The condition of immunity or resistance to the growth of an inoculable cancer is undoubtedly present in a certain percentage of animals. The mechanism of this resistance differs in many respects from the immunity of an organism against bacterial diseases. The presence of an antibody in the blood serum of a resistant animal cannot be demonstrated by any of the known methods. No irrefutable evidence is brought forward to indicate that a passive immunity may be induced by treatment of a normal animal with the blood serum of an immune one. The further fact, that unimpaired living tissue is generally required to immunize an animal against tumor growth, is the cause of the prevailing idea that immunity in cancer is due to a purely cellular activity of the organism of the host. But such hypothetical conceptions are hardly adequate to elucidate the phenomenon. Every kind of immunity represents in the final analysis the result of a cellular activity, but it is impossible to assume that the cells of a piece of fresh tissue placed under the skin and absorbed will induce an immunity in the host by their life activities. Indeed, the writer (1) has recently indicated in a series of investigations, that the same immunity may be induced by treatment with autolyzed tissue, i. e., tissue in which the cells are killed but the endocellular enzymes remain active. The fact that enzymes introduced into an organism may serve to induce immunity against growth of tumor indicates that there

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must be a certain similarity between this condition and immunity in bacterial diseases. Our present methods may be inadequate to demonstrate the mechanism which is at work. The writer is engaged in the further investigation of this subject. At the same time, it seems advisable to investigate the various cellular theories of resistance against the growth of tumor. If these theories are not helpful in the further study of the subject, they must be discarded, so that further investigation of the question of resistance to cancer growth, which may prove to be of the most vital importance in the understanding of the disease, may be continued unhampered.

Ehrlich's (2) hypothesis of athrepsia, which seemed to explain readily the main phenomena of the immunity to growth of cancer, was the subject of a recent study by Levin and Sittenfeld (3). The results of the investigation seem to indicate that the immunity of the rat and mouse against the growth of inoculable cancer cannot be explained merely on the basis of cellular nutrition, but tend to show an active inhibitory influence of the organism of the host on the cancer cells. Bashford (4), in his conceptions of immunity in cancers of rats and mice, denies that there is any direct influence of the organism of the host upon the inoculated cancer cell. According to his ideas, an inoculated piece of cancer tissue causes in the new host the formation of a specific connective tissue and vascular scaffolding. This connective tissue stroma surrounds the graft and furnishes it with the mechanical support and nutrition necessary for its development. Immunity then consists in the failure of the organism of the host to supply the specific stroma reaction. The immune organism does not produce a direct deleterious effect on the cells of the inoculated cancer, but alters its normal connective tissue cells so as to render them insusceptible to the chemiotactic properties of the inoculated cancer cells. Russell (5) seems to have furnished microscopic proof for this contention through his comparative microscopic studies of the "early stages" of tumor grafts in susceptible and in immune animals. In accordance with his results, there is no morphological difference noticeable in the appearance of the tissue surrounding the graft in the two kinds of animals during the first two days after a subcutaneous inoculation. But the picture changes greatly five to six days after inoculation. In the susceptible animal, there is seen an active division of the connective tissue cells of the host, which leads to the formation of a connective tissue stroma with abundant vascularisation. In the immune animal, on the other hand, there is no active proliferation of the host's fibroblasts, nor is there any development of new capillaries; the new host failing to supply a vascular stroma. Da Fano (6), in a recent investigation conducted in Bashford's laboratory, claims even to have observed a difference in the behavior of the various elements of the connective tissue of the immune host. The inoculated cancer cells retain their chemiotactic properties as against polymorphonuclear leucocytes and mast cells, but they are unable to influence the plasma cells and lymphocytes.
The question of the specificity of the reactive stroma formation after an inoculation of cancer tissue in white rats and mice, and its relation to the success of the graft, was the subject of a recent investigation by the writer (7). This study showed unmistakably that the formation of a connective tissue stroma surrounding the graft is not specific to inoculation of cancer, and is identical with the connective tissue capsule surrounding any foreign body introduced into the organism. Only when an inoculation is done subcutaneously does the transplantability of cancers of the white rat depend upon the formation of a connective tissue stroma around the implanted piece. The reason for it is to be looked for in the fact that, by the subcutaneous method, the graft is placed in a loose pocket at a distance from blood-vessels, and the prompt formation of a surrounding stroma is necessary for the support and nutrition of the inoculated cancer cells. When a piece of tumor is grafted into a parenchymatous organ, the cancer cells are placed near the blood-vessels of the organ and enabled to obtain immediately the necessary nutrition. In numerous experiments with inoculation of pieces of tumor into the brain, kidney, or testicle, there did not take place in a single instance the formation of a surrounding connective tissue stroma. The first visible step after such an inoculation into a parenchymatous organ is the proliferation of cancer cells themselves. They do not grow in a compact mass but infiltrate diffusely in all directions the parenchyma of the organ. There is no evidence of a small round cell infiltration and subsequent connective tissue stroma formation around the growing tumor. In view of these findings, it seemed of importance to investigate the behavior of both the inoculated cancer cells and the tissue of the host after an inoculation into the parenchymatous organ of an immune animal. The first question to be determined was, whether an animal rendered immune by a subcutaneous inoculation would also resist a subsequent inoculation into a parenchymatous organ. The second point for investigation was the microscopic appearance of the graft and the behavior of the neighboring tissue of the host. The present study was undertaken with these aims in view. As in the former investigation on the importance of the stroma formation, the experiments were conducted
with the same transplantable sarcoma of a white rat. Seventy-six
animals previously shown to be immune against repeated sub-
cutaneous inoculations of the tumor were used for the experiments.
The rat sarcoma is a very virulent tumor, and only about 10 per
cent. of the inoculated animals appear to be immune. Further-
more, since the testicle presents the best organ for the study of the
microscopic appearance of the graft, the majority of the animals
selected for this investigation were males. In view of all this, in
order to obtain suitable animals, a selection had to be made from
nearly two thousand rats which had been inoculated with the
sarcoma for other purposes. Of these seventy-six animals, fifty-
six, which were males, were inoculated in the testicle, and the other
twenty, which were females, were inoculated in the kidney. The
methods of operation were described in a previous publication (7).

These experiments showed that the tumor did not develop in a
single animal; consequently an animal rendered immune by a sub-
cutaneous inoculation of the tumor is resistant against a subsequent
inoculation into a parenchymatous organ. The microscopic ap-
pearance of the graft and the surrounding tissue in the two organs
are reported separately.

INOCULATION INTO THE TESTICLE.

The testicles were removed under ether anesthesia at periods of
one, two, four, six, nine, twelve, and twenty-one days after the
inoculation, hardened in formalin and Zenker's fluid, embedded in
paraffin, and stained with hematoxylin and eosin and Mallory's
anilin-blue stain. On microscopic examination, the pictures ap-
peared strikingly different from those observed after an inocula-
tion of the sarcoma into a testicle of a susceptible animal. The
sarcoma cells of the graft preserved their morphological character-
istics for the first two days only, after which they gradually de-
generated. On the sixth day, one could hardly find a single well
preserved cell. On the ninth day, the whole graft appeared as a
homogeneous, amorphous mass, and on the twenty-first day, all
traces of the graft had disappeared.

More remarkable still is the appearance of the neighboring tissue.
Twenty-four hours after the inoculation, the graft was surrounded
by a round cell infiltration. On the sixth day, the mass of degenerated tumor cells was surrounded by a connective tissue stroma and by a great number of newly formed blood-vessels. This new connective tissue not only immediately surrounded the graft but spread further away from the graft between the seminiferous tubules (figure 1). In some instances, this connective tissue apparently compressed the tubules and injured the epithelial cells. Some of these tubules sustained more injury than the tubules surrounded by actively growing sarcoma in a susceptible animal. The amount of newly formed connective tissue constantly increased, and, twelve days after inoculation, in two animals the whole testicle was found filled with connective tissue spread between all the tubules (figure 2). Twenty-one days after inoculation, both the original graft and the newly formed connective tissue were found to be completely absorbed, and in most of the animals the testicle appeared to be restored to its normal condition. Occasionally a remnant of the new connective tissue was found between the tubules.

INOCULATION INTO THE KIDNEY.

The kidneys were removed under ether anesthesia at periods of one, two, four, six, fourteen, and twenty-one days, prepared, and examined in the same way as the testicles. In no instance did the tumor develop in the kidney of the immune animals. The microscopic examination showed a condition similar to the one observed in the testicles. In the first two days, some of the inoculated tumor cells were preserved, but the beginning of a connective tissue formation was noticed. Fourteen days after inoculation, the tumor cells of the graft were completely degenerated and surrounded by a stroma consisting of connective tissue and newly formed blood-vessels, which invaded the neighboring parenchyma of the kidney (figure 3). Subsequently, the graft and most of the newly formed connective tissue disappeared. The kidney, however, as a rule, was not restored entirely to its normal condition. This is probably due to the very severe operation required for the inoculation of the tumor into the kidney.

The analysis of the results of this investigation shows that Bashford's conception that immunity in cancer is due to the failure of
the tumor cell to elicit in the host a specific connective tissue and vascular stroma formation is not capable of general application. An objection may be offered that the present investigation was conducted on a rat sarcoma, while in Bashford's laboratory carcinoma of the mouse was used, and that in the former there is usually less stroma formation than in carcinoma. This difference concerns only the specific connective tissue stroma between groups of cancer cells. Bashford, Russell, and Da Fano, in describing the connective tissue and vascular scaffolding, mean primarily the layer of fibrous tissue which surrounds and encapsulates the graft. The development of this latter stroma after a subcutaneous inoculation of a rat sarcoma is identical with that of a mouse carcinoma.

The fact that cancer cells, when inoculated into a parenchymatous organ of a susceptible animal, grow without a preliminary stroma formation may not militate entirely against Bashford's conceptions of immunity, because by this method of inoculation the proliferation of the cancer cells begins so promptly that no opportunity is given for the chemiotactic properties of the cells to develop. The theory that an immune animal is an organism which resists the chemiotactic properties of the inoculated cancer cells may still be correct.

But the phenomena observed on the inoculation of cancer cells into parenchymatous organs of immune animals cannot be brought into accord with Bashford's theories. If immunity in cancer is due to the failure of the host to supply a connective tissue stroma, then the inoculated cancer cells should grow in the parenchymatous organs of the immune animal as well as in those of the susceptible one, since no preliminary stroma formation is needed for such growth. Instead of this, the immune animal kills the cancer cells inoculated in the parenchymatous organs, but, unlike the susceptible host, forms an extensive connective tissue stroma around the graft. Bashford and Russell (8), in the course of their study, made an observation which did not agree well with their theoretical considerations. This observation is as follows: When a mouse tumor is inoculated into a rat, it grows for eight or ten days and is then gradually absorbed. Through this the rat is rendered immune against a subsequent inoculation of a mouse tumor. Upon micro-
scopic examination of a mouse tumor graft inoculated into this immune rat, the authors found that after two or three days there is hardly a single cancer cell left, but that there is an extensive connective tissue stroma formed around the dying graft. They explain this phenomenon by the aid of a supposition that the immunity of a rat against the growth of a mouse tumor is not a true cancer immunity, but an active immunity against mouse tumor tissue acting as a foreign proteid. As further proof of this supposition, they state that it is possible to immunize rats against mouse tumor by previous treatment with disintegrated mouse tumor. The results of the present investigation show that the mechanism of the immunity of a rat against an inoculation of a rat tumor into an organ is identical with the immunity of a rat against a subcutaneous inoculation of a mouse tumor. In either case, the tumor cells are destroyed and surrounded subsequently by an extensive connective tissue stroma. Consequently there is produced in either case, in accordance with the conceptions of Bashford and Russell, an active immunity which leads to the rapid destruction of the cancer cell. On the other hand, it is hard to conceive of the mechanism of immunity being different solely because at one time the tumor is inoculated into a parenchymatous organ and at another subcutaneously. Goldmann (9) has also shown in a recent investigation that an immune animal does not lose the capacity for the formation of a stroma. Mice rendered completely immune against carcinoma, but not against sarcoma, produced after a subcutaneous inoculation of the latter tumor a stroma just as rich in bloodvessels as that produced by susceptible animals.

The most plausible explanation of the reactive stroma formation, and the explanation which reconciles better than any other all the known facts, is one which does not accord any specificity to the phenomenon. A cancer graft when introduced into a mouse or rat acts at first as a foreign body and as such is surrounded by the cellular elements of connective tissue. When the graft is introduced subcutaneously, the cancer cells do not become adjusted to the new host and do not begin to proliferate until a new vascular stroma is formed. On the other hand, the cancer cells have a sufficient amount of vitality to withstand the phagocytosis of the
surrounding connective tissue cells. When the graft is introduced into a parenchymatous organ, the cancer cells immediately find nutrition, begin to proliferate, and become organically united with the organ. Such a graft does not act as a foreign body and does not elicit any stroma formation. But if the same graft is inoculated into a parenchymatous organ of an immune animal, then the host, by the aid, probably, of some substance present in the body fluid, destroys the introduced cancer cells. The degenerated cells act as a foreign body and as such induce the formation of a connective tissue stroma. Ultimately both the dead cancer cells and the newly formed connective tissue are completely absorbed. Borst (10), Schmidt (11), and Orth (12) describe the same mechanism in human pathology. A connective tissue stroma surrounds a group of cancer cells, gradually increases in size, compresses the cancer cells, and produces a local cure of the growth. According to Orth, the primary factor in this condition is a degenerative change in the cancer cells, while the connective tissue stroma is of the same nature as the connective tissue which forms around a foreign body. The identical process is described by Da Fano in spontaneous healing of mouse tumors. The comparatively inefficient formation of the connective tissue stroma, after a subcutaneous inoculation of a cancer graft in an immune animal, is apparently due to the fact that the destroyed cancer cells are absorbed more rapidly from the subcutaneous tissue than from the parenchymatous organs. There is consequently less of a foreign body left to induce the stroma formation and the whole process disappears sooner.

Thus this investigation, as well as the previous studies of the writer and the study by Levin and Sittenfield, furnish further evidence to show that immunity to cancer growth is due to an active inhibitory influence of some substance present in the body fluids of the immune host, and not to any purely cellular activity. Immunity to growth of cancer must be very similar to, though not identical with, immunity in bacterial diseases.

BIBLIOGRAPHY.

EXPLANATION OF PLATES.

PLATE 16.

Fig. 1. A tumor graft in a testicle of an immune animal. To the right of the figure is seen necrotic tumor tissue; to the left, connective tissue stroma; and further to the left, testicle tissue.

Fig. 2. A testicle of an immune rat. Newly formed connective tissue fills all the spaces between the tubules.

PLATE 17.

Fig. 3. A tumor graft in a kidney of an immune animal. To the right of the figure is seen necrotic tumor tissue; further to the left, testicle tissue.