

TUMOR INOCULATION INTO ORGANS AND THE
ANALOGY BETWEEN HUMAN CANCER AND
THE TUMORS OF WHITE MICE AND
WHITE RATS.*

By ISAAC LEVIN, M.D.

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

PLATES 13-15.

The experimental study of inoculable tumors of white mice and white rats promises to be very fruitful, not only in elucidating the various phases in the pathogenesis of human cancer, but also in establishing correct methods for the specific therapy of the disease. The basic conception for this whole field of experimental cancer research is the most generally accepted idea that the tumors of white mice and white rats are analogous to human cancer.

Recently a grave doubt was cast upon the correctness of this idea. von Hansemann (1), in discussing his own and Wassermann's papers on chemotherapeutic experiments with inoculable tumors of white mice, emphatically declares that there is no analogy between these tumors and human cancer. He bases his opinion on the following differential characteristics between the two conditions. (1) Human cancers never reach as large a size in proportion to the body weight as do the tumors in animals. The only tumors that reach such a size in man are the benign growths, like the lymph-angioma. (2) Animals do not suffer constitutionally from the tumors, *i. e.*, the state of cachexia is not observed. (3) The tumors in the mouse and rat appear as movable encapsulated nodules, which are easily removed surgically and do not recur after the operation. (4) A true metastasis does not take place in these animals. Conditions described as metastasis are simply multiple inoculations.

* Conducted at the expense of the George Crocker Special Research Fund. Read before the meeting of the American Association for Cancer Research, April 3, 1912. Received for publication, April 22, 1912.

When an emulsion of the tumor cells is injected subcutaneously, part of it may enter a blood-vessel and thus reach some distant part of the animal and form there a second tumor, which is, consequently, not a metastasis. (5) The inoculated tumors frequently retrocede, whereas human cancer does not. The apparent spontaneous cures described occasionally in human beings are due usually to a wrong diagnosis. (6) Morphologically the animal tumors resemble endotheliomata most nearly, and while it is not possible to prove absolutely the endothelial nature of the growths, the growths are not analogous morphologically to human carcinoma.

von Hansemann is one of the greatest authorities at present on the pathology of human cancer, and it is therefore imperative, in view of the great importance of the question for the future of experimental cancer research, to review the subject once more. The thesis the writer expects to sustain by the present investigation is that a white mouse or white rat bearing a tumor is suffering from a malignant disease which is analogous in every particular to human cancer.

SPONTANEOUS TUMORS.

In his discussion, von Hansemann apparently had in mind mainly the tumors artificially inoculated into previously healthy animals. In order to gain a true conception of the nature of the animal tumors, the analysis must begin with the spontaneously occurring tumors from which the material for the inoculation is taken.

The number of the morphologically different forms of these tumors that have been described in animals is nearly as great as the number of human cancers. Jobling (2) described cases of adenocarcinoma, cystadenoma, alveolar carcinoma, and sarcoma. Haaland (3) described carcinoma of the preputial gland, adenocarcinomata of the kidney and ovary, spindle-celled, round-celled, and polymorphous-celled sarcomata, melanoma, and fibromyoma of the uterus. It is evidently impossible to classify all these tumors as endotheliomata.

The best proof of the fact that these spontaneous tumors are malignant is the comparative frequency of the occurrence of metastases. Murray (4) in a study of sixty-eight mice with spontaneous tumors found metastasis in the lungs in twenty-seven cases, and in

the lymphatic glands in three cases. In studying twenty-six animals Jobling found metastases in five, and Haaland, in a study of 273 animals that had spontaneous tumors, found nodules clearly visible to the naked eye in the lungs of 38 per cent. of them.

Furthermore, most of the clinical characteristics of these tumors clearly indicate that the animals are suffering from a malignant disease. According to Haaland the average duration of life of the animals after the tumor has come under observation is about six weeks.

What is still more conclusive is the fact, also observed by Haaland, that in 54 per cent. of the animals with spontaneous tumors, the tumor recurred after an apparently radical operative removal. The same phenomenon was observed by Clunet (5). There can be no doubt, therefore, that a spontaneous tumor in a white mouse or white rat is malignant and consequently not only morphologically but also biologically analogous to human cancer.

It is impossible to define what is to be considered a condition of cachexia in these animals. The fact, however, that the animals die in the short period of about six weeks after the tumor is discovered, shows that the general health of the animal must have been deeply affected by the growth of the tumor.

INOCULATED TUMORS.

Tumors artificially reproduced through inoculation in previously normal animals are of greater importance for the experimental study of cancer than the spontaneous tumors. The reason is that the latter are comparatively rare, while inoculated tumors may be obtained in any desired number. These artificial tumors apparently seem to differ widely both from the spontaneous tumors of the same animals and from malignant growth in man. Although the material used for the inoculation is taken originally from a spontaneous tumor, *i. e.*, from an animal suffering from a malignant growth, the tumor that develops in the new host seems to be of a more benign character.

The transfer is usually made by a subcutaneous inoculation. As a result of this inoculation there forms under the skin a growth which may reach a very large size. In the majority of cases this

tumor is circumscribed, movable, and encapsulated. It usually does not infiltrate the surrounding tissue, it never infiltrates bone, peritoneum, or the pleura, and very seldom infiltrates the fascia and musculature underlying the skin. The skin itself, however, may become adherent to the tumor and ulcerate. Upon complete surgical removal, the tumor does not recur as does the spontaneous tumor. Thus the inoculated tumors apparently resemble benign growths rather than true cancers.

What is it that causes a tumor which was malignant in the first host to become apparently benign after transplantation? The recent studies of the writer (6) upon the behavior of inoculable tumors of white mice and white rats when implanted into various parenchymatous organs, convey the impression that the change in the behavior of the inoculated tumors is due to the method of transfer most generally employed. A subcutaneous inoculation may be the most convenient one for obtaining a sufficient amount of tumor material and is consequently useful for a number of different purposes. But in order to study the true behavior of the inoculated tumor and its influence on the general state of health of the new host, the inoculation should be made into the various parenchymatous organs.

INOCULATION INTO ORGANS.

For the inoculation into organs the writer employed the white rat mainly. Two tumors served for the inoculations: a spindle-celled sarcoma of the white rat, and an adenocarcinoma described by Flexner and Jobling (7). The tumors were inoculated into the brain, testicle, kidney, spleen, and liver. In the beginning of the investigation the organs were opened, the piece of tumor was placed inside, and the incision in the organ was closed with a suture. Subsequently the method was simplified. The organ was reached through an abdominal or lumbar incision, or by trephining the skull to reach the brain, and then a small piece of the tumor was placed in the center of the organ by means of a trocar needle. The bleeding even in the liver was minimal and ceased very soon. The abdominal or lumbar incision was then closed by a silk suture. The trocar needle does not cause any permanent injury to the organ.

When the tumor placed inside an organ fails to grow, it is with

difficulty that one can notice any change in the region where the tumor graft was placed, either by gross inspection or by microscopical examination. Consequently, whatever local change is found in the region of the organ surrounding the growing tumor is caused not by a mechanical injury through the operation, but by the interaction between the organ cells and tumor cells.

In order to compare the effects produced by inoculating a tumor into one of the organs of a susceptible animal, with the effects seen in human beings suffering from cancer, it is well to recapitulate the salient characteristics of malignant tumors. These characteristics are: (1) rapidity of growth; (2) peripheral extension, lack of capsule, and invasive infiltration of the surrounding tissue; (3) tendency to develop metastases and to recur after removal; and (4) cachexia and clinical malignancy. In the following pages a separate analysis will be made of these characteristics.

Rapidity of Growth.—If a white rat is inoculated successfully with either of the two tumors used in this investigation, it lives only ten to twelve weeks. The tumor produced by inoculation into an organ does not usually grow as large as that produced by a subcutaneous inoculation, but the number of days the animal survives is about the same after either form of inoculation. Apparently the large size which the tumors reach after a subcutaneous inoculation is not due to a peculiar character of the tumor cells, but to the fact that the subcutaneous tissue in these animals is very loose and consequently offers no resistance to the expansion of the tumor. It is much easier for the growing tumor to separate the skin from the underlying fascia than to invade the fascia.

Invasive Growth.—As stated above, when a tumor is inoculated subcutaneously it finds space for its peripheral extension in size by separating the skin from the superficial fascia and it usually does not invade the surrounding tissue. On the other hand, when a tumor is inoculated into an organ it does not increase its size by separating the parts of the normal organ tissue, but actually invades the latter and replaces it by tumor tissue.

Figure 1 shows a white rat with the abdominal wall removed and one lobe of the liver turned over; the other lobe shows in the center an inoculated sarcoma. The tumor apparently destroyed and re-

placed part of the liver tissue. Figure 2 shows two spleens inoculated with sarcoma. In the center of one spleen a small tumor growth is seen, but nearly all of the other spleen is replaced by a large tumor, the remains of the organ being noticed only at its two ends.

In some animals the tumor stays within the organ and the latter remains as freely movable in the abdomen as before the inoculation. In others the tumor invades and partly destroys the tissue or impairs the function of a neighboring organ; for instance, the stomach is injured when the inoculation is made into the liver or spleen. The autopsy findings in such an animal are analogous to those seen at the autopsy of a human being in which death was due to cancer in the peritoneal cavity.

This invasion of the neighboring organs cannot be ascribed in the present experiments to a multiple inoculation at the time of the operation, a criticism which was offered by von Hansemann to the experiments of Citron (8), who introduced into the wall of the stomach a thread permeated with a tumor emulsion. Here, apparently, there was a possibility for multiple inoculation. In the experiments of the writer, however, a solid piece of the tumor was introduced into the substance of the organ, and when the trocar was withdrawn, great care was taken to have the inoculated piece completely surrounded by the organ.

The microscopical appearance of the early stages of a sarcoma graft placed into the brain, testicle, or kidney was described by the writer in a former communication (6). The sarcoma cells infiltrate diffusely and replace the substance of the organ. In the present study similar experiments were performed with both sarcoma and carcinoma, the inoculations being made into the spleen, liver, and kidney.

Figure 3 shows the diffuse growth of a carcinoma in a spleen, and figure 4 the growth of a carcinoma inoculated into a liver. Carcinoma cells are seen to be growing diffusely between normal liver cells. Figure 5 shows an inoculation of a carcinoma into a kidney. The field shows kidney tubules surrounded everywhere by tumor cells. It is very clear that in this region most of the normal kidney tissue is replaced by the tumor. Thus it is evident that the invasive

infiltrating growth of an inoculable tumor implanted into a parenchymatous organ is similar to the growth of cancer in human beings. That the tumor behaves differently when inoculated subcutaneously is due then entirely to the nature of the tissue into which it is inoculated.

Formation of Metastases.—von Hansemann claims that a true metastasis was never described in inoculated tumors. Whenever a secondary tumor occurred in an animal in a region distant from the primary subcutaneous inoculation, it was due to the fact that part of the tumor emulsion used for the subcutaneous inoculation entered a blood-vessel, was transported to a distant part, and there formed a second tumor. The investigations of Levin and Sittenfield (9) do not bear out this assumption.

In the first place, even upon a direct injection of a tumor emulsion into the vascular system, the success of the formation of tumor nodules depends apparently upon the character of the tumor. While metastatic nodules were formed after an injection of an emulsion of carcinoma cells, similar experiments with sarcoma gave completely negative results. Furthermore, an inoculation of a solid piece of carcinoma into the bone marrow gave metastasis in the lung. The objection may be raised in regard to these experiments that a part of the inoculated piece of the tumor may have been torn off and carried readily from the bone marrow into the general circulation. The experiments of the writer given below seem to be free from all these objections.

Levin and Sittenfield did not succeed in producing metastases with rat sarcoma either by intravenous injection or by inoculation into the bone marrow. Of hundreds of animals inoculated subcutaneously with this sarcoma, metastasis was found only once and in this instance it was in the liver.

In the present series of experiments the sarcoma was inoculated into the livers of twenty rats, and the animals were kept alive for about four weeks. Metastasis was found in the lungs of three animals. The same sarcoma was inoculated into the spleens of twenty rats. Two of these animals developed metastases in the lungs and one in the liver.

Figure 6 shows one of the metastases in the lung. In all these

animals a solid piece of tumor was placed by means of a trocar in the substance of the liver or spleen. It is impossible then to suppose in these cases that the secondary nodule was formed at the time of inoculation. Apparently the metastatic nodule in the lung or liver was formed subsequently to the development of the primary tumor. Consequently the mechanism of the formation of a metastasis following an inoculation of a tumor into an organ is identical with that of the formation of metastases in spontaneous animal tumors and in human cancer.

Cachexia and Clinical Malignancy.—It is hardly possible to define the condition in a white rat or white mouse which would be identical with the cachexia in human beings, for the peculiar color and general condition of the skin, which is one of the main characteristics of the condition in human beings, cannot be obtained in these animals. But as was stated above, animals in which the inoculation was successful, do not survive the inoculation longer than about twelve weeks, and this is certainly direct clinical proof of the malignancy of these tumors. The following series of experiments gives further clinical proof of malignancy.

The writer (10) reported recently that the Flexner-Jobling carcinoma of the white rat does not grow when inoculated into a normal testicle, but it does grow in testicles which were treated previously with Scharlach R-oil or with ether water. Twenty-two animals in all showed a positive growth of the tumor. The testicles of these animals were removed surgically. During the operation the testicles did not appear to be adherent to the adjoining tissue nor were any small tumor nodules observed in the vicinity of the testicles. But in seven of these animals a secondary tumor developed near the operative field. The removal of these tumors had been as radical as any surgical operation for human cancer, but apparently microscopic groups of tumor cells remained in the neighborhood of the operation and a local recurrence took place.

SUMMARY.

The analysis of the experiments described above indicates that tumors of the white rat or white mouse inoculated into parenchymatous organs acquire a different biological character from those

inoculated subcutaneously. The latter are a great deal more benign in their behavior than human cancer or spontaneous tumors in the same species of animals. Tumors inoculated into organs, on the other hand, are quite identical in their biological behavior with the malignant tumors of animal and man. A conclusion must then be drawn, even *a priori*, that the method of inoculation into organs is a very important aid in the experimental investigation of cancer. It is true that the method is a great deal more complicated and time-consuming than the ordinary subcutaneous inoculation.

The subcutaneous method is satisfactory for a number of cancer problems. One of these is the study of general susceptibility and resistance of the organism of the host to the inoculation of the tumors, and this is a subject of paramount importance in cancer research. On the other hand, the investigations of the writer (10) have shown that an animal may be susceptible to a subcutaneous inoculation of a certain tumor and resist the inoculation of the same tumor into the testicle. Undoubtedly this method of inoculation will reveal the existence of a number of other phenomena.

The discovery of specific therapeutic measures is certainly the greatest problem in cancer research. A great deal of work has been done already on the subject, and the latest investigations of Wassermann on the chemotherapy of experimental tumors seem to be of great promise. But here also the therapeutic methods must be tried on animals in which the inoculations of tumor cells have been made into parenchymatous organs before the growths thus treated will have any analogy to human cancer.

In this connection one must bear in mind the fact that all the empirical so-called specific cancer remedies, which are continually being devised, are usually successful in treating localized skin cancers and fail utterly in the malignant growths of the internal organs. It is comparatively easy to produce a localized necrosis and softening in a circumscribed growth of the skin and subcutaneous tissue, but whether the same result will be produced on a diffuse and better nourished tumor growing inside of a parenchymatous organ cannot be decided *a priori*. To determine this it is necessary to have experimental proof on animals in which the tumor was inoculated into organs.

BIBLIOGRAPHY.

1. von Wassermann, A., von Hanseemann, D., Keysser, F., and Wassermann, M., *Berl. klin. Wchnschr.*, 1912, xlix, 222.
2. Jobling, J. W., *Monographs of The Rockefeller Institute for Medical Research*, 1910, No. 1, 81.
3. Haaland, M., *Scientific Reports of the Imperial Cancer Research Fund*, 1911, No. 4, 1.
4. Murray, J., *Scientific Reports of the Imperial Cancer Research Fund*, 1908, No. 3, 69.
5. Clunet, J., *Recherches expérimentales sur les tumeurs malignes*, Paris, 1910.
6. Levin, I., *Jour. Exper. Med.*, 1911, xiii, 604.
7. Flexner, S., and Jobling, J. W., *Monographs of The Rockefeller Institute for Medical Research*, 1910, No. 1, 1.
8. Citron, H., *Berl. klin. Wchnschr.*, 1912, xlix, 223.
9. Levin, I., and Sittenfield, M. J., *Jour. Exper. Med.*, 1911, xiv, 148.
10. Levin, I., *Jour. Exper. Med.*, 1912, xv, 163.

EXPLANATION OF PLATES.

PLATE 13.

FIG. 1. A white rat with the abdominal wall removed and one lobe of the liver turned over. The left lower lobe shows in the center an inoculated sarcoma.

FIG. 2. The top figure shows a spleen with a small sarcoma growth in the center. The second drawing shows a spleen, nearly all of which is replaced by a large tumor. At both ends can be noticed the remaining parts of the spleen.

FIG. 3. The center shows a diffuse growth of a carcinoma surrounded everywhere by normal spleen tissue. Low magnification.

PLATE 14.

FIG. 4. The top of the drawing shows the growth of a carcinoma, which invades the liver. High magnification.

FIG. 5. A diffuse growth of a carcinoma surrounding four kidney tubules. High magnification.

PLATE 15.

FIG. 6. To the left of the drawing is a metastatic sarcoma nodule in the lung. To the right is normal lung tissue. Low magnification.



FIG. 1.



FIG. 2.

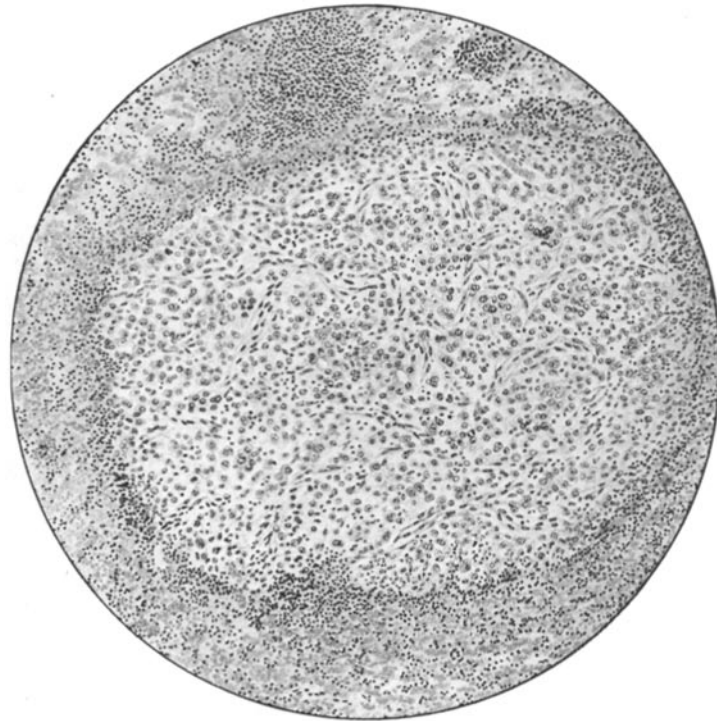


FIG. 3.

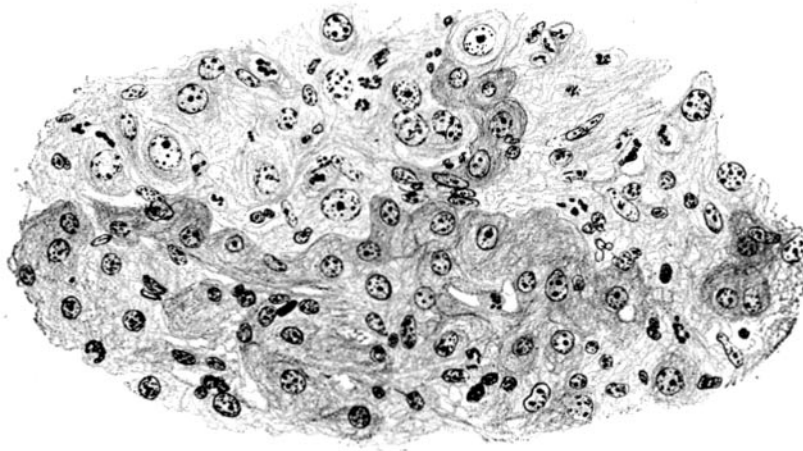


FIG. 4.

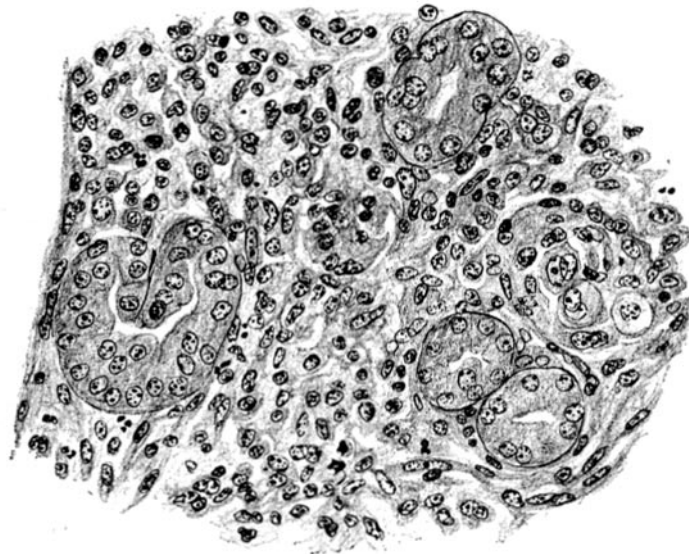


FIG. 5.

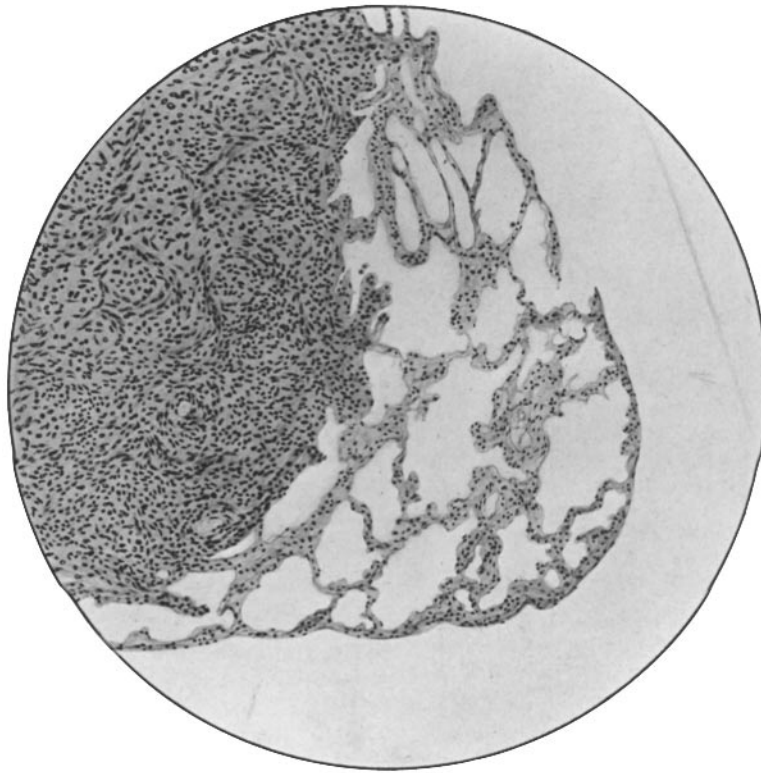


FIG. 6.