THE REACTIVE CELL PROLIFERATION IN THE WHITE RAT; AND ITS RELATION TO THE GENESIS OF TRANSPLANTABLE TUMORS.[1]

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There is hardly any doubt left in the minds of pathologists that the transplantable tumors in the white rats and mice, recently discovered by Hanau (1), L. Loeb (2), Jensen (3)and others, resemble clinically very closely human carcinoma and sarcoma. These tumors grow to large size, occasionally weighing as much as the animal on which they grow; they are malignant to the animal, and frequently fatal to the host. There were noticed local recurrences after extirpation of the original tumor and metastases, usually in the lungs.

The main feature distinguishing the tumors of white rats and mice from human cancer as well as from tumors of most other animals, lies in the fact that the latter cannot be transplanted into another individual of the same species, while in the former this can be accomplished not infrequently. The transplantability of these tumors may be due to the great inherent power of proliferation of the tumor cells. This inherent proliferating capacity would then be, not only much greater than in any normal cell, but also greater than the proliferating power of a cancer cell of any other animal species. This power would not depend on the character of the tissues to which the cell is transplanted.

On the other hand, it is possible that the cancer cell in a white rat or mouse is not different from a cancer cell in any other animal species, but the organisms of the former animals may react differently to tumor transplantations.

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If one accepts the first factor as the only one controlling the transplantability of the mice and rat tumors, then it would be logical to regard transplanted tumors as artificially produced metastasis. On the basis of that assumption one could not expect much information regarding the genesis of tumors from the experimental transplantation of tumors from animal to animal. But there are a number of facts in the experimental research of these transplantable tumors which seem to indicate very strongly that cellular, and possibly also metabolic reactions of the host, contribute a great deal to the success of the transplantation of these tumors. The powers of limitless proliferation ought to be equally inherent in every cancer cell, no matter to what animal species it belongs. A human cancer cell transferred anywhere through the lymph or blood circulation will proliferate and form a metastasis very frequently of a much larger size than the original tumor. But it is impossible to make this cell proliferate when transferred to any other organism. In the innumerable operations done for cancer, surgeons have most probably often enough cut themselves, and thus become inoculated with some cancer cells, and yet there is not one report to the effect that one was in this way infected with cancer. The cancer cell of the white mouse or rat *per se* is certainly not different from any other cancer cell, in as much as a tumor cell of a white mouse while growing readily when implanted in another mouse, grows slightly only when implanted in another animal, in a white rat for instance, a species which is very near the white mouse, and imperfectly even when implanted in a white mouse of a different race. Very interesting in this connection is the so-called zigzag-transplantation of Ehrlich and Apolant (4). One transplants a tumor of a white mouse into a white rat. The tumor grows only for a while and then ceases. A few days later he takes the tumor from the rat and implants it another mouse, where the tumor grows strongly. He then removes the tumor from the mouse and implants it again into a rat and the growth ceases after a while, and so on. Ehrlich (5), who is of the opinion that cancer growth is due to the proliferating power of the cancer cell, admits that for the growth of a mouse tumor there is required the addition of an X substance which exists in the mouse and is absent in the rat or any other animal.

The work of Gaylord and Clowes (6), Ehrlich (5), Michaelis (7), Bashford (8), Flexner and Jobling (9) seems to indicate that it is possible either to increase or to decrease artificially the success of the tumor transplantation in a white mouse or rat, produce a so-called "athreptic" immunity. Ehrlich calls this phenomenon "athrepsia" because he thinks that the difference in the success of a transplantation is due to the difference in the nutrition that the implanted cell receives in the new soil.

All these facts indicate very clearly that the success of tumor transplantation in the white mouse or rat is due to the difference in the reaction of the tissues to the implantation of cancer cells as compared with other animals or man.

While cancer cells are different from normal cells in their power of unlimited proliferation, this power manifests itself only when a certain specific stimulus is added by the host to the implanted cancer cells. More than that, some facts in the recent research with the transplantable tumors seem to indicate that the growth of a transplanted tumor is not necessarily due to the proliferation of the implanted cancer cells, stimulated to it by some factor derived from the host. In some cases the reverse may be the case and the growth of a tumor may be due to the proliferation of the normal cells of the host, stimulated to it by some factor derived from the implanted cancer cells. According to J. Orth (10), even human cancer may in some cases grow in this way. As he states it, there are cancers in which the transformation of preformed epithelial cells into cancer cells takes place.

C. Lewin (11) recently reported a series of experiments in which he transplanted human carcinoma of the uterine cervix into white rats. The implanted piece became necrotic and around it there developed a growth of granulation tissue. He succeeded in re-implanting these granulomata into two generations. In none of his tumors could he detect any microörganisms either by staining or by cultivation.

While different mechanical and chemical injuries may produce a granuloma, cancer tissue, in Lewin's opinion, acts differently inasmuch as it produces a transplantable granuloma. It is self-evident that in this case the stimulus acts on the cells of the host.

Quite similar to Lewin's results, seem to be the reported cases of transformation of a transplanted carcinoma into a sarcoma in subsequent generations. Apolant (12), L. Loeb (13), Bashford Murray and Haaland (14) have all reported such cases, and they all lately give practically the same explanation to the phenomenon. Through a specific stimulus (Reiz) produced by the implanted carcinoma cells, a new connective tissue stroma is formed by the host. this stroma in subsequent generations becomes ever richer in cells, and, finally, these cells of the host under the influence of something emanating from the implanted carcinoma cells become sarcomatous and transplantable. It is clear, then, that the cellular activity of the host contributes something to the success of transplantation of the tumors of the white mice and rats.

It seemed desirable to investigate whether the reactive cell proliferation of these animals will also be different from other animals, when the stimulus to this proliferation is given, not by an implantation of cancer tissue, but by a different agent.

While the transplantable tumors of the white mouse are usually carcinomata developing from the mammary gland, most of the tumors of the rat reported by Velich (15), Flexner and Jobling (16), Loeb (17), and Herzog (18) are sarcomata and of all the immense number of rat tumors examined there are only two cases of carcinoma reported, Hanau (19), Michaelis and C. Lewin (20).

It is always easier to produce a proliferation in cells of the connective tissue type, and consequently this investigation was conducted on white rats.

I reported previously the results of experiments with implantation of normal tissue (21) and also with introduction of aleuronat in the form of an emulsion or triturate tablets (22). The objectionable feature of aleuronat consists in the fact that it is a substance producing an inflammation and the formation of pus. There has lately been described an agent acting more directly on the proliferating power of the local fixed cell, without the aid of migrating leucocytes. In 1907, Fischer (23) reported a series of experiments in which he injected subcutaneously in a rabbit's ear, oil containing scharlach R, a fat-staining substance. He produced in this way an extensive irregular proliferation of epithelial

cells, giving the morphological appearance of an epithelioma. His results were corroborated by Jores (24), Snow (25) and Helmholz (26), who found besides the epithelial cells some proliferation of connective tissue cells also.

I used this substance on white rats in the following variety of methods:

I. Subcutaneous Injection of Scharlach R in Paraffin.—A saturated solution of scharlach R in soft paraffin was employed, and the usual surgical technique followed. Three weeks after the injection the paraffin with the tissue surrounding it was excised. In every experiment the paraffin was surrounded with a thick connective tissue wall from which chords were running in the paraffin. Both the wall and the chords consisted mainly of young round and spindle cells, with very little fibrous tissue.

II. Subcutaneous Injection of Scharlach R in Oil.—For these experiments a sterile saturated solution of scharlach R in oil was used. Five injections were made in the same place in every animal at intervals of forty-eight hours. Three days to two weeks after the last injection the animals were killed. There always developed in the place of the injection a cyst which contained in its center some unabsorbed oil. The walls consisted of the subcutaneous fat completely impregnated with young round and spindle cells, the latter frequently combined in regular rows.

III. Injection of Scharlach R-Oil in the Mammary Gland.—The same oil used for Experiments II was injected into the mammary gland through the nipple. Five injections were made in the same gland at intervals of forty-eight hours. Two weeks after the last injection the animals were killed. The glands were always found greatly enlarged and containing some of the oil in the center. Microscopically no change in the glandular tissue was detected, but the connective tissue was filled with round and spindle cells. The microscopical picture certainly resembled a great deal more a sarcoma of the breast than a chronic mastitis.

IV. Transplantation of Scharlach R-Mammary Glands.—Small pieces of the mammary glands of the animals from Experiment III were transplanted subcutaneously or in the peritoneum. Ten days after the transplantations the animals were killed. The

implanted pieces were always found somewhat enlarged. The peritoneal pieces were usually attached to the omentum. Microscopically the implanted pieces appeared necrotic and surrounded by a thick layer of round and spindle connective tissue cells. The cells appeared to grow into the implanted piece.

Dr. J. H. Larkin assisted me in the preparation of the microscopical specimens and I take great pleasure in extending my thanks to him.

The analysis of these experiments shows that the white rat reacts with a profuse connective tissue cell proliferation to different stimuli, whether it is implantation of normal rat tissue or human cancer tissue, whether an injection of aleuronat or scharlach R solutions. The latter substance produces in a rabbit mainly an epithelial proliferation, but in a white rat, no matter how or where employed, it acts only on connective tissue cells.

That it is impossible microscopically to determine in every instance whether we are dealing with a sarcoma or a granuloma, can be seen from the studies with the transplantable sarcoma in the dog. Sticker (27) and Ewing and Beebe (28) maintain that it is a sarcoma, while Apolant (29) and Bashford, Murray and Cramer (30) are inclined to consider it an infectious granuloma. Ehrlich considers it a microscopical proof of sarcoma when there are regular intersecting rows of spindle cells. But the same picture can be found in the formations resulting from the injection of scharlach R.

Ehrlich claims further that the sarcoma which developed in his case from carcinoma grew too fast for an ordinary granuloma. But the cysts developing after injection of aleuronat or scharlach R grow just as rapidly and may also attain a very large size.

After transplantation of pieces of scharlach R mammary glands, the new connective tissue cell formations were not as extensive nor as malignant to the host as after implantation of pieces of rat tumor. But the difference is apparently only quantitative. Not every sarcoma of a white rat is transplantable, and when it is, the weight of evidence seems to tend toward the explanation that in every new host it is the cell of the host that forms the new growth under stimulation by the implanted piece. There can cer-

tainly be no qualitative difference between the implantation of normal rat tissue or pieces of mammary gland that have previously been subjected to the influence of scharlach R, on the one hand, and the implantation of human cancer tissue, on the other. Still C. Lewin reports the development of malignant transplantable granuloma after implantation of a piece of human cancer in a white rat, which, by the way, took place only in two animals from a whole series of experiments.

It is interesting to note here that while all the pieces of normal tissue (skin, liver, spleen, testicle or mammary gland), when transplanted under the skin or in the peritoneum, retain their cellular structure, the transplanted pieces of scharlach R-mammary gland in the same length of time (ten days after implantation) become necrotic. This necrosis is not due to the injection of scharlach R, as the similar pieces of mammary gland without transplantation show no signs of necrosis. The same central necrosis took place in the pieces of human carcinoma reported by C. Lewin and also usually takes place in the retransplanted pieces of sarcoma of the white rat. Apparently the peripheral new cellular formation takes place more rapidly in transplantation of cancer tissue or of tissue subjected previously to the influence of scharlach R, than around normal tissue, and consequently the former is sooner cut off from the food supply and sooner becomes necrotic. In a transplanted sarcoma at least it is impossible to prove definitely that the new growth in the host is due to the proliferation of the implanted cells and not to the proliferation of the cells of the host, and the implanted piece acts only as a stimulant. It is not so easy to indicate the influence of the reactive power of the host in transplantation of carcinoma, but even there some facts point in the same direction. Apolant reports on a transplanted carcinoma which on a subsequent transplantation was transformed into adenoma. C. Lewin reports a case of carcinoma in a white rat which was transformed in subsequent generations into an adenoma, then again into a carcinoma, then into a sarcoma. Loeb saw a sarcoma change into an endothelioma. All such mutations of the morphological appearance of the tumors are very difficult to explain on the supposition that the growth of a transplanted tumor is due only to the

proliferation of the transplanted cells without the reactive proliferation of the cells of the host. This extensive reactive cellular activity of the organisms of the white rat and mouse is the most important factor in the modern biological investigation of the genesis of cancer and will contribute mainly to the elucidation of its problems.

As Bashford, Murray and Haaland state it, in connection with their report where an implanted carcinoma changed into a sarcoma, this cellular activity of the host puts us in a position to follow the formation of the actual malignant tumor from its very incipiency. On the other hand, if the growth of a transplanted tumor is due only to the proliferation of its own cells, then these tumors represent only metastasis, and the study of them could only elucidate the course of one of the later phases in the development of tumors.

The work reported here is still in progress and conditions may still be found under which a malignant transplantable tumor will be produced artificially in white rats or mice.

In conclusion I deem it a pleasant duty to express here my gratitude to Dr. T. Mitchell Prudden for the privilege of the laboratory and for the interest shown in my work.

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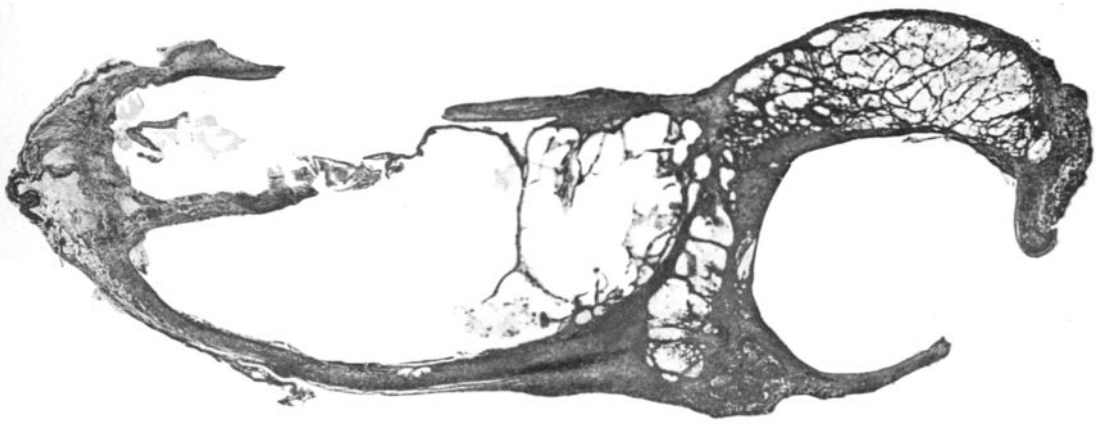


FIG. 1.

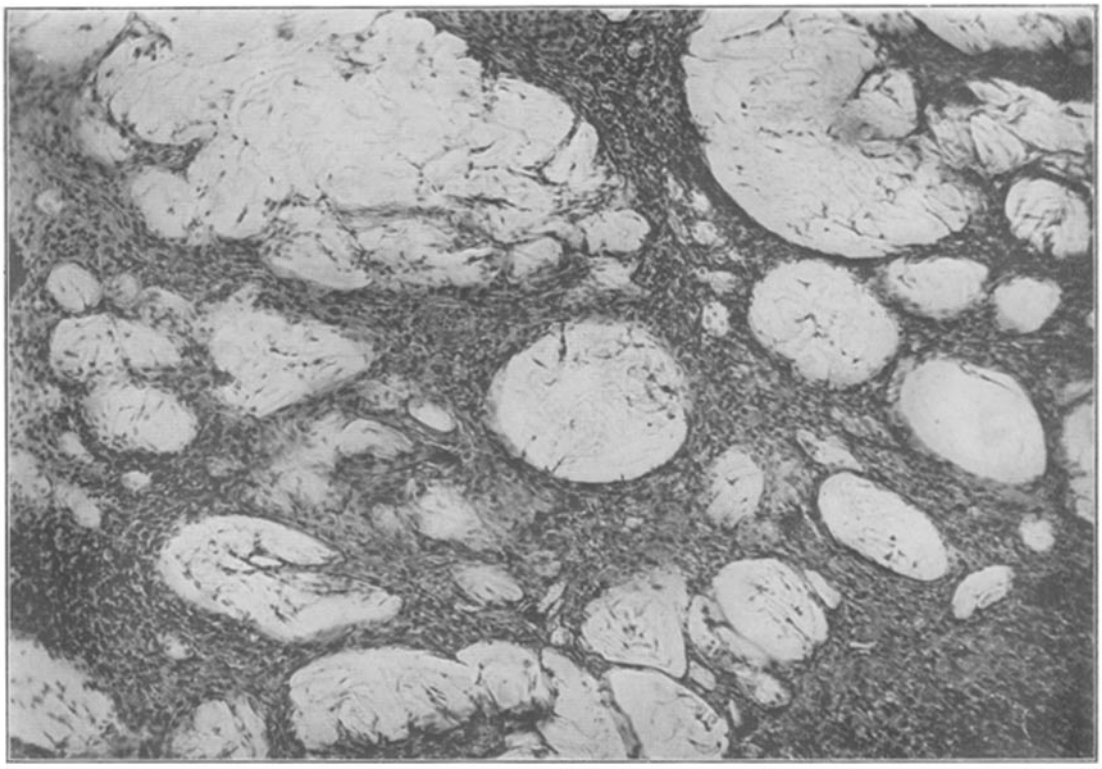
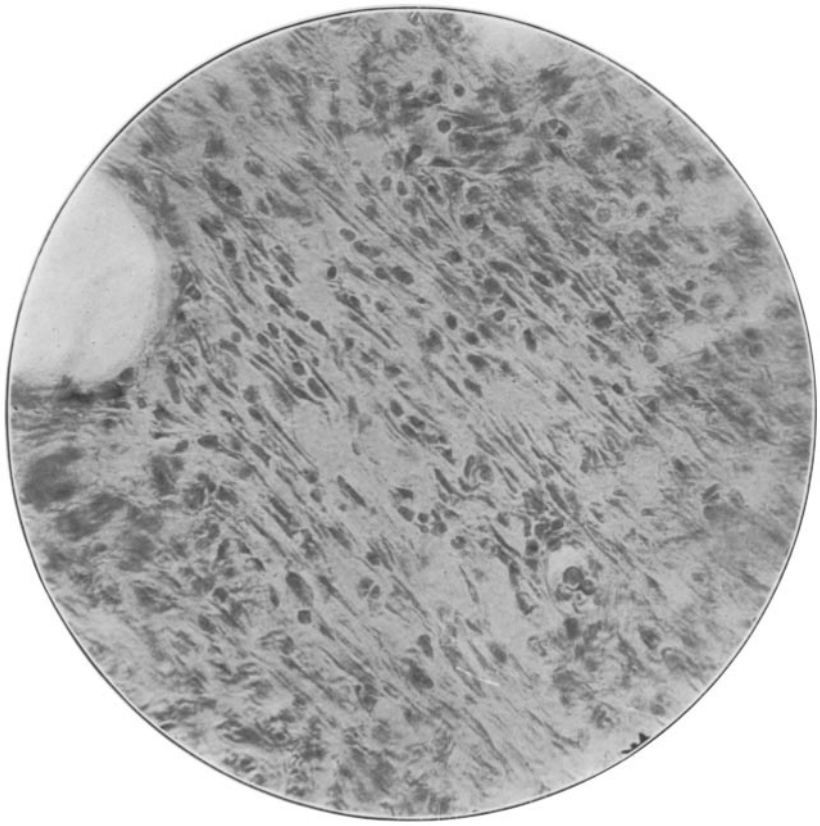


FIG. 2.



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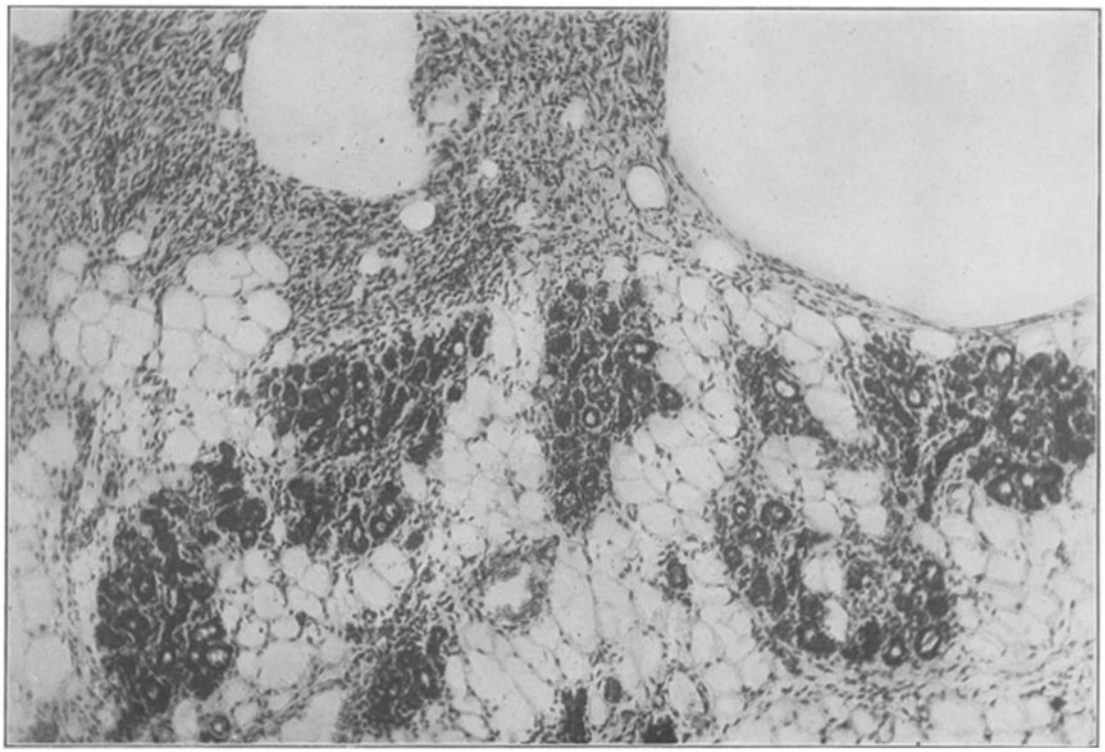

FIG. 4.

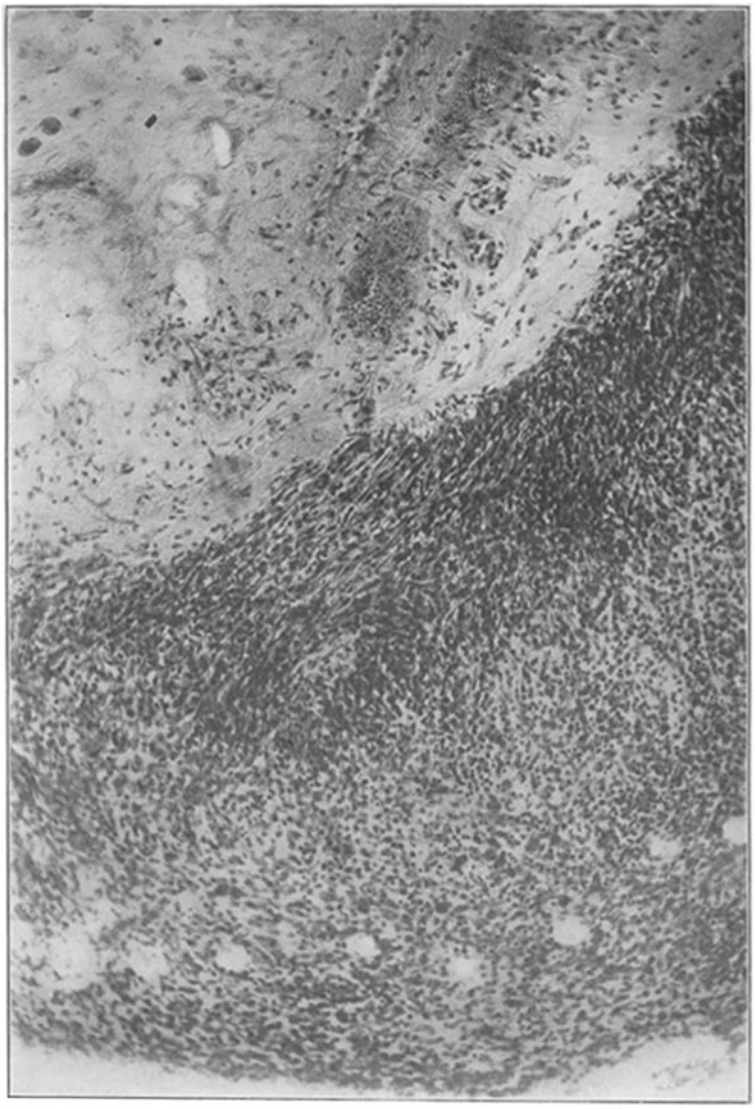

FIG. 5.

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EXPLANATION OF PLATES.

PLATE LI.

FIG. 1. Scharlach R-paraffin cyst. Low power shows part of the wall and the net of chords.

FIG. 2. Same as Fig. 1, but higher power; shows the round and spindle cells.

PLATE LII.

FIG. 3. Scharlach R-oil cyst. Subcutaneous fat impregnated with round and spindle cells.

FIG. 4. Mammary gland treated with scharlach R in oil. The connective tissue filled with round and spindle cells.

PLATE LIII.

FIG. 5. A piece of mammary gland treated with scharlach R in oil and then transplanted into the peritoneum of another rat. Specimen shows the necrotic implanted piece surrounded by a layer of round and spindle cells.