ON TRANSPLANTATION OF TUMORS.[1]

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A few transplantations of a carcinoma of a cow and a carcinoma of a mouse were made into the same animals respectively, in which, if the pieces healed in, only connective tissue was found after a few weeks. More successful was a series of consecutive transplantations of a sarcoma found in the thyroid gland of a white rat. This work extended over a period of fifteen months, 360 pieces being transplanted into about 150 animals. The original tumor was a cystic sarcoma with rather small cells. The cysts were produced through a gelatinous softening of the tumor cells. The adjoining sarcoma cells arranged themselves in a regular way around the cyst, forming an almost epithelial-like lining. The tumor tissue was rich in blood-vessels, but the cells did not show any definite relation to the vessels. The original tumor was about the size of a nut. After extirpation it recurred and made, in the course of a few weeks, local metastases, little nodules near the primary tumor. No general metastases were found when the animal, a short time later, died during an operation.

From this tumor pieces were transplanted into the subcutaneous tissue or into the peritoneal cavity at each operation, about five to eight pieces being inserted into three to four other animals. At various periods after the operation, from six hours until more than three months, pieces which had been transplanted were taken out for microscopical examination. To avoid accidental findings usually more than one piece, often four or five, of the same period were investigated microscopically.

The main result of this part of my investigations is that the largest part of the transplanted piece became necrotic.

[1] An almost complete review of the literature on this subject is given by Sailer in the "Journal of the Medical Sciences," 1900.

After six hours the nuclei began at some places to become pycnotic; after fifteen to twenty hours the nuclei were more or less pycnotic, the cells began to shrink. But a number of the nuclei were still well preserved. Around the periphery of the tumor I found a small zone of vesicular nuclei with mitoses. They may have been derived partially from the surrounding connective tissue, but probably also the peripheral tumor cells contributed to them, for usually we did not find them where connective tissue was transplanted with the tumor.

This zone of vesicular nuclei could also be seen for several days, but it could not longer be distinguished from the surrounding growing connective tissue. From the fifth to the eighth day we see a large zone of growing connective tissue around the inserted piece. Sometimes we find cysts in this connective tissue. It is difficult to decide if these originate in the zone of growing connective tissue itself through liquefaction in a similar way as liquefaction takes place in the tumor, or if they are caused by the connective tissue penetrating into the necrotic parts of the tumor which at the same time become dissolved.

Inside of the growing connective tissue zone we find a necrotic area; inside of this a row of polynuclear leucocytes. The number of these varies, this variation probably being dependent upon the presence or absence of infection. Frequently we see cells with pycnotic nuclei in direct contact with the growing connective tissue, and usually a number of these cells are included in the growing zone. In the centre of the transplanted piece we find an area of shrunken cells with pycnotic nuclei. The endothelial cells of the blood-vessels are desquamated. Among these pycnotic nuclei a few are occasionally somewhat better preserved, and among these we usually find a few mitoses at all times up to twelve and fifteen days after operation.

The possibility must be admitted of these mitoses being situated in cells which recently immigrated through the necrotic area into the centre of the piece. Still, on the whole, these cells in mitotic division show the same character as the

surrounding cells and belong, therefore, perhaps to the transplanted cells themselves, especially as no cells can be found in the surrounding necrotic area. From the seventh to the seventeenth day, in the majority of cases between the tenth and fourteenth days, the connective tissue zone around the necrotic area becomes transformed into the tumor, or else the tumor cells lying in this connective tissue framework give rise to the tumor. To determine somewhat better, if possible, the line separating the transplanted piece from the growing surrounding tissue, in a series of experiments, the pieces of tumor were placed inside sacs of gauze and were transplanted within these sacs and there allowed to grow for various periods and then removed for examination. Those examined in the course of the first week showed the contents of the sac to be putrid. Nevertheless, the majority of the pieces left in the animals for a somewhat longer period grew, although some of the large tumors afterwards cut open showed a putrid nucleus inside.

After eight days we found around some places of the gauze outside and inside of it, connective tissue very rich in cells with many mitoses, this being probably the starting point of the tumor. At other places connective tissue penetrated into the gauze sac. After a fortnight we saw the transplanted piece surrounded by dense fibrous tissue, outside of this we found growing sarcomatous tissue which contained a few small cysts, then followed the section through the distended gauze sac. The fibres of the gauze were usually surrounded by somewhat denser fibrous tissue with giant cells. This denser fibrous tissue was at some places directly connected with the denser fibrous tissues forming the capsule around the whole newly growing tumor and through a few connective tissue septa also with the fibrous tissue surrounding the transplanted piece. At some places the tumor tissue itself surrounded the gauze and contained giant cells. Outside of the gauze followed the larger part of the newly formed tumor, also containing cysts, and around this the dense fibrous capsule. The tumor grew mainly on one side of the transplanted piece. The other side of this piece and

of the gauze was only secondarily surrounded by the tumor. The new growth originated at a few points, and later these nodules united. In a piece examined after five weeks, we found the gauze sac and the zone surrounding it filled by fibrous tissue; inside was also some necrotic tissue, the growing tumor being further removed.

When the tumor had started to grow, which usually occurred between the tenth and the fifteenth day, its further development usually followed a definite course. Up to the eighteenth or twentieth day it grew almost as a solid tumor, increasing rapidly in size, so that about sixteen to twenty days after the transplantation the tumor was perhaps fifteen to twenty times larger than the transplanted piece. Then the degenerative changes (mainly myxoid softening), which had already begun before that time, increase, especially in the centre of the tumor, and gradually transform it into a cyst. The peripheral parts continue to grow. Therefore the cyst continues to increase in size until it reaches almost the size of the whole animal. Usually before that time the skin ruptures and ulceration sets in, and the fluid of the cysts may coagulate under the influence of the infection. Still the peripheral parts of the tumor continue to grow, until the animal dies in a very anemic state, many hemorrhages usually taking place in the growing tumor.

A number of experiments were made to determine whether the tumor could be transmitted in any other way. It was impossible to transmit it by feeding rats with pieces of tumor. Six times cystic fluid of the tumor was injected into the peritoneal cavity of rats. The amount of the injected fluid varied from one to ten cubic centimetres. In three cases abscesses formed, the injected fluid had been infected, but in three other cases multiple tumors developed in the peritoneal cavity, which after perhaps eight weeks had (some of them at least) developed into large sarcomatous masses.[1] These tumors grew repeatedly through the diaphragm. Microscopic examination showed that their character was the

[1] In the last days I again had, sixteen days after injection of about half a cc. of cystic fluid, a positive result.

same as that of the original tumor; they were cystic sarcomata.

In another set of experiments threads of silk or cotton, or pieces of agar, were introduced into the tumor with the view of determining if the sarcomatous cells behaved towards these foreign bodies like ordinary connective tissue cells. After eleven days we saw cells which were in direct connection with the tumor cells penetrate into the agar and form epithelioid and giant cells. The connective tissue which was further removed from this part of the tumor also adjoined at some places the agar and penetrated into it, and the cells emanating from this source were very much like the cells immigrating into the agar directly from the tumor. The two facts, (1) that the tumor cells were at many places so much nearer the agar than the connective tissue, and (2) that the invading cells were a direct continuation of the tumor cells, make it probable, although not yet certain, that the sarcoma cells entered the agar and formed epithelioid and giant cells. Around threads frequently found in the centre or in the periphery of the tumor, giant cells were invariably found; sometimes the fibrous tissue was relatively somewhat increased in the neighborhood of the threads, in other cases there was no increase of fibres between the cells around the foreign bodies.

During the operation a piece exposed to the air, or coming in contact with the skin during the process of transplantation, frequently becomes infected with bacteria. Another piece may have contained some microorganisms before the transplantation. Many observations have proven, that such an infected piece can, after transplantation, cause a tumor formation. The infected part may be separated by connective tissue from the healthy growing tumor and break as a putrid or suppurating mass through the skin, and the tumor may then be absolutely freed from the infected part; the wound where the perforation took place may afterwards heal. In other cases a communication between the growing tumor and the infected piece remains. In the first stages of growth, the healthy growing tumor is very resistant towards

the action of bacteria; but as soon as, in the normal course of development, the degenerative processes increase, the infection progresses, until only the periphery of the tumor remains growing. In the case of the occurrence of ulceration or infection of a tumor some interesting changes can be observed. A normal tumor frequently, although not invariably, grows as one large mass. An infected piece, if the infection took place early, frequently takes on a lobulated character, probably through increased activity of the surrounding connective tissue, or through an unequal growth of the tumor or cells under the influence of the microorganisms. Not only a division into larger lobules takes place, but quite frequently, near the place of ulceration, a separation of multiple small nodules from the main tumor occurs through the surrounding connective tissue. If the separation is complete (and that is frequently the case), we have at the side of the infected tumor healthy local metastases formed, which in some cases may in their further growth again unite with the main tumor and so also become infected, but frequently grow independently. These formations of local metastases were so frequent under the influence of ulceration and infection of a part of the main tumor, that a mere coincidence can be excluded. It might be suggested that in other tumors also ulcerations may be a cause for the separation of small tumor nests in the surrounding connective tissue, and therefore, indirectly, of a recurrence of the tumor after operation.

Sometimes transplanted pieces began to grow somewhat later than usually. I noticed this especially in infected pieces. They began to grow perhaps between the eighteenth and the twentieth day. In a few cases tumors did not begin to grow until many weeks after transplantation or after a large piece had been taken out of a formerly transplanted piece during an operation. Such a growth was then found quite unexpectedly. There were some pieces which did not grow at all, although no ulceration was present, and there were other pieces which did not continue to grow after growth had started.

Some experiments were made to determine, if possible, the

cause of the failure to grow. In one case a tumor in the back of a female rat had ceased to grow for a long period. Half of the tumor was cut out and transplanted into the other side of the back of the same animal. In the usual time (after ten to fifteen days) both pieces began to grow at a rapid rate, and it would have been possible to transplant from them many tumors. In this case the opening of the original tumor, probably its liberation from the tension of the surrounding connective tissue, sufficed to make the tumor grow. Discontinuation of growth, therefore, need not mean that the cells lose their power to multiply. A similar result was obtained in another case. Here the original tumor had not only ceased to grow for a considerable time, but even had diminished in size. One piece was taken out and transplanted into the peritoneal cavity of another rat. After fifteen days this piece had markedly increased in size, and afterwards a number of tumors were successfully transplanted from it. But the original tumor began, nevertheless, to decrease rapidly in size, and soon only a few very small nodules were left.

In two other cases accidental circumstances (suppuration in one case) prevented the growth of the transplanted piece. In a fifth experiment only a small piece was taken out from a cyst, which decreased in size. The remaining piece closed again so that a cyst was found, but did not resume its growth. If pieces from such tumors, which had ceased to grow, were examined microscopically, the cells were found to be smaller than usual, but the number of mitoses was not markedly decreased. Perhaps the pressure of the surrounding connective tissue was the cause of this decrease in size.

The structure of the tumor in these cases was more or less preserved; but in the last mentioned case, of the small cyst which continued to decrease in size, no more mitoses could be found.

This long series of transplantations offered an opportunity to approach the study of the causes which determine the structure of this tumor. Only a few facts can be mentioned here. Although the tumor cells were not derived from the

blood-vessels, they frequently after some time took a definite position towards the blood-vessels, being either concentrically arranged or showing a regular radiating arrangement around the capillary blood-vessels as a centre.

From the first to the last piece examined, the character of the tumor was preserved during these fifteen months, even after so many pieces had been successfully transplanted. The same cysts were formed, with the same characteristic arrangement of the connective tissue cells. Still a number of variations took place. For instance, twice the tumor cells took on quite a different character. They looked like large endothelial cells, sometimes with more than one nucleus. In one of these two cases this tumor, after transplantation, yielded again the old typical tumor (which under certain conditions appeared as an ordinary spindle-celled sarcoma); in the second case transitional stages between these cells and the ordinary tumor cells could be seen.

The peripheral tumor cells which penetrated into the neighboring tissue were usually spindle-cells, with well-developed fibres in connection with the cells. At the margin of the tumor we found in most cases more or less round cell infiltration. The unstriated muscle of the intestines was usually hypertrophic near a growing tumor. Occasionally multiplication of the nuclei of striated muscles could be seen between the advancing tumor, but degenerative changes in the neighborhood were the usual result.

Just as the morphological character of the tumor was on the whole preserved, so was its physiological character. Neither the original tumor nor any of the transplanted pieces made general metastases, neither through the blood nor through the lymph vessels, though I saw in one case the tumor cells penetrate into a blood-vessel. Many lymph glands near the tumor were examined. They were frequently hypertrophied, showed quite commonly endothelial cells inside the lymph sinuses, but never any tumor was found to develop. Still the tumor grew into the pleural cavity several times through the diaphragm. The rate of the growth of the tumor remained throughout about the same.

Local metastases were of quite frequent occurrence. We even found them in the original tumor after an operation. We found them frequently around the transplanted pieces. They formed in two ways. The one way was by little nodules growing out of the main tumor, the neighboring connective tissue contributing to this formation of nodules by separating these little particles of tumor from the main mass of tumor cells. New nodules frequently pushed the first-formed one still further out, and the connective tissue in a short time separated such a nodule entirely from its base; it thus looked as if such a nodule had originated independently of the tumor. It has been mentioned before that such a formation of secondary nodules can be found where ulceration of the main tumor takes place. Local metastases may from the beginning be independent nodules. We noticed this especially after a piece was cut off from a tumor, or even a short time after a piece had been inserted in the subcutaneous tissue. These independent nodules are in the latter case found under the skin, near the opening of the skin, which the tumor touched during the process of insertion into the subcutaneous tissue. We never found it in the opposite direction, following the lymphatics.

We found local metastases after implantation of a piece into the peritoneal cavity, in the subcutaneous and muscular tissue, at the place of incision. In a similar way, we found such *contact metastases* where a large tumor inside the peritoneal cavity touched the muscles at the side of the spinal column. Here little independent nodules began to grow.

In one case as early as seven days and seventeen hours after the contact in implanting a tumor had taken place, distinct nodules were growing.

Old and young, female and male rats were used in these experiments. It was possible to get positive results from all kinds. It may, nevertheless, be the case that a certain difference exists between different varieties of rats, that it might be more difficult to make tumors grow in certain rats than in others. This will be determined in further experiments.

There has not so far been any marked decrease in the viru-

lence of the transplanted pieces. By virulence is understood the rate of growth of the tumor.

Many experiments were made to determine if pieces could not be transplanted into guinea-pigs. All these experiments were unsuccessful. Microscopically, the transplanted pieces behaved not very differently from pieces transplanted into rats. The nuclei became pycnotic. Mitoses could be seen inside this pycnotic area. The immigration of leucocytes was perhaps more pronounced. (But this is a variable factor, depending perhaps in its degree on the presence or absence of infection.) Equally unsuccessful were transplantations into white mice and into hens. Two transplantations into a hybrid between a white and a wild gray rat were successful.

Experiments were begun on the action of chemical substances on the growth of the tumor. Iodide of potash injected at different periods into the peritoneal cavity, or a number of injections made into the tumor itself, did not produce a decrease in the size of the tumor cells or in the number of mitoses.

There are certain questions which cannot yet be definitely decided by these experiments. Although the fact that the morphological and physiological character of the tumors, even of those produced by injection of cystic fluid, on the whole remained unchanged, favors the view that in every case tumor cells were transplanted and started the new growth, still we must take into consideration the fact that the tubercle bacillus, *e.g.*, always changes connective tissue cells into epithelioid and into giant cells.

The view that in every case the original tumor cells were transplanted is also supported by the fact that all transplantations into other animals were unsuccessful. But neither does this fact absolutely disprove the possibility of a transplantation of micro-organisms as a cause of the tumor growth. If it is at least probable that the same cells were always transplanted, and if we consider that in the cases in which cystic fluid was injected one cell would be sufficient to start a new growth, the idea suggests itself that not only the germ cells can be immortal, but that perhaps also the so-called somatic

cells, like connective tissue cells, might under certain conditions live for a long period, much longer than the individual life of the organism of which they were a part, that they might perhaps also be immortal in the same sense as the ovum is.

[I wish to express my thanks to Dr. M. Herzog, to whom I am indebted for the rat with the sarcoma of the thyroid; to Dr. McCoy, who sent me a white mouse with a carcinoma; and to Dr. J. W. Walker and to Dr. W. L. Wilson, who kindly assisted me in a number of my experiments.]

PLATE II.

FIG. I. — Growing tumor cut in two parts. Piece transplanted February 6, subcutaneously. Growing tumor as it appeared February 23; a and a_1, indicate the size and location of the transplanted piece. It was necrotic and surrounded by a fibrous tissue capsule b, from which fibrous bands pass into the growing part. Small cysts visible.

FIG. II. — One small piece transplanted into the subcutaneous tissue of each side, February 1. Tumor with secondary nodules as they appeared February 18.

FIG. III. — Tumors, developed in the peritoneal cavity after injection of cystic fluid about two months previously; a, sarcomatous mass in the left hypochondrium passing beneath the liver e; b, tumor connected with the omentum; c, stomach; d, spleen.

PLATE II*a*.

FIG. IV. — Section through original tumor of thyroid gland; aa, cysts; bb, blood-vessels; c, hydropic cells; d, blood in a cyst. Zeiss oc. 4. Obj. A.

FIG. V. — Piece of a tumor passing through the diaphragm. This tumor had formed within 6 weeks after injection of cystic fluid in the peritoneal cavity. Animal died Nov. 7, 1900; aa_1, cysts; bb_1, blood-vessels; c, extravasated blood; dd_1, muscle fibres of the diaphragm separated by the growing tumor. Zeiss oc 4. Obj. A.

PLATE II*b*.

FIG. VI. — Growing tumor, 14 days after subcutaneous transplantation. The piece was included in a gauze sac at the time of transplantation; a, the transplanted piece, which had become necrotic; b, leucocytic infiltration, c, connective tissue capsule from which septa d, and d_1, pass into the tumor; e, section through gauze sac; f, and f_1, growing tumor with cysts.

[The drawings from which these figures were produced were very faint. — EDITOR.]

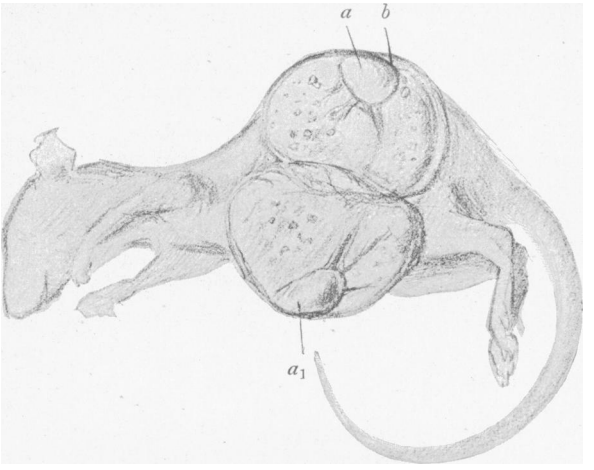

FIG. 1.

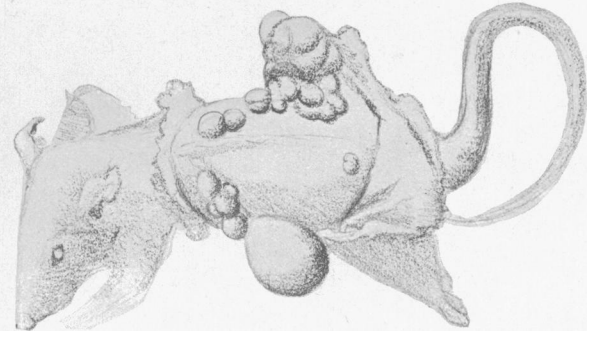

FIG. 2.

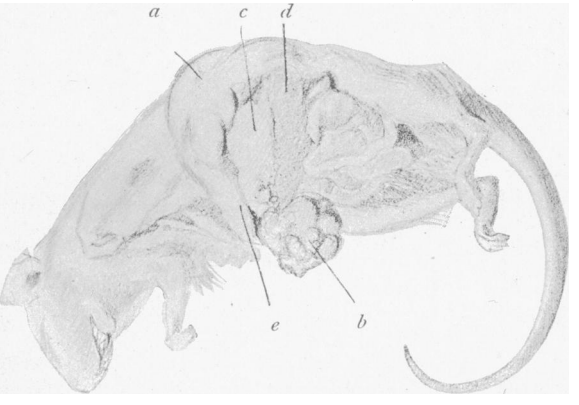

FIG. 3.

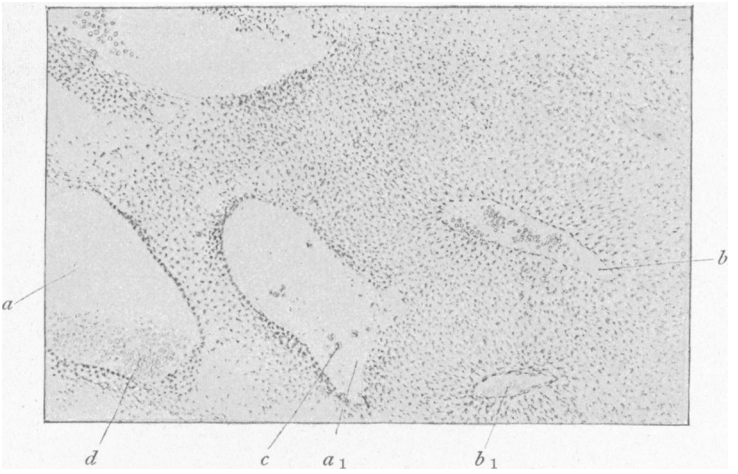

FIG. 4.

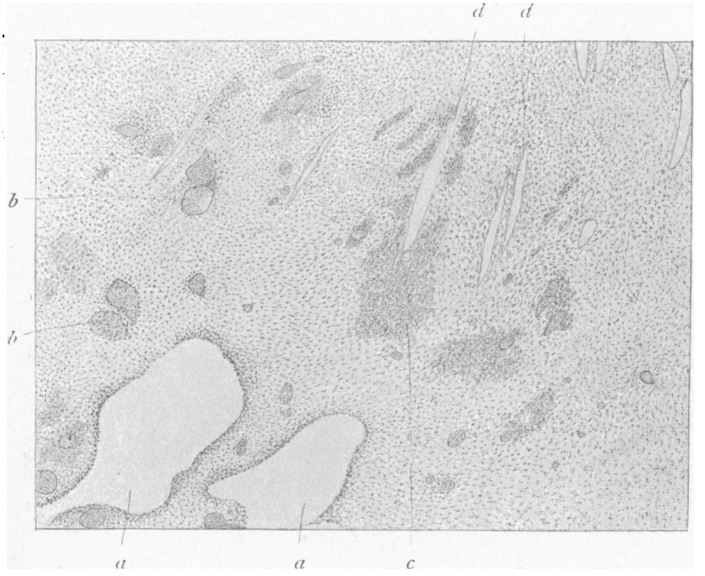

FIG. 5.

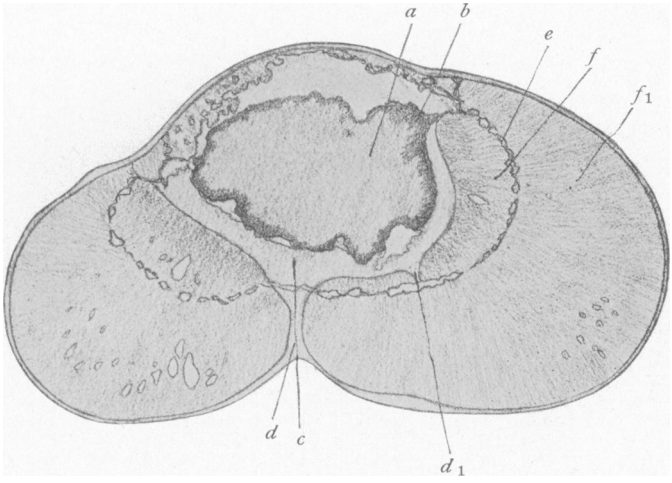

FIG. 6.