ON THE MECHANISM OF THE FORMATION OF METASTASES IN MALIGNANT TUMORS.*

AN EXPERIMENTAL STUDY.

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PLATES 18 AND 19.

The formation of secondary metastatic growths is characteristic of carcinoma as well as of sarcoma and presents the chief element of the malignancy of these tumors. A true metastatic growth is a secondary nodule formed in a distant organ or part of the body not contiguous to the primary tumor, and it should not be confused with even the most extensive local dissemination.

The only conceivable mode of formation of metastasis is the proliferation of a group of cancer cells, which have been transported, through the blood and lymph channels, to distant parts or organs of the body. These pieces of cancer tissue act as emboli and, finding lodgment in some parts of the organism, form secondary metastases.

Handley (1) attempted to prove recently that, at least in metastasis of cancer of the breast, embolism has no significance. According to this conception, every metastasis is formed by a process of " lymphatic permeation." In reality, the author makes use of the mechanism of the formation of local disseminations. The tumor cells grow along the lymphatic vessels until they reach the nearest lymph glands. From these glands the cells enter the next lymphatic vessel in the same manner as a fluid used for lymphatic injection, such as mercury. This process is continuous, and the appearance of an apparently isolated tumor nodule is due to the fact that a perilymphatic fibrosis destroys the permeated lymphatic vessels which form the lines of communication. Handley ascribes the formation of metastases in distant organs to the proliferation of cancer cells which escape from the subserous lymphatic plexuses into the serous cavities, pleura, or peritoneum. These cells are then distributed through these cavities under the influence of gravity and of visceral movements, and implanted on the

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serous surface of the viscera. Handley's theory is inadequate to explain all the phenomena of formation of metastasis even in cancer of the breast. Only the conception of embolic transportation of tumor cells accords with the main factors of the process both in carcinoma and sarcoma.

The main difficulty in the correct interpretation of all the phases of the formation of metastatic tumors lies in the fact that both the frequency of the occurrence of metastasis and its localization are different for the various tumors. This difference is very pronounced in carcinoma and sarcoma. Until recently it was generally accepted that the difference is due to the fact that sarcoma metastasizes through the blood-vessels and carcinoma through the lymphatics. Von Recklinghausen (2) was the first to indicate that carcinoma may also metastasize through the blood-vessels. The most thorough study, however, of the subject of the relation of blood-vessels to the formation of metastasis in carcinoma was made by Goldmann (3) and Schmidt (4). Goldmann proved that there is an extensive invasion of tumor cells into the coats of blood-vessels in carcinoma as well as in sarcoma. The paths of entrance of the cancer cells into the vascular coats appear to be the vasa vasorum, since the blood-vessel walls do not contain any lymphatics. This supposition is in accord with the fact that in arteries, the tumor cells rarely proceed further than the outer coat, whereas in veins they are generally found beneath the intima. The same difference takes place in the distribution of the nutrient vessels in the arteries and veins. While in the former the vasa vasorum usually remain within the limit of the outer coat, they extend in the veins beyond the middle coat into the region of the intima. The same investigator has shown further in his experiments with injection in the jugular vein, that the injecting fluid enters with greatest ease into the adjacent lymph glands. The conclusion must be drawn from Goldmann's investigations that there is a very intimate relation between the lymph and blood-vessels, and that carcinoma cells enter blood-vessels just as readily as sarcoma cells. Indeed, a tumor cell may penetrate a vein with greater ease than a large lymphatic vessel, since in the latter the vasa vasorum do not reach as near the inner coat as in a vein.

Schmidt examined forty-one cases of carcinoma of the abdominal viscera selected at random from his autopsy material for possible metastases in the lungs. In fifteen cases, he found, on microscopic examination, small nodules of carcinoma within the lumina of the blood-vessels of the lungs. These nodules are either surrounded by a stroma of connective tissue formed apparently by the endothelium of the blood-vessel, or else consist of a free group of cancer cells, which seem to have been transported more recently. The coats of the small pulmonary arteries, in which the cancer nodules were usually found, showed no lesion. The tumor cells apparently did not break through the wall of the blood-vessel from a focus of local dissemination, but were transported from the primary tumor or a distant metastasis. Schmidt inclines to the opinion that the carcinoma cells found in the lungs were transported through the lymphatic vessels, thoracic duct, subclavian vein, and pulmonary circulation. He finds support for this supposition in the fact that in three cases where nodules were found in the lungs there were visible metastases only in the regional lymph glands. Furthermore, he actually found small carcinoma nodules within the lumen of the thoracic duct. He does not exclude, however, the possibility that in certain cases,

in accordance with Goldmann's findings, the cancer cells may have entered a minute blood-vessel either within the primary tumor or within the metastatic tumor of the regional lymph gland, and in this way have reached the pulmonary vessel. Schmidt arrived at the conclusion that in carcinoma of abdominal organs, cancerous embolism of the small arteries of the lungs occurs with great frequency. Only a small proportion of these emboli give rise to metastatic tumors, because most of them are encapsulated and rendered harmless. A certain number of these emboli may, however, pass the pulmonary circulation and form metastases in distant organs.

This proof of the transportation of carcinoma cells through blood channels offers a better explanation for certain peculiarities in metastasis formation, than the purely lymphatic theory. In some instances, as in the formation of bone metastases in the carcinoma of the prostate, recourse had to be taken to the theory of retrograde transportation of cancer cells, to explain the formation of secondary tumors. All these investigations seem to have established firmly the fact that cells of all malignant tumors may be transported either through the lymphatics or the blood-vessels.

The difference in the channels of transportation cannot explain then the specific localization characteristic of the various malignant tumors. Different theories are offered in explanation of this phenomenon. Neusser (5) and Bamberger and Paltauf (6) believe that certain organs possess a peculiar affinity to cells of a certain malignant tumor. This selective affinity may be due to some peculiarity in the chemical constitution of the organ. Other pathologists, again, (Schmidt, Lubarsch (7), and von Recklinghausen), think that the phenomenon is caused by differences in the morphological structure of the organs. Von Recklinghausen, for instance, believes that the formation of bone metastases in carcinoma of the prostate is due to the fact that the veins and capillaries of the bone marrow have thin walls and are not collapsible. These morphological peculiarities favor the accumulation of carcinoma cells within these blood-vessels.

Lubarsch is also of the opinion that the relative size of the cancer cells and minute blood-vessels of an organ is of importance for the success of the formation of metastases.

All the investigations analyzed here were conducted on autopsy material and, as was stated above, they indicated the paths through

which the cancer cells are transported into distant parts. The real mechanism of the formation of metastases, the reason for the difference in their frequency and location for various tumors, and the reason why all cancer emboli do not form metastases, cannot be studied by mere observation on anatomical material. Only experimental investigations can throw some light on the subject. All former experiments with intravascular injection of human tumor material into animals either gave negative results or were entirely untrustworthy. The inoculable malignant tumors of white rats and mice should offer good material for the study of the subject, but there is in the literature no report of any systematic study of the mechanism of metastasis of these tumors.

In view of all this, and in view of the further fact that the study of the mechanism of the formation of metastasis may be helpful in the elucidation of many general factors in the genesis of tumors, the writers have undertaken a systematic study of the subject.

The experiments were conducted on various tumors of white rats and mice and consisted mainly of an injection of tumor emulsion into blood-vessels or joints. By the latter method, as is shown by similar experiments with bacteria, the tumor cells are introduced into the lymphatic system. A preliminary communication was published recently (8) on the methods and results of the intravascular injection of tumor emulsions. In the following report details are given of the methods of study for each tumor.

SARCOMA OF THE RAT.

This tumor is very malignant, grows locally to a large size after a subcutaneous inoculation, and usually kills the animal in about ten weeks. The formation of metastases after a subcutaneous inoculation is very rare. Though hundreds of animals were inoculated and subsequently autopsied by the writers, local dissemination was observed in not more than half a dozen cases, and once only was metastasis found in the liver.

Intravenous Injection.[1]—An emulsion of this tumor was prepared by cutting it in Haaland's mincing machine, grinding it in

[1] Ether anesthesia was employed throughout the experiments.

normal salt solution, and filtering it through a layer of coarse gauze. The milky opalescent fluid contains a sufficient number of living cells to produce a tumor growth. Control experiments with a subcutaneous injection of this emulsion showed the usual percentage of successful takes. Thirty-six rats received an injection of this emulsion into the jugular vein. The animals were killed at periods ranging from eight days to four weeks after the injection, and a thorough search for metastasis was made in all the organs. No microscopic study was made of the organs which appeared normal on gross inspection, since this investigation is not concerned with the place where the tumor cells circulating in the blood find lodgment, but with the question whether such cells transported into a certain organ will form there a visible metastasis. All suspicious nodules found anywhere and all lungs appearing abnormal on gross inspection were examined microscopically. Metastatic tumors were not found in a single animal, which is the more remarkable, since after a subcutaneous inoculation of the same emulsion about 90 per cent. of the animals grew the tumor.

Intra-Arterial Injection.—Six rats received an injection into the carotid artery, and the animals survived. This method is very difficult of execution and the animals usually die from respiratory paralysis a few minutes after the injection, though the heart continues its action a few minutes longer. No animal showed the formation of metastatic nodules.

Subcutaneous Inoculation of Tumor and Blood.—In order to test the possibility that the negative results were due to the deleterious effect of the blood on the tumor cells, a mixture was prepared of the tumor cells and defibrinated blood. The mixture was injected subcutaneously into thirty rats. Seventy-five per cent. of the animals took the tumor as against 85 per cent. in the control animals. The difference is too insignificant to indicate any influence of the blood on the tumor cells. The same subject was tested in the following series of experiments.

Inoculation into the Bone-Marrow.—The method of operation in these experiments consisted in opening the marrow of the left tibia and placing a small piece of tumor in the marrow. The opening in the bone was closed with wax and the skin sutured.

Fifteen animals were operated on in this manner, and of these, five died before an examination could be made. In eight of the remaining ten animals, a large tumor grew at the site of inoculation. But in no instance was there found any metastatic tumor. In these experiments, the inoculated cells were closely intermingled with the circulating blood, and still local tumors formed in 80 per cent. Circulating blood has consequently no appreciable deleterious effect on the tumor cells. Experiments with injection of a tumor emulsion into the joint were not done with this tumor, since a large local growth develops so promptly after an inoculation. A large tumor growth at the joint would have been so intimately connected with blood-vessels that it would have been difficult to differentiate the rôle played by the lymphatic system.

CARCINOMA OF THE RAT.

This tumor, an adenocarcinoma of the white rat described by Flexner and Jobling, seemed by its character to be more favorable for the study of metastasis than the sarcoma of the rat. The Flexner and Jobling (9) tumor possesses to a high degree the property of invading adjacent tissue, muscle, fascia, and even cartilage and bone. This tumor also frequently forms metastases after a subcutaneous inoculation. The authors described metastases in the sternum, cartilage of the ribs, in the lungs, kidney, the musculature of the heart, and in later generations even in the lymphatic glands.

Intravenous Injection.—Sixteen rats received an injection of an emulsion of this tumor into the jugular vein. In three animals, metastasis was found in the lungs (figure 1). No other organ showed any metastasis.

It must be stated here that in the intravascular injections of any of the tumors described here there has never developed a local growth at the site of the inoculation. In the experiments with the sarcoma described above, this occurred in a few animals and they were discarded as faulty experiments.

Intra-Arterial Injection.—Six rats received an injection into the carotid and survived the operation, and of these animals, one showed metastasis in the lungs. Another rat, in which the injection was made in the carotid against the stream of blood, was

found dead twelve days later.   At the autopsy, a nodule was found on the wall of the left ventricle.   The animal remained in the cage over night and the specimen was greatly deteriorated; still the nodule resembled microscopically the picture described by Flexner and Jobling of a metastasis found by them in the heart.

Inoculation into the Bone-Marrow.—Seven rats received the inoculation.   Four animals developed a local growth in the marrow and showed no metastasis.   Three animals showed no local growth and in two of these metastases were found in the lungs.

Injection into the Knee Joint.—The operation consisted in a small longitudinal incision of the skin over the knee joint and an injection of three minims of the tumor emulsion under the patella. Twenty-four rats received the injection into the joints.   Eleven animals developed a small local growth in the knee joint, and in three of these, metastases were found in the lungs.   Thirteen rats showed no local growth in the joint, and of these, one animal showed metastasis in the lungs.

SARCOMA OF THE MOUSE.

This tumor grows locally to a large size after a subcutaneous inoculation, but it does not form any metastases after a subcutaneous inoculation in mice.   Ehrlich has shown that when this tumor is inoculated subcutaneously into a rat, a small nodule forms, which remains for eight to ten days, and is then absorbed.   No metastasis formation of this mouse tumor after a subcutaneous inoculation into a rat was ever described.   Experiments with intravascular injection of this tumor were performed both in mice and rats.

Intravenous Injection into Mice.—Twenty mice received an injection of an emulsion of the tumor into the jugular vein.   No metastatic tumor was found anywhere in any animal.

Intravenous Injection into Rats.—Twelve rats received an injection of an emulsion of the tumor into the jugular vein.   The rats were killed in periods of four to eight days, and in two of the animals metastatic nodules were found in the liver (figure 2).   In no other organ was any metastasis found.

Injection into the Knee Joint of Rats.—Fifteen rats received the injection of this tumor emulsion in the knee joint. No local growth developed in the joints. One animal showed metastasis in the liver —another animal showed a very striking condition. The inguinal lymphatic glands of the opposite side to the one where the injection was made appeared suspicious on gross inspection. Microscopic examination showed that the glands were normal, but the minute blood-vessels of the fat tissue immediately surrounding the gland were completely filled with tumor cells. Figure 3 presents the condition; figure 4 is placed for comparison and shows an inguinal gland and similar blood vessels of another rat without the tumor cells. No other animal showed any metastasis anywhere.

The occurrence of metastasis in inoculable tumors of the white mouse and rat subsequent to the formation of a primary local growth is rare as compared with human cancer. The present investigation shows that it is just as difficult to induce in a healthy animal the formation of a metastatic tumor by a direct introduction of cancer cells into the lymph or blood channels. The experiments indicate further that, at least in these animals, the cancer cells, no matter how introduced, enter the blood-vessels, and through these only do they form metastases in distant organs. After an injection into blood-vessels or joints, metastases were found only in the lungs and the liver, and these organs could only be reached through the blood channels. The fact that cancer cells were found within small vessels after an injection into a joint, affords further proof for this assertion. This investigation, therefore, affords experimental proof for the supposition that metastases in malignant tumors of the rat and mouse occur through the blood-vessels. Even in the carcinoma of the rat, in which metastases were found in the lymphatic glands, Flexner and Jobling consider the blood-vessels the only paths for the formation of metastases. Carl Lewin (10) thinks that the fact that in these animals the blood-vessels are the channels for the transportation of metastases, is the reason for its rare occurrence, since the blood exerts a deleterious effect on the tumor.

The experiments with the inoculation of a mixture of tumor cells and blood, and the experiments with inoculation into the bone

marrow, indicate that direct contact of the tumor cells with blood does not impair the proliferating power of the former. It is possible, however, that the blood is capable of neutralizing the few cells which enter as emboli or are introduced as an emulsion into the circulation, but that it cannot influence the larger groups of cells introduced during an inoculation into the marrow.

Ehrlich explains the rare occurrence of metastasis in the tumors of the rat and mouse by his athreptic theory. The tumors in these animals are very malignant and grow to a large size. The cells of the primary tumor use up all the specific food found in the organism of the host, and the cells transported from the primary tumor to other regions of the organism do not find the necessary nourishment and consequently cannot proliferate. This explanation does not accord well with the facts as shown in the present investigation. It is true that the rat sarcoma gave completely negative results and that it is also the most virulent of the tumors used. None of the animals used for the experiments had any local sarcoma growth anywhere and consequently the sarcoma cells introduced into the circulation could find all the necessary specific food. No metastatic tumors, however, were formed. The most probable reason for the differences in the frequency of the occurrence of metastasis is the one which, as will be seen later, explains the differences in its localization.

The most interesting phenomenon observed in the course of these experiments is that while the Flexner-Jobling tumor found lodgment in the lungs upon intravascular injection, the mouse sarcoma, when injected into the rat, produced metastasis only in the liver. The methods of transportation were identical in each instance, and in each instance the experiments were performed on normal animals. Consequently, the differences in the morphological structure of the channels of transportation could not play any rôle. It would seem then that the most plausible explanation for the different localization of metastasis in the different kinds of malignant tumors is that it is due to a specific affinity between cancer cells and cells of certain organs. It is very interesting to note, that while the cells of the mouse sarcoma obtained lodgment, in a few instances, in the liver of the rat, they failed to produce metastasis in the mouse. As far

as it is permissible to use results obtained in experiments on lower animals for the explanation of phenomena in human pathology, it seems reasonable to suppose that there also the specific affinity of the cancer cells to the cells of different organs is the only reason for the specific localization of metastases.

The fact, then, that carcinoma forms metastases in the lymphatic glands is not due to the difference in the channels of transportation in the carcinoma and the sarcoma, but to the specific affinity between carcinoma cells and the cells of lymphatic glands. In the case related above, where sarcoma cells were found filling the blood-vessels immediately adjoining a lymphatic gland, no metastasis formed in the organ. The sarcoma cells had easy access to the gland, but apparently could not find there a favorable medium for their proliferation. The relation of this affinity to various factors in the genesis of malignant tumors and the changes in this relationship which may be noted when the parenchymatous organs are abnormal, are subjects of further research by the writers.

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EXPLANATION OF PLATES.

PLATE 18.

FIG 1. A metastatic growth in the lung of the Flexner-Jobling carcinoma of the rat formed after an intravenous injection of the tumor emulsion. In the centre of the figure is seen a group of carcinoma cells.

FIG. 2. A metastatic growth of mouse sarcoma in the liver of a rat. The upper margin of the specimen shows a mass of growing sarcoma cells.

PLATE 19.

FIG. 3.   Numerous minute blood-vessels in fat tissue, filled with sarcoma cells, in the vicinity of a lymphatic gland.   A part of the lymphatic gland is seen to the left of the figure, the blood-vessels to the right.

FIG. 4.   Numerous minute blood-vessels in fat tissue in the vicinity of a lymphatic gland.   The figure presents a morphological picture identical with the one shown in figure 3, but without the tumor embolism in the blood-vessels.
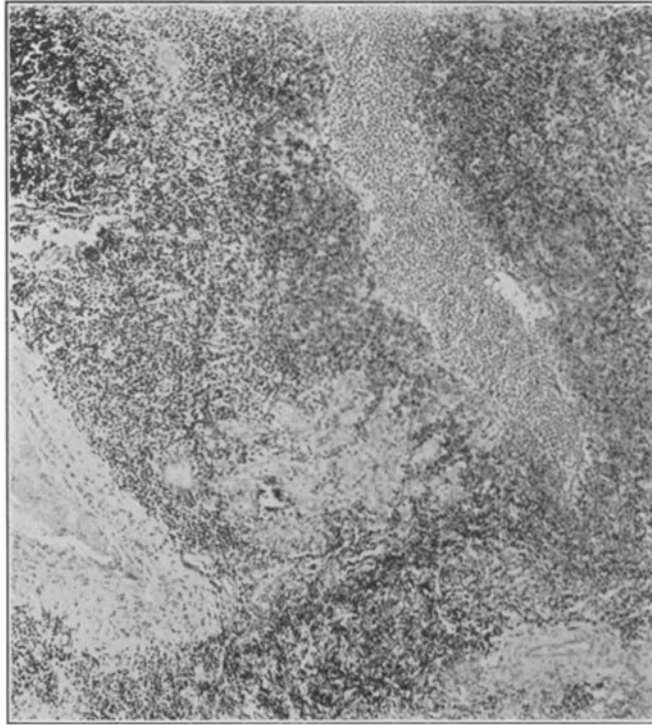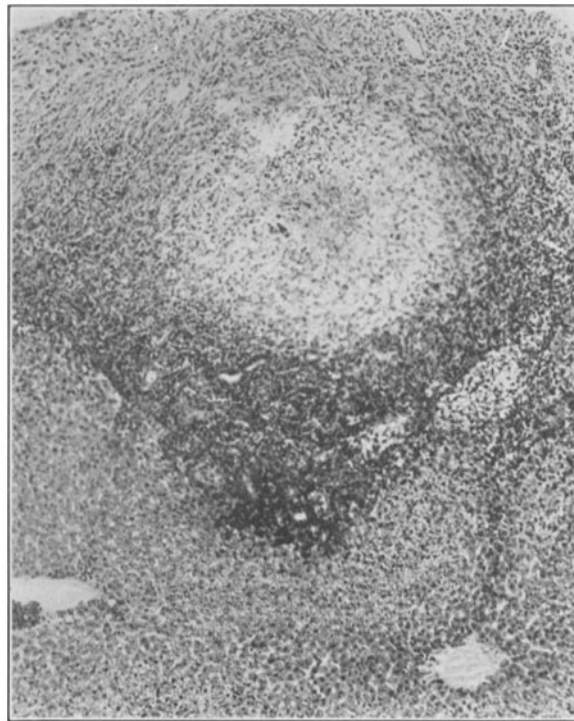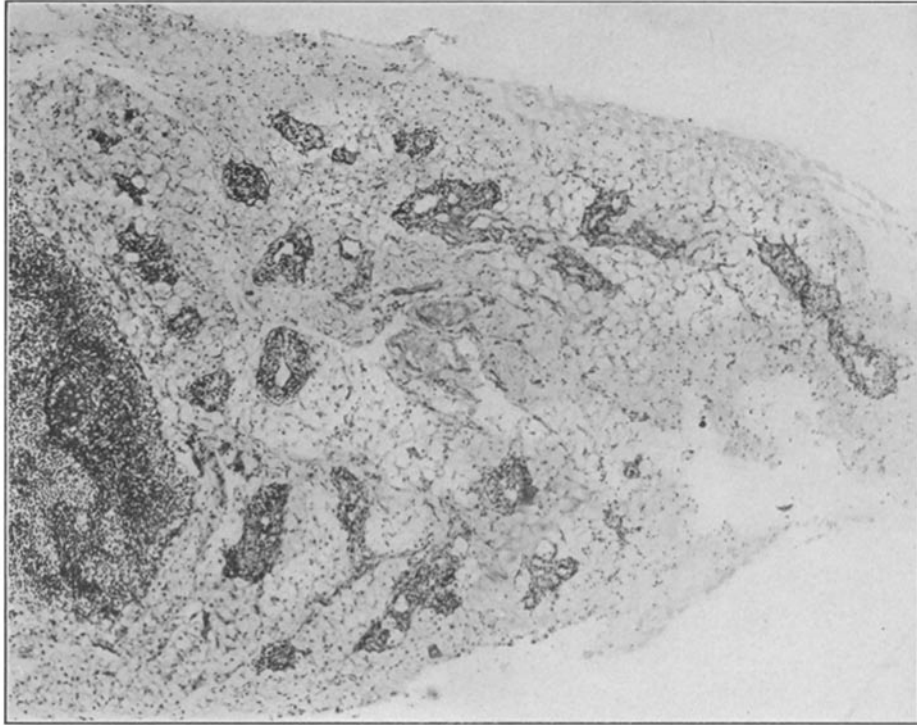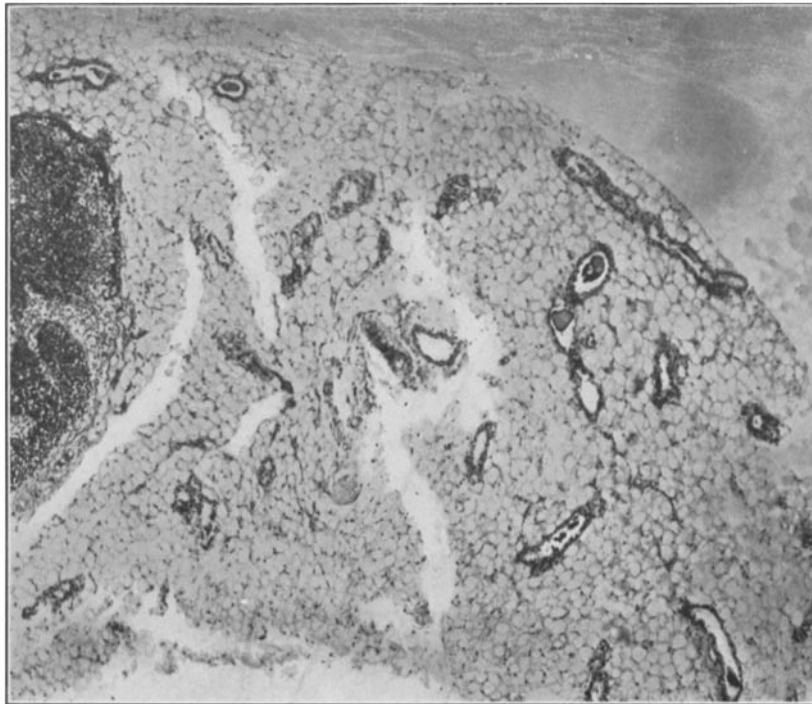
FIG. 1.

FIG. 2.

FIG 3.

FIG. 4.