FACTORS OF RESISTANCE TO HETEROPLASTIC TISSUE-GRAFTING.

STUDIES IN TISSUE SPECIFICITY. III.*

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PLATES 56 TO 60.

Previous observations have tended to show conclusively that tissues cannot be transplanted from one species to another, even though these be closely related. Two theories have been brought forward to explain this failure in heteroplastic grafting. The two schools are still at variance and neither has been able to produce evidence conclusive enough to convince the other. The first and most prominent theory is that of Ehrlich, termed athrepsia.[1] The experimental foundation for this hypothesis is the so called zigzag transplantation of tumors between rats and mice. It was observed that a mouse tumor when grafted into a rat, or *vice versa,* would survive and proliferate for six to eight days, but would later fail rapidly and be absorbed. If, however, the mouse tumor was removed during the proliferating stage and reinoculated into a mouse it continued to grow actively. After a period of six or eight days' active growth in the mouse it could again be grafted into a rat. This zigzag grafting could be carried on indefinitely with no apparent effect on the tumor tissue or in lessening its activity of growth. The interpretation, suggested by Ehrlich, is that each species provides its tissues with a specific food substance (X) which is necessary for its maintenance and growth. The temporary survival of the mouse tissue in the rat is due to the amount of this specific food carried over with the graft. When this is exhausted the graft dies unless returned to its native species, where it will accumulate a fresh supply of the specific food and again be able to survive for a time in a foreign species.

* Received for publication, March 24, 1914.

[1] Ehrlich, P., *Arb. a. d. k. Inst. f. exper. Therap.,* 1906, No. 1, 84.

The chief opponent of this theory is Bashford[2] who rests his objection on the findings in an experiment in which rats were inoculated a second time with mouse tumor. Under these conditions the second graft, although containing an equal amount of the hypothetical food substance, would survive only two to three days. From this fact he concludes that there is an active immunity developed against the cancer cell as a foreign proteid. The time of survival of the first graft he considers the time required for the development of the active immunity. Bashford[3] claims that the immunity to homoplastic grafting is an entirely different process and that it depends entirely on the blood vessel and stroma reactions. The merits of the two theories will not be discussed; they are quoted to give an idea of the present views on the subject.

LYMPHOCYTIC REACTION IN RELATION TO TISSUE GRAFTS IN IMMUNE ANIMALS.

The occurrence of a lymphocytic reaction around tissue grafts in immune animals has been pointed out by numerous observers[4] and arises whatever the type or source of the animal's immunity. The immune states are: the natural immunity possessed by an animal individually, or because of variety of species; the acquired immunity which is present in animals that have recovered from a primary or implanted tumor; and finally the so called artificial immunity which can be induced by one of several procedures. The small round cell or lymphocytic infiltration is present when there is healing in a spontaneous tumor, and is seen around the edge of slowly growing cancers in man. The importance of these cells in the immunity reaction to tissue grafts would seem evident, yet they have received scant attention.

HETEROPLASTIC GRAFTING IN A NON-RESISTANT ORGANISM, THE CHICK EMBRYO.

In a previous communication[5] it has been pointed out that the avian embryo has no defensive mechanism against the growth of

[2] Russell, B. R. G., *Third Scientific Report of the Imperial Cancer Research Fund*, 1908, 341.

[3] Bashford, E. F., and Russell, B., *Lancet*, 1910, i, 782.

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

[5] Murphy, Jas. B., *Jour. Am. Med. Assn.*, 1912, lix, 874.

tissues of a foreign species. The tumor tissue of a rat, for instance, by transference from embryo to embryo could be kept growing in the chick for an indefinite period.[6] The rat tissue underwent no marked change during its long sojourn in the chick embryo, as was shown by the fact that at any time during this period it could be replanted successfully into its native species but was promptly disintegrated when grafted into the adult chicken. It was later shown[7] that a defensive mechanism developed rapidly in the embryo at about the time of hatching, quickly destroying any foreign tissue that might be present. The foreign species tissue growing in the embryo shows a total absence of a round cell reaction. The lymphoid cells around the graft first become evident at about the time that the defensive mechanism begins to show its effect. Other than this there is no great change in the embryo to account for this sudden development from a susceptible to a highly resistant organism. If this change is the result of the sudden activity of a tissue or organ formerly quiescent, it should be possible to provide the embryo with this necessary tissue or organ by grafting various adult tissues into the chick embryo.[8]

ACTION, IN VITRO, OF TISSUES IN HOMOLOGOUS PLASMA ON THE GROWTH OF HETEROLOGOUS TISSUE.

Lambert and Hanes[9] have shown that the tissues of one species are capable of growth in the plasma of certain other species. As a preliminary step to the experiment suggested above an attempt was first made to determine the interaction of tissues *in vitro*. Bits of a rapidly growing rat sarcoma were placed in drops of chicken plasma and to these were added in series bits of various adult chicken tissues. The cultures were mounted in hollow slides according to the well known method. The rat tissue in chicken plasma grew remarkably well and was not affected by adult chicken connective tissue, kidney, or liver in close proximity. When, however, a bit of adult chicken spleen was growing in the same drop

[6] Murphy, Jas. B., *Jour. Exper. Med.*, 1913, xvii, 482.
[7] Murphy, Jas. B., *idem*, 1914, xix, 181.
[8] Murphy, Jas. B., *Jour. Am. Med. Assn.*, 1914, lxii, 199.
[9] Lambert, R. A., and Hanes, F. M., *Jour. Exper. Med.*, 1911, xiv, 129.

of plasma there was practically a total inhibition of the growth of the rat sarcoma. The only other tissue showing a similar effect was the bone marrow, which caused definite retardation, but not so marked an inhibition as that brought about by the spleen.

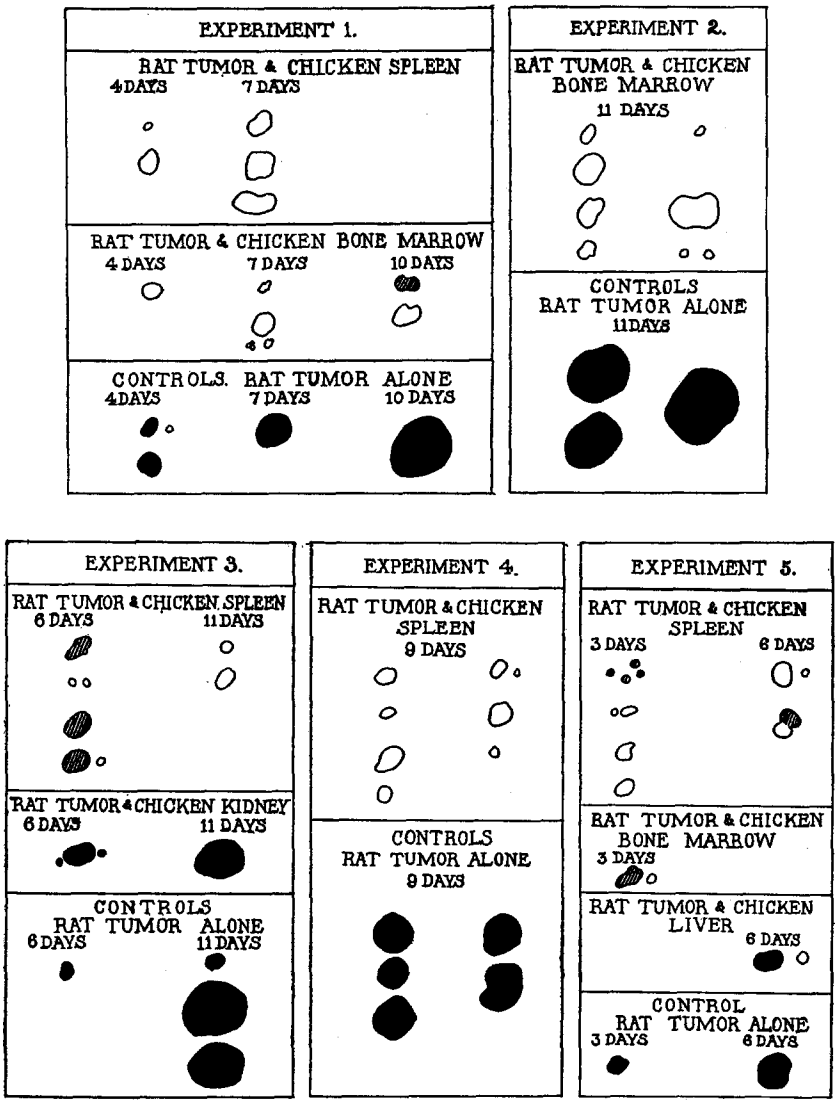
THE EFFECT OF ADULT CHICKEN TISSUE GRAFTS ON THE HETEROPLASTIC GRAFTING IN THE EMBRYO.

Since it is possible to graft various adult tissues into the embryo[10] the above experiment was repeated *in vivo*.

In the first series, comprising twenty experiments and over 150 embryos, grafts of rat sarcoma and bits of adult chicken tissues were placed side by side in the outer membrane of seven day chick embryos, according to the method previously described. The adult chicken tissues used were spleen, kidney, liver, bone marrow, and connective tissue. The eggs were returned to the incubator, and at intervals up to the eighteenth day of incubation part of each lot was opened and the grafts were removed for microscopic examination. Text-figure 1 shows the results in a few of these experiments. In the instances 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 one to two centimeters. 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 (figure 1). Chicken liver grafts generally caused a widespread necrosis of the membranes of the chick. When bits of the liver graft survived they were found to consist of a few scattered bile capillaries. 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 had no effect on the rat tumor cells in the embryo, although the connective tissue itself grew well.

The striking result was obtained when grafts of adult chicken spleen were inoculated with the rat tumor. The resulting tumors instead of being well rounded, greyish, and semitranslucent were flat, often mottled, yellowish, and opaque. Microscopic examination of specimens removed after three or four days showed the

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



TEXT-FIG. 1. 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.

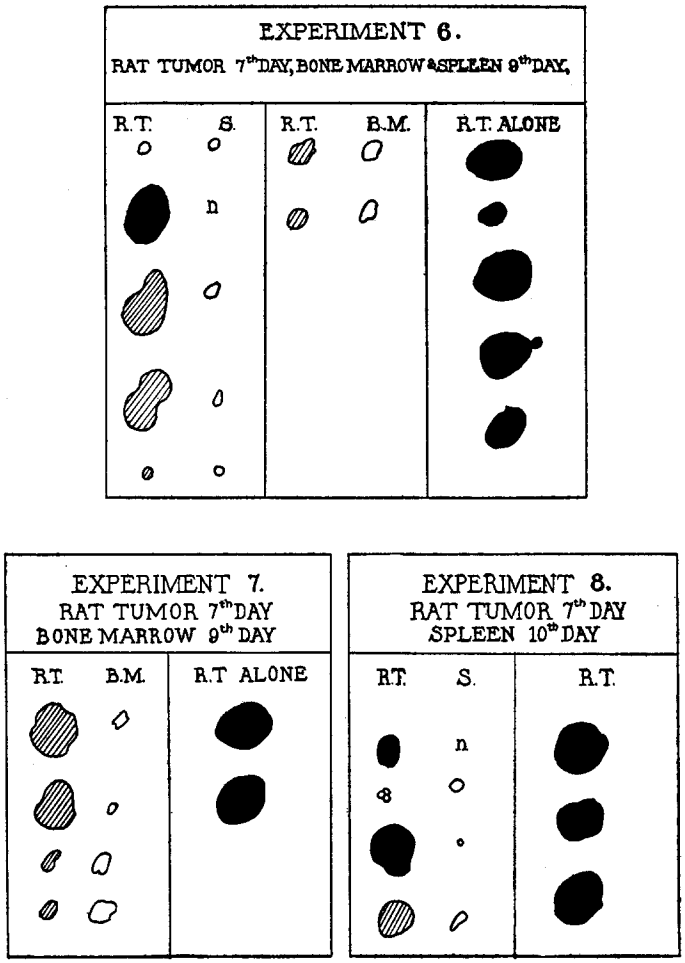
spleen graft well established, made up of the typical spleen cells. The rat tissue was found much degenerated, surrounded by collections of small round cells and largely replaced by connective tissue (figure 2). Later stages show the rat tissue to be dead, embedded in a mass of connective tissue with clumps of small round cells scattered throughout the surrounding tissue (figure 3). This condition offers a strong contrast to the picture shown by the controls of the rat tumor inoculated alone. Here the rat cells are practically devoid of stroma and the edges show no reactive tissue (figure 4). The final stages of rat tumor and chicken spleen showed the spleen graft to be in good condition, but with no evidence of the rat tissue remaining (figure 5).

The action of bone marrow resembled that of the spleen (figure 6), but was slower and less complete. The bone marrow grafts were composed for the most part of fat cells and collections of lymphoid cells.

THE EFFECT OF CHICKEN SPLEEN AND BONE MARROW ON ESTABLISHED GRAFTS OF FOREIGN TISSUES IN THE EMBRYO.

The fact that the tissues in the foregoing experiments were growing side by side and often intermingling presents a difficulty in the interpretation. The present series of experiments was planned to avoid the contact action and to give the spleen and bone marrow a more severe test. The kidney (figure 7) and other tissues mentioned above have no evident effect even in contact and they were therefore not used in this experiment.

Series of eggs were inoculated with rat sarcoma in the usual way on the seventh day of incubation. Two or more days later an opening was made on the opposite side of the eggs, and an adult chicken spleen or bone marrow graft was placed in the outer membrane. Some of the results are shown in outline in text-figure 2. On examination of these specimens eleven days later, the controls inoculated with rat tumor alone showed, almost without exception, large, well rounded, semitranslucent masses at the point of inoculation. 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 (figure 8). Microscopic ex-



TEXT-FIG. 2. 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 eighteenth 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.

amination of the tumors showed massive collections of lymphocytes around the edges and in clumps associated with the blood vessels throughout the tumor (figures 9 and 10). There was a great increase in the connective tissue surrounding and replacing the rat tissue. The rat cells showed many degenerated forms and mitotic figures were rare. It would seem therefore that the adult spleen and bone marrow are capable of supplying a defensive mechanism to the chick embryo, even though the graft of spleen or bone marrow be some distance from the foreign tissue and introduced after the foreign tissue is established and actively growing.

DISCUSSION.

I shall make no attempt to discuss these findings in relation to the theories already brought forward to explain the failure of heteroplastic tumor grafts. The constant result obtained in a score of experiments or more shows conclusively that the adult spleen and bone marrow are capable of supplying a defensive mechanism to the chick embryo, which under ordinary conditions offers no resistance to the growth of foreign species tissue. Furthermore, the embryo bearing such grafts of spleen and bone marrow defends itself in the same way as the adult, if we may judge from the histological picture. The cells common to the graft of bone marrow and spleen, to the reaction around the foreign species graft in the adult, and to the embryo supplied with a defensive mechanism is the small lymphoid cell. It is therefore natural to suppose that this is an active agent in the defense.

Whether or not these lymphoid cells are the important factors in the failure of homoplastic grafts under certain conditions remains to be seen. Certainly a large preponderance of the evidence points in this direction. They are present often in large numbers around grafts of transplantable cancer in immune animals of the same species, regardless of the type of immunity. A recent communication of Apolant's[11] adds weight to this idea. He has shown that in splenectomized animals only a slight or very transient immunity can be developed to transplantable tumors. Oser and Pribram[12]

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

[12] Oser, E. G., and Pribram, E. E., *Ztschr. f. exper. Path. u. Therap.*, 1912, ii, 295.

have shown also that transplantable tumors grow more rapidly in splenectomized animals. The results reported by Baeslack[13] indicate that the number of small lymphocytes in the circulating blood of a tumor-bearing animal has a definite relation to the rate of growth of the tumor, falling rapidly in a susceptible animal and increasing steadily in an animal showing resistance.

SUMMARY.

It has been shown that the chick embryo offers suitable conditions for the growth of implanted tissues, whether these be embryonic or adult, of the same species or a foreign one. The chick at about the time of hatching develops a defensive mechanism against the tissue of foreign species. This resistance can be supplied to the embryo in the early stages if grafts of adult spleen or bone marrow are implanted. Under these conditions the embryo exhibits the same resistance to foreign tissue as does the adult, and presents the same histological manifestations about the graft. Furthermore, the same tissues, spleen and bone marrow, when grafted into an embryo with an established and growing rat tumor, bring about a retrogression and absorption of the foreign tissue. Other adult tissues do not supply this power to the embryo.

EXPLANATION OF PLATES.

PLATE 56.

FIG. 1. The edge of a tumor resulting from a ten days' growth of a Jensen rat sarcoma and adult chicken kidney inoculated into the outer membrane of a seven 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.

FIG. 2. A section of a tumor resulting from a simultaneous inoculation of adult chicken spleen and a rat sarcoma, after five 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.

PLATE 57.

FIG. 3. The remains of the rat sarcoma cells eight days after inoculation into an embryo which at the same time received a graft of adult chicken spleen. The cell structure of the rat tissue is entirely lost and the whole is embedded in a thick connective tissue mass. Small round cells are seen scattered through the section. Compare with figure 4.

[13] Baeslack, F. W., *Ztschr. f. Immunitätsforsch., Orig.*, 1914, XX, 421.

FIG. 4. A section of a tumor resulting from an inoculation of the rat sarcoma alone, after eight days' growth in the chick embryo. Several mitotic figures are seen.

PLATE 58.

FIG. 5. The resulting tumor from a simultaneous inoculation of a seven day embryo with a rat sarcoma and a graft of adult chicken spleen, after eleven 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.

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

PLATE 59.

FIG. 7. A drawing, somewhat enlarged, showing a tumor in the outer membrane of an eighteen day old embryo, resulting from a simultaneous inoculation eleven 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. 8. 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 eleven 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 two days later a graft of adult chicken spleen was added (pink nodule).

PLATE 60.

FIG. 9. 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. Compare with figure 4.

FIG. 10. Section of rat tumor in a chick embryo which had at some distance away a graft of adult chicken spleen. A = dense round cell infiltration at edge. B = degenerated rat cells lying in a mass of connective tissue. Compare with figure 4.

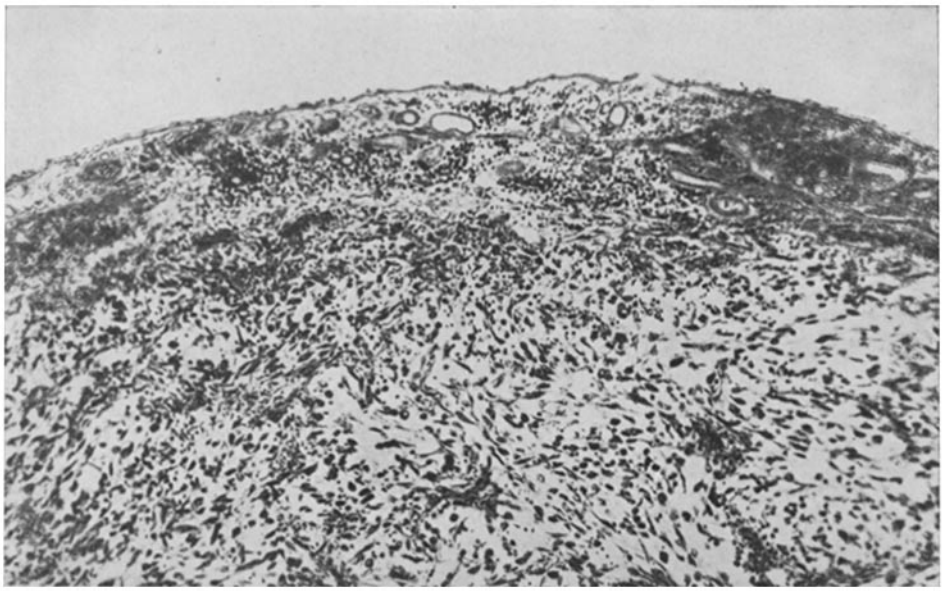


FIG. 1.

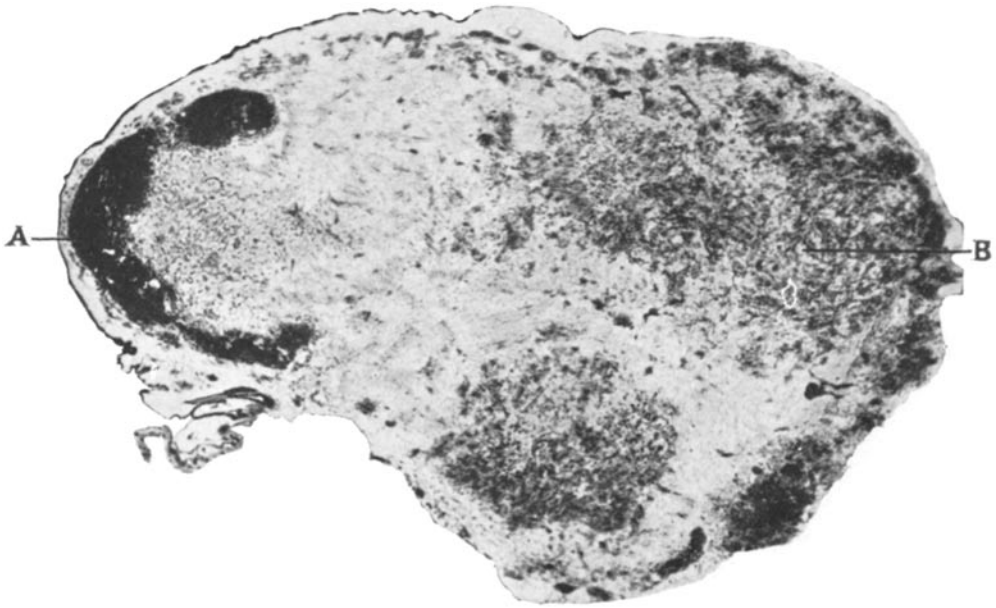


FIG. 2.

(Murphy: Factors of Resistance to Heteroplastic Tissue-Grafting.)

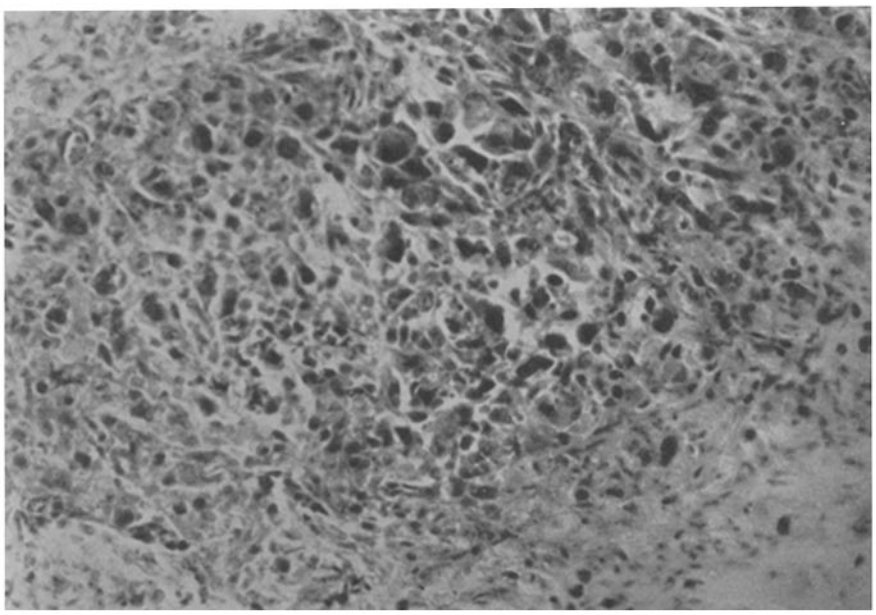


FIG. 3.

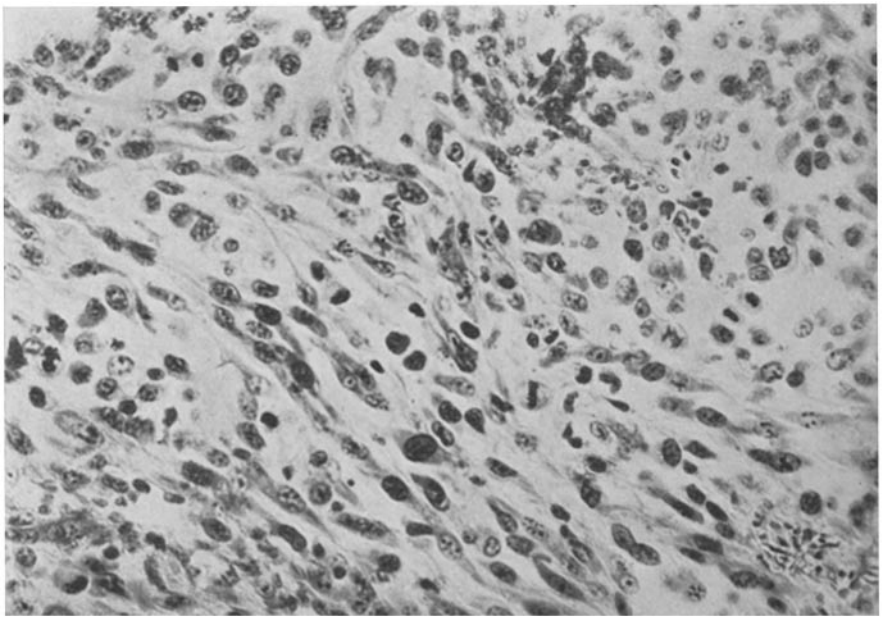


FIG. 4.

(Murphy: Factors of Resistance to Heteroplastic Tissue-Grafting.)

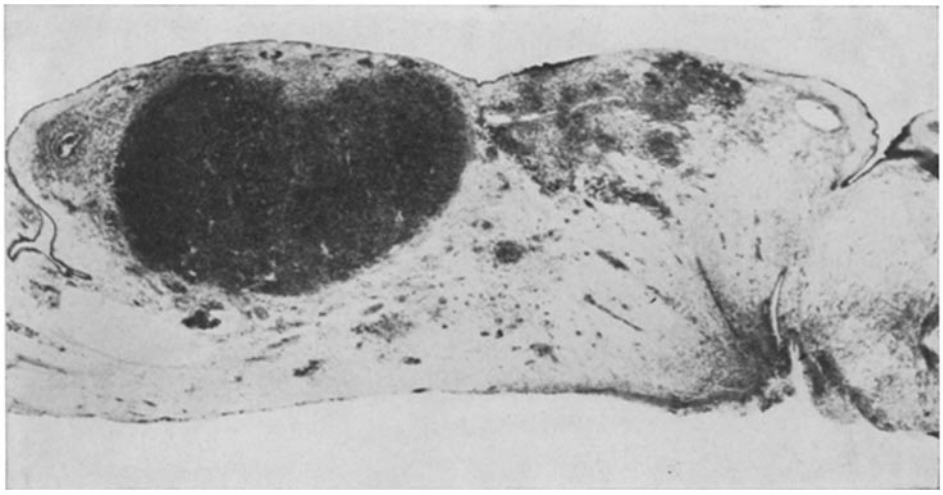


FIG. 5.

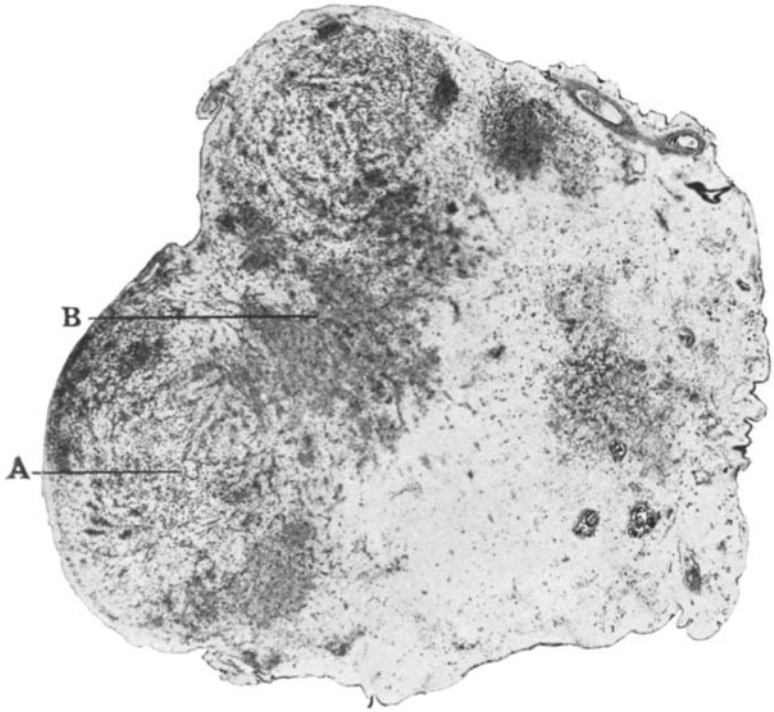


FIG. 6.

(Murphy: Factors of Resistance to Heteroplastic Tissue-Grafting.)

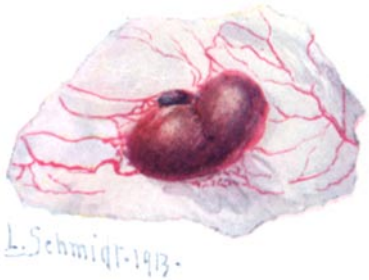


FIG. 7

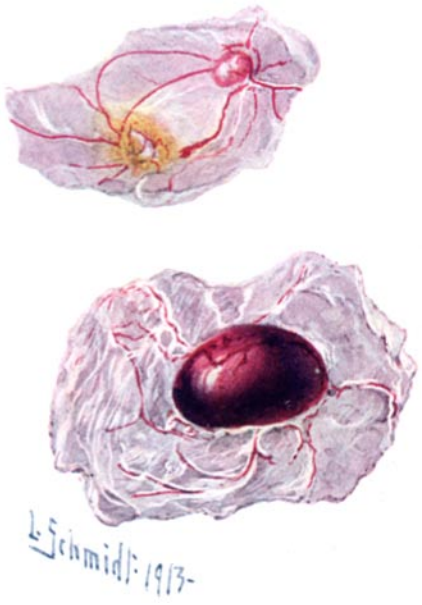


FIG. 8.

(Murphy: Factors of Resistance to Heteroplastic Tissue-Grafting.)

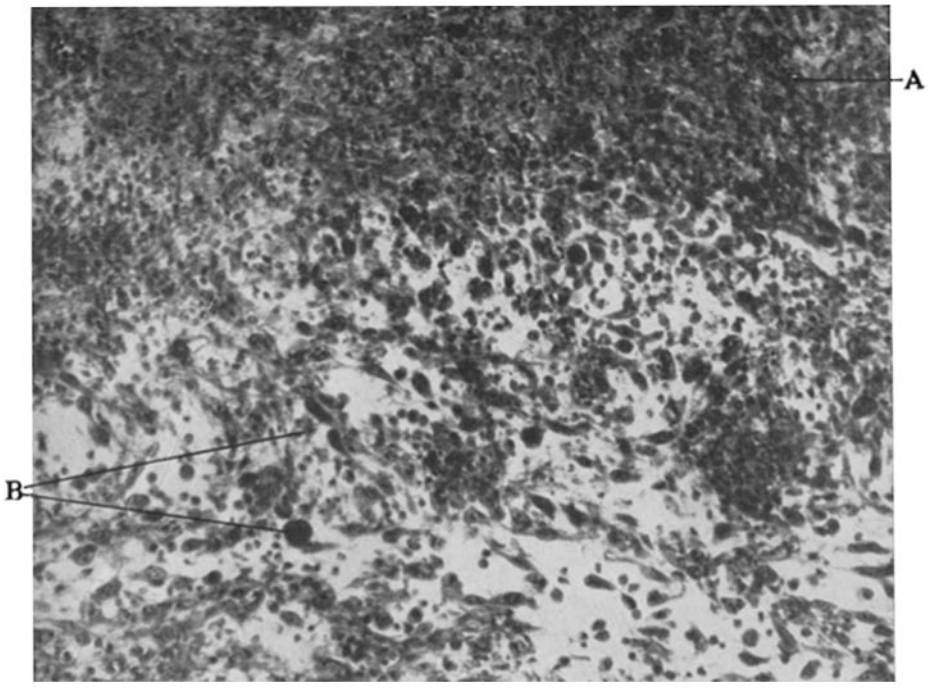


FIG. 9.

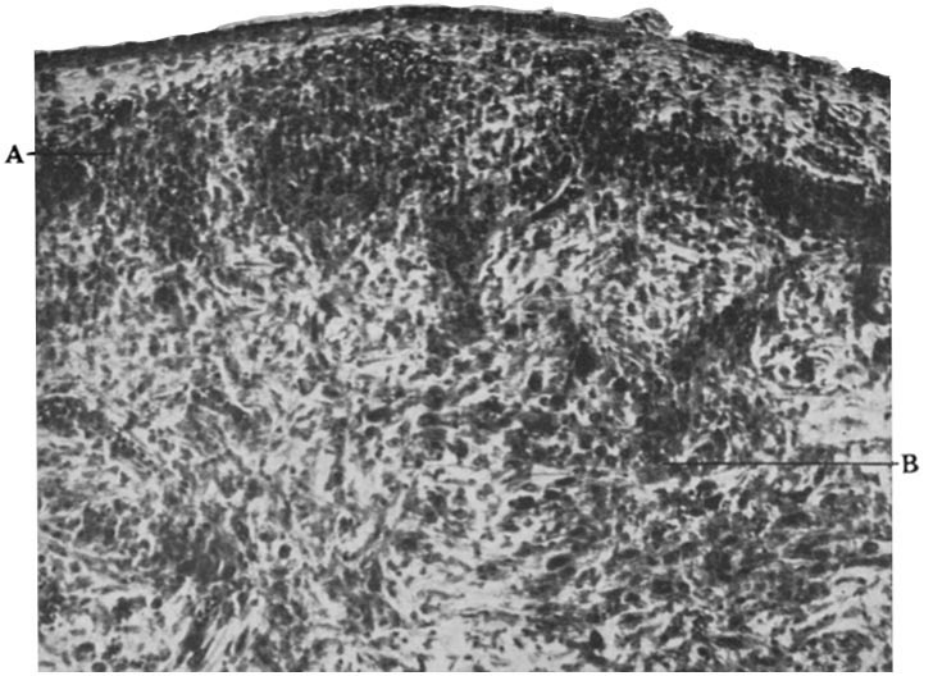


FIG. 10.

(Murphy: Factors of Resistance to Heteroplastic Tissue-Grafting.)