TRANSPLANTABILITY OF TISSUES TO THE EMBRYO OF FOREIGN SPECIES.

Its Bearing on Questions of Tissue Specificity and Tumor Immunity.*

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Plates 84 to 89.

Gardeners and arboriculturists have known for centuries that plants of one species could be grafted on to another without in any way affecting the essential characters of either. In more recent years it has been shown that a like condition exists in certain of the lower animals. For example, Joest[1] working with worms found it possible to bring about a permanent union between *Lumbricus rubellus* and *Allolobophora terrestris,* each of the segments retaining its individual characteristics. Born,[2] Harrison,[3] and Morgan[4] have extended these observations to tadpoles. They have noted that even when the grafted portion is small and absolutely dependent on the major component for its circulation, nervous system, etc., it retains the characters of the original species from which it was derived. Formerly it was considered possible to graft certain tissues from lower animals[5] to human beings, but a more careful consideration and repetition of the experiments which suggested this have shown them to be erroneous.

In recent years much has been added to our knowledge of the laws underlying tissue grafting in warm blooded animals through the study of the transplantable tumors. These in general are governed by the same principles as those that govern the transplantation of

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[1] Joest, E., *Arch. f. Entwcklngsmechn. d. Organ.,* 1897, v, 419.

[2] Born, G., *Arch. f. Entwcklngsmechn. d. Organ.,* 1897, iv, 349.

[3] Harrison, R. G., *Arch. f. Entwcklngsmechn. d. Organ.,* 1898, vii, 430.

[4] Morgan, T. H., *Biol. Bull.,* 1899, i, 7.

[5] Allen, W., *Lancet,* 1884, ii, 875.

normal tissues. Not only do tissue grafts from warm blooded animals fail to survive when placed in a foreign species, but the barrier of tissue specificity also divides the different varieties of the same species. For example, certain tumors of the white mouse can not be successfully transplanted into grey, brown, or black mice of the same species, nor can certain tumors of the white rat be propagated in any other than white rats. Leo Loeb[6] has shown that the skin of a white guinea pig can be grafted on to the ear of a black guinea pig, but it eventually undergoes replacement with an inwandering of pigmented cells. A more recent and striking example of the strictness of the laws of specificity is that of a chicken sarcoma[7] which failed to grow in any other than blood-related animals during its first few transfers, later grew only in pure stock animals of the same variety, and would not grow in chickens of another variety until the malignancy had become greatly enhanced. This remarkable phenomenon of tissue specificity has been the subject of extensive investigation but its cause has not yet been determined. A general impression exists among experimenters that one of the immunity reactions is directed against the invasion of the foreign cells. The literature dealing with hemolysins and cytolysins need scarcely be gone into as the results reported offer no conclusive evidence. A theory of some interest, which explains the facts along other lines, is the athrepsia theory of Ehrlich,[8] which accounts for the failure of grafts in a foreign species by the lack of some specific food substance.

In the course of an investigation on the Rous chicken sarcoma in relation to chick embryo, it was observed that this tumor grew as well in the duck and pigeon embryo[9] (figure 1) as in that of the chicken, whereas it would not grow in the adults of these foreign species. This fact suggested to me the possibility that the chick embryo might serve as a suitable host for mammalian tissue as well. Inoculations of rat, mouse, and human tissue were made into the chick embryo and it was found that they grew as rapidly if not

[6] Loeb, L., *Arch. f. Entwcklngsmechn. d. Organ.*, 1898, vi, 1.
[7] Rous, P., *Jour. Exper. Med.*, 1911, xiii, 397.
[8] Ehrlich, P., *Arb. a. d. k. Inst. f. exper. Therap.*, 1906, No. 1, 84.
[9] Murphy, J. B., and Rous, P., *Jour. Exper. Med.*, 1912, xv, 119.

more so than in the adult of the native species[10] (figure 2). A typical experiment of this kind will be quoted.

Experiment 1.—A large rapidly growing Jensen sarcoma[11] 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 twelve chick embryos on the sixth day of incubation. On the eighteenth day of incubation the eggs were opened. Of the eight remaining alive, one showed no evidence of tumor; all the others showed a spherical mass projecting from the membrane at the point of inoculation. They varied in size from 0.1 to 1.6 cm. in their greatest diameter (figure 3). Histological examination showed these tumors to be made up of cells like those of the original tumor.

Bits of this tissue were inoculated into rats and there developed as a result tumors of the Jensen type.

Many experiments like the foregoing have been done. The growth of the Jensen tumor in the chick is always rapid. An accurate comparison of the rate of growth in the foreign embryo and the native adult would be of great interest, but this is rendered impossible on account of the nature of the injection site in the embryo. A certain proportion of the material injected into the egg escapes into the cavities and the amount clinging to the membrane and giving rise to the tumor varies on this account considerably. The results are sufficient, however, for the statement that the rate of growth is at least as rapid in the chick embryo as in the adult rat. It is of interest to note in this connection that while the normal temperature of the rat is 37.9°, the eggs were incubated at about 40° C.

DESCRIPTION OF RAT TUMORS IN THE CHICK EMBRYO.

For the work described in the present paper the Jensen sarcoma of the rat was used, a tumor widely known and the subject of much experimental work. Our strain gives a rapidly growing tumor in a high percentage of the animals inoculated. This tumor when introduced into the chick embryo grows readily where inoculated in the various membranes or in the body of the chick itself. For the general purposes of the experiment it has been found advantageous to use the outer membrane (fused allantois and chorion) for the reason that it lies just under the shell membrane and, therefore, can

[10] Murphy, J. B., *Jour. Am. Med. Assn.*, 1912, lix, 874.

[11] Jensen, C. O., *Ztschr. f. Krebsforsch.*, 1909, vii, 45.

be inoculated with a minimum amount of trauma. Furthermore, this membrane is the respiratory organ of the chick at this period and is rich in lymphatics and blood vessels. The inoculations were made between the fifth and seventh day of incubation and were allowed to grow till the eighteenth day.[12] Tumors resulting from such an inoculation of the Jensen sarcoma are found in the membrane as large globular masses lying in or suspended from the inner surface of the thin membrane by a broad pedicle. In the ten to twelve days of growth they sometimes attain the remarkable size of 2.1 cm. in diameter. The protruding masses are covered by a continuation of the chick membrane which gives them a smooth and glistening surface. Numerous dilated vessels are seen coursing through the membrane and penetrating the semitranslucent greyish tissue of the tumor itself. On section the nodules are found to be made up uniformly of this semitranslucent tissue, rarely with a small area of necrosis in the center but more frequently small areas of hemorrhage scattered here and there through the tissues.

Microscopically the cells making up these tumors resemble closely 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. They are so characteristic that there is no difficulty in distinguishing the tumor tissue from that of the chick even at the margin of the growth. The pattern of the tumor in the chick generally shows some variation from that in the rat (figures 4 and 5). In the latter the cells are seen in compact bundles coursing in various directions through the section, with a fair number of scattered, thin walled vessels. In the chick the arrangement of the cells is less compact, in some cases forming a loose network with clear spaces between, while in others the picture more closely approaches 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 in almost every microscopic field, and as many as five mitotic figures have been seen in a single field of an oil immersion lens (figure 6). This finding is more striking when it is remembered that in the chick-grown tumors the number of cells

[12] Rous, P., and Murphy, J. B., *Jour. Am. Med. Assn.*, 1911, lvi, 741; Murphy, J. B., and Rous, P., *Jour. Exper. Med., loc. cit.*

per field is much less than in the rat-grown ones. The vessels are much more numerous in the tumors of the embryo, here occurring in two forms, either ingrowths as clusters from the chick membrane (figure 7) or as individuals scattered throughout the tumor (figure 8). Apart from the thin continuation of the chick membrane which covers the tumor and the ingrowth of vessels with their scant accompanying stroma, there is no histological evidence of reaction on the part of the embryo to the invasion of foreign tissue. Certainly there are none of the sort attributed to a defensive reaction under similar conditions in the adult host.

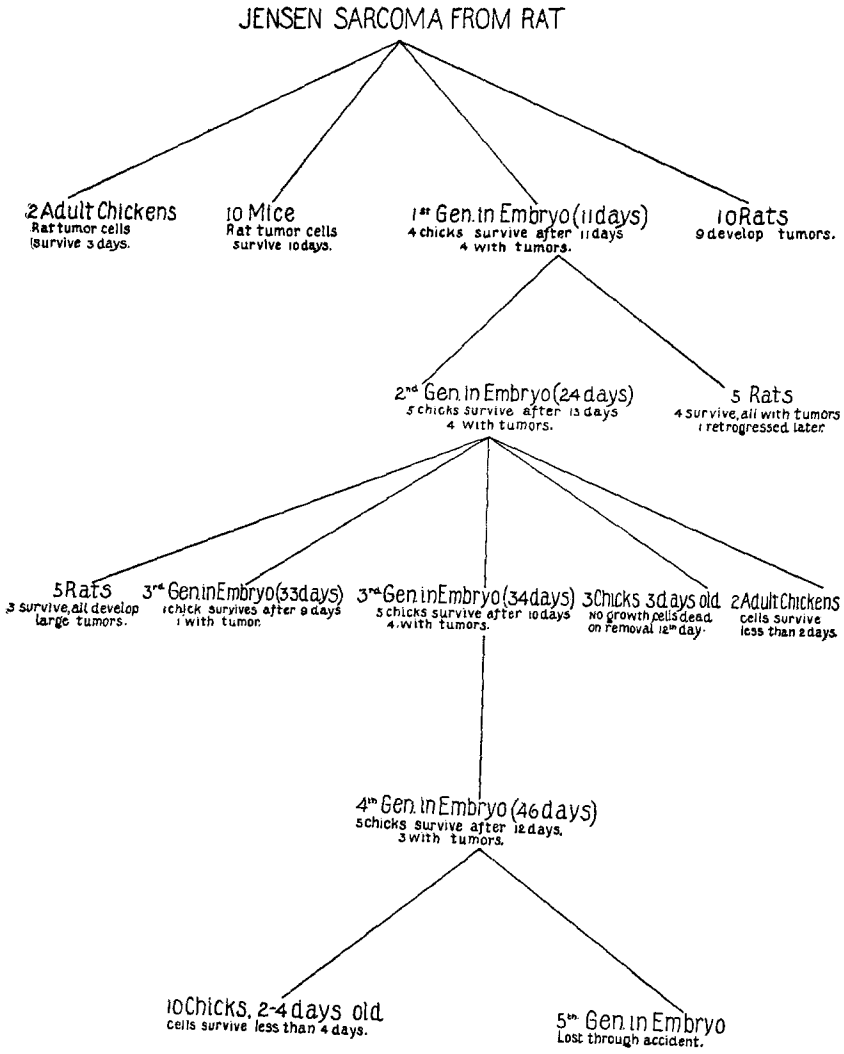
A microscopic study of the tumors occurring in the rat from inoculations with the chick-grown rat tumor shows them to be identical with those propagated in the usual way from rat to rat.

PROLONGED GROWTH OF RAT TISSUE IN CHICK EMBRYO.

The results of the experiments so far reported do not establish beyond question the utilization of the chick food material in the nourishment and growth of the rat cell, but they leave little doubt that this is the case. At the end of the twelfth day in the chick when the specific food carried with the graft from the native species must have been largely exhausted, there is no sign of lessened activity on the part of the tumor cells. Partly to settle this question beyond dispute, but more particularly to determine the changes, if any, that would 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 in rapid growth here, but they grew in turn when transplanted to a third or fourth series (figure 9). There seems to be no reason why by repeating transplantation growth cannot be prolonged indefinitely. Such an experiment with its controls will be quoted.

Experiment 2.—(Text-figure 1.) First generation. A rapidly growing Jensen rat sarcoma was finely hashed and injected into a series of seven day chick embryos. As control some of the same material was inoculated into the following animals: (1) ten rats, nine of which subsequently developed tumors; (2) ten mice, one of which was killed every forty-eight hours and whose tissues on microscopical examination showed survival of the rat cells till about the tenth day when they practically disappeared; (3) two adult

chickens, ten grafts in each, removed at twenty-four hour intervals, whose tissues showed survival of rat cells till the third day.[13] The eggs inoculated were opened on the eighteenth day of incubation, eleven days after the injection; four remained alive, all with large tumors.



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

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

Second generation. The tumors from the above series of embryo were hashed and inoculated as follows: (1) into five rats, four of which survived and all developed tumors; and (2) into a second lot of embryos on the sixth day of incubation. These were opened on the nineteenth day (thirteen days later), five remaining alive, four with tumors

Third generation. The material from the last series was hashed and inoculated into the following animals: (1) five rats, three of which survived, all developing tumors; (2) three young chickens, hatched less than three days before, all developing small nodules which on removal twelve days later were found to be made up of reactive tissue with no rat cells present; (3) two adult chickens, ten grafts each, which on removal at forty-eight hour intervals showed no rat cell surviving even after the first forty-eight hours; and (4) into a lot of eight day embryos, five of which survived till the eighteenth day, four with tumors. The total number of days in the embryo was thirty-four.

Fourth generation. All the material from the above series was used to inoculate another lot of six day embryos. On the eighteenth day five chicks remained alive, three with tumors. Total number of days in embryo, forty-six.

Fifth generation. The material from the above was inoculated into: (1) ten newly hatched chicks about three days old, grafts from which were removed at forty-eight hour intervals beginning with the fourth day. None of the grafts showed surviving rat cells. It was also inoculated into (2) a lot of seven 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 generation in the chick were similar in practically every respect to those grown in one chick for twelve days. The cells showed no morphological changes, but the tissue was perhaps more compact than in the early tumors.

This experiment, showing as it does the ability of the rat cells to survive and grow actively in the chick embryo for at least forty-six days, makes it certain that the rat cells utilize successfully the food offered by the blood of the foreign species. The question of adaptation therefore comes up for consideration. During this growth in the chick, have the rat cells acquired a real adaptation to the strange host? Apparently not. Morphologically there are no changes in the cell (figure 10). The tumors transferred from chick embryo to chick embryo grow neither better nor worse in the later periods than in the first chick to which they were transplanted. The material when returned to the rat caused a rapidly growing sarcoma which is always of the same type as the original tumor.

A comparative study of the fate of rat cells taken directly from the rat and those grown for a period in the embryo, when implanted in an adult, has proved of interest. Some of these results are men-

tioned in experiment 2, and numerous other experiments have been carried out along the same line. As previously mentioned the cells taken directly from the rat and inoculated into the adult chick will survive about three days. The period for the newly hatched chick is about the same. When, however, the rat cells have been grown for a period in the chick embryo and are then placed in the adult chicken, no evidence of them can be found after twenty-four hours. Practically the same results are obtained when newly hatched chickens are used as hosts. Instead of an adaptation to the new conditions the rat cells appear to have become less resistant during their growth in the chick embryos. All these findings show that the tumor cells have retained their essential characters despite the strange environment. Whether a longer dependence on a foreign species embryo would affect an adaptation remains to be seen, but certainly the present results offer little encouragement in this direction.

The conditions that make a chicken an unsuitable host for the growth of a foreign species cell seem, according to these experiments, to develop during the last two days of shell life, for it is certainly not present before that time and is present at the time of hatching. An attempt is being made now to establish the nature of this reaction and the exact time of its development.

GROWTH OF OTHER TISSUES IN THE CHICK EMBRYO.

Besides the rat sarcoma a variety of other tissues have been grown in the chick embryo for longer or shorter periods. Highly organized tissues of the chick itself, which fail to grow or grow poorly on transplantation to the adult, will grow in the embryo. Such tissues as kidney (figure 11), testicle, ovary, bone, cartilage, etc., have been grown for a period of from seven to ten days, but as yet no attempt has been made to carry them farther. Embryomata formed by inoculation of hashed chick embryo can be carried through several generations. They resemble the embryomata seen in the adult. After several transplantations of the embryomata the epithelial elements are less evident, the bone, cartilage, and connective tissue predominating.

Several other tissues of foreign species were grown with success,

among them the Ehrlich sarcoma and chondroma of the mouse, embryomata of the rat and mouse, a mammary carcinoma of the mouse (figure 12), and the Flexner-Jobling adenocarcinoma of the rat. Attempts with human tissue met with only moderate success. due perhaps to the time that elapsed between removal from the body and the introduction into the eggs. However, with this exception the tissue grew actively, with numerous mitotic figures and a copious blood supply from the embryo.

DISCUSSION.

The question of an adaptation of the rat cells to a new host is naturally of great interest. The evidence of change in the tissue is slight, if present at all. Instead of becoming capable of a longer survival in the adult or even newly born individual of the foreign species after the long dependence on its embryo, the rat cells have become more susceptible to the unfavorable conditions existing in these hosts. As mentioned above, this retention of characteristics has an analogy in the plants and lower animal forms. The branches of one species of tree may be grafted into the trunk of another, but though it depends upon the sap supplied by the roots of a foreign species, it retains all its own characteristics. In the lower animals where it is easy to graft parts of closely related species, it is observed that where small pieces of one species can be grafted on to another, the minor component will retain its characteristics. When a part of this minor component is removed, the regeneration that takes place will be like the minor component, although dependent on the major component of a foreign species for its nervous system, alimentary tract, and vascular system.

The fact that rat cells will grow in the chick embryo almost until the time of hatching, and when transferred to second, third, and fourth lots are found at the end of forty-six days of continuous growth in the foreign species embryo to be growing as actively as the same tissue in the rat, leaves no possibility of doubt that the avian food is just as suitable as the native food for the maintenance and growth of the tissue. The inability of the embryo to elaborate a defensive substance against this invasion is not surprising when it is remembered that in very young animals certain of the immunity

substances, as hemolysins, can only be developed to a very slight extent or not at all.[14]

With the evidence at hand from these experiments, it would seem that the athrepsia theory of Ehrlich does not apply to this special case. We have evidence of the ability of the rat cells to utilize nutritive substances from the embryo of a foreign species. It might be suggested that the embryo offers a food of less specific composition and therefore utilizable by a foreign species cell. The evidence presented by the experiments in adults would favor another interpretation. If the temporary survival and growth of the cells when transferred to an adult of a foreign species is to be accounted for by the specific food carried with the graft from the native animal, we should expect the tissues to live an equal length of time whether in a closely related or more distantly related species. But such is not the case. I have shown here that while the rat cells can live for from nine to eleven days in the mouse, they die off in the adult chicken by the third day. It would seem much more likely that there is a defensive mechanism whose strength and rapidity of reaction depend upon the degree of relationship, being more prompt and violent the more foreign the tissue introduced. We have at least one example in immunity reactions of the fine gradation between species, namely, in the precipitin phenomena.[15] Here a precipitin developed for the sera of one animal will give a heavy precipitate with all sera from animals of the same species and a progressively fainter reaction with sera of other species, depending on the distance of relationship.

The type of reaction that prevents the growth of a tissue in a foreign species adult is as yet a matter of speculation. That it is a property which is developed rather than one naturally present in the adult is strongly suggested by the experiments of Russell.[16] He found that the cells of a mouse survived and multiplied in a normal rat for more than nine days, while in a rat previously immunized the

[14] Famulener, L. W., *Collected Studies from the Research Laboratory, Department of Health, New York,* 1911, vi, 199.

[15] For the literature and a comprehensive review of the subject, see Nuttall, G. H., Blood Immunity and Blood Relationship, Cambridge, 1904.

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

graft was rapidly disintegrated, all cell outlines being obliterated by the fourth day. Lambert and Hanes[17] have added the observation that rat and mouse tissues will grow almost as well in plasma from an alien as from the native species. They have further shown[18] that if the animal from which plasma is obtained is previously immunized with the living cells of the foreign species, the plasma will inhibit or actually prevent any such activity. It has been suggested that this may be due to a cytotoxin. We have little conclusive evidence either from these experiments of Lambert and Hanes or from mine as to the exact nature of this defensive mechanism. Little more can be said at present than that such a defense exists.

SUMMARY.

Inoculation of the Jensen rat sarcoma into the developing chick embryo gives a rapidly growing tumor at the site of inoculation, whether in the membranes or in the body of the chick itself. These tumors by transfer from embryo to embryo can be kept going for as long as forty-six days, and perhaps indefinitely in the foreign species. The rat cells show no morphological change even after a very long dependence. Their biological characters are also retained, as is shown by the fact that the cells when replanted in the rat, after a prolonged sojourn in the chick, will produce a rapidly growing sarcoma of the Jensen type. These rat tissues grown for long periods in the chick show no adaptation to the new species, being destroyed even more rapidly when placed in the adult chicken than cells taken directly from the rat. Morphologically the cells retain a close resemblance to those in the original tumor.

Other tissues grown in chick embryo are various embryonic cells from the chicken, mouse, and rat, the Ehrlich sarcoma and chondroma of the mouse, a mammary carcinoma of the mouse, the Flexner-Jobling adenocarcinoma of the rat, and a human sarcoma.

EXPLANATION OF PLATES.

PLATE 84.

FIG. 1. A chicken sarcoma growing in the yolk sac of a pigeon embryo (*A*), seven days after inoculation.

[17] Lambert, R. A., and Hanes, F. M., *Jour. Exper. Med.*, 1911, xiv, 129.

[18] Lambert, R. A., and Hanes, F. M., *loc. cit.*, p. 453.

FIG. 1.

FIG. 2.

(Murphy: Transplantation of Tissue to Embryo.)

FIG. 3.

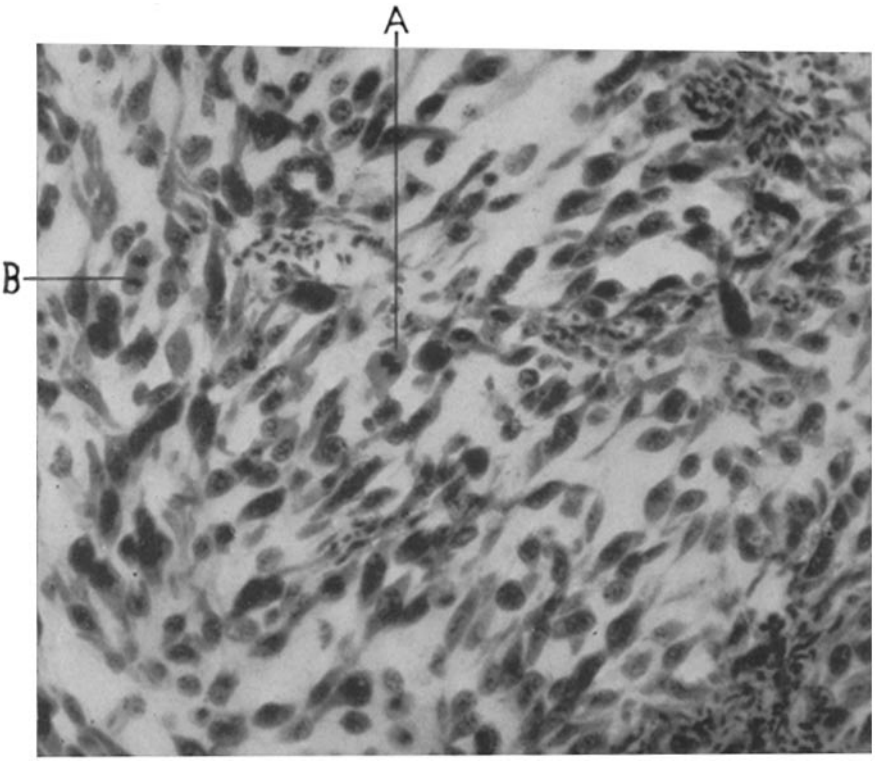


FIG. 4.

(Murphy : Transplantation of Tissue to Embryo.)

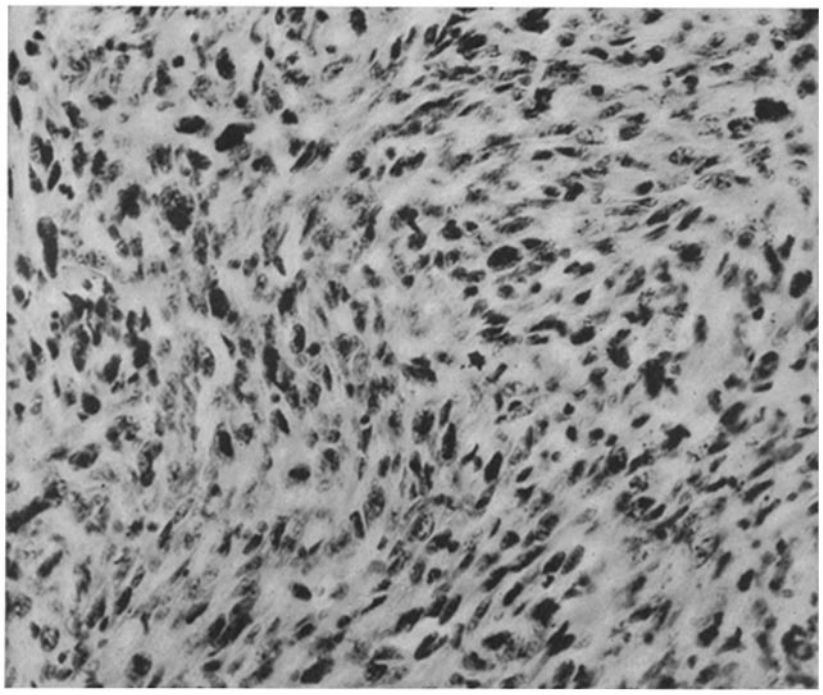


FIG. 5.

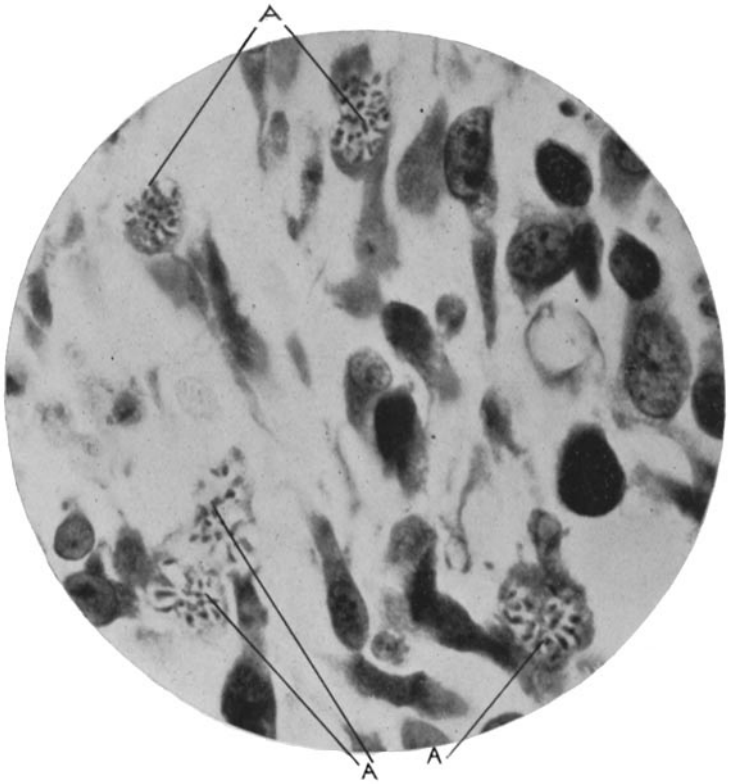


FIG. 6.

(Murphy : Transplantation of Tissue to Embryo.)

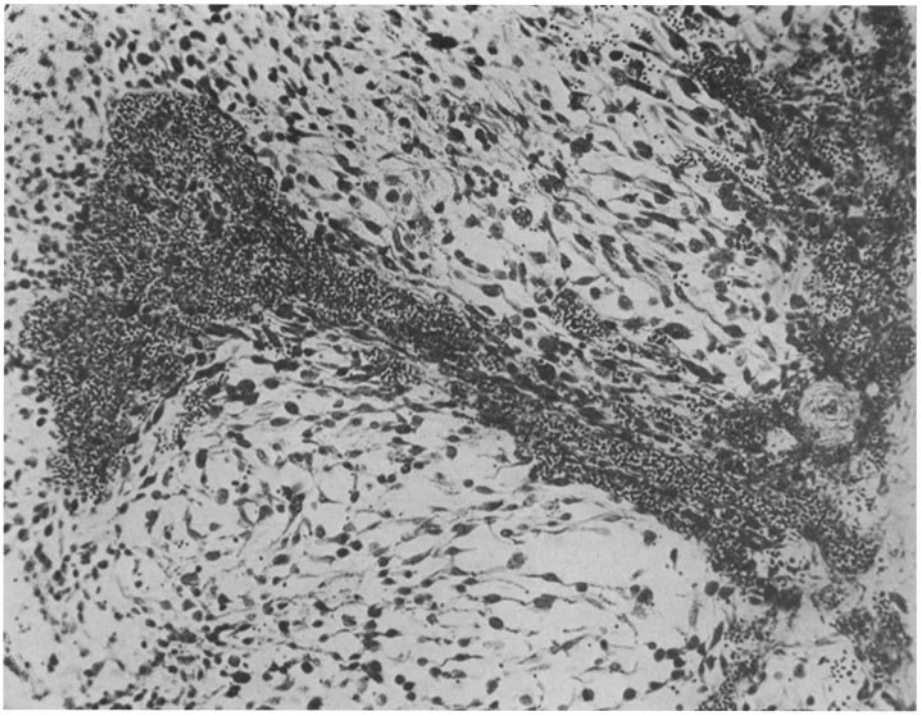


FIG. 7.

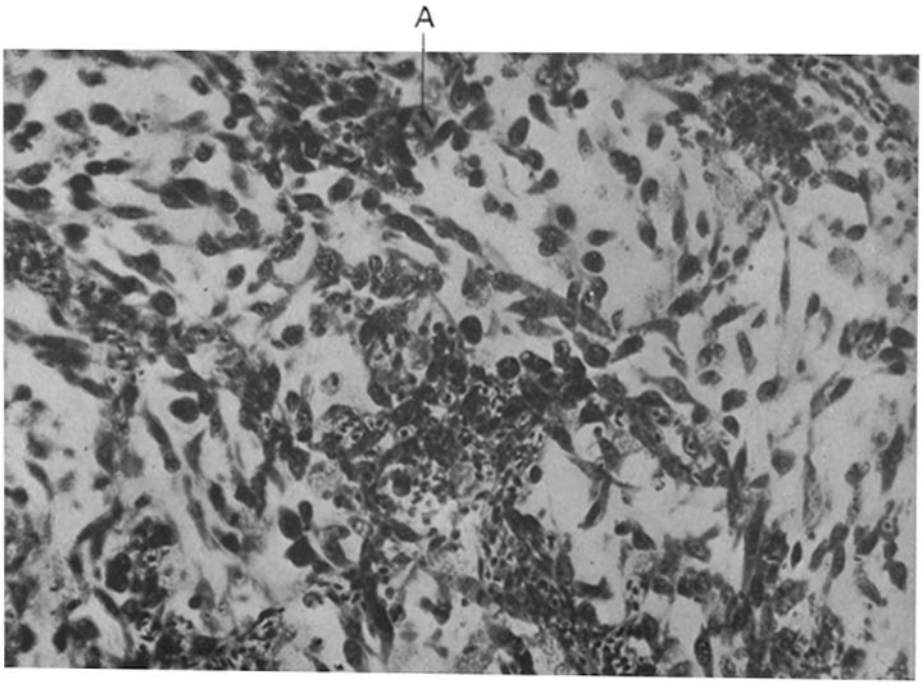


FIG. 8.

(Murphy : Transplantation of Tissue to Embryo.)

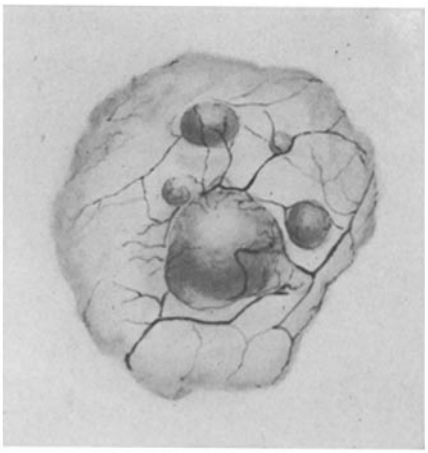


FIG. 9.

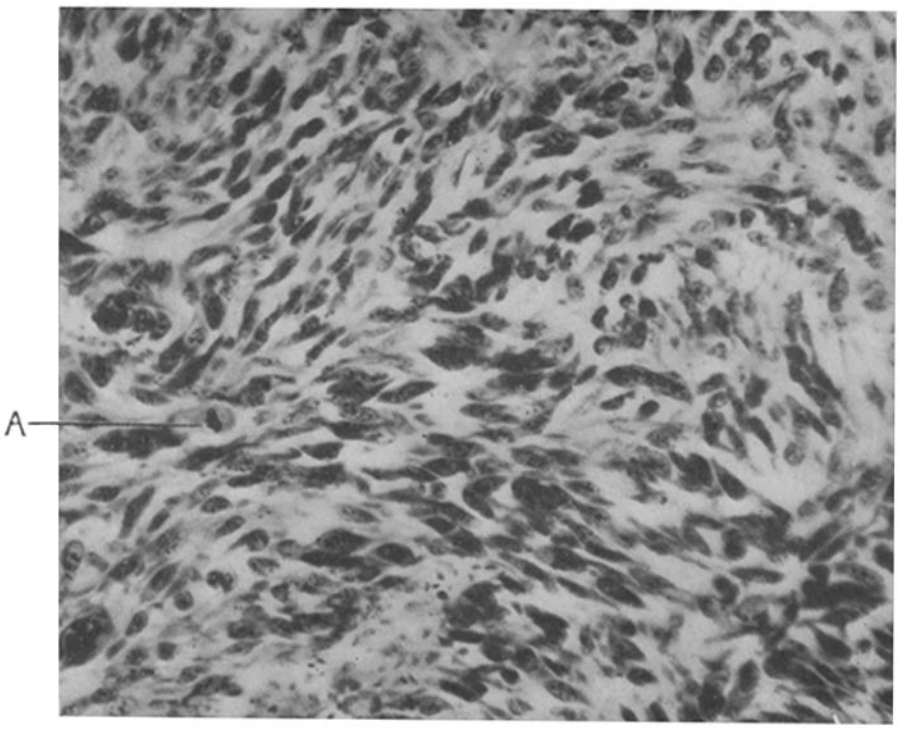


FIG. 10.

(Murphy : Transplantation of Tissue to Embryo.)

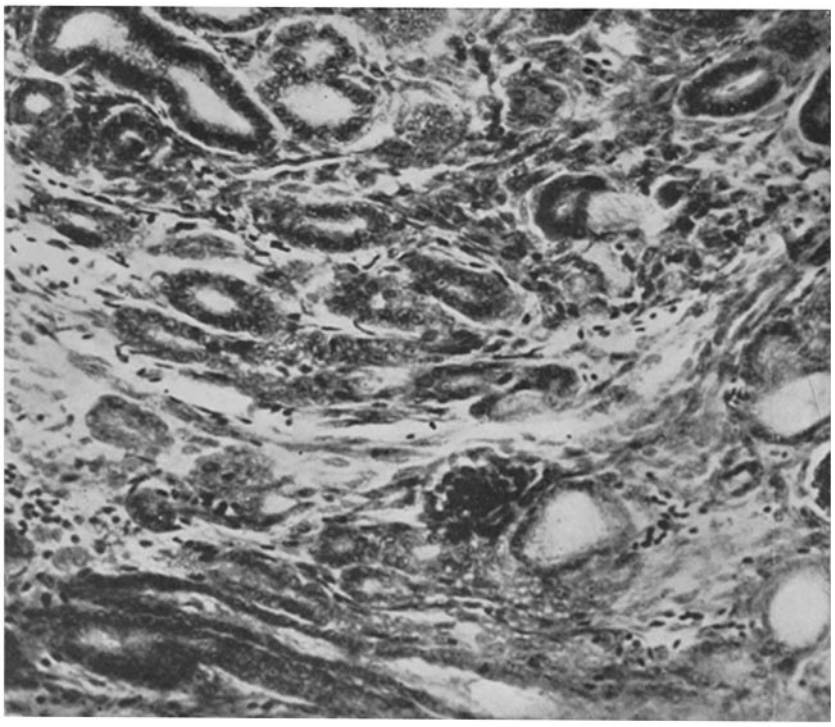


FIG. 11.

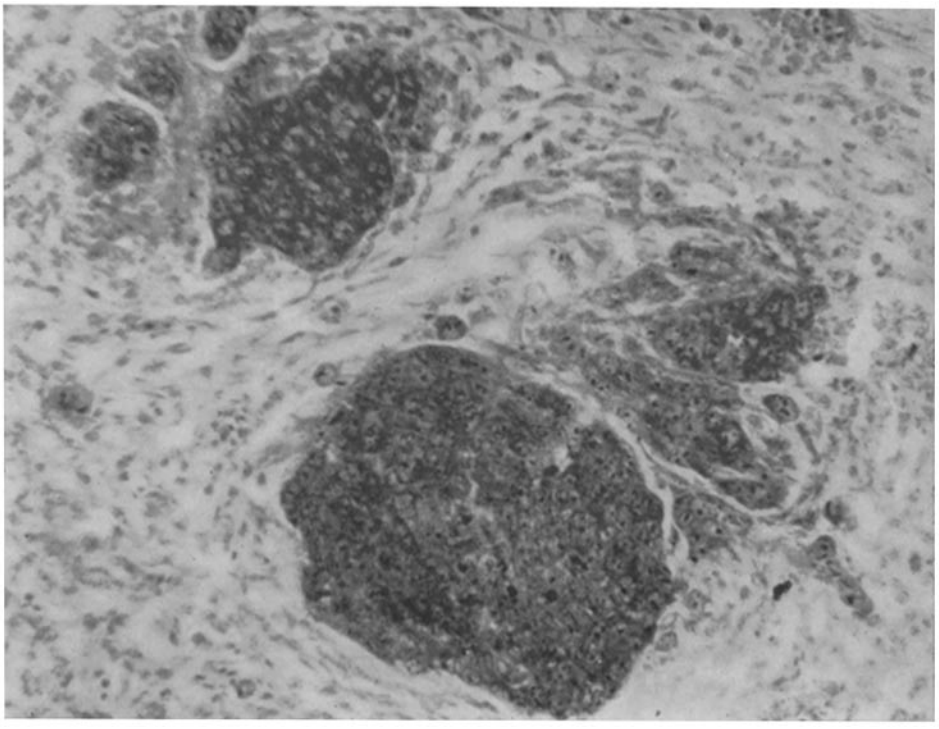


FIG. 12.

(Murphy : Transplantation of Tissue to Embryo.)

FIG. 2. The Jensen rat sarcoma in a chick embryo. This animal was killed on the eighteenth day of incubation, seven days after inoculation. The tumor lay partly in the cranial cavity, extending through an opening in the skull and protruding outward.

PLATE 85.

FIG. 3. The Jensen rat sarcoma in the outer membrane of an eighteen day embryo, eleven days after inoculation. The tumor measured 1.7 by 1.5 cm.

FIG. 4. A section of rat sarcoma after twelve day's growth in a chick embryo. *A* and *B* = mitotic figures.

PLATE 86.

FIG. 5. The Jensen rat sarcoma as it appears growing in the rat.

FIG. 6. A rat sarcoma after eleven days in a chick embryo, showing five mitotic figures.

PLATE 87.

FIG. 7. A tuft of vessels from the chick membrane growing into a rat tumor which it supports. The membrane is on the right side.

FIG. 8. A second and more common type of vessel distribution in a rat tumor growing in chick embryos. The rat cells are seen clustered around the small scattered vessels. *A* = mitotic figures.

PLATE 88.

FIG. 9. A group of rat sarcomata in a chick membrane, second generation in the embryo, with a total of nineteen days of continuous growth in the foreign species. The central tumor measured 1.4 by 1.4 cm.

FIG. 10. A section of rat sarcoma after four generations in chick embryos (forty-six days). *A* = mitotic figures.

PLATE 89.

FIG. 11. Growing kidney tubules in the outer membrane of an eighteen day chick, resulting from the inoculation of embryonic kidney.

FIG. 12. A mammary carcinoma from a mouse growing in a chick embryo. The epithelial cells are seen in clusters.