TRANSPLANTATION OF BENIGN TUMORS

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While the conditions under which carcinoma and sarcoma can be transplanted are on the whole fairly well known, thanks to a large number of contributions published in the last sixteen years, our knowledge concerning the growth and transmissibility of non-malignant tumors is as yet very restricted. An answer to the following problems would be of great theoretical interest. How far do benign tumors differ from cancer in conditions of experimental growth? How does their growth compare with that of normal tissues? Is it easier to bring about by experimental means the transformation of a benign into a malignant tumor than to effect a similar transformation of normal tissues? These questions are to a great extent still unanswered, owing to the limited number of investigations concerned with these problems. The present paper is a contribution to this field of study. It is the direct sequel of two papers on the growth of benign tumors previously published by one of the authors. At the outset it may be stated that while the results thus far obtained are of interest, much additional work will be required for the definite solution of these problems.

The first recorded experimental investigation into the growth of a non-malignant tumor was published by one of us in 1902, and was concerned with an adenofibroma of the mammary gland of a rat (1). After autotransplantation, the graft remained alive in toto but grew only during pregnancy; after homoiotransplantation, only very small peripheral areas remained alive for a time, and showed transitory regenerative growth.
The second publication (2) concerned an adenochondromyxofibroma of the mammary gland of a dog. Here again after autotransplantation the tissue remained alive almost completely, while it perished after homoiotransplantation.

H. Ribbert's experiment (3), which followed in 1910, confirmed our result. This investigator was able to transplant a fibroma of the dog into the same animal in which it had originated, but not into another dog. It began to grow in the first dog after a relatively long period of latency.

In this connection it will be of interest to refer to the behavior of normal tissues after serial regeneration. One of us found that it is possible to transplant serially, through a number of generations, normal epithelium of the guinea pig. Under these conditions an increase in the proliferative power of the epithelium is not observed, and after the typical period of regenerative growth the epithelium returns to its old equilibrium (4). C. V. Craster (5) subsequently reported similar experiments in which the skin of the rat was transplanted serially. No increase in virulence was observed. This author observed living cells as late as sixteen days after the first transplantation; he found no proliferation of the transplanted tissues. It is of interest to note that while Loeb in the main employed autotransplantation, in Craster's experiments the skin was serially transplanted into other individuals.

**FIRST TUMOR**

(See chart)

A rat was received from Dr. J. W. Jobling, of Chicago, April 20, 1911. The animal had a tumor on the left side of the posterior part of the abdominal wall. The tumor was in the region of the posterior mammary gland.

On April 22, 1911, a piece of the tumor was removed and pieces about the size of half a pea were inoculated with a trocar into the right axilla of fifteen rats of different ages, females and males. On May 2, the inoculated pieces could be felt; eight days later (eighteen days after inoculation), they had become smaller or had disappeared. Four weeks after inoculation none
Chart representing transplantation of the first tumor into four generations

I. Generation

CI

5-18-11
CIA, o o o o (14 days)

6-1-11
CIB, o o +

4-22-11
C1, o o o o o o o o o o o +

6-10-11
CIP, o +

II. Generation

11-24-11
CIB, o o o o o +?

III. Generation

3-25-12
o + (+ ?)

IV. Generation

7-16-12
o o o o
of the inoculated pieces was growing. However, between this latter time and the following August the inoculated piece in one of the rats began to enlarge and had reached a considerable size by August 17 (three and one half months after inoculation), on which date the animal was found dead. The tumor measured approximately 43 by 20 mm.

The tumor of the dead rat was removed and inoculated into nine rats on August 17, 1911. On September 6 (twenty days after inoculation) the transplanted pieces could still be felt, but on October 10, they had disappeared. On November 7, seven of the rats were still alive and on February 5, 1912, three. In none of them did a tumor develop.

On April 22, 1911, at the time of the first inoculation, a piece of the tumor was also transplanted subcutaneously into the rat in which the tumor had originated. This piece did not grow during a period of observation lasting thirty-nine days.

On May 18, 1911, another piece was removed from the original tumor and inoculated into four rats. One of these rats died nine days later; the other three fragments were removed fourteen days after inoculation. At this time the transplanted grafts had not noticeably changed in size. (Pieces A₁, A₂, A₃.) At the time of this last inoculation a piece of tumor was transplanted into the original tumor rat also. This likewise was removed two weeks later. It had not grown at the time of removal.

On June 1, 1911, a third piece of the original tumor was removed and was inoculated into two female rats and one male rat. Three weeks after inoculation, the grafts could no longer be felt through the skin, but on August 17 (two and one-half months after inoculation) the pieces in the two female rats were found to have grown to considerable size. At that time (August 17) one of these rats gave birth to a litter of four young. In the male rat no tumor developed during the period of observation, which lasted until October 10, four months and nine days after transplantation.

The first of the two tumors (B) which developed in the female rats, continued to grow from August 17, when it measured 27
by 27 mm., to September 6, a period of twenty days, at the end of which time it measured approximately 33 by 30 mm. On the latter date, a piece of this tumor was removed and was used for inoculation into a second generation of rats (eight of which were males and two females). A piece which had apparently not grown, was removed from a rat used in the last inoculation; this rat died five weeks after operation (October 9, 1911). The majority of the inoculated rats were still under observation two months after inoculation (November 7). On that date no tumor had developed.

The tumor which had grown in the second female rat of the last lot of the first generation (B3), continued to grow definitely during the period from August 17 to September 6. On the latter date it measured approximately 25 by 25 mm. From then on, until October 10, no further increase in size was noticeable. On October 10, a part of the tumor was used to inoculate twelve rats. Two rats among this lot apparently showed a slight growth twenty days after inoculation, but they died soon afterwards. In one of the surviving rats (C1), a growing nodule could be felt (January 18, 1912) more than three months after the inoculation; it continued to grow definitely until March 5, 1912. On that date a part of the tumor was removed and used for the inoculation of thirteen rats of the third generation; at the same time a piece was transplanted into the same rat (C1). This rat (C1) was observed until the death of the animal on July 10, 1912. On that date, neither the remaining part of the tumor nor the autotransplanted piece had grown. On February 6 (almost four months after inoculation), in another rat of this lot, a nodule appeared which measured approximately 11 by 8 mm. It grew slowly until March 15, at which time it measured 18 by 16 mm. The last observation was made on May 8. Among the fifteen rats of the third generation inoculated with tumor of rat C1 on March 25, 1912, a few small nodules could be felt and were extirpated three months later (middle of June, 1912). Another graft, which had slightly enlarged, was taken out on October 4, 1912 (almost six and one-half months after inoculation), and the final nodule was removed.
from the last rat of this lot on November 6, 1912 (seven and one-half months after inoculation). This latter piece had grown slightly and measured 8 by 4 mm.

On July 16, some pieces which could be felt were removed from five rats of the third generation and inoculated into four female rats of the fourth generation. One of these died six weeks later without tumor; of the remaining three animals, one had young in the latter part of August, 1912. It showed a very small nodule on October 4, more than two and one-half months after inoculation. At that date the animal was killed and the nodule extirpated. On November 6, 1912, one of the other rats also showed a very small nodule, which may have been the remnant of the inoculated piece. The fourth rat, which had young ones early in September, 1912, had no tumor at the time of the last examination, November 6, 1912.

The tumor B₃ of the first generation, from which a large piece had been removed on October 10, 1911, enlarged again and was inoculated into three rats on November 24, 1911, six weeks after it had been used for the first implantation; of these three rats of the second generation, one died soon after inoculation. Rat B₃ died on January 28, 1912. At the time of death only a small nodule was present. Another of the rats of the second generation died without a tumor on March 12, 1912, three and one-half months after inoculation. The third rat died with small nodules August 17, 1912, about eight and one-half to nine months after inoculation.

On June 10, 1911, the last piece was removed from the original tumor rat and inoculated into a pregnant rat and into another female rat which was apparently not pregnant. On June 18, eight days after inoculation, the pregnant rat littered. On the following day (June 19), the inoculated piece, which had begun to grow, probably under the influence of the pregnancy, and which measured 10 by 5 mm., was removed and inoculated into two female rats. No tumor growth resulted from this inoculation; two months later the inoculated pieces could no longer be felt.
MICROSCOPICAL EXAMINATION

a. Original tumor

1. Piece removed May 18, 1911 (no. 3). There are many mitoses in the epithelium, and some also in the connective tissue; but they are more frequent in the epithelium than in the connective tissue. The acini and ducts are surrounded by dense fibrous tissue with fairly numerous fibroblasts containing vesicular nuclei. In some places the acini are somewhat irregularly arranged, and there is a great deal of gland tissue which is rather diffusely distributed without the formation of definite lobules. Some of the ducts are somewhat dilated.

2. Piece removed June 1, 1911 (no. 5), used for inoculation. There are no mitoses visible either in the epithelium or the connective tissue; the lobules filled equally throughout with acini. The latter are surrounded by a dense fibrous connective tissue. These lobules are separated from each other by collections of connective tissue cells which do not contain epithelial structures. Ducts with small lumina are numerous. There is no irregularity in growth, and no dilatation of the ducts. Inoculation with these pieces led to the development of tumors in several of the rats, although no mitoses were present in the piece examined microscopically.

b. Piece autotransplanted into original tumor rat

This remained fourteen days in the original tumor rat—May 18 to June 1, 1911. The central parts are hyaline and necrotic; the peripheral parts contain cellular connective tissue. Toward the center, connective tissue cells as well as epithelium decrease around the ducts, the connective tissue becomes quite hyaline and compresses the ducts. In some areas the epithelium is lost and the connective tissue almost lost. There are probably a few mitoses in the connective tissue, but this is not certain. In this piece, the ducts are regularly branching and are very numerous.

A₂ (no. 1) inoculated May 18, 1911, and removed fourteen
days later. In some places the connective tissue is very rich in fibroblasts, at others it is clear and hyaline. The tumor consists of lobules, in the centers of which there is a system of glandular ducts surrounded by connective tissue. Where the connective tissue is very hyaline, the ducts are often dilated. There are a number of mitoses, and some amitotic divisions in the cellular connective tissue and also in the ducts, but no mitotic figures in the neighboring glandular epithelium.

A₃ (no. 6), fourteen days after transplantation. In the peripheral areas the connective tissue is well preserved; nearer the center, there is some hyaline tissue with a few connective tissue cells and gland ducts diminishing in number. Arrangement in lobules which are somewhat irregularly dilated, and surrounded by connective tissue which tends to become hyaline. The interstices between the lobules are filled by cellular connective tissue strands, which may be invasions from the host's tissue. In the peripheral parts an unusually large number of mitoses is found in the epithelium and in the connective tissue. In the latter the number is even larger than in the epithelium. The interstices contain, also, collections of small round cells, especially where the neighboring tissue becomes necrotic. They penetrate from here into the adjacent glandular structure.

A₁, May 18 to June 1, fourteen days after transplantation. Nodules of hyaline connective tissue some of which contain ducts. There is much round cell infiltration. The ducts are rather shrunken; on the whole there is very little epithelium left, and some of the epithelial cells are desquamated. There were pneumonic areas in the lung.

Piece (no. 4) transplanted into a pregnant rat June 1, 1911, removed on June 19, one day after delivery. The periphery is alive, but toward the center the graft is necrotic. Numerous mitoses occur in the epithelium; there are some mitotic figures in the connective tissue also, but here they are less numerous. The acini are surrounded by dense hyaline connective tissue containing some vesicular nuclei. Some of the ducts are dilated and irregular.

Piece inoculated April 22, 1911, removed May 10, 1911,
(eighteen days). In the center there is dense hyaline connective tissue; at the periphery, glands surrounded by cellular connective tissue. The tissue is shrunken, without any sign of proliferation.

B₁ (no. 11), June 1, 1911 to October 10, 1911. Examined four months and nine days after transplantation. The graft had been of considerable size two and one-half months after inoculation. It is composed of mammary acini and ducts, concentrically surrounded by dense hyaline connective tissue which is not very rich in cells. Some of the gland cells are vacuolated, and a few small mononuclear cells are occasionally found in the ducts. No mitoses are visible; there is some colloid in the acini. At some places the connective tissue is almost, or quite necrotic. In certain areas the structure is fairly typical, while others contain only isolated ducts without the typical ramifications.

B₂ (no. 12). The same tumor, after it had been used for two transplantations and only a small nodule had been left. Piece taken out after the death of the animal, June 1, 1911 to January 28, 1912 (seven months, twenty-one days). This fragment has the same structure as the first; the ducts and acini are surrounded by dense fibrous connective tissue. There are some fibroblasts in the connective tissue. Apparently no growth had taken place.

**Pieces of the second generation**

1. No. 13, rat inoculated with piece of tumor B₁ September 6, 1911, died October 9, 1911 (thirty-three days after inoculation). The specimen consists of hyaline connective tissue containing fibroblasts and a few blood-vessels, but no epithelial elements. No growth had taken place.

2. No. 14, a male mouse, October 10, 1911 to July 10, 1912, was found dead after nine months. The tumor had grown very slowly. Postmortem changes are present; the nuclei are shrunken, and the tumor consists of necrotic material and hyaline connective tissue containing some glandular structures. Concretions are to be found in some of the ducts.

3. No. 15, autotransplanted into previous rat March 25.
On July 10, 1912, the animal was found dead, eight months and sixteen days after inoculation. This piece, which corresponds to the third generation, consists mostly of necrotic dense fibrous tissue without nuclei. Postmortem changes had taken place. In one area the fibrous tissue contains well preserved acini, surrounded by fibroblasts; here the connective tissue is more cellular.

4. No. 16, October 10, 1911 to August 20, 1912 (ten months, ten days). A very small nodule, mostly necrotic; some of the lobules contain shrunken desquamated epithelium (possibly due to postmortem changes). The ducts and acini, where present, are surrounded by connective tissue containing nuclei, and in some of them concretions are to be found. Where the epithelium is lacking, the connective tissue is often necrotic, and the greater part of the tumor is entirely devoid of cells. In one epithelial cell there appears to be a mitotic figure. Some dilated ducts are present.

5. No. 17, November 24, 1911 to August 17, 1912 (eight months, twenty-four days). The animal was found dead. There are vestiges of acini in the dense hyaline connective tissue, but the fragment was necrotic, apparently as a result of postmortem changes.

Pieces of the third generation.

1. No. 21, March 25, 1912 to November 6, 1912 (seven months, eleven days). The small tumor, removed during the life of the animal, contains hyaline connective tissue arranged concentrically around ducts or acini often devoid of their epithelium. In some areas there are no nuclei in the connective tissue. Preservation of the connective tissue and the epithelium has run a parallel course. In some places all the ducts and acini in a lobule are preserved. Some of the acini are dilated, and at such places more fibroblasts are present in the connective tissue. The epithelium consists of relatively large cuboidal cells with nuclei rich in chromatin, a number of which are in mitosis. Where the epithelium is undergoing mitosis, the connective tissue is not so densely hyaline, but rarified, and connective
tissue cells are present in larger numbers; a little further away from such areas, however, the connective tissue surrounding the ducts is again densely hyaline. In the large ducts and also in the acini there is some colloid material. Around isolated ducts, the connective tissue is dense and contains fewer fibroblasts. Cyst-like dilatations of ducts are also present, directly surrounded by cellular connective tissue. Occasional mitoses are to be found in the connective tissue.

2. No. 20, March 25, 1912 to October 4, 1912 (six months, nine days). A small nodule which had apparently grown somewhat, composed of dense hyaline fibrous tissue, mostly necrotic, with a few nuclei, and without epithelial structures. Some of the cells in the connective tissue may have grown in from the outside.

3. No. 19, March 25, 1912 to June 17, 1912 (two months, twenty-three days). A very small necrotic nodule, with hyaline connective tissue. In the centers of areas of concentric hyaline connective tissue lie some shrunken ducts. It is impossible to state to what extent postmortem changes are responsible for these appearances.

4. No. 18 March 25, 1912 to June 13, 1912 (two months, nineteen days). A fragment consisting of connective tissue and shrunken vessels which is almost entirely necrotic.

Piece of the fourth generation

No. 23, inoculated July 16, 1912 to October 4, 1912 (two months, eighteen days). A small inactive nodule of the third generation which had been used for transplantation. It consists of hyaline connective tissue, mostly necrotic, and without nuclei; in certain areas at the periphery some nuclei are to be found, which, however, had probably immigrated from the surrounding tissue of the host.

FOURTH TUMOR

A rat with a large tumor under the skin covering the sternum, was received from a Philadelphia dealer on October 4, 1911. On October 10, three rats obtained from Philadelphia and three
from Chicago were inoculated, and a piece of the tumor was transplanted into the original tumor rat. The original tumor rat died on October 19. The autotransplanted fragment was not yet very firmly fixed. On November 7, four weeks after inoculation, no tumors had appeared in the inoculated animals, and soon afterwards the majority of the rats died. In the only one that survived longer, no tumor had developed when it died on March 5, 1912, almost three months after inoculation. A small nodule was, however, still present at the place of inoculation.

The microscopical examination of a piece of the original tumor, taken out some time after the death of the animal, showed merely dense fibrous tissue with a necrotic center.

The piece that had been transplanted into the original tumor animal consists also of fibrous tissue which is mostly necrotic, but in some peripheral areas numerous connective tissue cells are present. In this, as well as in the original tumor that had not been transplanted, some of the peripheral cells may possibly have immigrated into the tumor from the peripheral tissue of the host. A nodule removed from the rat which was found dead on March 5, 1912, has the same structure; nowhere is there any indication of growth.

SECOND TUMOR (GRANBY)

The rat bearing this growth was received in the spring of 1913 from Granby, Mass. The tumor was situated in the right axilla toward the back. On May 6, 1913, a fragment was removed from the tumor, which was very firm; two grafts were autotransplanted into the dorsal subcutaneous tissue of the tumor rat, and six normal rats were inoculated. None of the pieces grew.

On July 8, two months after the inoculation, the homoio-transplanted grafts, which had apparently not grown, were removed, as well as a piece (no. 406), which had been transplanted into the tumor rat. At the same time another was removed from the original tumor and fragments were autotransplanted at three different sites in the original tumor rat. Six normal
rats were also inoculated. Sixteen days later (July 22, 1913), the transplanted pieces were removed from two normal rats (no. 405), and one autotransplanted piece from the tumor rat. On August 20 (one month, twelve days after the second inoculation), the grafts were removed from two of the inoculated rats (no. 407), and one autotransplanted piece from the tumor rat (no. 403) was extirpated. The tumor rat died on the day following the last operation. The original tumor, from which pieces had been removed on two occasions, had grown noticeably. After the death of the rat it was dissected out and found to consist of two parts, a firm white portion (no. 401) from which the pieces had been previously removed, and a larger soft, pink mass (no. 402). It is not improbable that the latter represents that portion of the tumor which had grown since the operation, and perhaps as a result of the stimulating effect of the operation. On sectioning, the soft part shows a distinctly lobulated arrangement, and there was a sharp line of demarcation between the hard and the soft regions. The lungs presented a smooth appearance, white and pink areas alternating with each other. The remaining organs were normal. Six rats were inoculated with grafts from the soft part, but no growth took place.

The microscopical examination showed the following:

1. The hard part of the original tumor, removed at the time of the animal’s death, consists of dense hyaline fibrous tissue with slender nuclei, arranged in bundles, and containing compressed ducts lined with low epithelium. Some of these ducts seem to have atrophied under the influence of the pressure. The connective tissue, which at various places contains fat cells, is concentrically arranged around the compressed ducts.

2. The soft part of the tumor is very much richer in ducts than the firm portion; their lumina are larger and their epithelium higher, but mitoses are absent. Though the connective tissue is, on the whole, hyaline, it contains a larger number of nuclei. At some places fibroblasts are so numerous that the absorption of hyaline connective tissue has been brought about. Here again the connective tissue is usually arranged around the ducts as centers of lobules. Some ducts are much dilated; in other
regions they are the seat of irregular outgrowths. Large vessels occur occasionally in the tumor.

3. The first autotransplant (No. 406), May 6 to July 8, removed after two months, has the same structure as the original tumor. At the periphery, some of the ducts are preserved, and the hyaline connective tissue is concentrically arranged around them. In some of the ducts the epithelium is higher than in others and some ducts are surrounded by more connective tissue cells than others. Mitoses are nowhere visible. From the peripheral tissue of the host, connective tissue cells have immigrated into the tumor tissue and separated the individual lobules. In the center of the piece, ducts as well as connective tissue cells have disappeared.

4. An autotransplant from the second inoculation (no. 404), July 8 to July 22, removed after two weeks, has on the whole a similar structure, but there is evidence of infection at one point, and this may have injured the tissue to some extent. No mitoses are seen. The connective tissue is dense and hyaline, and contains nuclei, and narrow compressed ducts, either without a lumen or with a very small one. At the periphery, connective tissue from the host is growing into the piece.

5. The corresponding homoiotransplant (no. 405), removed after two weeks, has, at the periphery, well preserved ducts, some of which contain mitotic figures. The connective tissue around them is poor in nuclei, or the latter may be entirely missing. There is some connective tissue from the host growing between the hyaline tumor lobules. In the center of the graft the ducts are degenerating. The epithelial cells swell and become vacuolated and the nuclei become paler. In the center of the piece connective tissue has also been lost. The proliferation in the glandular structure, which we find in this case, may have been the result of wound stimulus.

6. An autotransplant (no. 403) from the second inoculation, removed after six weeks, contains hyaline connective tissue with relatively few nuclei, and some narrow compressed ducts in the center of it. In some places, the connective tissue is absent altogether. Mitoses are nowhere visible. In the center
the ducts seem to be degenerated, but some ducts are preserved even well toward the center.

7. A corresponding homoiotransplant (no. 407) of the same period contains hyaline connective tissue with very few connective tissue cells, and small narrow ducts which usually have vesicular epithelial cells with deformed nuclei. In some areas, the ducts are somewhat better preserved. The nuclei of the connective tissue cells are to a great extent lost.

In this case we have to deal with the same kind of adenofibroma of the mammary gland as in the first animal. Although two weeks after transplantation there was some proliferation of the ducts, no definite growth took place in them in the homoiotransplanted pieces as a whole. The ducts determine, as is usual in these tumors, the structure of the whole neoplasm. In both autotransplanted and homoiotransplanted pieces central necrosis is present, but the area which remains alive directly after transplantation is in these tumors much larger than in the case of either mouse carcinoma or rat sarcoma. The fibrous connective tissue is apparently more resistant, and also preserves the enclosed ducts to some extent. While no proliferation took place after transplantation, a considerable part of the tissue, at least, was preserved.

THIRD TUMOR

The rat was probably about two years old when received. In the right groin of the animal there was a tumor of lobulated structure, consisting of glandular ducts surrounded by fibrous and largely hyaline connective tissue. In some of the ducts the epithelial cells are somewhat deformed and vacuolated, while in others they are well preserved. A gland duct with connective tissue concentrically arranged around it forms lobules. Between the latter there are ramifying strands of connective tissue cells without ducts, which have probably pushed in between the lobules from the surrounding normal tissue.

1 This rat was received from the Wistar Institute in Philadelphia through the courtesy of Dr. H. H. Donaldson.
On October 16, 1914, pieces of the tumor were transplanted into the tumor rat, into ten rats belonging to the same family as the tumor rat, and into several non-related rats. Five days after the operation the tumor rat died. The inoculated animals were observed during the remainder of their lives, some during a period of almost six months, at the expiration of which they all had died. No tumor developed in any of them. This case constituted another instance of an adenofibroma of a rat, in which inoculation was unsuccessful.

FIFTH TUMOR

A second rat with a tumor was obtained from Philadelphia on October 4, 1911. The tumor was situated on the back, in the region of the left fore leg. The rat was a female. On October 10, a part of the tumor was removed and six rats were inoculated; another piece was transplanted into the tumor rat. On February 6 (almost four months after the inoculation) three rats were still alive. No tumor had developed; the remaining three animals had died without tumors. On October 20 (ten days after the inoculation), the autotransplanted piece had grown a little, and then remained for a short time apparently stationary. On November 15, however (thirty-six days after inoculation), it had become decidedly larger, measuring 10 by 12 mm. It continued to enlarge until on November 24 (forty-three days after inoculation) it measured 15 by 17 mm. The following day a part of the autotransplanted tumor was removed and a second piece transplanted into the rat with the original tumor, which had in the meantime showed active growth. At the same time three other rats were inoculated. On March 7, 1912 (almost three and one-half months after inoculation) no tumor had developed in the two rats which had survived up to that time, and one lived until May 8 without developing a tumor. Neither did the autotransplanted piece, inoculated on November 25, show growth at the time of animal's death on February 24, 1912, three months after inoculation.

After the second transplantation, on November 24, the original tumor decreased somewhat in size during the following two and
one-half months, and on February 6 measured 15 by 15 mm. The animal became thin and emaciated, and the first autotransplanted piece also diminished in size.

On February 13, 1912, the greater part of the original tumor was removed and pieces were inoculated into four rats. On the day following the last operation the tumor rat died. No growth developed in the inoculated rats, six of which were observed until June 25, 1912 (four months, twelve days).

The microscopical examination of the first autotransplanted piece, taken out forty-five days after inoculation, showed a very cellular, myxoid connective tissue with rather large vesicular nuclei; the tumor was traversed by capillary blood-vessels. A number of mitoses were present, some of which were irregular. At other places the tumor was necrotic. A few polymuclear leucocytes were seen in the tissue. The tumor consisted entirely of connective tissue, resembling the fourth tumor; it was not an adenofibroma, as were the first three tumors. In this case we are perhaps no longer dealing with an entirely benign tumor. It is of interest to note that an autotransplanted piece grew, while the homoiotransplanted piece did not grow, an observation in agreement with our previous results. It is furthermore of interest that when the general health of the original tumor rat suffered, the original tumor and the first autotransplant decreased in size and a second piece autotransplanted during this period did not grow.

SIXTH TUMOR

In this connection it might be of interest to mention a rat which was received from Granby, Massachusetts, in June, 1912. This animal had a large mammary tumor consisting mainly of mammary gland tissue, in which the cells are filled with vacuoles, probably containing fat, and arranged in the form of solid alveoli. At other places there are acini with wide lumina and cells showing a homogeneous protoplasm, or filled with vacuoles. The alveoli and acini are arranged in lobules which are separated by strands of dense fibrous tissue. We are evidently in this
case dealing with a hypertrophic secreting gland which has failed to undergo involution.

CONCLUSIONS

1. We may conclude from these studies that it is possible to transplant benign tumors, specifically the adenofibroma of the mammary gland of rats, through several generations. We succeeded in demonstrating growth through three generations, not only by following the increase in size, but also by demonstrating proliferation microscopically many months after inoculation. It is quite possible that if we had been able to carry out the experiments on a large scale, the propagation could have been continued into further generations. It is of interest in this connection to recall the fact that in the case of normal tissue (skin) one of us was able to obtain successful "serial transplantations," but the transplanted normal tissue very soon regained its old equilibrium and did not acquire a long continued expansive growth, in contradistinction to benign tumors, which may show a long continued growth after transplantation. Thus each tissue after transplantation retains, on the whole, the characteristics which it possessed before transplantation.

2. If we compare the growth in the different generations, we notice an apparent decrease in the growth energy in each successive generation. The latent period increases, and the subsequent microscopically determined growth becomes slower in the second and third generations. While we must take into consideration the fact that in the first generation also variations occur in the number of growing tumors in the different sets of inoculations, still the gross difference between the various generations is sufficiently pronounced to give to this conclusion a considerable degree of probability. In the first generation we find several large tumors within a period of time varying between two and four months; in the second, the largest tumor obtained is considerably smaller five months after transplantation than the tumors of the first generation after two and four months. In the third generation we find a growing nodule with mitoses
seven months after transplantation, but it is still small even at this time.

We may then conclude that in all probability benign and malignant tumors differ after transplantation in a definite way. As Loeb has found, sarcoma as well as carcinoma generally show upon transplantation a definite increase in growth energy, which is not dependent on a selection of the most virulent cells, as Ehrlich maintained, but upon a direct stimulation of the tumor cells by the conditions associated with transplantation. The curve of this increase differs in different tumors. In benign growths, on the other hand, this increase in growth energy seems to be absent; on the contrary, there is a gradual decrease, and they seem, therefore, to stand in this respect somewhere between normal tissues and cancers. It would be of great interest to attempt an increase in the growth energy of originally benign tumors also.

3. In the case of our first tumor the period of latency is relatively great, comprising several weeks even in the most favorable transplants. Microscopically, however, we may find numerous mitotic figures nine to fourteen days after transplantation. While it is possible that in some cases this marked proliferation was essentially regenerative in character, it is probable that such regenerative growth in the case of benign tumors exceeds that of normal tissue at corresponding periods after transplantation. There is therefore added to the mere regenerative external stimulus a condition within the tumor that leads to cell proliferation.

4. There is a certain variability in the results of transplantation in the first as well as in the succeeding generations. Especially noticeable is the fact that the first inoculation with pieces of the first original tumor (April 22, 1911), did not lead to as good a result as the later inoculations. It is possible that the stimulating effect of the first extirpation of pieces from the original tumor made the latter more effective in the following inoculations, just as the extirpation may have had a stimulating effect on the proliferation in the original tumor. We also notice that the piece inoculated into a pregnant rat on June 10, 1911, grew definitely within a short period of time. This is an observation which
accords with the earlier one of Loeb on the stimulating effect of pregnancy on the growth of mammary adenofibroma. It is of interest that in the benign mammary tumor the same substances which regulate the growth of the normal gland should be still active. The chemical constitution of the adenofibroma shows, therefore, the same specificity as the normal tissue from which it is derived. While chemical factors evidently play a certain rôle in the growth of the adenofibroma after transplantation, such favorable substances are not limited to female rats; the tumor growing most actively in the second generation was observed in a male.

5. At various periods after transplantation mitotic figures can be found, which are occasionally more numerous in the connective tissue than in the epithelial structures. We may therefore conclude that the increase in proliferative power found in the adenofibroma affects both the epithelial and the connective tissue structures, perhaps independently of one another. In this respect we should have, therefore, not only an adenomatous, but also a fibromatous tumor, and the designation "adenofibroma" would be appropriate. The epithelial components, on the other hand, are the determining factors as far as the structure of the tumor is concerned; in the center of the lobules we find glandular ducts or acini around which the connective tissue is arranged in a more or less concentric fashion. While, therefore, epithelium and connective tissue show an independent tendency to cell proliferation in the case of our tumor, a correlation between the two tissues still exists.

6. Tumors II and III, which also were adenofibromata of the mammary gland of the rat, did not yield growing tumors. Neither the auto- nor the homoiotransplanted pieces grew, but a considerable part of the transplanted pieces remained alive. We can not, of course, exclude the possibility that if a still greater number of rats had been inoculated and if the observation had extended over a still longer period, some positive results might have been obtained. But at present it appears at least probable that exactly as with malignant tumors, there is also among benign tumors a difference in transplantability; some appear to
be more readily transplantable than others. As Loeb stated in the report of his first transplantation of rat sarcoma, a similar structure in tumors may be associated with differences in biological characteristics.

7. Those pieces of adenofibromata which do not grow after transplantation seem at least to remain alive for a considerable period of time, and this applies to the auto-, as well as to the homoiotransplanted pieces. In those pieces that do not show any definite growth some mitoses may be found within the first few weeks after transplantation, probably in response to the regenerative stimulus of the experiment.

It is of interest that the area that remains alive after transplantation of these adenofibromata of the rat is considerably larger than the area that survives after transplantation of carcinomata or sarcomata in the rat or mouse. But in all cases the central parts of the tumors become necrotic even after autotransplantation. This confirms the previous observations of Loeb in the case of transplantation of benign tumors.

While, however, in the former transplantations of adenomata or fibrochondroadenomata of the mammary gland in the rat and dog, a considerable part or all of the transplanted tissue remained alive only in the original tumor animal, in contradistinction to other individuals of the same species where the pieces soon became necrotic in toto, in the present series of experiments we find that even in certain other individuals a considerable part of the peripheral tumor tissue remains alive. But while in our earlier work in the rat even the central necrosis was absent after autotransplantation, we find in this third series of experiments indications of central necrosis in many transplanted pieces.

The increase in the area of living tissue after auto-, or, in certain cases even after homoiotransplantation of tissues, may be due in part to the greater resistance of the fibromatous tissue which shields also the included glandular structures. In addition, less actively growing tissues are less sensitive to injurious influences, and actively growing cancer cells are more sensitive—they do not survive as readily as cells of benign tumors. Furthermore, the preservation of blood-vessels in the transplanted tissue
which join with the new capillaries formed by the host, may insure in the adenofibroma a more rapid restoration of circulation than in the carcinoma, where many or all of the irregular, less well-formed blood-vessels perish soon after transplantation.

8. Two purely fibromatous tumors, the second of which approached a sarcomatous nature, were not successfully transplanted into other rats. In the case of the second of these tumors it is interesting to note that while the homoiotransplanted pieces did not grow, the autotransplanted piece grew and showed mitoses six and one-half weeks after transplantation. This is an occurrence in accordance with the first observations of Loeb, who showed that very often tumors grow or remain alive after autotransplantation, while they die after homoiotransplantation.

It is furthermore of interest to note that the fate of both the original tumor and the autotransplanted pieces may depend upon the general health of the animal. Just as in the case of homoiotransplanted pieces the general state of health is one of the factors that determine the rate of growth of the inoculated pieces, so we find that in the case of the second fibromatous tumor the general condition of the animal determined a decrease in size of the autochthonous tumor and at the same time prevented the growth of the second lot of autotransplanted pieces.

9. If we compare the fate of the various non-malignant tumors after transplantation, we find some interesting variations. In the first mammary adenoma of the rat, on which one of us reported in 1902, the autotransplanted piece remained alive in whole, or at least in the greater part, while after homoiotransplantation only a small peripheral part remained alive and showed temporary regenerative growth. In the adenochondrofibroma of the dog, the pieces remained alive almost in toto after autotransplantation, while after homoiotransplantation they became necrotic. No growth took place.

In a similar manner we found growth in one Philadelphia myxofibroma only in an autotransplanted piece, not in the homoiotransplanted pieces. In the Chicago adenofibroma large parts remained alive and grew even after homoiotransplantation;
and in the Granby adenofibroma parts of the homoiotransplanted tumor apparently remained alive for some time, although we do not find growth in any of the pieces. In two cases we find an influence of the ovarian internal secretion on the growth of the adenofibroma of the rat; in our previously reported case in the autotransplanted piece, and in the Chicago tumor in the homoiotransplanted piece.

It is apparent that there are variations in the resistance to homoiotoxins as well as in the growth energy in the case of different non-malignant tumors.

10. It is of interest that the transplantation of adenofibroma of the mammary gland into pregnant rats seems to favor its growth during the period of pregnancy, much as the growth of the transplanted normal mammary gland is favored through pregnancy. If, on the other hand, carcinoma of the mammary gland of the mouse is transplanted into other pregnant mice, it usually does not grow. Normal embryonal tissue behaves in a manner similar to carcinoma in the mouse, but apparently not in the rat.

We are in these cases evidently dealing with an equation containing four variables—namely, (a) the specific affinity of the transplanted tissue for a certain growth substance given off by the ovaries. This affinity is greatest in the case of normal mammary gland tissue and of adenofibroma of the mammary gland; it is less marked in the carcinoma of the mammary gland and lacking in the ordinary embryonal tissue. (b) A factor injurious to tissue growth operating in pregnancy. This may be either a directly injurious substance or a shortage of ordinary food-stuffs due to the growth of the embryo. There are certain facts which suggest the first alternative rather than the latter. (c) Homoiotoxins seem to strengthen the second injurious factor, while their absence seems to favor the first aiding factor. (d) There seem to be variations in the strength of one or several of these variable factors in various species. In the mouse the injurious factors seem to be relatively stronger than in the rat. The manner in which these variables combine determines the end result.
11. There are certain structural abnormalities in the growth of the adenofibroma which distinguish its growth from that of the normal mammary gland. On the whole they are slight; they correspond to a slightly increased growth tendency under abnormal conditions. It was occasionally found in pieces of the original tumor as well as in transplanted pieces. We observed irregularities in the structure of the acini, due either to ingrowth of some cells or to the disappearance of the walls separating neighboring acini.

This increase in growth energy implies a somewhat greater intensity of growth processes, even in the absence of specific growth stimuli, and also the failure to attain perfect retrogression at periods of rest, which characterizes the normal gland. Here again slight morphological peculiarities go hand in hand with slight biological differences and the former are probably the expression of the latter. However, biological as well as morphological peculiarities are such as are associated with benign tumors rather than with cancerous growths. We must, however, remember that sharp demarcations between these conditions do not exist, that the demarcation is within certain limits arbitrary. In our case the deviation from the normal is as yet relatively so slight that our tumor cannot be classed among the carcinomata.

REFERENCES


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PLATE I

FIG. 1. ORIGINAL TUMOR

c, dense fibrous tissue; d, a somewhat irregularly formed gland. a, part of the tumor which contains a relatively small number of glands. b, part of the tumor rich in glandular structures.
PLATE II

Fig. 2. Transplanted Tumor, 3d Generation

a and a', glandular structures in the periphery of the transplanted piece. a', collections of glandular structures in which are situated formations which are reproduced in higher power in figures 3 and 4. b, area toward the center of the transplanted piece; here the number of living glandular structures decreases and necrosis begins.
c, surrounding connective tissue between the glands; in the center of the drawing between the acini the connective tissue is becoming dissolved; a, irregular gland convolutions; m, mitosis in a gland cell.
PLATE IV

Fig 4. Irregular Gland Structures from the Same Place as Fig. 3

b, surrounding connective tissue; a, epithelial structures filling out a considerable part of the cavity; c, 2 mitoses. l, immigrated lymphocyte.