STUDIES ON IMMUNITY IN CANCERS OF THE WHITE RAT.

THE "PRECANCEROUS STATE" AND THE MECHANISM OF THE ORGAN RESISTANCE TO TUMOR GROWTH.*

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PLATES 23–25.

For all future investigation of the cancer problem, it is of the utmost importance to ascertain whether the disease is, in its early stages, a purely local condition and whether all the clinical symptoms are subsequent to the dissemination of the tumor, or whether it is, even in its incipiency, a general parasitic or constitutional disease. Most pathologists and clinicians are inclined to consider cancer, in its early stages, a local disease. Those who favor the parasitic theory of cancer must, of necessity, consider it a condition immediately affecting the whole organism. Neither clinical nor anatomical investigations on human material have succeeded in settling the problem. Were it possible to approach the subject by the aid of experimentation with inoculable tumors on white rats and mice, a way would be opened for the ultimate understanding of the pathogenesis of cancer.

It is hardly necessary to dwell upon the fact that inferences and analogies to the human organism may be drawn from the study of cancers of white rats and mice, in the same way that inferences are drawn in bacteriology from studies on lower animals. Both spontaneous and inoculated tumors produce in these animals clinical as well as pathological manifestations akin to those observed in human beings. An objection is raised, however, that in the inoculated

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animals one is dealing not with a true primary tumor, but with a metastasis. The reason for this lies in the fact that the growth of cancer in these animals is from a group of cancer cells transferred from another host. But the inoculated tumor is the first and only manifestation of cancer in the new host and is not secondary to another tumor in the same organism, as is metastasis. Consequently, if it is assumed that there is a qualitative difference between a spontaneous and an inoculated tumor, the supposition is that a spontaneous tumor begins with a proliferation of a group of normal cells and not with cells changed previously in their character.

There seems to be little doubt that cancer growth must be due to two factors: (1) the presence of a group of peculiarly changed cells, and (2) the reactivity of the host. In the white rat, where it is comparatively easy to induce the unlimited growth of inoculated tumor cells, all experiments with inoculations of adult or even embryonic tissue have failed. Ribbert has always been the strongest advocate of the theory that a cancer cell is not different in any way from a normal cell, and that the only changed condition is in the surrounding connective tissue. It is significant that in his latest publication (1) he inclines to the view that not only are the cells of fully developed cancer different from normal cells, but that the beginning of the process of cancer formation may be due to a primary change in a group of cells. In the concluding sentence of his book, he says: "Das Karzinom entsteht auf Grund einer durch Epithelprodukte bewirkten, die Differenzierung des Epithels vermindernden und sein Tiefenwachstum auslösenden subepithelialen Entzündung." He admits that the changes in the subepithelial connective tissue, which formerly served as the basis for his cancer formation theory, appear frequently without a subsequent development of cancer. According to his latest conception, therefore, only certain specific kinds of subepithelial inflammatory processes may induce the formation of cancer. This specific precancerous inflammation of connective tissue is caused by products of the degenerative changes in the same epithelial cells, which subsequently lose their differentiation and acquire the proliferating power. In other words, they become cancer cells.

If investigators like Ribbert admit that the growth of cancer begins from a group of abnormal cells, then it would seem that there is no fundamental qualitative difference between an inoculated cancer of white rats and mice and the spontaneous tumors of man and animals.

The next question, then, is to determine the experimental methods by which it may be ascertained whether cancer begins as a local or as a general disease Since the disease always originates from a group of abnormal cells, and the causation of the latter is completely unknown, recourse must be taken to the influence of the host on the development of cancer cells. If cancer begins as a local disease, then purely local conditions must be found in the different organs and tissues of the host, which either favor or inhibit the proliferation of the group of spontaneous or inoculated cancer cells. Thus far, in all the experimental studies on the inoculable tumors of white rats and mice, attention has been paid only to the general susceptibility of the whole organism of the host to the growth of cancer.

The writer's (2) studies of the mechanism of resistance or immunity to the growth of tumors when inoculated into parenchymatous organs, seemed to indicate a method for the study of the local organ reactivity of the host. The investigation was conducted on an adenocarcinoma of a white rat. Before reporting the experiments, it will be advantageous to describe briefly the characteristics and behavior of this tumor.

THE FLEXNER-JOBLING MIXED TUMOR OF THE RAT.

This tumor, for a transplant of which the writer is indebted to Dr. Simon Flexner, was first described by Flexner and Jobling (3), in 1907, as a polymorphous sarcoma. Subsequent study (4) showed, however, that the tumor contained, from the beginning, epithelial structures. At present, it grows either in the form of a simple alveolar carcinoma, or an adenocarcinoma. Upon subcutaneous inoculation, the tumor does not grow to as large a size as the rat sarcoma, and it very soon degenerates, leaving only a thin capsule of growing tissue. The degenerating part of the tumor at present frequently shows the condition of coagulative

necrosis. Although, as stated, the inoculated tumor does not reach great size, it presents a high degree of invasive power, penetrates nearly all tissues, muscle, fascia, bone, and cartilage, and often forms metastases. In other words, it is completely analogous to human carcinoma.

In the course of their investigations, Flexner and Jobling observed that this tumor rarely grows when inoculated into the testicle. The results of this study, in their own words, are as follows:

"In all the experiments except one the result was a circumscribed necrosis of the testicle, with more or less formation of fibrous tissue, but no tumor growth. In one experiment tumor nodules were produced. . . . In the successful experiment two rats developed tumor nodules the size of small shot, just beneath the tunica of the testis and penetrating between the tubules. It was only in the periphery of the nodule that preserved, multiplying tumor cells, lying in a fibrous stroma, were observed. The centers even of these small nodules were necrotic, and consisted partly of degenerated tubular elements."

In view of this report and in view of the ease with which a rat sarcoma grows when transplanted into the testicle, it seemed that the phenomenon described by Flexner and Jobling might offer a method to study the local reactivity of the host to inoculation of cancer. The first requisite was to repeat the experiments on a sufficiently large number of animals so as to exclude the possibility that the failure of growth in the testicle was due to the fact that the selected animals were generally resistant to the growth of this tumor. Then if the resistance should appear to be of a purely local nature, the next problem that presented itself was to see whether local conditions could not be created within the testicle, which would make it more adaptable to the growth of the tumor.

INOCULATION INTO THE NORMAL TESTICLE.

The experiment was repeated on forty animals. In half the animals, an emulsion of the tumor was injected into the testicle, and in the other half, a piece of the tumor was placed in the center of the testicle by means of a trocar needle. In only one of the forty animals was growth of the tumor observed.

All the testicles were examined microscopically. Of the unsuccessful experiments, six testicles presented a normal appearance. But in a small region, apparently where the tumor was placed,

there was noticed new connective tissue formation surrounding the tubules, the cellular elements of which appeared to be perfectly normal. In six more testicles, the connective tissue formation was more extensive, and in some tubules the spermatozoa-forming cells either completely disappeared or were greatly changed, but the Sertoli cells appeared quite normal. In the majority of the tubules, however, there was no abnormality to be noticed. The other twenty-seven testicles in which the inoculation did not succeed seemed to have undergone the condition of coagulative necrosis without any formation of a reactive inflammatory connective tissue. The tunica albuginea and the fibrous part of the tubular membranes were preserved, but all the cellular elements were changed into an amorphous mass of coagulative necrosis.

It must be noted here for the understanding of the descriptions that follow, that the parenchyma of the normal testicle of the rat consists of tubules with hardly any indication of an interstitial connective tissue. The microscopical picture of the testicle in which there was a growth of the tumor, resembled the condition found by Flexner and Jobling. The tumor nodule was situated near the tunica albuginea, and there was an extensive inflammatory connective tissue formation between all the tubules. The cellular elements of nearly all the tubules underwent a more or less deep alteration, and the tubules immediately adjoining the tumor growth were in the state of coagulative necrosis described above.

The results of this series of experiments show clearly that the failure of the tumor to grow in the testicle is not due to a general refractory state of the organism of the rat. Thirty-nine out of forty animals cannot be resistant to this tumor, because, upon subcutaneous inoculation, the tumor grows in at least 80 per cent. of the animals used. Nor is it a general characteristic of the testicle not to allow the growth of an inoculable tumor, since, as stated above, a rat sarcoma grows well in the testicle. Experiments by the writer with inoculation of mouse carcinoma in the testicle were also successful. The resistance of the testicle to the growth of this rat tumor must be due then to a certain interaction between the inoculated cancer cells and the parenchyma of the testicle. The nature of this interaction will be discussed later.

The next step in this investigation consisted in an experimental change of the condition of the testicular parenchyma previous to the inoculation of the tumor.

TESTICLES TREATED WITH SCHARLACH-R OIL.

Fischer (5), in 1906, succeeded in inducing an extensive proliferation of epithelial cells of the skin of a rabbit's ear by a subcutaneous injection of oil containing Scharlach-R, a fat-staining substance. The results were confirmed by several other investigators. The investigations of the writer (6) showed that in a white rat Scharlach-R oil, upon an injection into the subcutaneous tissue or the parenchyma of the breast, induces a profuse proliferation of connective tissue cells. Ribbert and his followers, as has been stated, contend that a certain proliferating activity of the connective tissue is a primary etiological factor in the formation of cancer. It seemed advisable, therefore, to investigate whether an injection of Scharlach-R oil in the testicle would increase the inoculability of the Flexner-Jobling carcinoma into the organ.

The experiment was repeated on thirty-two rats. Two minims of Scharlach-R oil were injected into the center of the testicle by the aid of a hypodermic syringe. Only one testicle was treated in each animal. Six days later the tumor was inoculated into the same testicle. Growth of the tumor was observed in nine testicles, or in 28 per cent. of the experiments.

All of the thirty-two testicles treated were subjected to a microscopic examination. Sixteen testicles presented a condition of a coagulative necrosis without any connective tissue reaction identical with the appearance of the normal testicles inoculated with the tumor. Not in one of these testicles was there a growth of the tumor. The remaining sixteen testicles showed an extensive inflammatory connective tissue formation between the tubules, while the cellular elements of the latter presented conditions varying from normal to different degrees of changes in the cells, and finally complete coagulative necrosis of all the cells of the tubules. Of these sixteen testicles, nine showed tumor growth, and, what was most significant, the tumor grew in the testicles where the degeneration of the parenchymatous cells was most pronounced. Usually

the tubules surrounding the growing tumor were in a state of coagulative necrosis.

Figure 1 shows this condition of the tubules surrounding an early growth of the tumor. Figures 2 and 3 show the appearance of the tubules in a testicle in which there was extensive interstitial connective tissue growth, but in which the tumor did not grow.

The results of this series of experiments indicate that the success of the inoculation of the tumor in a treated testicle depends upon two factors: (1) the newly formed connective tissue, and (2) the degeneration of the parenchymatous cells of the tubules. *A priori,* it seemed probable that the success of the inoculation could be increased still more by other methods.

TESTICLES TREATED WITH ETHER.

Askanazy (7), in his studies on the growth of inoculated embryonal tissue, maintained that an addition of a 4 per cent. mixture of sulphuric ether in water enhances the growth. Since the writer has shown previously that all methods that induce proliferation of cells in the white rat usually act on connective tissue, it appeared advisable to investigate whether a preliminary treatment of a testicle with ether water will render it more susceptible to the growth of the Flexner-Jobling carcinoma. The experiment was repeated on twenty rats. Three millimeters of a 4 per cent. mixture of sulphuric ether in water were injected by means of a hypodermic syringe into the center of the testicle. Forty-eight hours later the tumor was inoculated into the same testicle. Growth was observed in thirteen testicles, or in 65 per cent. of the experiments.

The microscopic examination showed in only four testicles a condition of coagulative necrosis without any connective tissue reaction. These four testicles showed uniformly an absence of the tumor growth. The remaining sixteen testicles showed an extensive inflammatory connective tissue formation between the tubules, and a varying degree of degeneration of the cellular elements of the latter. The degeneration and necrosis of the cells of the tubules were much more marked in these testicles than in the testicles treated with Scharlach-R oil.

This difference in the results after the treatment with the two

substances could actually be expected *a priori*. Ether is a cellular poison and must injure the cells of the tubules. The connective tissue is probably formed subsequently under the influence of the degenerated tubular cells. Scharlach-R, on the other hand, is not a poison and probably induces primarily the proliferation of connective tissue between the tubules. The connective tissue subsequently injures, to a certain degree, the tubular cells. Of the sixteen testicles that showed both a connective tissue formation and a degeneration of the tubular cells, thirteen presented a growth of the tumor. Again the tubules adjacent to the tumor growth showed the most marked alteration.

DISCUSSION.

A general analysis of the experiments shows that the normal testicle of the white rat is resistant to the growth of the Flexner-Jobling carcinoma, while the rest of the organism of the same animal may be susceptible to the growth of the same tumor. The proof of this assertion may be found in the fact that thirty-nine rats out of forty resisted the intratesticular inoculations; while, with a subcutaneous inoculation, the tumor would have grown in at least thirty-two animals. Another instructive proof is shown in figure 4. It represents a testicle that was treated with Scharlach-R oil and underwent the process of coagulative necrosis without any connective tissue formation. The tumor did not grow in the testicle. During the inoculation, the needle injured the tunica albuginea and lodged a few tumor cells there. The tumor grew within the connective tissue of the tunica.

The failure of the growth of this tumor in the testicle is not due to a general condition of the organism, but to a local action of the testicle on the tumor cells. Nor is it due to the mechanical influence of the tunica interfering with the expansion of the organ by the growing tumor, nor to the lack of nutrition. Investigations of the writer (8) have shown that other inoculable tumors grow perfectly well in the testicle of a generally susceptible animal. The mechanism of this local resistance can be due only to an inhibitory action upon the tumor cells of the tubular cells, aided probably by the product of cellular metabolism. The connective tissue found

in twelve of the normal testicles inoculated was apparently formed subsequently to the destruction of the cancer cells, the latter acting as a foreign body. The same mechanism was described by the writer in the inoculation of the sarcoma into the testicle of a generally resistant animal. In the twelve testicles in which the connective tissue was formed and no growth of the tumor took place, the tubular elements appeared to be fairly normal. In the one normal testicle in which the tumor grew, there was noticed a very extensive connective tissue formation, but also a marked degeneration and necrosis of the tubular cells. Apparently, in this testicle some microörganism or other agent injurious to the cells of the testicle was introduced with the tumor, and the testicle cells became abnormal before their inhibition of the tumor cells became effective.

The coagulative necrosis of the whole testicle without any connective tissue formation, noticed in so many testicles in which the tumor did not grow, cannot be due to a fault of the technique. In the first place, in all the writer's previous experiments with inoculation of different tumors in both resistant and susceptible animals, this condition was never observed. Further, in the experiments with treated testicles, ether water, which gave the greatest number of successful inoculations, also gave the least number of testicles showing complete coagulative necrosis. This condition can possibly be explained by the fact that the Flexner-Jobling tumor always undergoes very extensive coagulative necrosis. When the whole graft is destroyed by the testicle cell, the former becomes necrotic and then probably affects in the same manner the parenchyma of the testicle.

This investigation shows also that it is possible to render the same testicle artificially susceptible to the growth of the tumor. Such a testicle in the extent and character of the newly formed connective tissue, resembles the "precancerous stage" in the tissues surrounding an early growth of cancer, which was described by Ribbert, Borrmann (9), Bonney (10), and others. But the present experiments also show that the newly formed connective tissue in the testicle is not capable alone of rendering the organ susceptible to the growth of the carcinoma. The tumor grows only when simultaneously the parenchymatous cells of the organ become de-

generated and unable to inhibit the cancer cells. It is true that the tumor does not grow in a testicle in which all the parenchyma is necrotic and no connective tissue is formed, but there the failure of growth is due purely to lack of nutrition. A testicle treated with Scharlach-R oil may contain a great amount of connective tissue, but if the cells of the tubules are fairly well preserved the tumor cells fail to proliferate. It must be understood, however, that this is true only for the early stages of the growth. If the inoculated tumor cells are placed near necrotic tubules and the growth of the tumor advances, then the latter is enabled to replace normal tubules as well as the sarcoma described by the writer does. Figure 5 illustrates such a condition. The testicle is nearly all replaced by the tumor, and only at the periphery tubules are seen that appear to be quite normal.

Thus, the mechanism by which a resistant testicle is rendered susceptible to cancer growth consists in the impairment of the inhibitory action of the parenchymatous cells on the cancer cells. The connective tissue is a secondary formation of an unspecific nature, as maintained by the writer (2) in his previous investigations, and serves only for the nutrition and support of the cancer cells.

It has been indicated above that the Flexner-Jobling carcinoma of the white rat resembles very closely human cancer. A testicle rendered susceptible to the growth of this tumor resembles still more closely the "precancerous stage" adjacent to the early stages of cancer growth described in human tissues. It seems proper to suppose by analogy that in human pathology also the process of the beginning of cancer growth consists in the degeneration and loss of inhibitory powers of the parenchymatous cells adjacent to the groups of cancer cells. The abnormality in the parenchymatous cells may be so slight as to escape notice. The changes in the connective tissues may also be only secondary, unless, as in the case of epithelioma of the skin, which was extensively studied, the subepithelial connective tissue represents the nearest affected parenchyma.

Growth of cancer, then, presents a loss of equilibrium between the inhibitory power of the parenchymatous cells and the proliferating power of the cancer cells.

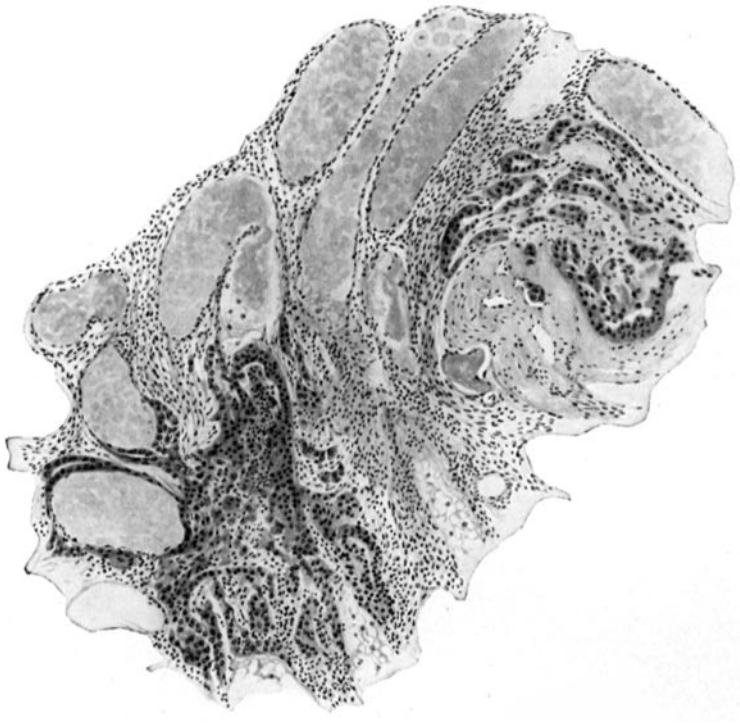

FIG. 1.

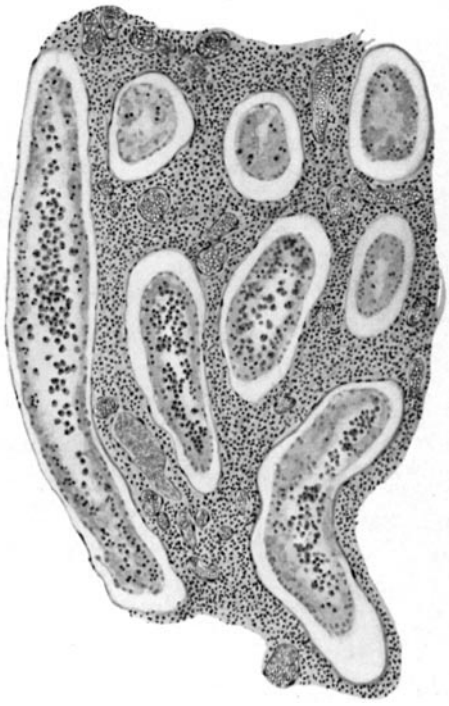

FIG. 2.

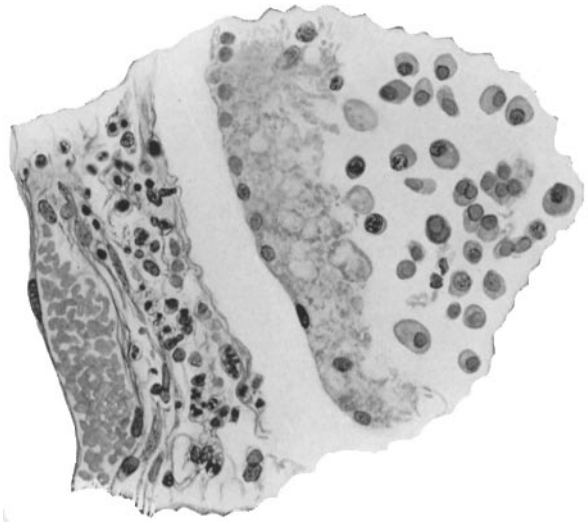

FIG. 3.

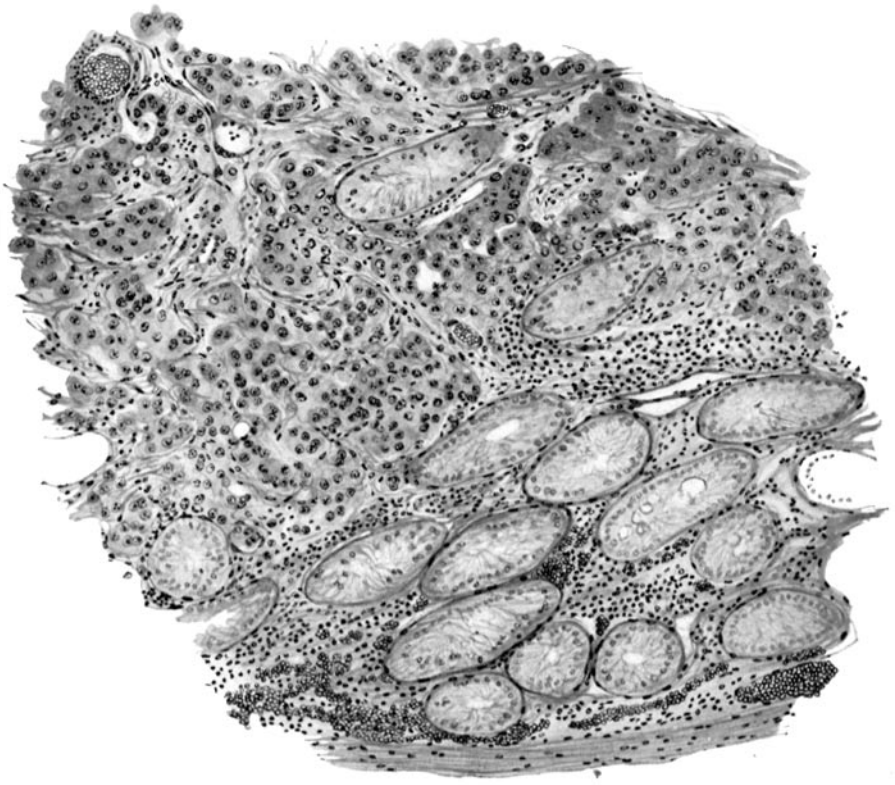

FIG. 4.

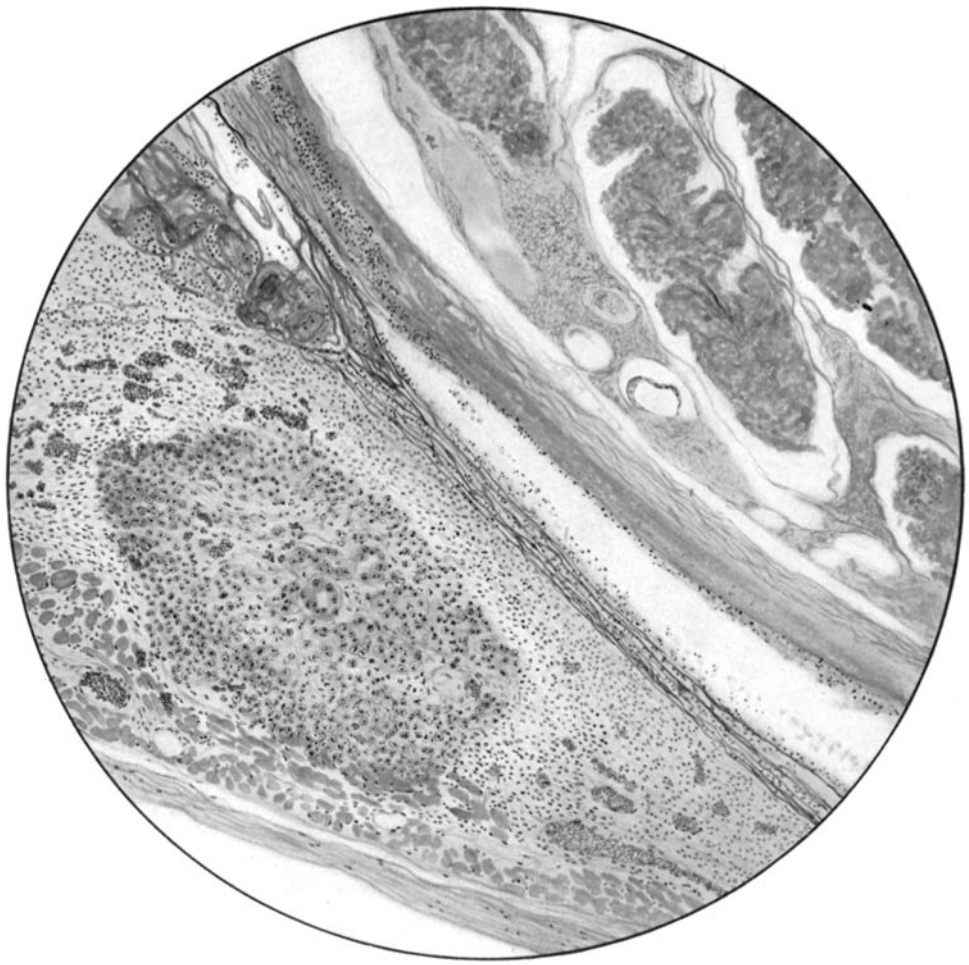

FIG. 5.

It will require further study to decide whether the same cause that injures the parenchymatous cells at the same time frees the proliferating power of other previously normal cells, as adherents of Ribbert believe, or whether the causes for the two phenomena are different.

The value of the present investigation consists in the fact that it presents the first experimental proof of the existence of a local "precancerous stage." It also shows that there are still many points in the etiology of cancer which it will be possible to elucidate through animal experimentation.

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EXPLANATION OF PLATES.

PLATE 23.

FIG. 1. Two groups of cancer cells surrounded by tubules in the state of coagulative necrosis. (Low magnification.)

FIG. 2. A testicle treated with Scharlach-R oil, in which the tumor did not grow. There is extensive interstitial connective tissue formation and a great many newly formed blood-vessels. The tubules show the Sertoli cells fairly well preserved. There are apparently no normal spermatozoa-forming cells. (Low magnification.)

PLATE 24.

FIG. 3. The same condition as in figure 2. To the right of the drawing is seen a tubule; to the left, newly formed connective tissue; further to the left, a blood-vessel. (High magnification.)

FIG. 4. Extensive tumor growth in a testicle treated with ether water. Tubules, in a fair state of preservation, are seen within and below the tumor mass. (Low magnification.)

PLATE 25.

FIG. 5. The upper right side of the drawing shows a testicle in a state of coagulative necrosis. The lower left side shows the tumor growing in the tunica albuginea. There was no growth in the parenchyma of the testicle. (Low magnification.)