EXPERIMENTAL BIOLOGY

FACTORS OF OSTEOGENESIS AND THE SECRETORY FUNCTION OF TRANSITIONAL EPITHELIUM

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The question of the character of the histogenetic influences of epithelium in the development of osteogenic tissue is still unsolved. Meanwhile the appearance of bone tissue in phylogenesis, in the form of scales and integumentary ossification, and the formation of rudiments of corresponding structures in the present-day forms are undoubtedly connected with the formative action of the epithelium [3, 9].

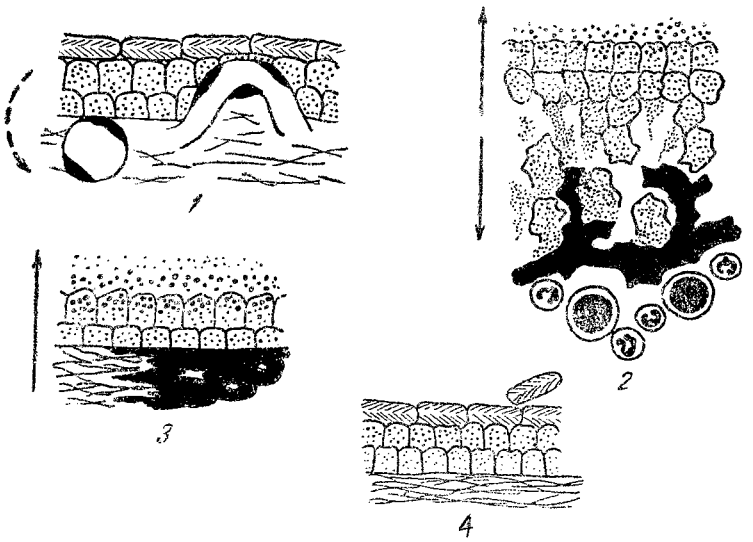
In this connection attention is drawn to the fact, known for some time, of the ectopic formation of bone near the transitional epithelium of the urinary organs – in the wall of the renal pelvis after ligation of the renal vessels [2, 12, 13] and in transplantates of the mucosa of the urinary bladder [11]. No satisfactory explanation of these facts exists.

In the present paper the results are given of an experimental, histogenetic, and histochemical analysis of these phenomena, which allow a possible explanation to be postulated.

EXPERIMENTAL METHODS AND RESULTS

The first series of experiments consisted of homografting of finely cut fragments of the mucous membrane of the urinary bladder of guinea pigs beneath the fascia of the rectus abdominis muscle. At times from 3 to 35 days the transplantates were fixed and, in addition to the routine histological study, examined histochemically for the detection of ribonucleoproteins, calcium salts, and polysaccharides [4-6].

Analysis of the histogenetic processes in the grafts enables one to determine the morphological expression of the osteogenetic action of transitional epithelium on connective tissue. It was found that large foci of osteogenic tissue, very reminiscent of typical rudiments of lamellar bones, are induced in the grafts. This induction is actually taking place on the 10th day after transplantation, and the differentiation of the connective tissue of the recipient into bone is maintained for 25 days, i. e. until the time of absorption of the transplanted homograft as a result of the immunological incompatibility of the tissues of the donor and recipient. Bone tissue develops in direct contact with the proliferating transitional epithelium, usually forming casings of bone around the epithelial cysts developing in the grafts. A morphological study showed definite signs for distinguishing those areas of the epithelial layer under which foci of osteogenesis were forming, i. e. active areas in the sense of their osteogenetic influence on the underlying connective tissue. Epithelium in a state of disturbed differentiation is active, but adult epithelium is not. The most characteristic form of relationship between this epithelium and the underlying connective tissue was found to be atypical growth of the epithelium with detachment of its cells from the layer, to become buried in the connective tissue, and also a series of other phenomena leading to the liberation into the connective tissue of the contents of epithelial cells at a definite level of differentiation. In the absence of these conditions mentioned, i. e. during normal differentiation of the transitional epithelium in the grafts, a basal membrane is formed between these and the underlying connective tissue, and the transposition of cells in the epithelial layer in the course of differentiation is effected from



Scheme of osteogenesis under the influence of transitional epithelium. 1 – Normal complete differentiation of transitional epithelium in the wall of the urinary organs; secretory activity possibly directed into the capillaries in intimate contact with the epithelium; surface of the epithelium formed by integumentary cells; secretion does not take place through the surface; 2 – atypical relationship between transitional epithelium and the underlying connective tissue during grafting and ligation of the renal vessels; secretion into the underlying tissue with induction therein of osteogenic and myeloid tissue; incomplete differentiation of the epithelium with absence of integumentary cells; secretion into the cavity of the cyst or the pelvis (secretion of pelvin); 3 – incomplete differentiation of epithelium with absence of integumentary cells from the wall of the cysts in the transplantates or in the renal pelvis during ligation of the vessels; epithelium lies on an already formed bony foundation or on dense connective tissue; secretion into the cavity of the cyst or the pelvis (secretion of pelvin); 4 – normal differentiation of transitional epithelium in the grafts; formation of integumentary cells and desquamation of these cells into the cavity of a cyst; development of a basal membrane underneath the epithelium.

the basal layer to the integumentary layer, so that the contents of the cytoplasm of the epithelial cells do not fall into the underlying tissue. Under these circumstances bone tissue never forms around these cysts, just as in the normal morphogenesis of the organs of the urinary system.

Histochemical detection of the polysaccharides (Schiff reaction) showed that the changes in the differentiation of the epithelium leading to osteogenesis are associated with disturbances of the polarity of the epithelial layer and the entry of intermediate products of metabolism into the underlying connective tissue [6].

In atypical conditions of growth in the grafts, polysaccharides from cells corresponding to cells of the transitional layer of epithelium are secreted into the underlying tissue. It is in these places, where there is secretion of polysaccharides into the tissue beneath from the epithelium, that the development of osteogenic tissue is induced. Histochemical reactions (Schiff reaction and for RNA) show that in addition to polysaccharides, substances of a protein nature, entering into the composition of the cytoplasm of cells of the intermediate layer of epithelium, are also secreted from the epithelium. Hence, it follows that the osteogenetic properties of transitional epithelium are probably due not to the appearance of new substances in it during transplantation, absent under normal conditions, but to the transfer from epithelium to connective tissue of certain substances which are probably always present in the epithelium but which usually do not reach the underlying connective tissue.

The above-mentioned changes in the differentiation of transitional epithelium, from what has been said, may be interpreted as evidence of some form of secretory process directed from the epithelium towards the underlying connective tissue; an osteogenetic factor is evidently secreted in the course of this secretory process taking place under the conditions of the experiment. In addition to the secretion into the underlying connective tissue just described, obvious secretion is observed in the grafts also, into the cavity of cysts through the surface of the epithelial layer where it is lacking in integumentary cells. The secretion consists of droplets of polysaccharide (not staining with mucicarmine) and a flocculent protein substance; as a result a translucent yellowish fluid accumulates in these cysts. This is not a question of secretion by individual goblet cells or their complexes, forming the glands of varying complexity in the composition of the mucous membrane, but of a secretory function of the whole epithelial layer. Secretion of mucus may also occur in the grafts, but induction of osteogenesis never takes place around such cysts [6].

In natural conditions the study of the secretory activity of transitional epithelium is made difficult by the fact that the products of secretion, if this takes place in the direction of the interior of the urinary organ, must be dissolved and excreted in the urine. This difficulty may, however, be overcome experimentally.

In a second series of experiments the vessels of the left kidney were ligated in rabbits, followed by ligation of the ureter in its lower third or by division of the ureter and suture of its proximal and distal ends to fatty tissue. Ligation of the vessels causes cessation of urine formation in the kidney and degeneration of its medullary and cortical layers. Viability is preserved only of the transitional epithelium of the pelvis, which undergoes proliferative changes, since vascularization of this structure is effected by means of vessels ascending in the wall of the ureter, and this is not cut off by ligation of the renal vessels. In these conditions, as is known [2, 13, 12], the development of osseous and medullary tissue is induced in the hilum of the kidney. It might be expected that if the transitional epithelium secretes into the cavity of the pelvis, the secretion can be collected in the lumen of the ligated ureter emerging from the kidney whose vessels have been ligated. When the end of this ureter is fixed by a suture, the secretion must flow along it, down to the place where it is fixed; the place of fixation of the distal end of the divided ureter served as a control. The operation on the ureter was carried out from 4-15 days after ligation of the renal vessels. From 10-215 days after ligation of the vessels the animals were killed. The left kidney, the left ureter and the place of suture of its ends, after fixation, were examined histologically and histochemically for the presence of polysaccharides. Smears were made of the contents of the ligated ureter. A detailed histogenetic analysis of this material must await further reports.

As a result of ligation of the vessels considerable proliferative changes develop in the epithelium of the pelvis, and as early as the 16th day, in places where there is infiltration of the transitional epithelium into the underlying tissue, the development of bone and of myeloid tissue is induced in it. The morphological expression of the osteogenic activity of the epithelium is composed in general of the same phenomena which were described in osteogenesis in transplantates of the urinary bladder [7]. It is relevant to stress the close genetic relationship between osteogenic and hemopoietic tissue [1]. Their simultaneous induction cannot therefore give cause for surprise.

In the conditions created by ligation, secretory processes are clearly revealed in the transitional epithelium of the pelvis [7]. They are directed, in the first place, into the underlying connective tissue and they are brought about as a result of those atypical relationships which are established by the infiltration of the transitional epithelium into that tissue. In these places foci of osteogenesis appear on the 16th-18th day after ligation of the vessels. In the second place, in those situations where the epithelium is thickened on account of proliferation of the intermediate type of cells, but without the formation of typical integumentary cells, the secretion is directed through the surface of the epithelium into the cavity of the pelvis. Under these circumstances there is clearly seen secretion from the apical parts of the superficial cells of flocculent (in fixed preparations) masses of polysaccharide and protein, and also droplets of glycogen. Often from the same areas of the epithelial layer it is possible to observe both secretion into the cavity of the pelvis through the surface of the layer, and secretion of the same substances of polysaccharide nature into foci of osteogenesis in the underlying connective tissue. In such areas, on the 20th to the 200th day after operation (and possibly even longer) there takes place accumulation and secretion from the apical parts of the cells of the whole layer of discrete, large drops of secretion, giving an intense Schiff reaction which is not removed by amylase; the secretion does not stain at all with mucicarmine. After liberation from the cells, the drops of secretion lose their proper shape, and in the cavity of the pelvis they form a flocculent protein mass which also gives a positive reaction

for polysaccharides. The name "pelvin" is suggested for this secretion.

Ligation of the ureter, when carried out after ligation of the renal vessels, has no essential effect on the changes brought about by ligature of the renal vessels alone (secretion, formation of bone and bone marrow). However, under these circumstances accumulation of translucent yellowish contents takes place in the ureter above the point of ligation. Morphologically the secretion of this substance by the transitional epithelium of the pelvis can be demonstrated perfectly clearly. On the slides it is quite similar to the secretion whose production by the pelvis was described above and also to the contents of the epithelial cysts in transplantates of transitional epithelium, where osteogenesis is induced [6, 7]. From 3-5 days after aspiration with a syringe, the contents accumulate once again.

Suture in fatty tissue of the proximal end of the divided ureter leading from the kidney whose vessels have been ligated, leads to the formation, on the 20th day, at the site of fixation, of multinuclear symplasts filled with small granules of polysaccharide, and around these – the appearance of foci of myeloid hemopoiesis. Control suture of the distal end of the ureter did not cause any such reactions. When the proximal end of the ureter has been sutured the contents of the pelvis flow down along it and accumulate in the ligated ureter. It must be emphasized that the formation of bone marrow at the proximal end of the divided ureter cannot be explained in any other way than by the histogenetic action of the contents of the pelvis that have flowed down to that point. In its morphology this process is similar to the induction of myeloid tissue in the region of the pelvis; under these circumstances at the place of suture of the distal end of the ureter, whose stump is in no way different from that of the proximal end except that no pelvic contents reach it, the development of myeloid tissue does not take place.

The findings described show that in experimental conditions an obvious secretory activity is shown by transitional epithelium. This secretion may be directed into the cavity lined by the transitional epithelium, or into the underlying connective tissue. In the latter case the development of bone and of bone marrow is induced underneath the epithelium.

With all its variety the morphology of the secretory processes demonstrates the similar character of the secretion into the cavity and into the underlying tissue, which is observed both in the case of formation of cysts in transplantates of transitional epithelium and after ligation of the renal vessels. In other words it can be considered that substances causing osteogenesis and hemopoieses are present in the secretion (pelvin) which may accumulate in the cavity of the pelvis. The fact that drainage of the pelvic contents through the ureter into connective tissue can cause myelopoieses confirms this conclusion.

As already emphasized, secretion by the transitional epithelium into the cavity through the surface of the layer is effected by means of imperfectly differentiated epithelium, in particular in the absence of typical integumentary cells. In normal urinary organs in contact with urine the differentiation of the transitional epithelium in complete,and conditions for secretion through the surface of the layer are evidently not created.

There arises, however, the question of physiological analogs of this secretion from the epithelium into the underlying tissue,which is observed in experimental conditions. It seems unlikely that histogenetically active substances, important for the body, should disappear (run to waste) in the process of normal development of the transitional epithelium. In this connection some preliminary observations may be made, which will require further confirmation, but which even now may be found useful in an analysis of the histogenetic factors of osteogenesis and hemopoiesis.

We may postulate the presence of a secretory (or incretory) process, directed from the transitional epithelium of the pelvis into the capillaries. It is possible that under these conditions the transitional epithelium secretes a certain factor (corresponding to the active principle of pelvin), which possesses a histogenetic action in respect to myeloid or osteogenic tissue. This form of incretory activity of the transitional epithelium, in A. N. Studitskii's opinion, may be connected with the hemopoietic function which is performed by the kidney in the lower vertebrates and in the earlier ontogenesis of the higher. In this connection it is interesting to observe that the mesonephros and the metanephros of birds evidently contain some substance which has a skeletogenic activity [10], and also to bear in mind the intimate contact with the capillaries that is so characteristic of transitional epithelium.

From the point of view of the hypothesis which we have propounded, a satisfactory explanation could be obtained of experiments on the induction of bone and of bone marrow by the action of transitional epithelium,

which are not so far understood. It is obvious that certain experimental conditions (transplantation, ligation of vessels) disturb the natural mechanisms of absorption of the factor – pelvin – secreted by the transitional epithelium into the blood stream, and it is deposited locally in the underlying connective tissue, where it induces the corresponding histogenetic processes.

SUMMARY

The author conducted an experimental, histogenetic and histochemical analysis of the transitional epithelium bone-forming activity in the transplantates and in conditions of ligature of the renal blood vessels. It was demonstrated that in such conditions substances of polysaccharide origin are secreted by the epithelium into the underlying tissue, as well as over the surface of the epithelium layer. It is suggested to name this secretion "pelvin". The introduction of pelvin in the connective tissue causes the appearance of myeloid hemopoiesis in it. A hypothesis is put forward of the presence of the incretory function in the transitional epithelium. The explanation of its bone-forming activity in experimental conditions is suggested.

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