THE RELATION OF THE REACTIVE STROMA FORMATION TO THE TRANSPLANTABILITY OF THE CANCERS OF THE WHITE RAT.*

By I. LEVIN.

(From the Department of Pathology of Columbia University, College of Physicians and Surgeons, New York.)

PLATES LXXX–LXXXIII.

Every malignant growth, whether carcinoma or sarcoma, consists of two parts: parenchyma, composed of cancer cells; and a connective tissue ground work, or *stroma,* which is distributed around and between the cells of the parenchyma. The true significance of the latter structure is still under discussion.

Ribbert (1) considers the stroma of paramount importance in the genesis of cancer. He believes that every normal tissue cell possesses an inherent power for unlimited proliferation, and may change into a cancer cell. The only condition for this transformation is a preliminary change in the surrounding connective tissue, which impairs the normal association of the cell with the rest of the organism. Bonney (2), in a recent investigation on the connective tissue in carcinoma, reaches very similar conclusions, *i. e.,* that the formation of carcinoma is always preceded by a precarcinomatous state, which consists in various inflammatory processes in the surrounding tissues. This precarcinomatous process assists the progress of the growth of the tumor. According to these investigators, the changes in the connective tissue are the primary etiological factors in the formation of cancers.

Hansemann (3), Heidemann (4), Borst (5), and many other pathologists, on the other hand, deny the great importance of the connective tissue for the formation of cancer. According to the view of these writers, the main functional part in cancer is the parenchyma, and the stroma is only formed subsequently under the influence of the cancer cells. This opinion is based on the following grounds:

The beginning of a new growth is not always accompanied by changes in the surrounding connective tissue. Furthermore, many characteristics in the morphological appearance of malignant tumors indicate that the connective tissue

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does not influence the proliferation of the cancer cells, but the latter, on the contrary, induce in some manner the formation of a connective tissue stroma. The amount of connective tissue between groups of cancer cells varies greatly in the different tumors. It is minimal in sarcoma, small in medullary carcinoma, and very large in scirrhous carcinoma. In the majority of cases, the connective tissue in a metastasis of a malignant tumor retains its morphological characteristics. This factor can be explained only on the basis of a *specific* influence of the cancer cells upon the surrounding connective tissue. Further proof of the specific influence of the cancer cells upon the connective tissue can be found in the fact that certain cancers act only on specific forms of connective tissue. While the stroma of a cancer usually consists of newly formed fibrous connective tissue and blood vessels, von Recklinghausen (6) and Askanazy (7) have shown that, in the bone metastasis of the carcinoma of the prostate, the cancer cells induce a formation of new bone tissue. Ehrlich (8) and Gierke (9), in their studies on hemorrhagic tumors of the white mice, noted that these tumors on every subsequent implantation produced in the new host a stroma formation consisting mainly of blood vessels.

These brief preliminary considerations show that the study of the connective tissue of malignant tumors is important, not only from the standpoint of morphological differentiation, but also for the elucidation of many factors in the growth and genesis of cancer.

The experimental study of the transplantable cancers of white mice and rats offers a better field for the investigation of the subject than the mere anatomical study of human cancer.

Jensen (10) indicated in his first publication the possibility that the new stroma is formed by the host. Bashford and his collaborators have paid a great deal of attention to this phase of the subject in their studies on cancers of the white mice. Bashford, Murray, and Cramer (11) have shown that when a piece of a mouse carcinoma is inoculated into another animal the implanted stroma becomes necrotic, and a new connective tissue stroma is formed by the host. According to Bashford, the stroma and vascular structures are a reaction on the part of the successive hosts, whereby the parenchyma is nourished and supported.

This stroma reaction of the host is perfectly specific for every tumor, *i. e.*, in all the subsequent inoculations the stroma retains the structural characteristics of the primary tumors. On the other hand, when two tumors are inoculated into the same animal, the host provides for each tumor a stroma of a different structure identical morphologically with the structures of the stroma of the original tumors used for inoculation. The same is still more apparent in the mixed tumor which appears in the white rat as a result of an injection of an emulsion of sarcoma and carcinoma cells. In the

same tumor one notices a minimal stroma formation in the parts showing the structure of sarcoma, and a great deal of stroma in the carcinomatous parts of the tumor.

Thus it is shown experimentally that the connective tissue stroma of the transplantable tumors of the white mice and rats is supplied by the new host under the influence exerted by the implanted cancer cells.

In his studies on the same subject, Russell (12), a collaborator of Bashford, goes still further in his conclusions, and tries to show that the growth of a cancer inoculated into a new host, depends upon the ability of the latter to supply the connective tissue stroma. His investigation consisted in a microscopical study of the "early stages" of tumor grafting in normal and immune animals. Small particles of tumor tissue were inoculated with a trocar into the subcutaneous tissue. After varying periods of six, twelve, twenty-four hours, etc., the grafts were removed and examined microscopically.

The results showed that while during the first two days the changes in the graft are similar in normal and immune animals, a great difference is noticed five or six days after inoculation. In the normal animal there appears an active division of the connective tissue cells of the host, leading to the development of a cellular reaction tissue, which penetrates between the islets of parenchyma. This connective tissue stroma formation is accompanied by a rich development of new blood vessels. In the immune animal, on the other hand, there is no active proliferation of the host fibroblasts, nor is there any development of new capillaries, and the new host fails to supply a vascular stroma.

This absence of "the specific stroma reaction" in immune animals is due, according to Russell and Bashford, to the suppression of the chemiotatic properties of the cancer cells. This suppression robs the cancer cells at the same time of their powers of assimilation and growth, and to this phenomenon Bashford ascribes a very general importance in the explanation of the condition of immunity, or resistance to growth of an inoculated cancer. To put it in his own words: ". . . a refractory condition may indeed not have anything to do with an action against the cancer cells, but, on the contrary, be due to an alteration of the connective tissue of the host, which hinders it from supplying the necessary connective tissue reaction."

The question whether the success of tumor transplantation in the white mouse or rat is due to the different reactions of the tissues of these animals to the implantation of cancer cells as compared with other laboratory animals or with man, was the subject of a series of investigations by the writer (13). Experiments with inoculations of normal tissue, with injections of aleuronat, scharlach R-oil, etc., have shown that the white rat reacts with a profuse connective tissue cell proliferation to all the different stimuli. All these facts and also

the remarkable phenomenon observed by Apolant (14), L. Loeb (15), and Bashford, Murray, and Haaland (16), that the new stroma found in the host under the influence of an implanted carcinoma may itself change into a sarcoma and subsequently suppress the carcinoma, indicate the importance of the stroma of cancer.

Conditions may yet be found under which a malignant transplantable tumor will be produced artificially in white mice or rats, and work with this end in view is in progress by the writer.

Nevertheless there will not be offered sufficient proof to the existence of a correlation between the reactive stroma formation and the transplantability of the cancers of the white rat or mouse, unless it be shown that a preliminary connective tissue stroma formation presents the only method whereby a tumor may be successfully inoculated. The following observation drew the attention of the writer forcibly to this question. The tumor which served for this investigation, a spindle-celled sarcoma of the white rat, grows, upon subcutaneous inoculation, in a manner similar to the one described by Russell for mouse carcinoma. There forms through the proliferation of the connective tissue cells of the host an extensive reactive stroma around the graft. This stroma is well vascularized, but in view of the sarcomatous structure of the inoculated tumor, the newly formed connective tissue does not penetrate to any appreciable extent between the parenchymatous cells of the tumor. Figure 1 shows very clearly this new stroma formation around a tumor graft three days after a subcutaneous inoculation. In its further development the tumor retains this surrounding stroma, does not infiltrate the musculature, fascia, or skin, and always remains a strictly encapsulated growth. This tumor, while very malignant, rarely forms metastases, and then usually in the lungs. In one animal, numerous metastatic nodules were observed in the liver. These metastases were not surrounded with a capsule, but showed a diffuse infiltrative growth in the parenchyma of the liver (figure 2). Thus the same tumor shows great differences in its manner of growth, when appearing in different tissues of the same organism.

The question then presented itself, whether different results in regard to reactive stroma formation could be obtained if the inoculation of the tumor were made into the parenchymatous organs

instead of subcutaneously. The present investigation was undertaken to answer this question.

Notwithstanding the great amount of research done during the last decade on transplantable cancers of the white rat and mouse, there have been very few attempts at inoculation of tumors into parenchymatous organs. Ehrlich (17) simply mentions the fact that Goldman successfully inoculated tumors for him into different organs. Similar successful inoculations are reported by Kraus, Ranzi, and Ehrlich (18), and by Graf (19), but none of these investigators seem to have paid any attention to the character of the stroma. Flexner and Jobling (20) twice succeeded in causing a rat tumor to grow in the testicle. They say that in the periphery of the tumor nodules multiplying tumor cells were lying in a fibrous stroma. Stumpf (21) inoculated mouse carcinoma into the kidney, and noted a certain diminution in the reactive stroma formation. His experiments did not seem to be very successful; he reported that in 80 per cent. of the operations the tumor fell out of the kidney and grew in the peritoneal cavity, and he stated also that it was very difficult to evade sepsis after the operation.

As was stated above, the present investigation was made with a transplantable sarcoma of a white rat, and the tumor was inoculated into the testicle, brain, and kidney. The following is a description of the experiments.

INOCULATION INTO THE TESTICLE.

This experiment was repeated on thirty animals. At first the operation was done with surgical technique, *i. e.*, a small incision was made in the tunica albuginea, a piece of the tumor was placed in the centre of the testicle, and the opening in the capsule was closed with a suture. In subsequent experiments a piece of the tumor was placed in the centre of the testicle simply by the aid of a trocar needle. The testicles were removed and examined at periods varying from twenty-four hours to four weeks after the inoculation.

On gross inspection of the organs, one notices that the tumor grows centrifugally from the inoculated piece to the periphery of

the organ, gradually replacing the testicle tissue. The organ increases in size under the pressure of the growing tumor, which ultimately breaks through the capsule and grows outside of the organ. The percentage of cases where the tumor did not grow in the testicle corresponds closely with the percentage of unsuccessful takes on a subcutaneous inoculation of the same tumor.

But the most striking picture presents itself on the microscopical examination of this sarcoma. The sarcoma cells spread diffusely between the seminiferous tubules replacing the strands of connective tissue cells of the organ. There is no indication of any connective tissue reaction, small round cell infiltration around the growing tumor, or any stroma formation. The tumor cells do not grow in one compact mass, but infiltrate diffusely and branch out into the different spaces between the tubules. Figure 3 illustrates the appearance of the tumor three days after the inoculation. The proliferation of the tumor cells is apparently just as active as it is three days after a subcutaneous inoculation (figure 1). But while in the latter case there is an extensive stroma formation around the group of proliferating tumor cells, there is no such connective tissue reaction in the testicle.

INOCULATION INTO THE BRAIN.

The operation consists in drilling an opening into the skull over the area of the right or left hemisphere. The hole is made large enough to permit the introduction of the trocar needle used for the inoculation. By the aid of this needle, a piece of the tumor is introduced into the brain, and then the skin incision is sutured.

The experiment was repeated on ten animals. One animal died from the operation and the rest were killed at periods ranging from twenty-four hours to ten days after the inoculation. On gross inspection one notices again, as in the testicle, that the tumor grows centrifugally, and replaces the brain tissue so as to occupy practically the greater part of a hemisphere. The microscopical picture is similar to the one observed in the testicle. The sarcoma cells infiltrate diffusely the brain substance. The margins of the growth show an extremely irregular form, and there is no indication of any

connective tissue stroma formation around the growth. Figure 4 shows the appearance of the tumor three days after inoculation into the brain and clearly illustrates all the points mentioned above.

All the animals developed symptoms of intracranial pressure. By placing the pieces of the tumor into different regions of the brain (which is technically easy of performance) it is possible that a great deal of information may be gained on the pathology of intracranial tumors. This subject will be considered in a future investigation.

INOCULATION INTO THE KIDNEY.

This operation is performed in the following way. The kidney is exposed by a lumbar incision and brought into the operative field by counter-pressure from in front. An incision is made with a fine scalpel along the convex border lengthwise of the kidney. A small piece of the tumor is inserted with forceps, the slit in the kidney closed with one suture of very fine silk, and the incision in the skin closed with a continuous suture.

The experiment was repeated on twenty-five animals. The percentage of cases, where the tumor did not grow corresponded with the percentage of unsuccessful takes on subcutaneous inoculation. In no instance did the implanted piece of tumor fall out of the kidney, nor did a single animal die as a result of the operation. The gross appearance of the tumor growth in the kidney is similar to the one observed in the testicle and brain. Microscopically one notices again complete lack of reactive stroma formation around the growing tumor. The sarcoma cells infiltrate the spaces between the tubules or glomeruli. When the tumor grows to a larger size, groups of glomeruli may be observed surrounded everywhere with sarcoma cells. Figure 5 illustrates the diffuse spreading of the tumor cells in the kidney and absence of connective tissue reaction around the growing edges of the tumor.

In attempting to analyze the significance of the results obtained by this investigation, one must bear in mind the fact that the connective tissue groundwork in tumors consists of two distinct parts. One is the specific connective tissue stroma between groups of cancer cells. This stroma, as was stated above, retains its structural

characteristics for each type of tumor, and its formation is probably due to a specific chemiotactic influence of the cancer cell upon the adjacent fibrous connective tissue or endothelium of the blood-vessels. In an artificially inoculated tumor, the formation of this specific stroma begins only after the implanted cancer cells become ingrafted and are enabled to use the nourishment of the new host. Only then do the cancer cells develop their power for unlimited proliferation and their chemiotatic influence upon the neighboring connective tissue cells. This latter part of the stroma plays a very subordinate rôle in the formation of a sarcoma, but it is demonstrable. Figures 7 and 8 show that this intercellular connective tissue stroma appears to be identical whether the sarcoma was inoculated subcutaneously or into the testicle. The other part of the connective tissue groundwork presents a layer of fibrous tissue, frequently accompanied by a small round cell infiltration, which surrounds and encapsulates the whole tumor and walls it off from the surrounding normal tissue. The presence of this surrounding stroma is not a general characteristic in human malignant tumors, and while it is very extensive in scirrhous, for instance, it may not be present at all in medullary carcinoma. Nor is the significance of the presence of this part of the stroma as uniform as the intercellular part, which latter in all cases apparently serves both for the support and nourishment of the cancer cells. In certain conditions, the formation of the surrounding stroma is a result of an inflammatory process which precedes the development or further growth of the tumor. This "precarcinomatous state of the surrounding connective tissue," as Bonney calls it, may facilitate the further growth, or even give rise to the formation of the tumor.

On the other hand, Borst (22) and Orth (23) have shown, that in certain tumors a connective tissue stroma may surround a group of cancer cells, gradually increase in size, compress the cancer cells and produce, as it were, a local cure of the process. In this case, Orth thinks that the primary factor is a degenerative change in the cancer cells, while the connective tissue stroma which forms subsequently is of the same nature and origin as the connective tissue capsule which forms around a foreign body or a conglomeration of

dead cells. The same explanation must be given apparently to the formation of the connective tissue capsule which was described by Schmidt (24) as taking place around a tumor embolus which penetrates into the lumen of the blood vessels.

In transplantable tumors, provided the inoculation is subcutaneous, the success of grafting depends upon the formation of a connective tissue stroma around the implanted piece. By the subcutaneous method, the piece of tumor is placed in a loose pocket at a great distance from the blood vessels either of the skin or the muscles. Unless the inoculated piece is surrounded promptly by a round cell infiltration and by fibroblasts, and unless a connective tissue and vascular stroma is formed, the tumor cells can not become ingrafted, and die from starvation. But the formation of this primary surrounding stroma is not due to any *specific* influence of the cells of the graft. As was stated above, this specific chemiotactic influence of the implanted cancer cells develops only subsequently, and then serves to form the specific intercellular stroma. A piece of normal tissue or any foreign body is surrounded by a similar vascular connective tissue stroma when introduced subcutaneously into a rat. Whatever the cause of the formation of primary stroma after a subcutaneous inoculation, the influence upon the cells of the host is identical, whether a piece of normal tissue or a piece of a malignant tumor be used for inoculation.

Levin and Sittenfield (25) have shown, in a recent communication, that the success of a tumor implantation depends upon two factors: first, whether the implanted piece becomes ingrafted, and second, on the capacity of the implanted cells to proliferate indefinitely in the new host. The formation of the surrounding stroma is of importance only for the success of the first step, the ingrafting, and it has no influence upon the subsequent proliferation of the inoculated cells and the formation of a malignant tumor. Figure 6 shows extensive connective tissue stroma formation around a piece of a mouse sarcoma inoculated subcutaneously into a rat. The cells of this mouse tumor obtain at least as good a primary surrounding stroma as they would in a mouse, and they even proliferate for a few days, but ultimately they become absorbed and do not form a malignant tumor in the foreign host.

But what is of much more importance, the present investigation shows conclusively that the formation of a primary surrounding stroma is not a necessary condition for the success of the ingrafting. When a piece of tumor is inoculated into a parenchymatous organ, it is placed so near the blood-vessels of the organ that it is enabled to obtain immediately the necessary nutrition. As a result, the first visible step after such an inoculation is not the formation of new connective tissue, but the proliferation of the cancer cells themselves. The specific intercellular stroma is formed later, after the implanted cancer cells become adjusted to the conditions of nutrition in the new host.

The present investigation tends to show further, that the absence of "the specific stroma reaction" in immune animals can not have a general application in the explanation of the phenomenon of the resistance of certain animals against the inoculation. It may serve only to explain the success or failure of the grafting of a tumor subcutaneously. Not only does this theory fail to explain the further growth and development of the inoculated tumor, but it is also inadequate to explain the first stage of grafting when the inoculation is made into a parenchymatous organ, since a stroma formation does not represent the first step of such an inoculation.

The study of the fate of tumor tissue when inoculated into the parenchymatous organs of immune animals will be the subject of further research.

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EXPLANATION OF PLATES.

PLATE LXXX.

FIG. 1. A graft of sarcoma three days after subcutaneous inoculation. The right side of the figure shows the part of the graft which became necrotic, and the centre, the proliferating part of the tumor which is surrounded to the left, above and below, with connective tissue stroma.

FIG. 2. The centre shows a metastasis of the sarcoma infiltrating diffusely into and surrounded by normal liver tissue.

PLATE LXXXI.

FIG. 3. A sarcoma graft in a testicle, three days after introduction. The centre of the figure shows the sarcoma cells infiltrating diffusely between the seminiferous tubules.

FIG. 4. A sarcoma graft in a hemisphere of the brain, three days after introduction. The centre of the figure shows the sarcoma cells diffusely infiltrating the brain substance. There is no indication of formation of stroma.

PLATE LXXXII.

FIG. 5. A sarcoma graft in a kidney, three days after introduction. The right side of the figure shows the necrotic part of the graft, and the centre, the proliferating part of the tumor, which invades diffusely the kidney tissue to the left. There is no indication of stroma formation.

FIG. 6. A graft of mouse sarcoma in a rat, three days after subcutaneous inoculation. The centre of the figure shows a small group of sarcoma cells surrounded everywhere by a connective tissue stroma. Numerous giant cells are seen.

PLATE LXXXIII.

FIG. 7. A sarcoma graft in a kidney, three days after inoculation. It shows a few fibrillæ between the sarcoma cells and around the kidney tubules. No con-

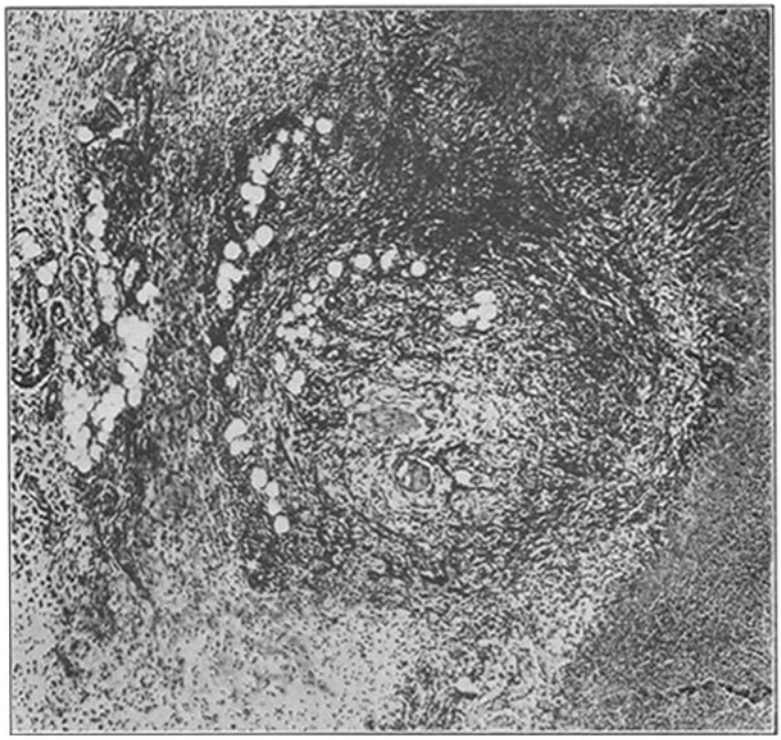


FIG. 1.

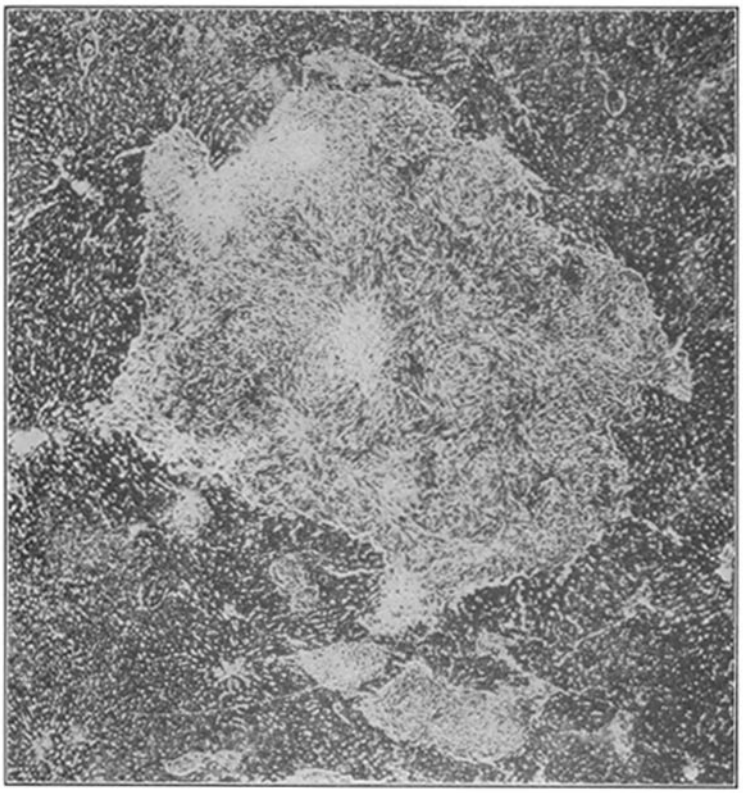


FIG. 2.

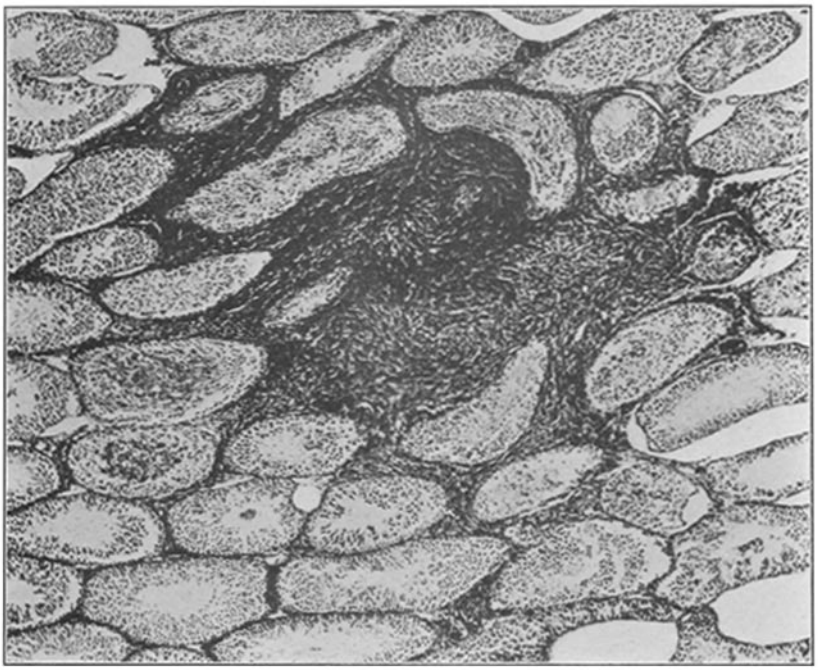
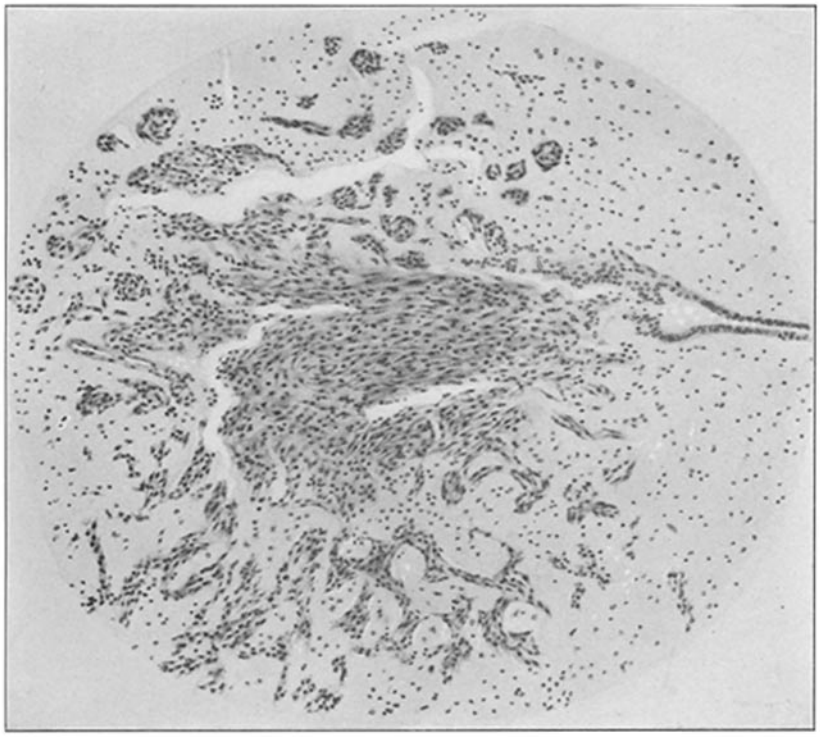


FIG. 3.



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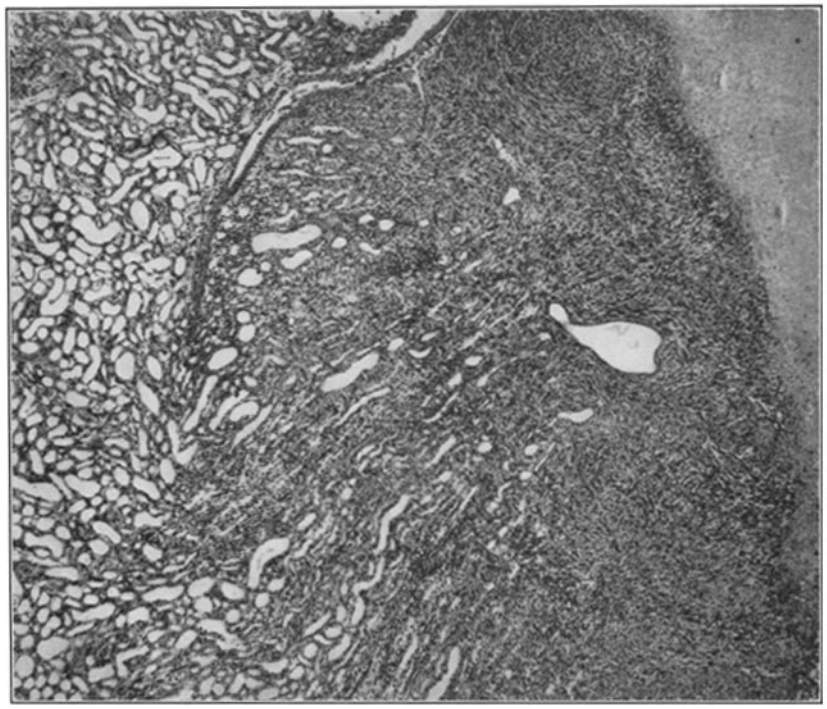

FIG. 5.

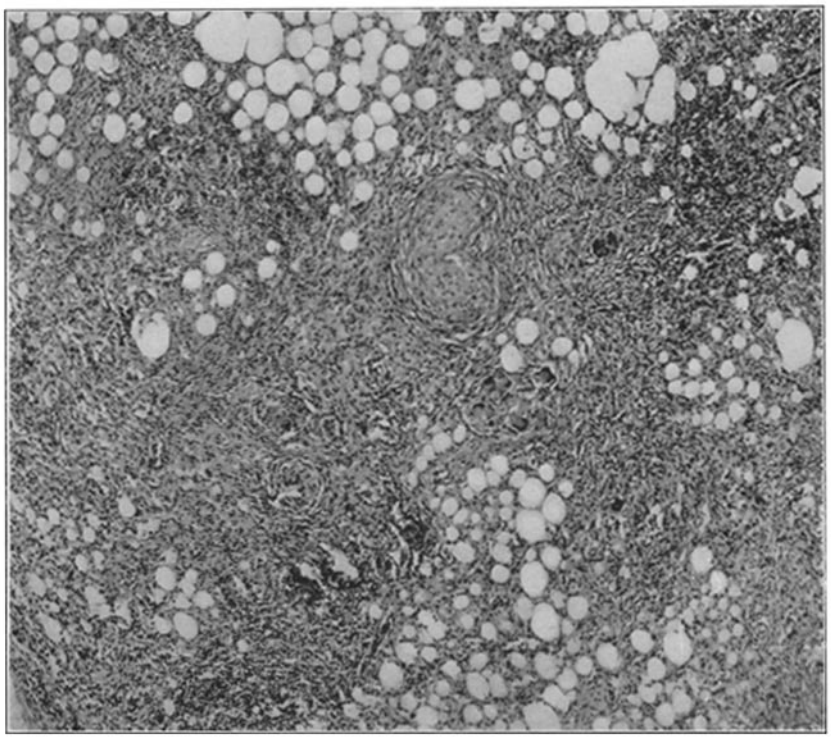

FIG. 6.

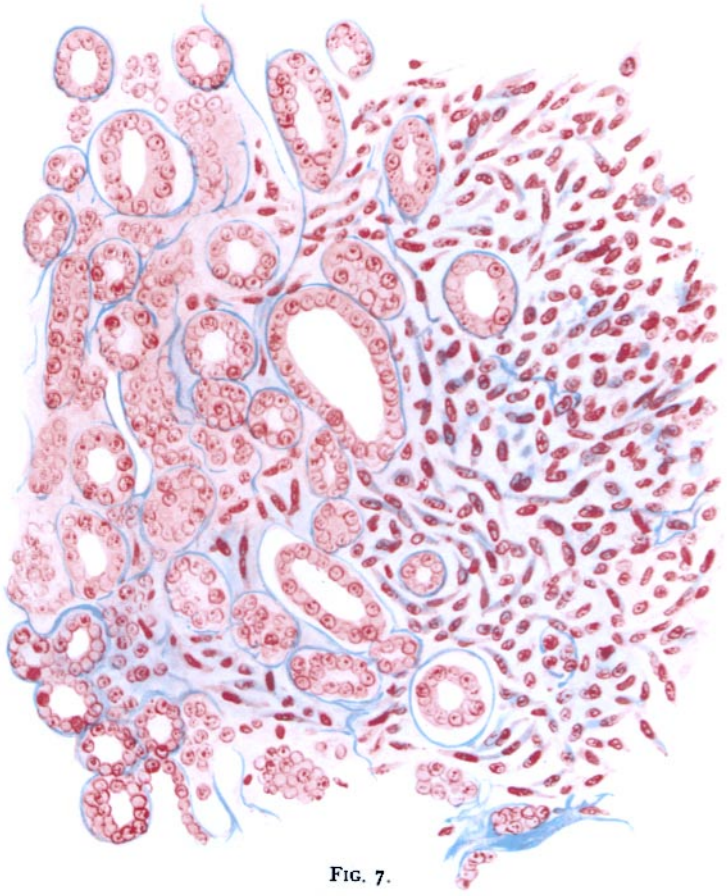

FIG. 7.

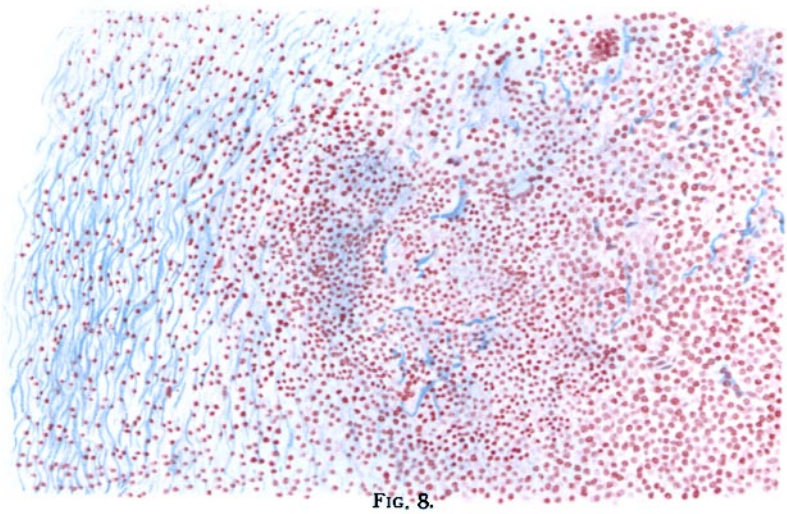

FIG. 8.

nective tissue rich in fibrillæ divides the tumor from the normal kidney. Stained with Mallory's anilin-blue.

FIG. 8. A graft of sarcoma, three days after its subcutaneous introduction. The right side of the figure shows sarcoma cells which are surrounded to the left by a small round cell infiltration and still further to the left by a connective stroma formation. The latter contains an abundance of fibrillæ. A few intercellular fibrillæ are seen between the sarcoma cells. Stained with Mallory's anilin-blue.