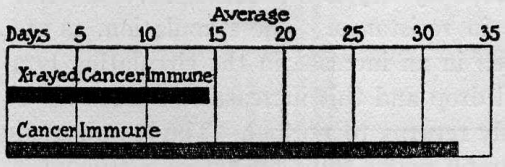
The average time of survival after inoculation for the cancer-immune mice was 36 days (30 mice), and for the controls 17.5 days (20 mice). A typical experiment of this kind showing the length of life of the individual animals is illustrated by Text-fig. 35.



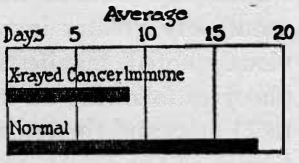
TEXT-FIG. 35. The horizontal lines show duration of life after inoculation of normal and cancer-immune mice with tubercle bacilli. The average time of survival of the normal animals was 20.3 days, and of the cancer-immune animals 47.7 days.

Effect of Destruction of the Lymphoid Tissue in Cancer-Immune Mice on Their Resistance to Tubercle Bacilli.—If the lymphocytic reaction in the cancer-immune animals is an important factor in determining the increased resistance to tuberculous infection, as reported above, the destruction of these cells in a cancer-immune animal should reduce the resistance to tubercle bacilli. This proved to be the case as may be seen from the following experiments.

Experiment.—A group of mice were immunized against and 10 days later inoculated with cancer grafts. After 2 weeks half of the cancer immunes were started on daily exposures to x-ray and this was continued for 7 days. At the end of this



TEXT-FIG. 36.



TEXT-FIG. 37.

TEXT-FIG. 36. The average duration of life of cancer-immune mice subjected to repeated small doses of x-ray before inoculation with tubercle bacilli compared with the duration of life of intact cancer-immune animals inoculated with the same infecting agent.

TEXT-FIG. 37. The average duration of life of cancer-immune animals subjected to repeated small doses of x-ray before inoculation with tubercle bacilli compared with the duration of life of normal mice inoculated with the same infecting agent.

time, which was 3 weeks after the cancer inoculation, these mice together with non-radiated immunes were inoculated intraperitoneally with 2 mg. of a culture of tubercle bacilli. The average duration of life after inoculation for the x-rayed tumor-resistant mice was 14.5 days, while the tumor-resistant animals which received no x-ray averaged 32.5 days (Text-fig. 36).

In another experiment the details of which were similar to that above, the resistance of x-rayed tumor-immune mice was compared with that of normal animals. The former averaged 8.9 days of life after inoculation and the latter 18 days (Text-fig. 37).

The results of these experiments definitely answer the question. Animals with an active lymphoid system such as we find in tumor-resistant animals for a time after the introduction of the tumor graft have a resistance considerably higher than normal mice. Fur-

thermore if this lymphoid activity is destroyed by exposing the animals to x-ray, the resistance to the tubercle bacilli is reduced to a point even below that of normal animals.

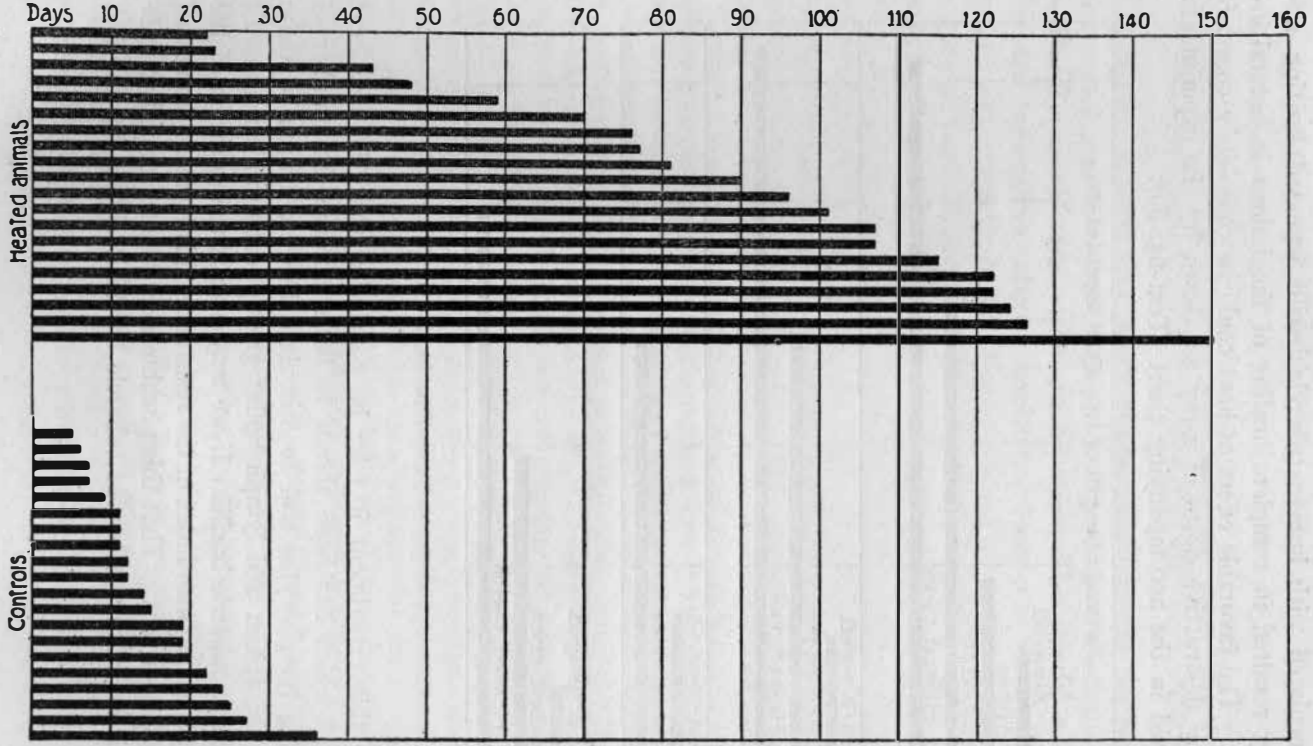
EFFECT OF DRY HEAT ON RESISTANCE TO TUBERCULOSIS.

The association of the lymphoid tissue with resistance to tuberculous infections has been further tested by investigating the fate of animals stimulated by means of dry heat. The amount of stimulation induced by this method is considerably greater than that induced by any other means so far discovered. If there is a quantitative relationship animals stimulated by dry heat should have a correspondingly greater increase in resistance. The stimulation, as previously noted, manifests itself in an increase in the circulating lymphocytes following an initial drop and this increase continues for 14 to 21 days and then gradually returns to normal. The lymphogenic tissues of the spleen and lymph nodes show an active participation in the stimulative process. A typical experiment is described below.

Experiment.—A number of mice were subjected to a temperature ranging from 55 to 65°C. for 5 minutes according to the method already described. 1 week later these animals with an equal number of controls were inoculated intraperitoneally with 2 mg. of a bovine strain of tubercle bacilli (4 week culture), suspended in 0.5 cc. of normal salt solution. The mice were then placed in individual jars so as to minimize the occurrence or spread of an epidemic. Careful autopsies were performed on all mice at the time of death and the peritoneal fluid, liver, kidneys, lungs, and heart's blood examined for the presence of tubercle bacilli. The organism was found widely disseminated in all of the animals, leaving no doubt as to the cause of death.

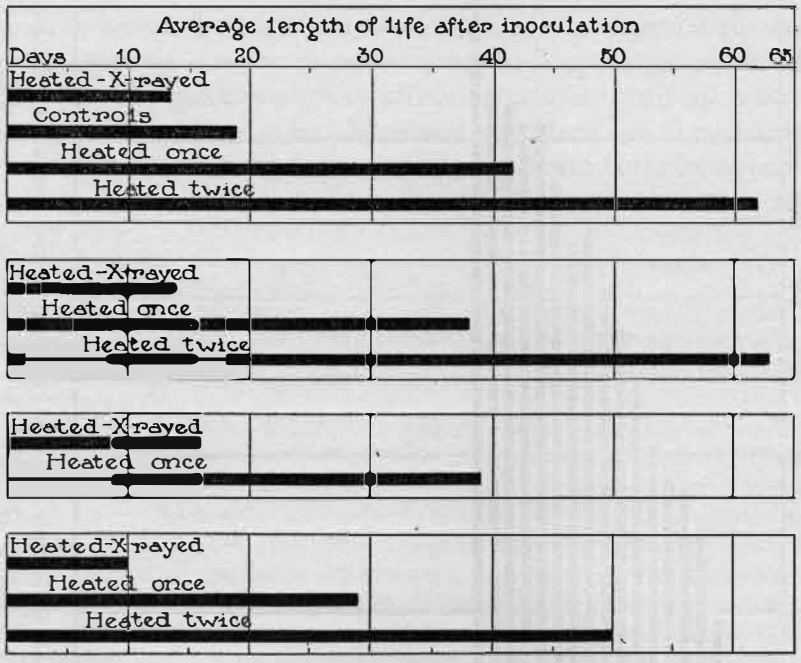
Three experiments of this nature were carried out. Combining the results, the average length of life after inoculation for 54 control animals was 23 days while the 52 mice subjected to the heat treatment 1 week prior to inoculation averaged 76 days of life. The duration of life of the individual mice in one of the experiments is illustrated in Text-fig. 38.

Several variations of the above experiment have yielded results of interest. By repeating the heat treatment at a period when the effect of the first treatment is declining, the length of life of the mice may be still further prolonged by 50 to 75 per cent. In guinea



TEXT-FIG. 38. Each horizontal line represents the time of survival of a mouse after inoculation with bovine tubercle bacilli. The first group was subjected to an exposure of dry heat 1 week before inoculation. The second group was untreated.

pigs inoculated with human tubercle bacilli repeated heating apparently resulted in complete healing of the lesions in several instances. The favorable effect of heat could be completely counteracted by destructive doses of x-ray as shown by the experiments illustrated in the accompanying chart (Text-fig. 39).



TEXT-FIG. 39.

From this experiment it may be concluded that the dry heat treatment which has been shown to both increase the number of circulating lymphocytes and to stimulate the production of these cells in the spleen and lymph nodes greatly enhances an animal's resistance to tubercle bacilli. If we may accept the duration of life after inoculation as an index of the resistance, this increase amounted to over 200 per cent. That this phenomenon is not confined to mice and their resistance to bovine tubercle bacilli is shown by the fact

that the resistance of guinea pigs to the human strain of the organism may be influenced just as markedly by the heat treatment.[1]

GENERAL LEUCOCYTIC RESPONSE DURING THE REACTION OF ARTIFICIAL IMMUNITY IN EXPERIMENTAL TUBERCULOUS INFECTION.

In further experiments on the relationship between the lymphoid tissue and resistance to tuberculous infections, observations have been made on the circulating leucocytes of guinea pigs in which resistance to virulent tubercle bacilli had been raised by a previous inoculation of relatively non-virulent tubercle bacilli. The method employed was that first carried out by Trudeau (7) and subsequently elaborated by Baldwin, Krause, and others.

Method.—In our experiments a preliminary inoculation of non-virulent tubercle bacilli, Saranac Strain R1, was followed, after a proper interval of time, by an inoculation of virulent tubercle bacilli, Saranac Strain H37. As controls some animals were inoculated with Strain R1 or H37 alone. Guinea pigs were used throughout the observations.

Leucocyte counts both absolute and differential were made twice weekly on a number of normal guinea pigs over a period of a month. These blood specimens, as well as all others to be referred to, were obtained from a vessel of the ear. The absolute counts were made according to the well known standard technique with the added precaution of using the same pipette for a given animal for each successive observation. Any animal showing an erratic tendency in the leucocyte count was discarded, and all animals were kept under the same living conditions. Therefore, when the experiment was begun, conditions were standardized as far as controllable variables permit, and were maintained throughout the observations.

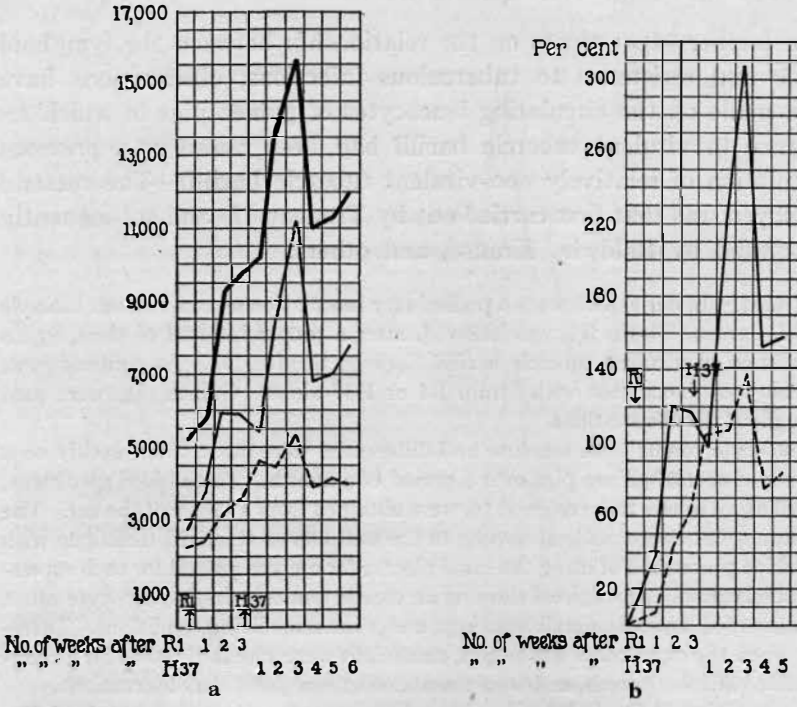
A suspension of Strain R1 tubercle bacilli was prepared so that a stained film preparation showed not more than ten individual bacilli to a microscopic field. 0.2 cc. of such suspension was inoculated subcutaneously into the left groin, and about 3 weeks later these animals were inoculated with 0.1 cc. of a suspension of the virulent strain, H37, so standardized as to show not more than two bacilli to a microscopic field.

For normal guinea pigs weighing between 200 and 250 gm. the average absolute leucocyte count was 5,247 per c.mm. of blood: 52.1

[1] This observation was made by R. G. Hussey in this laboratory. It is of interest to note that the treated animals tended to develop the more chronic type of disease. The only late manifestations were found in the lungs showing a pathology similar to that seen in man.

per cent, or 2,734 of these cells being lymphocytes, and 43.1 per cent, or 2,261, polymorphonuclear cells.

The inoculation of Strain R1 produced a gradual but definite increase in the total leucocyte counts (Text-figs. 40 and 41), so that at



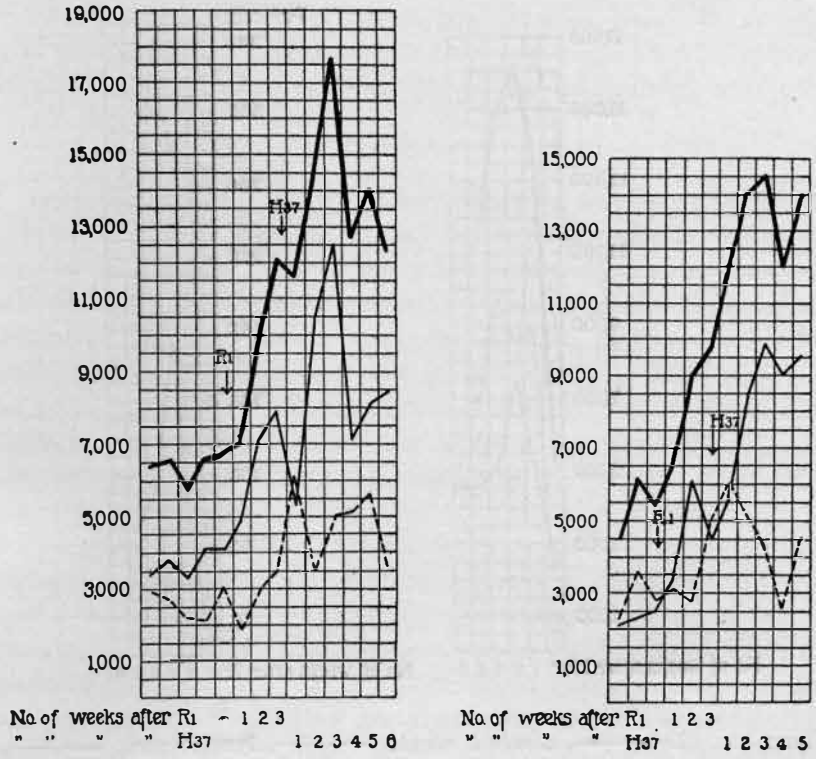
TEXT-FIG. 40. ———, absolute count; ———, lymphocytes; ----------, polymorphonuclears.

A. Composite absolute and differential white cell counts of all test animals plotted at intervals of 1 week following inoculation and reinoculation.

B. The percentage variation of white cells following inoculation and reinoculation. The standard average of 2,734 lymphocytes and 2,261 polymorphonuclears is indicated as zero.

the end of 3 weeks, just before the inoculation of Strain H37, this increase amounted to 80.5 per cent, representing an increase in lymphocytes to 60.5 per cent. Polymorphonuclear cells, though actually increased, made up only 34.6 per cent of the total white cells, a relative decrease of 8.5 per cent.

These guinea pigs, upon inoculation of the virulent strain, H37, showed for the 1st week a slight fall in the lymphocytes and an increase in the polymorphonuclear cells, and during this time the animals appeared quite sick. Then an exaggerated increase in the

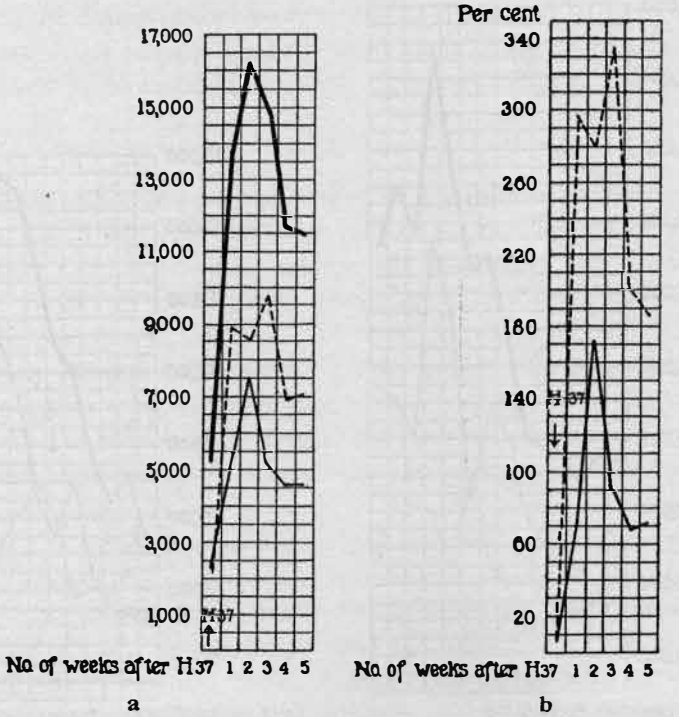


TEXT-FIG. 41. ————, absolute count; ————, lymphocytes; ----------, polymorphonuclears.

Absolute and differential white cell counts of two individuals used in the experiment, plotted at weekly intervals before and after inoculation and reinoculation.

lymphocytes occurred which at its height during the 3rd week, reached a point 306 per cent above their initial number. Polymorphonuclear cells were 9.2 per cent below their normal relative average. After the peak of the curve was reached, there was a drop especially of the lymphoid cells.

The control guinea pigs inoculated with virulent tubercle bacilli alone showed a definite increase in the total leucocytes, but the relative proportion of polymorphonuclear and lymphoid elements was erratic. Lymphocytes increased greatly during the 2nd and 3rd



TEXT-FIG. 42.———, absolute count; ———, lymphocytes; ———, polymorphonuclears.

A. Composite absolute and differential white cell counts of individuals plotted at weekly intervals following an inoculation of a heavy suspension of virulent tubercle bacilli.

B. The percentage variation of white cells in the counts plotted in A.

weeks after which they progressively decreased with a corresponding polymorphonuclear increase.

Ten guinea pigs were inoculated with a much heavier suspension of Strain H37. The reaction of these animals was distinctly different from the response of others which were inoculated with the

smaller dose. The lymphocytes increased during the 1st week but fell rapidly afterwards while the polymorphonuclears reacted much more intensely and remained much above their normal level during the progress of the disease (Text-fig. 42).

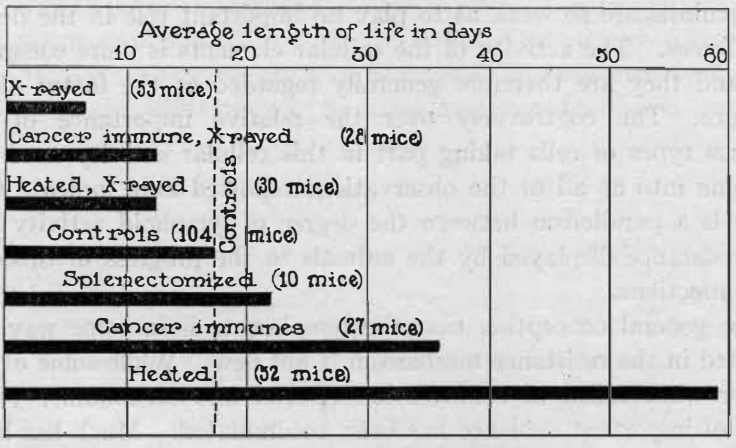
These results indicate a parallelism between lymphoid activity and resistance to tuberculous infection and suggest again an association of the lymphocytes with the factors determining resistance.

DISCUSSION.

There is almost uniformity of belief that the antibodies present in tuberculosis are so weak as to play no important rôle in the defensive forces. The activity of the cellular elements is more conspicuous and they are therefore generally regarded as the factor of resistance. The controversy over the relative importance of the various types of cells taking part in this cellular activity need not be gone into as all of the observations reported here indicate that there is a parallelism between the degree of lymphoid activity and the resistance displayed by the animals to the progress of tuberculous infections.

The general conception that the lymphocyte is in some way implicated in the resistance mechanism is not new. While some of the earlier work failed of confirmation (Bartel and Neumann), yet a mass of important evidence has been accumulated. Much has been made of the absence of characteristic blood changes in the disease but the present indications are that when the blood counts are analyzed on the basis of the type of case, the stage of the disease, the presence or absence of massive secondary infections, some definite relationships will appear. For example in generalized miliary tuberculosis and in tuberculous meningitis, conditions where it may be assumed that resistance is very low, the circulating lymphocytes are far below the number found in normal individuals. In the rapidly advancing pulmonary type, according to many observers, this is also true; but where resistance is indicated by the healing of the lesions, the blood counts show an increase of lymphocytes over the normal level. It is not surprising that some confusion has arisen when it is considered that the counts have rarely been analyzed

according to the condition of the patient or the subsequent history. Certainly the experimental blood studies presented above give definite evidence of close parallelism between resistance and the number of lymphocytes in the circulating blood. More recently this relationship has been emphasized by the study of Cunningham, Sabin, Sugiyama, and Kindwall (8), who conclude on the basis of a careful analysis that "high monocytes and low lymphocytes have been found associated with active tuberculosis, while low monocytes and high lymphocytes have been found associated with arrested tuberculosis... We therefore consider that the study of the development



TEXT-FIG. 43.

of immunity to tuberculosis must include an intensive analysis of the lymphocytes."

The constant presence of the lymphoid cell in the local reaction to infection with tubercle bacilli adds further emphasis to the possible value of these cells in the defensive mechanism. Added to this evidence we have the facts brought out in the present publication, that experimental depletion or stimulation of the lymphoid tissue results, on the one hand, in the lowering and, on the other, in the augmenting of resistance. Furthermore the degree of resistance seems to have a definite quantitative relationship to the amount and activity of the lymphoid tissue. For comparison, the results of all

of the reported experiments have been reduced to a common ratio on the basis of the average number of days of survival of the inoculated controls for each group (Text-fig. 43). Arranged according to the amount of lymphoid activity the x-rayed animals would all be below the average for the normal animals, while the splenectomized mice at the time of inoculation would be slightly above the normal. Next in order would be the cancer immunes and finally the heated animals which show the greatest amount of stimulation. Arranged according to the average length of life after inoculation with tubercle bacilli the groups were found in the same sequence.

With all of this evidence it would seem plausible to assume that the association of the lymphocytes with resistance is more than an associated reaction, and that these cells are at least an important if not the important resisting force of the organism, a purposeful phenomenon.

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VIII. GENERAL SUMMARY AND DISCUSSION.

Investigators in determining the function of an organ or a type of cell have as a rule resorted to indirect methods. Much information has been gained from the study of pathological conditions, where function has been interfered with, or by study of deficiencies after the removal of an organ. Rarely has it been possible to vary experimentally the size or activity of an organ or tissue, conditions which would yield more or less direct evidence of function.

The lymphoid tissue considered as an organ has been particularly difficult to investigate. It not only composes largely the spleen and numerous lymph nodes but small deposits are scattered through most of the organs and tissues of the body and in addition large numbers of the cells are to be found in the circulation. If all this tissue could be brought together in a single mass it would represent an organ of considerable size. It is not surprising that removal of the spleen, the largest single deposit, representing however only a fraction of the total, is so quickly compensated for that no definite deficiencies are detectable.

The development of methods by which the total lymphoid tissue may be varied at will, practically depleted on the one hand with repeated small doses of x-ray or stimulated by single small doses of x-ray, dry heat, and unsaturated fatty acids, has opened up possibilities for the study of the function of this tissue. The methods were developed primarily to determine the importance of the lymphoid reaction in tissue grafting and in resistance to cancer, and the study has been confined with two exceptions to these subjects. The two exceptions are the part played by these cells in antibody formation and their rôle in resistance to certain infections where humoral antibodies are of little moment. One of these studies, namely that dealing with resistance to the tubercle bacillus, has been reviewed here as an example of the application of the methods.

Heteroplastic Tissue Grafting.—The association of the lymphoid type of cell with the mechanism by which an animal prevents the

growth of an implanted tissue from another species and which eventually brings about the death of foreign cells is a subject which has long attracted the attention of pathologists. The possibility of utilizing the tissues of related species in human repair surgery and in organ deficiencies has been attempted even on a large scale. In spite of certain reported successful clinical results the consensus of opinion is that it is impossible to graft tissues from one species to another in the higher animals and that rarely does such grafting succeed from one individual to another even of the same species. The very sensitive mechanism through which an organism rejects the cells of another species even though those cells be capable of supplying a real deficiency in some vital function is as yet little understood.

In Section I we have reviewed several theories which have been brought forward to explain tissue specificity but none of these have stood the test of adequate investigation. While the new evidence presented here is not intended to furnish a basis for a new theory, it does suffice to indicate that the round cell infiltration so characteristic of the reaction to foreign tissue grafts is a purposeful reaction and that these cells are the agent through which the mechanism exerts its force. The evidence on which this conclusion is based is as follows: The avian embryo and the adult brain have proved to be without defense against heteroplastic grafts and in both there is a notable absence of the round cell reaction. From this it might be supposed, as suggested by a number of investigators, that the round cell reaction only appeared about cells injured by some other agent, but this conception is eliminated by the finding that the avian embryo and the adult brain are rendered completely resistant when supplied with lymphoid tissue. Furthermore the adult organism may be rendered non-resistant to heteroplastic tissue by destruction of the major portion of the lymphoid system and the power to rid itself of the foreign tissue is regained only when the lymphoid tissue has regenerated approximately to the normal amount. What other interpretation therefore are we to assume, than that these round cells are a necessary factor in this phenomenon and are to be considered at least as the guardians of the species' individuality?

Resistance to Transplantable Neoplasms in Mice.—The extensive experimental study of the relationship of the lymphocyte to re-

sistance against the transplanted malignant tumor has yielded strong evidence that in the lymphoid elements we have an important link in the process of so called cancer immunity. First we have the facts brought out by the study of the natural progress of the inoculated tumor. When the host is resistant this is evidenced by a local round cell reaction about the graft, a condition entirely absent in the susceptible animal. This local reaction of resistance is accompanied by a great increase in the circulating mononuclears, and back of this, in turn, is an increased proliferation in the lymphogenic centers. On the other hand the lymphoid tissue of a susceptible animal supporting a growing tumor shows marked degenerative changes which progress with the enlargement of the tumor. In other words the response to cancer inoculation is a general one, and the degree of resistance seems to depend on the reactibility of the lymphoid tissue.

So closely is resistance associated with the lymphocyte, that experimentally-produced variation in the amount and activity of the lymphoid tissue is accompanied by a variation in the resistance of the animal to cancer; increased activity resulting in increased resistance, depletion in decreased resistance. This statement is based on the following facts. Mice rendered potentially immune by the injection of homologous defibrinated blood may be reduced to a susceptible state by destruction of the lymphoid tissue (x-ray) or by the suppression of its activity (olive oil). On the other hand stimulation of the lymphoid tissue by three types of agents, biological, physical, and chemical, results in enhanced resistance to the transplanted tumor. As far as can be determined by minute study the only biological activity shared in common by these various agents is the stimulative effect on the lymphoid tissue.

A point probably not without significance is the apparent quantitative relationship between the amount of stimulation and the degree of resistance to tumors. This has been brought out not only by comparing the effectiveness of the resistance induced by the various agents but by the results of inoculation at various phases of the stimulation. The fact that the result of cancer inoculation made soon after the administration of the stimulus, particularly x-ray, indicates no increased resistance on the part of the animal, but results

in a neutralization of the stimulative effect of the agent on the lymphoid tissue, suggests an antagonistic action between the cancer and the stimulative agent acting on the lymphoid tissue. Other minor observations of this nature might be mentioned, all of which point in the same direction and strengthen the evidence for our final deductions in regard to the activity of the lymphoid cells.

It is probable that the majority of intact normal animals possess sufficient lymphoid tissue to combat a cancer graft but in the susceptible animal the mechanism by which these cells are attracted to the locality of the graft is for some reason imperfect. Some of the experiments quoted in Section V show that even in susceptible animals where a sufficient local lymphoid reaction is induced, by processes unrelated to general resistance, a high degree of local resistance results. Precisely what the mechanism is which determines whether or not a local reaction is to take place about a cancer graft and how the lymphocyte exerts its influence on the cancer cell are points on which we have been able to gain no light.

Resistance to Spontaneous Neoplasms in Mice.—The general opinion that one is not justified in drawing conclusions in regard to cancer as a disease from the results of the study of the transplanted cancer has some foundation. The host of a transplanted cancer acts only as a supporter of the actively growing cells from another individual and there is no evidence that the cells of the host ever become malignant by contact. It is probable that an animal may react to and destroy the inoculated cancer more readily than it would to one arising in its own body. Nevertheless the mechanism by which the destruction of cancer cells is brought about seems to be the same regardless of whether the tumor be a transplanted one or has developed spontaneously. The principal methods by which resistance to the transplanted cancer may be enhanced (x-ray, heat, and fatty acids) are shown in Section VI to be equally effective in augmenting the defense of the cancerous animal to its own spontaneous tumor. These observations leave no doubt that the principles and the deductions drawn are not confined to the experimentally transplanted tumors only but apply equally to cancer as a natural disease.

Resistance to Neoplasms in Other Species.—Although the main facts of our investigation have been arrived at through the study of

mice, selected because of their great susceptibility, there is considerable evidence from other sources that the relationship between lymphoid reaction and cancer resistance is not confined to this species. Ribbert (1) called attention to the association between the round cell reaction and retrogressing areas in human cancer, and more recently MacCarty (2) has demonstrated the prognostic importance of the amount of lymphoid infiltration, particularly in breast cancer.

The histological study of the fate of grafts of transplantable tumors of rats (3), chickens (4), and rabbits has shown that the local lymphoid reaction is just as constant an accompaniment of resistance as it is in mice. Varying the lymphoid content by means of x-ray (5) has been shown to exert the same influence on the fate of rat tumors as it does with mouse cancer. The recent studies of Ewing (6) and of Alter (7) indicate that the cell reaction induced by x-ray and radium is probably the intermediate agent in bringing about the favorable therapeutic result in human cancer, a conclusion analogous to the one we arrived at from the study of mouse cancer. In view of all these observations it would seem that the association of the cellular reaction with resistance to neoplastic growths is not a peculiarity of the mouse and also that the results could be duplicated in other species than those studied.

The question as to why cancer metastases grow so regularly in the lymph nodes, particularly in man, is a point which has not yet been adequately investigated. The dissemination to the lymph node is probably a mechanical affair. One possible explanation of the growth of the cancer in the presence of antagonistic cells, is that the lymphocyte as it exists in the node is inactive and is capable of functioning only when it migrates into the tissues. There is a somewhat analogous phenomenon in the polymorphonuclear leucocyte, which is unable to perform one of its important functions, *i.e.* phagocyting, inside the vessels. Another explanation, more plausible and with some supporting evidence, is that the lymph node only becomes the seat of an active metastasis when it has become exhausted. In addition to this consideration there seems little doubt that the lymphogenic function of an involved node is largely obliterated and the number of lymphoid cells is markedly decreased as

the growth progresses. There is at present not sufficient evidence at hand to answer the question inasmuch as it has not been studied from this point of view.

The mechanism by which the various agents bring about stimulation of the lymphoid system and induce resistance to cancer is a point on which we have no direct knowledge. One suggestive fact only is available to indicate a possible explanation, namely that the serum collected from an animal after an exposure to x-ray or dry heat has a definite stimulating action on lymphoid cells *in vitro*. From this fact it may be assumed that some chemical change has been produced in the body fluids which in turn is capable of acting on the lymphoid cell. The presence of a stimulative substance in the blood of oil-injected animals or those treated with homologous tissue has not been tested so that there is wanting evidence for a more general conclusion on this point.

In this connection it is of interest to note, à propos of the recent discussion of whether or not x-ray exerts stimulative properties, that our studies have demonstrated beyond doubt that this physical agent under proper conditions may induce a real stimulation of an animal tissue.

Other questions similar to the above which have not been subjected to experimental verification because of their comparative unimportance may be mentioned. One finds occasionally among the mice with spontaneous tumors a disease similar to lymphatic leucemia and a like condition has been described in man. Under these circumstances there seems no more reason to expect the abnormal lymphocyte to perform its normal function than for a cancer cell to produce a normal secretion. We have also noticed that an animal with an initial high lymphocyte count is no more apt to be resistant than one with a low count. Presumably the normal number of these cells in the circulation is maintained to meet the requirements of the animal. When called on to meet an additional burden, such as an inoculation with cancer, the degree of success seems to depend on the ability of the animal to produce more cells and the animal with a low initial count is just as apt to react as one with a high count. The production of a blood lymphocytosis without stimulation of the lymphogenic tissue, particularly the transitory lymphocytosis fol-

lowing the administration of certain drugs, is without effect on the resistance to cancer. This is not surprising when it is considered that the increase in numbers of the cells is caused by contraction of the spleen with a forcing out of the lymphocytes in the pulp spaces and that these quickly disappear from the circulation.

Bearing of These Studies on the X-Ray Therapy of Cancer.—In the course of our study of the function of the lymphoid tissue, certain points have been developed, incidentally, which seem to have a bearing on x-ray therapy of cancer and may at least offer an explanation of some of the clinical results from this form of treatment.

An occasional observation has been noted of instances where malignant tumors have apparently grown more rapidly and disseminated more freely after x-ray treatment. This fact has led to a controversy as to whether under certain conditions x-ray may not actually stimulate tumor cells rather than destroy them. So far there has been no experimental evidence to support this idea, but in Section III we have shown that doses of x-ray sufficient to deplete the lymphoid system, definitely lower an animal's resistance to implanted tumor. This is true more or less regardless of the type of resistance, whether it be natural, induced, or acquired. May not some such interference with the defensive mechanism result from the intense x-ray dosage used in man? There is no doubt that the amount of irradiation used in some cases is sufficient to reduce the circulating lymphocytes to a point indicating a very widespread destruction of this tissue and at least comparable to the condition in mice representing a state of reduced resistance to cancer.

If it be true that the human cancer cell is no more susceptible to x-ray than the mouse cancer cell (8), and there is very good reason to believe that this is true, how is one to account for the high percentage of cures in certain types of skin cancer? If this result depended on an unusual degree of sensitiveness of this group of cancer to the direct action of x-ray, it would be expected that the metastatic cells in a superficial lymph node would be almost as easily influenced by the ray as the cells in their original location, but this is not true. The dose of x-ray utilized in the treatment of skin cancer is given over too small an area to induce any general effect on resistance of the type described in mice.

The experiments described in Sections V and VI offer an explanation which at least has some experimental basis. The x-ray treatment, as shown by these tests, confers a local immunity on the exposed skin and renders it an unsuitable soil for the support of tumor growth. This resultant refractory state was found to be confined to the skin layers and did not extend even to the underlying connective tissue (see Text-fig. 23). The cellular reaction resulting from the treatment was also confined to the skin layers. This result led us to suggest that the therapeutic action of x-ray in cancer depended on the cellular reaction induced in the normal tissues surrounding the growth.

This idea was more exhaustively tested with spontaneous cancers of mice. Cancers growing in the skin disappeared promptly after a mild erythema dose of x-ray but if after exposure *in situ* the tumor was removed and replanted in an unexposed area of skin in the same animal growth progressed as uninterruptedly as if no x-ray had been given. A histological study of the fate of a cancer graft inoculated into an area previously exposed to x-ray, shows a series of degenerative changes identical with those which have been repeatedly described as the result of the direct action of x-ray on the cancer cells. Yet these particular cells had received no x-ray.

While there is not conclusive evidence that the above observations would hold true for man, practically the only study on human material carried out from this point of view supports the idea of the importance of the induced cellular reaction. Ewing based his investigation on a large number of cases treated with both x-ray and radium and concludes that there is little or no evidence of direct destruction of cancer cells, but that the subsequent cellular exudate appears to exert a damaging effect on the neoplasm. He considers the latter as an essential factor in the curative process.

To summarize: there is no evidence that cancer cells are more easily destroyed by x-ray than normal cells; x-ray in a mild erythema dose renders the exposed area highly resistant to a subsequent cancer implant; this resistance seems to depend on the amount of cellular reaction induced; x-ray in large generalized doses definitely lowers an animal's resistance to implanted cancer.

GENERAL CONCLUSION.

In drawing any general conclusion from the experimental results recorded here, the possibility cannot be absolutely excluded that other organs, physiological processes, or chemical reactions are influenced by the treatments but with present methods common changes other than that induced in the lymphoid tissue have been undetectable. With this possible limitation we have been led to conclude that in mice resistance to malignant tumors, whether transplanted or spontaneous, is closely associated with the lymphoid tissue and there are indications that the same is true of other species including man. The nature and the extent of the evidence is such that we are forced to conclude that this association is not without purpose and that we have in the lymphoid elements an important link in the chain of the process which determines resistance to cancer.

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