ON THE KINETIC AND INVASIVE POWER OF REGENERATING TISSUE AND ON SIMILARITIES IN THE BEHAVIOR OF THYROID TRANSPLANTS AND CARCINOMAS

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Received for publication, December 22, 1919

The following observations are of interest because they clearly demonstrate that some of the fundamental characteristics of cancerous growth can be found in regenerative growth. In addition, they bring out very sharply some of the factors which, under ordinary conditions, restrain regenerative proliferation.

I. INVASION OF BLOOD VESSELS BY REGENERATING THYROID TISSUE

In the course of a study of the factors which determine regeneration of the thyroid gland and the fate of thyroid transplants, we compared autotransplantation of one thyroid lobe in cases in which the second lobe had been left intact and in which it had been extirpated previous to the transplantation. We also compared the fate of autotransplants and homoiotransplants at various stages.

In an animal, a female guinea-pig (no. 758) weighing 370 grams, we transplanted one lobe of the thyroid subcutaneously into the same animal. The lobe of the other side was left intact. Seven days later the transplant was taken out for examination. The recovered piece was relatively large and cut into serial sections. Microscopic examination showed the following:

A connective tissue capsule rich in fibroblasts surrounds the piece; it encircles a ring of acini, which, however, is not complete, being interrupted at such places where fat tissue constituted the periphery of the transplant. The peripheral acini are the larg-
est ones; they contain often well formed colloid. Other acini are filled with blood and a few contain some polynuclear leukocytes. Towards the center the acini become smaller, and especially is their lumen diminished. At various places the inner acini seem to end in cell strands without lumen. These cell strands accompanied by growing fibroblasts grow towards the center of the piece, which latter consists in the main of thyroid tissue which, being deprived of blood supply after transplantation, had become necrotic and in part hemorrhagic. In the necrotic walls of the central necrotic acini some disintegrating polynuclear leukocytes are seen. In one small area of the necrotic center a somewhat larger collection of disintegrating polynuclear leukocytes can be observed. The acini as well as the more centrally situated epithelial strands contain relatively frequent mitoses. In the peripheral concentrically arranged capsule of connective tissue we find some thyroid tissue in the form of extensive ducts; they may send off branches in various directions. On the whole they follow the direction of the connective tissue capsule in which they are embedded. These ducts are perhaps acini which regenerated in the connective tissue capsule, and under the influence of the growing connective tissue assumed a concentric growth similar to that of the connective tissue itself. Mitoses are also found in these ducts. They are situated in the periphery of the ring of acini. There is on the other hand a possibility that they took their origin in epithelial ducts which occur even in the normal thyroid.

In the periphery of the transplant and inside of the connective tissue capsule we find running parallel with the capsule a large vessel. It had been transplanted with the thyroid and may perhaps have partly recovered after transplantation. It consists of two layers, an inner endothelial and an outer muscle layer. It is situated at a place where the thyroid ring is interrupted by fat tissue. This vessel gives off branches which enter septa of the transplanted thyroid and begin to take a course radially towards the center.

At one place the vessels connect with a large capillary which, accompanied by fibroblasts, traverses the greater part of the
Fig. 1

Fig. 2
necrotic center of the thyroid and here ends as narrow capillary tubes. This capillary again sends off branches at various places. There is actual growth taking place in these vessels, as evidenced by the presence of mitoses in the endothelial cells. It is furthermore probable that the coat of unstriated muscle cells which surrounds the larger vessels gradually extends towards the periphery, certain pictures suggesting that migration of cells may play a part in this growth process.

Fibroblasts accompany the growing vessels and organize the necrotic tissue which the vessels traverse. These fibroblasts gradually invade more and more the necrotic center and would in time have organized it.

At several places solid strands of thyroid tissue which are arranged in a radial centripetal direction grow towards the end ramifications of the capillary vessel. At two places they come in close contact with it. At one of these two places we see parts of the thyroid strands outside of the vessel but in close approximation to it. At one place such a strand breaks through the capillary wall and enters the lumen of the vessel (figs. 1 and 2). And from here on one can follow in serial sections how these strands of thyroid tissue fill the greater part of the lumen of the capillary, then enter the large peripheral vessel which we described above (fig. 3) and which connects with the capillary; at last they enter into the various branches of this vessel (fig. 4). Altogether considerable masses of newly formed thyroid tissue fill these vessels. Active proliferation continues in these strands within the blood vessels. We could find several mitoses in the thyroid cells. While in the capillary the thyroid tissue forms thin strands (fig. 1), in the larger vessels it is present in wider aggregates of tissue in accordance with the greater width of the vessels at the periphery of the thyroid (figs. 3 and 4).

While near the place of entrance into the capillary vessel the strands of thyroid tissue are lying free in the lumen, a little higher up they are covered by a layer of endothelial cells which are derived from the endothelium of the vessel. Wherever the thyroid strands are in touch with the vessel wall, endothelium begins to migrate over the strange tissue; thus in the course of time a cer-
FIG. 3

FIG. 4

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tain inhibiting effect will be exerted upon the invading tissue by the endothelium of the vessel wall. This inhibiting influence is however as yet unable to prevent mitotic proliferation. More towards the periphery of the transplant within the larger vessel, the thyroid strands form irregular gyrations which are all covered by an endothelial coat. Between these strands of thyroid tissue within the larger vessels spaces are left in which the blood is able to circulate. We see here considerable masses of well preserved blood corpuscles. Further toward the center of the transplant in the end piece of the capillary we find mainly polymorphonuclear leucocytes. Here evidently stagnation in the blood current occurred and in consequence of this the white corpuscles collected in larger numbers. A similar aggregation of polymorphonuclear leucocytes occurs, whenever the circulation is slowing down at a certain place.

We must assume that at least temporarily the invading tissue has come to a standstill within the vessel; otherwise an investment with endothelium would not have been possible; nevertheless it is very probable that a shifting of the tissue in a peripheral direction continued to take place as a result of mitotic proliferation and perhaps also of continued invasion in the capillary in the center of the transplant. Furthermore considering the presence of circulating blood within the larger vessel a breaking off of certain parts of the thyroid strands is a possibility. Such broken off tissue might be carried into capillary vessels of distant organs and perhaps remain alive for a certain period of time.

II. INVASIVE AND GROWTH POWER OF REGENERATING AND CARCINOMATOUS TISSUE

These observations are of general interest in the first place because they demonstrate the marked similarity between regenerating and cancerous tissue, and secondly because they prove that the potentiality of forming new tissue inherent in regenerating tissue is in many cases considerably greater than could be assumed from the amount of tissue which is actually formed.
Cancerous tissue shows an increase in proliferating energy over the normal tissue from which it is derived; it likewise very often shows an increase in ameboid activity. As the result of both these processes an invasion of the surrounding tissue takes place as well as an invasion of blood and lymph vessels with subsequent formation of metastases. The stimulus acting on the cancerous cells may be very strong or the condition under which the stimulus reaches the cells may be abnormal. These abnormalities find expression in certain morphological changes which we often find in cancerous growth, such as a simplification in structure, irregularity in mitoses and in the structure and size of nucleus and cell and amitotic division of nuclei.

None of these changes are however entirely characteristic of cancer. An increase in proliferative energy and ameboid activity is found in regenerating tissue. We have formerly shown (1) that regenerating tissue may likewise show an increase in its power of infiltrating neighboring tissue especially after the latter has become less resistant. Thus we saw regenerating tissue entering blood clots, coagulated blood serum, and agar, and branching within these media in various directions. We found it breaking through cartilage and surrounding particles of cartilage and carrying them along on their migration. We may presume that in this case the cartilage had been previously injured, perhaps through a temporary infiltration with leucocytes. It is known that a simplification of structure is often observed in regenerating tissue and under certain conditions morphological abnormalities may be found in regenerating tissue similar to those observed in cancerous tissue.

We now can add to these characteristics the invasion of blood vessels on the part of regenerating tissues and at least the possibility that such invading tissue may become detached from its connections with the surrounding tissue and be carried through the blood stream to a distant organ. Whether here a real metastasis could ever be formed under these conditions appears doubtful considering the transitory character of the increase in proliferative energy which is characteristic of regenerating cells. The possibility can, however, not be denied that under certain
conditions an additional stimulus, such as might perhaps be exerted by the abnormal environment, in which the transferred cells find themselves, may cause a retention of their increased growth energy and ameboid activity at least for a certain period of time, and that thus a metastasis consisting of apparently normal thyroid tissue may occur. Metastases of apparently not cancerous thyroid tissue have been noticed by various observers.

In a number of cases it has been observed that particles of apparently normal parenchyma entered the blood vessels and were retained in small blood vessels of the lung. Thus particles of bone marrow, liver cells and chorionic villi have been found (cf. O. Lubarsch (5)). There can be little doubt that in such cases we have to deal with a mechanical misplacement of tissue usually due to trauma of some kind. In no case is such an embolism the result of an active invasion of blood vessels such as we have observed.

The difference between regenerating and cancerous tissue is then mainly one which is quantitative as far as the intensity as well as the duration of changes are concerned, in cancer the increase lasting considerably longer than the stimulus and often becoming at least potentially perpetual or eternal. As a result of the action of proliferative stimuli a new cell equilibrium has been established in which through inner cell mechanisms those proliferative stimuli are furnished which originally had been called forth by external stimulation.

We see then that cancerous as well as regenerating cells are stimulated cells and, both show therefore similar reactions. The differences between regenerating and cancerous cells are mainly of a quantitative character as far as the energy and time factor are concerned and we must assume that in the cancer cells secondary inner regulations have taken place which are absent in the normal regenerating cells.

Our observations indicate that the amount of tissue which is actually produced during regeneration is considerably less than the amount which could be produced if certain restricting influences were absent. Thus we find that of the numerous cell strands which form at various places at the inner circumference
of the thyroid ring only those situated at one or perhaps two places are able to produce large amounts of tissues, viz., those situated in close approximation to growing blood capillaries. They break into the vessels and encountering in the lumen of the vessel less resistance than outside, they extend over a considerable area partly through mitotic proliferation and in all probability also through cell movements. If other cell strands had been situated equally favorably near growing capillaries, they likewise might have penetrated into the lumen of the vessels and produced much new tissue. Then the total amount of newly formed tissue would have greatly exceeded the amount actually produced.

At all other places the strands found mechanical obstacles to their proliferation and extension. The necrotic and hemorrhagic material filling the center of the transplant and at some places perhaps also fibrous tissue opposed the epithelial growth. The expansive energy of the regenerating epithelium can evidently be neutralized by a certain amount of resistance which depends upon the mechanical properties of the environment. It is certain that the expansive energy of carcinomatous tissue being lastingly increased would be able to overcome a resistance which proves too strong in the case of regenerating tissue. Moreover in many cases the expansive power of carcinomatous tissue is not only extending over a longer period of time but is also more intensive. This is, however, not necessarily so in every instance. Thus we found in a case of multiple carcinoma of the skin in a young man the corium offering so strong a resistance to the expansive tendency of the cancerous tissue that at one place the latter was forced to proliferate towards the outside of the skin (2).

III. INVASION OF MUSCLE AND FAT TISSUE BY REGENERATING THYROID

These observations concerning the kinetic and invasive power of regenerating thyroid tissue do not represent exceptional or isolated occurrences. Thus we have found another transplant in which the regenerating acini contained in their lumen particles
of fat tissue. They evidently had surrounded cells of fat tissue which were situated near them and closed around them to form acini.

Very frequently remnants of epithelial ducts or perhaps also peripheral acini which are situated in the growing fibroblastic tissue proliferate with the latter and form extraordinarily large epithelial spaces, which run parallel to the connective tissue. They may branch in various directions towards the central thyroid tissue as well as towards the surrounding proliferating connective tissue, and often multiple and large papillae are produced in these ducts as the result of the fibroblastic proliferation. We have mentioned such a duct in the first thyroid which we described in this paper.

In another case in which a thyroid had been removed seven days following homoiotransplantation (guinea-pig 670) we saw growing acini running parallel to proliferating connective tissue and advancing towards the outside and infiltrating the muscle tissue of the host; during this process the acini were accompanied by growing connective tissue and capillaries. At one place the lumen of an acinus contained a piece of the muscle tissue which had been surrounded by the growing thyroid strands and included in the acinus in a similar way to the fat tissue mentioned above. Many mitoses were found in this proliferating thyroid tissue.

IV. ACINAR AND ALVEOLAR TYPE OF GROWTH IN THYROID AND CARCINOMA

There is another similarity between the regenerating thyroid and certain carcinomata of the mammary gland in mice which however is perhaps in part at least more apparent than real. It is a very common occurrence that such carcinomata are structurally of a mixed character and of special frequency is a combination of acini in certain areas of the carcinoma and of solid alveoli in others. The alveoli often show a marked mitotic activity and they may thus enlarge; but it can be shown that there is still another mode in which alveoli grow; the apparently simple alveoli are in many cases merely conglomerations of cell strands, which
form gyrate structures. Gradually as the result of the pressure, exerted by the epithelium, intervening areas of connective tissue become smaller, form a more or less homogeneous mass and ultimately disappear, and thus we have in the end to deal with apparently homogeneous alveoli, whose mode of origin from cell strands is no longer discernible.

In the regenerating thyroid several authors mention a formation of structures not unlike cell nests consisting of squamous epithelium. It is assumed that such cell nests are derived from proliferating acini. If this interpretation were correct, we had in this case to deal with a process analogous to what apparently occurs in carcinoma, viz., a transition from acinar to alveolar structures. Our observations make it however very probable that such a transition is not the mode or at least not the only one in which these alveolar structures originate. We found in the normal thyroid of the guinea-pig ducts which may branch and are surrounded by dense fibrous tissue. Even in the not transplanted thyroid such ducts may occasionally produce solid cell nests. The production of such cell nests from ducts seems to be a frequent occurrence after transplantation of the thyroid. The ducts accompanied by proliferating fibroblastic tissue grow actively and produce a very widely spread, branching network of solid strands, all connected with each other. The connective tissue around them becomes fibrillar and fibrous. In several cases we have been able to trace such a network of alveoli towards a central duct surrounded by dense fibrous tissue, such as we find in the normal thyroid. There can be very little doubt that in these cases the ducts and not the acini give origin to the alveolar structures. The process is analogous to what I have observed in the transplantation of mammary gland where also similar alveolar structures occur, and where they do not owe their origin to a transformation of gland acini, but to a proliferation of the excretory ducts of the gland. Thus all these alveoli are connected with each other.

It is possible that the alveolar structures found in the mammary carcinomata may also be derived from gland ducts rather than from acini. If this should be so, the similarity between the be-
behavior of regenerating thyroid and certain carcinomata would be
great. If on the other hand the alveoli of the mammary carcinoma are derived from acini, then the similarity would be in
part at least more apparent than real. However at present we
do not wish to exclude the possibility that the cell strands which
take their origin in thyroid acini may also be able to produce
solid alveolar instead of acinar structures under certain condi-
tions. Thus in the lumen of the blood vessels the proliferating
cell strands, which we described above, proliferate as solid strands
and not as acini. It seems however probable that in most cases
the typical network of large alveoli sometimes showing approxi-
mation to pearl formation is derived from ducts and not from
acini.

In another respect however a certain similarity between the
alveoli of mammary carcinoma in mice and the alveoli in the
regenerating thyroid may exist. The former, as we have seen,
may be composite structures. The same may hold good in the
case of thyroid alveoli. Solid cell strands growing from different
directions towards each other meet, include at first parts of
the stroma, which later disappears. This condition was for in-
stance observed in guinea-pig 499 in which a homiotransplan-
tation of a lobe of thyroid had been carried out eight days pre-
viously. It was a rather young guinea-pig, weighing 427 grams.
There was found in this case a noticeable infiltration with lym-
phocytes and much hemorrhage in the center which was perhaps
in part due to the rupture of newly formed capillaries. In the
center we found solid alveoli. In serial sections it was seen that
all or at least the majority of these strands were connected with
each other (fig. 5). There is marked mitotic proliferation in
these cells. This contributes to the enlargement of the alveoli,
but in addition it appears that at certain places neighboring cell
strands approach each other, and exert pressure on the inter-
vening stroma and that thus processes of solution take place in
the connective tissue. At first one may still be able to discern
connective tissue strands within the apparently homogeneous
alveoli but ultimately the strands disappear and then it is no
longer possible to recognize the origin of these conglomerate
alveoli. Simultaneously with these changes in the epithelial structures changes take place in the accompanying stroma. The fibroblasts form fibrillae and often dense fibrous tissue around these strands and alveoli and it may be that the changes from fibroblastic to fibrous tissue are in part responsible for the lessened nourishment and secondary changes in the center of the alveoli which correspond to similar changes which take place in the epidermis whenever it is further removed from the source of nourishment.

These solid cell strands grow especially towards the center and not within the peripheral ring of acini. This is evidently due to the fact that the fibroblasts grow from the peripheral ring towards the center of unorganized material which they organize. It is the movement of the fibroblasts which determines the direction in which the epithelial structures move.
These cell strands cannot under any circumstances succeed in reestablishing the normal thyroid structure. They will always remain epithelial tissue dissociated from neighboring normal tissue; they represent "cell nests." And yet growth within them ceases and they do not produce carcinoma. There is therefore one essential difference between regenerating and carcinomatous tissue. In the latter the growth stimulus continues to act independently of the environment in which the growing cells are, as long as the cells obtain sufficient nourishment; in regenerating tissue the stimulus leads to growth processes which can be represented by a curve which earlier or later approaches the base line, although normal conditions may not yet have been reestablished.

V. ON THE INTERACTION BETWEEN GROWING GLAND TISSUE AND FIBROBLASTS

In the cases mentioned above there can be little doubt that it is the growing fibroblastic tissue which causes proliferation of the thyroid tissue and at the same time determines the direction in which the epithelium proliferates and moves. It determines the shape of the thyroid cell strands, and the degree and direction of their proliferation in the center of the transplant. It likewise determines the proliferation and the structure of the concentric canals in the periphery of the thyroid and the secondary formation of papillae within these canals. In all those cases the proliferating fibroblastic tissue does not only not prevent, but even stimulates the epithelial growth; at the same time it influences the direction in which it takes place. In accordance with these observations on the beneficial effect of fibroblastic proliferation on epithelial growth we often notice that after transplantation of an epithelial tissue the most active proliferation takes place in that part of the graft which is in contact with actively moving and proliferating fibroblasts of the host, while that part of the transplant which is at a place more distant from proliferating fibroblasts is often not exhibiting any noticeable growth. In the latter case, however, we cannot be quite certain which is the
primary process, the fibroblastic proliferation stimulating the epithelial growth in the graft, or the epithelial growth attracting and stimulating the fibroblasts. On the other hand in the observations to which we referred above there can be no doubt that the fibroblastic tissue is the primary factor.

While thus it is certain that fibroblasts may through their activity stimulate epithelial growth, the reverse effect also occurs. We have formerly described the difference in the character of the stroma in resting and in active mammary gland of the guinea-pig (3). In this case there is no doubt that the epithelial activity is the primary factor which stimulates secondarily the connective tissue. Growing epithelial tissue usually stimulates the surrounding stroma. A similar stimulating effect may be exerted by the growing parenchyma on the blood vessels. In growing carcinoma the proliferation of the epithelial structures quite commonly stimulates the activity of the fibroblasts and of the blood vessels.

While thus the activity of epithelial tissue stimulates fibroblastic and vascular activity in the area adjoining the epithelium, the latter possesses, as we have pointed out previously, the power to prevent the penetration of fibroblasts into the epithelial structure and the epithelial tissue may even actively restrict the fibroblastic growth (4). It seems that it is the "autosubstance" produced in the epithelium which has this power in the highest degree and that it is partly lacking, whenever homoiostases are produced through the interaction of transplant and body fluids of the host.

The interaction between epithelial and fibroblastic tissue is therefore evidently complex. Epithelial activity stimulates fibroblastic activity and fibroblastic activity may under certain conditions stimulate epithelial activity. The epithelium possesses mechanisms through which it restrains the invasive power of connective tissue. Under certain conditions epithelium loses to some extent this power (for instance, under the influence of homoiotoxins) and then the connective tissue exerts a destructive influence upon the epithelium. Fibrous tissue restrains the activity of the epithelium and metabolically inactive or pathologically functioning epithelium may favor the transformation of the surrounding fibroblastic into fibrous tissue.
1. Regenerating tissue of the thyroid may possess an invasive power not unlike that of cancerous tissue. Strands of regenerating thyroid can invade blood vessels and advance and proliferate within their lumen. They may also invade fat and muscle tissue and include in the lumen of the acini particles of such tissue. This invasive power is less intense than that exhibited by very active carcinomata.

2. Growing transplants of thyroid tissue may apparently show a transition from acinar to alveolar structure. Similar transitions can be found in the case of mammary carcinoma in mice. In both cases the formation of alveoli may be due to a conglomeration of cell strands. At first parts of stroma are still included between the epithelial constituents. Secondarily these become dissolved and disappear. It can be shown that in many cases the alveolar network in the center of the transplanted thyroid does not take its origin in acini, but in ducts included in the thyroid. In a similar way alveolar structures in transplants of the mammary gland originate in ducts.

3. The kinetic and invasive activity of regenerating thyroid tissue is associated with fibroblastic activity. There are indications that under certain conditions epithelium and connective tissue exert a mutually stimulating influence, while under other conditions their effect upon each other is antagonistic.

4. The power of regenerating tissue to proliferate is much greater than could be foreseen from the amount of tissue which is actually produced. Usually the environment exerts a restraining influence upon the regenerating activity of the transplant.

5. In contradistinction to carcinomatous growth regenerating growth of the thyroid comes to a standstill, although the typical structures of the thyroid may not yet have been reestablished and the cell strands are as yet without their normal connections with neighboring epithelium.
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