THE MECHANISMS OF METASTASIS FORMATION IN EXPERIMENTAL CANCER.*

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PLATES 25 AND 26.

The term metastasis was originally used to indicate the transportation of any substance, whether metal or coal dust, normal cells (placenta, bone marrow), bacteria, or cancer cells from one part of the organism to another. von Recklinghausen (1) was the first to restrict the meaning of the term and used it only in connection with the transportation of pathological substances. More recently the word metastasis has been used only in connection with the secondary tumor nodules which occur so frequently in malignant growths. This restricted use of the term was caused by the generally recognized fundamental difference which exists between a multiple granuloma of an infectious disease and a true metastatic growth of a malignant tumor. While in the former only the bacteria or the noxious agents are transported into the distant parts of the organism where the granuloma is formed from the local cells, a metastatic tumor growth originates in every case through the proliferation of a group of cancer cells detached and transported from the primary tumor.

The mechanism of the formation of metastasis in malignant growths consists of the following phases: (1) the detachment of a group of cancer cells from the primary tumor; (2) the transportation of this group of cells through the blood or lymph channels into distant parts of the organism; and (3) the proliferation of these cells in the new location and the consequent formation of metastatic tumors. It is well known that both the frequency of the

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occurrence of metastases and the localization of the metastatic growths varies widely in different malignant tumors.

Most pathologists (von Recklinghausen (2), Lubarsch (3), and Schmidt (4)) consider the difference in the channels of transportation to be the main reason for the difference in the frequency and localization of the metastatic tumors. The causes are purely mechanical, namely, the differences in the morphological structure of the organs, the relative size of the cancer cells, the capillaries of a certain organ, and so on.

More recently Albrecht (5) and Ehrlich (6) advanced the hypothesis that the success of the development of a metastatic tumor is due to the chemical metabolic conditions of the entire organism. Their idea is that the growth of the secondary tumor depends upon the character of the primary growth. If the latter is very malignant and reaches a large size, then it uses up all the specific food required by the multiplying cancer cells, and none of it remains for the cells of the secondary growth. On the other hand, when the primary tumor is growing slowly, a sufficient amount of the specific food is left for the development of the metastatic tumor. Recent investigations of the writer (7) indicate that neither the purely mechanical conceptions nor the athreptic theory of Ehrlich are adequate to explain all the phenomena of the formation of metastasis.

The investigations were conducted mainly on two inoculable tumors of the white rat,—a spindle cell sarcoma which was studied originally by Jensen, and an adenocarcinoma which was described by Flexner and Jobling (8). The sarcoma is very malignant, grows to a large size, and apparently, in accordance with Ehrlich's theory, does not form any metastases after a subcutaneous inoculation. When an emulsion of this tumor was injected intravenously into a healthy animal no tumor growth formed anywhere. In these experiments lack of specific food could not play any part since the injected animals had no primary tumor. On the other hand, when the same tumor was inoculated into a parenchymatous organ, the spleen or the liver, metastases formed in a certain number of cases.

The second tumor, the Flexner-Jobling adenocarcinoma of the white rat, induces the formation of metastases when inoculated subcutaneously. An intravenous injection of an emulsion of this tumor induced the formation of metastatic nodules in parenchymatous organs.

In the two series of experiments the conditions within the host, both as regards the mechanical structure of the organs as well as nutrition, were identical; all the animals were normal. The

methods of experimentation were also identical and the only difference consisted in the different character of the cancer cells. The sarcoma cells injected into the circulation did not develop into a metastatic growth, while the adenocarcinoma cells did. The formation of metastasis upon the inoculation of the sarcoma into the liver and spleen was apparently due to the change of character which the sarcoma cells underwent during their sojourn in the parenchymatous organs. Such changes in the character of the cells after a lodgment in a different organ may occur also in human pathology, as was shown by Hansemann (9) and others.

In the present investigation the writer undertook to study the influence of the organism of the host upon the development of secondary metastatic tumors, while the character of the cancer cells used for the experiments was not changed. The research was conducted with the same two tumors of the white rat described above.

SUBCUTANEOUS INOCULATION AND SUBSEQUENT INOCULATION INTO A PARENCHYMATOUS ORGAN.

In a previous investigation (10) it was shown, in agreement with other investigators, that it is possible to inoculate successfully under the skin a tumor in an animal bearing in a different region a previously inoculated subcutaneous tumor. In order to approach more nearly the conditions obtained during the formation of metastasis, inoculations were made into a parenchymatous organ of animals in which a subcutaneous inoculation was done previously. In a former investigation the writer (11) has shown that when an animal is resistant to a subcutaneous inoculation of a tumor it will also resist in every case a subsequent inoculation into an organ. In the present study an inoculation was done into parenchymatous organs of animals of a tumor which was previously inoculated into the same animal subcutaneously.

With regard to the method used in the experiments the following explanation should be made. A normal rat, as has been shown by the writer in a number of investigations, stands a laparotomy and inoculation of a tumor into an organ very well and there is practically no postoperative mortality. But tumor animals are a

great deal more vulnerable, and a number of them succumb to the operation. A description of the experiments follows.

Experiment 1.[1] *Sarcoma in the Liver.*—A laparotomy was done on eighteen rats which were previously inoculated subcutaneously with a rat sarcoma. A small piece of the same tumor was introduced by the aid of a trocar needle into the liver. Of the thirteen animals that survived the operation for two weeks, six developed a large subcutaneous tumor and also a fairly large sized tumor in the liver. Figure 1 shows one of these animals. Seven animals developed only a small nodule subcutaneously and none of them developed any growth in the liver.

Experiment 2. Carcinoma in the Liver.—An inoculation of the adenocarcinoma into the liver was done on twenty-six rats which were previously inoculated subcutaneously with the same tumor. Sixteen animals survived the operation and of these five developed a large subcutaneous tumor and also a fairly large tumor in the liver. Eleven animals developed a small subcutaneous nodule and showed no growths in the liver.

Experiment 3. Sarcoma in the Spleen.—A laparotomy was done on nine rats which were previously inoculated subcutaneously with a rat sarcoma. A small piece of the same tumor was introduced into the spleen by the aid of a trocar needle. Of the six animals that survived the operation for two weeks, three developed a large subcutaneous tumor and a fairly large tumor in the spleen Three animals developed a small nodule subcutaneously and showed no growth in the spleen.

Experiment 4. Carcinoma in the Spleen. The same operative method was employed as in experiment 3. The carcinoma was inoculated in the spleen of six rats in which a subcutaneous inoculation of the same tumor was previously done. The three rats that survived the operation for two weeks developed a large subcutaneous tumor and a fairly large tumor in the spleen.

The results of the series of experiments with subcutaneous inoculations and subsequent inoculations into a parenchymatous organ are significant inasmuch as they clearly indicate the influence of the organism of the host on the growth of metastases. The subsequent inoculation into an organ of a tumor-bearing animal represents an artificially induced formation of a metastasis. The results of the present as well as the former investigations of the writer show that an inoculation into an organ will not succeed in an animal into which a previously attempted subcutaneous inoculation failed entirely or in which there developed only a small subcutaneous nodule. On the other hand, in the animals in which the subcutaneous inoculation produced the formation of a large subcutane-

[1] All the experiments were done under ether anesthesia.

ous tumor, the subsequent inoculation into an organ was also invariably successful. It is a well established fact that the success or failure of growth of a primary tumor inoculated in a normal animal is due to the conditions of general susceptibility or resistance of the organism of the animal to the growth of the tumor. Apparently the same general phenomena influence the growth of the secondary inoculated tumor, or, in other words, influence the formation of the artificial metastases.

SUBCUTANEOUS INOCULATION AND SUBSEQUENT SIMULTANEOUS INOCULATION INTO TWO PARENCHYMATOUS ORGANS.

The formation of metastases is a complex phenomenon. Not only does the frequency and localization of metastatic growth differ in the various tumors, but even the same tumor may form metastases in different organs in different individuals. In order to imitate experimentally this phase in metastasis formation, a simultaneous inoculation was done into two parenchymatous organs.

Experiment 5. Sarcoma in the Liver and Spleen.—The same operative methods were employed as in the previous experiments. A laparotomy was performed on sixteen rats which received two weeks previously a subcutaneous inoculation of the sarcoma. A small piece of the tumor was introduced subcutaneously into the liver and the spleen. Of the fifteen animals that survived the operation for two weeks, six developed only a small subcutaneous nodule and in these there was found no growth either in the liver or in the spleen. Nine animals developed large subcutaneous tumors, and of these, three showed a growth only in the liver, and three showed growth only in the spleen.

The results of this series of experiments show that, on the whole, the results of the multiple inoculations also depend upon the general conditions of resistance or susceptibility of the organism of the host. When the tumor fails to grow it fails everywhere, and when it succeeds, it succeeds in every organ. On the other hand, in those instances where the tumor grew both subcutaneously and in one organ and failed to grow in another organ there must have been an additional factor; namely, while the organism of the animal as a whole was susceptible to the inoculation both subcutaneously and into one organ, the other organ, the spleen or the liver, resisted the growth of the tumor. The possibility of such a local organ resistance in an animal of which the general organism is

susceptible to the growth of the same tumor was proven by the writer (12) in a recent investigation.

The Flexner-Jobling carcinoma of the white rat grows readily when inoculated anywhere in the organism, but fails to grow when inoculated in a normal testicle. But when an inflammatory lesion is produced in the testicle previous to the inoculation of the tumor, then the tumor inoculation is successful. The mechanism apparently causes the failure of growth of a tumor in one organ, while the same tumor grows subcutaneously and also in another organ.

SURGICAL REMOVAL OF A SUBCUTANEOUS TUMOR AND SUBSEQUENT INOCULATION INTO PARENCHYMATOUS ORGANS.

Uhlenhuth, Händel, and Steffenhagen (13) have shown recently that when a subcutaneous tumor is removed surgically and the same tumor inoculated subcutaneously in a different place, the second inoculation will not succeed if the removal of the first tumor was radical; but when there is a recurrence after the removal of the first tumor, then the second inoculation also succeeds. These striking results were confirmed by Meidner (14), Händel and Schönburg (15), and Uhlenhuth, Dold, and Bindseil (16). Apolant (17) seems to have reached different results and claims that there is no relation between the recurrence of the first tumor and the success of the second inoculation.

The influence of the surgical removal of a primary tumor upon the growth of metastases is of great practical and theoretical importance. In view of this and also of the contradictory results of the work of the different investigators it seemed advisable to repeat and enlarge upon these experiments by removing surgically the subcutaneous tumors and subsequently inoculating the same tumor into one or two parenchymatous organs.

Experiment 6. Carcinoma Removal and Inoculation in the Liver.—In ten animals a longitudinal incision was made at the inner side of the subcutaneous tumor and the latter was enucleated *en masse.* Immediately following this step the peritoneum was opened and a small piece of the animal's own tumor was inoculated into the liver. Of the five animals that survived the operation the primary tumor recurred in three, and in these there was a positive growth in the liver; in two animals there was no subcutaneous recurrence and no growth in the liver.

Experiment 7. Sarcoma Removal and Inoculation into the Liver and Spleen.—The method of operation was identical to the one described in experiment 6. In eleven animals the subcutaneous tumor was radically removed and then the same tumor was inoculated simultaneously into the liver and spleen. Of the ten animals that survived the operation, in seven there was no recurrence of the subcutaneous tumor and in these the inoculation into the organs failed invariably. In three animals the subcutaneous tumor recurred and of these animals in one the growth was positive in both liver and spleen (figure 2), in one growth was found only in the liver, and in the last animal a growth was found in the spleen.

Experiment 8. Carcinoma Removal and Inoculation into the Liver and Spleen.—The method of operation was the same as in the previous series of experiments. In twenty-three animals the subcutaneous tumor was radically removed and then the same tumor was inoculated simultaneously into the liver and spleen. Seventeen animals survived the operation and in eight of these there was neither a recurrence of the subcutaneous tumor nor any growth in the organs. In three animals there took place a recurrence of the subcutaneous tumor and positive growth both in the liver and the spleen. In the last six animals there was a recurrence of the subcutaneous tumor and a positive growth only in the spleen.

This series of experiments in the first place corroborates the findings of Uhlenhuth and his collaborators, that a subsequent inoculation succeeds whenever there is a recurrence of the primary tumor, and fails when the removal of the primary tumor remains radical. The experiments show further, like the previous experiments of the present study, the influence of the general and local resistance or susceptibility of the organism of the host on the growth of the secondary metastatic tumors. They also emphasize the relation between the postoperative recurrence of the primary tumor and the formation of metastasis. When the organism possesses a certain amount of resistance it will neutralize the few cancer cells which must necessarily be left behind after the apparently most radical operation; there will not take place any local recurrence and for the same reason the secondary or metastatic tumor will fail to grow. While, on the other hand, when this resistance of the organism is lacking, notwithstanding the most painstaking operation both a local recurrence and the formation of metastasis will take place.

The greater frequency of the growth of carcinoma in the spleen than in the liver may be due to the greater vulnerability of the former organ. The spleen of animals inoculated with the car-

cinoma frequently shows the condition of an amyloid degeneration, and, as has been shown by the writer in experiments with the testicle, a diseased organ is more susceptible to growth of tumor than a normal one. The writer is at present engaged in the study of this question.

The analysis of the results of the present investigation shows clearly that the differences in the frequency of the occurrence and in the specific localization of metastases are not due to the ease of detachment of the cancer cells from the primary tumor. Nor does the difference in the mode of transportation play a part. The main factors in determining the localization and frequency of metastases are the character and malignancy of the cancer cell on the one hand, and the general and local susceptibility of the organism of the host on the other. The failure or success of the proliferation of a group of cancer cells transported from the primary tumor into a distant organ is a result of the interaction of these two causes.

The validity of this conclusion will be further enhanced when it is considered that the mechanism and cause of growth of the primary tumor and the metastasis are identical. In inoculable tumors this identity is certainly complete since even the primary tumor is caused by the proliferation of a group of cancer cells introduced from outside. But even in a spontaneous tumor of the same animal, or in human cancer, the difference between a primary tumor and a metastasis consists only in the fact that in the former one must consider the additional unknown factor, namely, the transformation of a normal cell into a cancerous one. Otherwise the further growth and development of the primary tumor, as well as the metastasis, depends upon the correlation of the two factors mentioned above.

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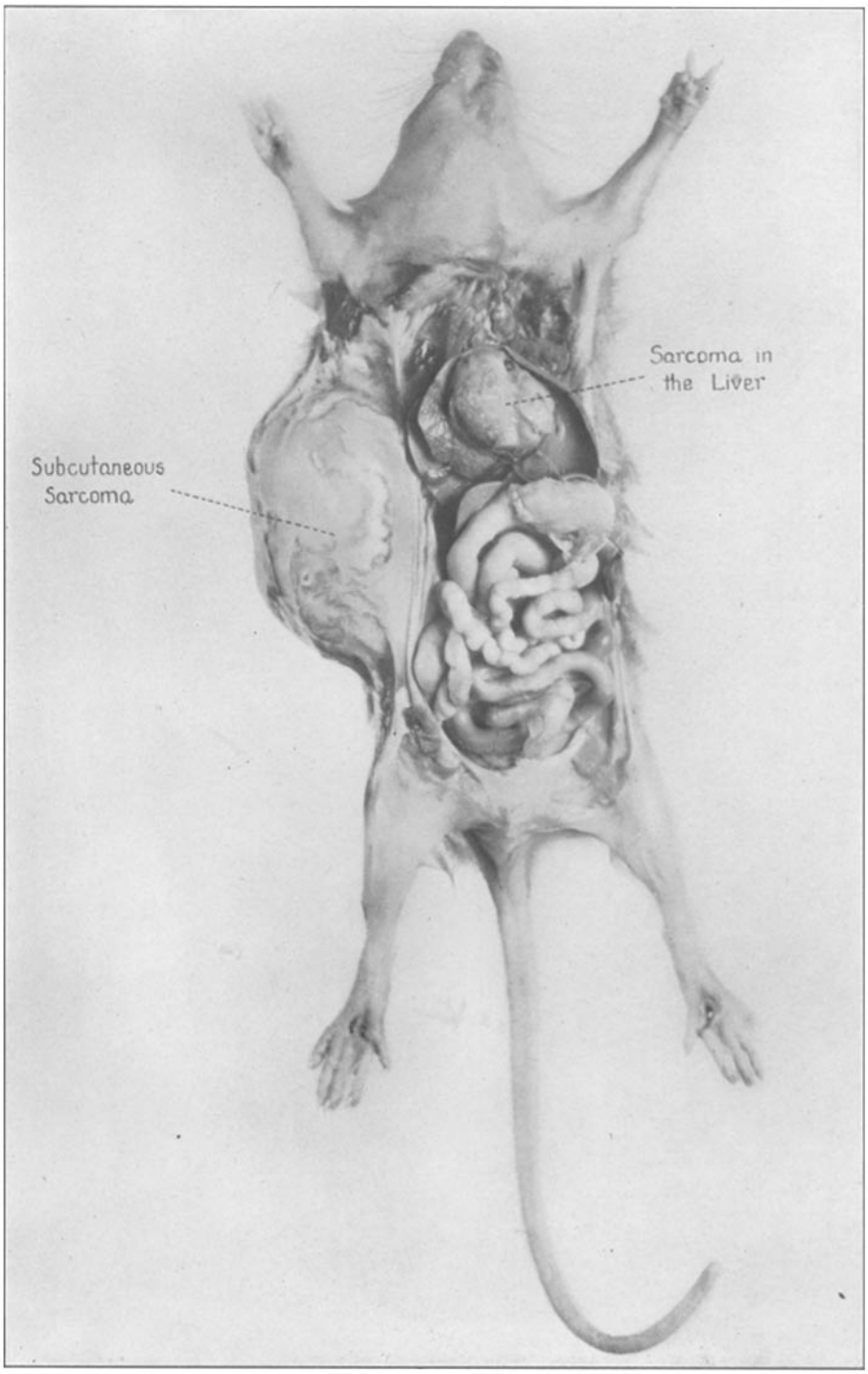

FIG. 1.

(Levin: Metastasis Formation in Experimental Cancer.)

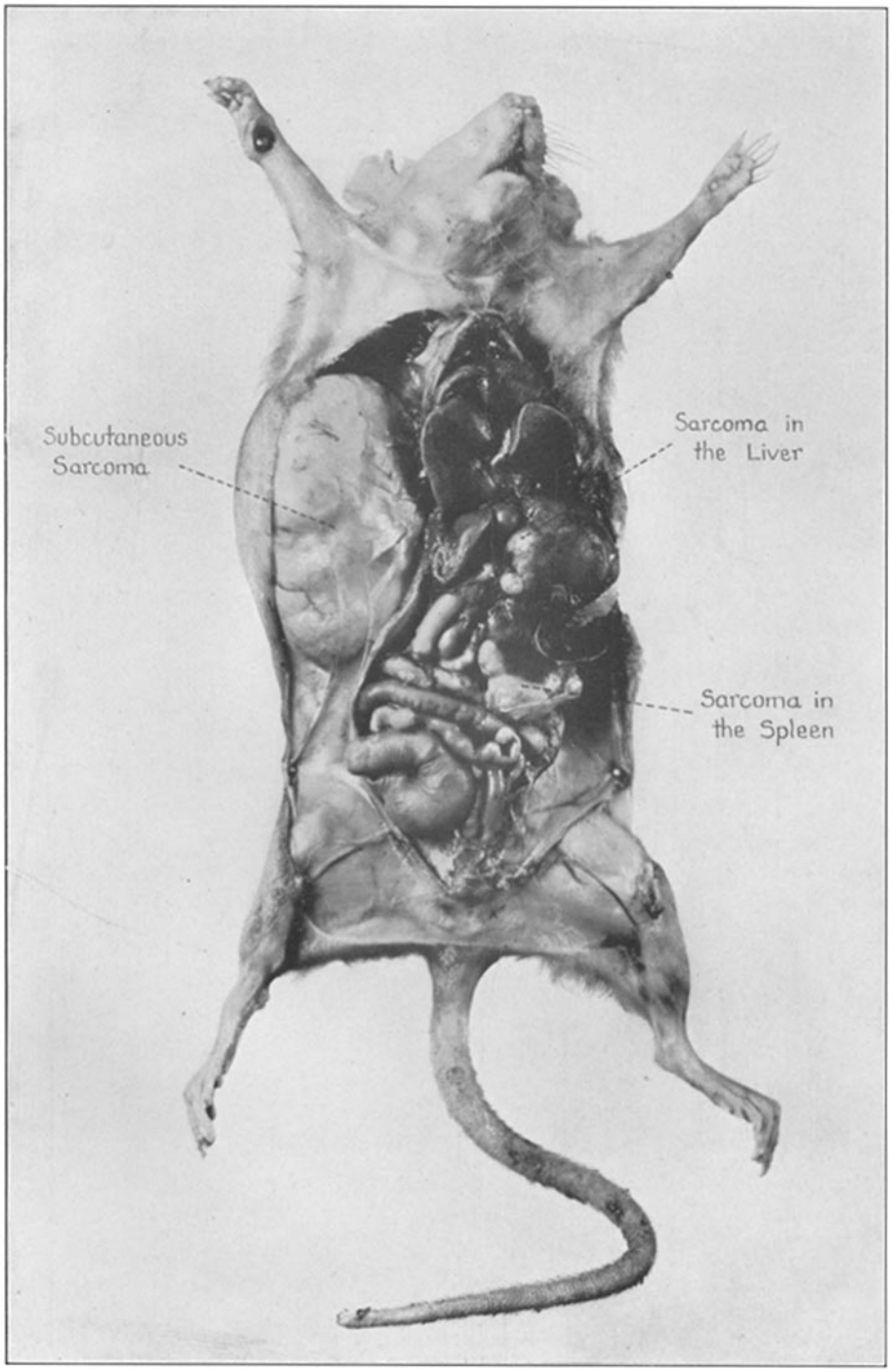

FIG. 2.

(Levin: Metastasis Formation in Experimental Cancer.)

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EXPLANATION OF PLATES.

Plate 25.

Fig. 1. Sarcoma of the white rat. A large subcutaneous tumor and a fairly large tumor in the liver.

Plate 26.

Fig. 2. Sarcoma of the white rat. A large subcutaneous tumor and a tumor growth in the liver and in the spleen.