THE CONDITIONS FOR AND THE HISTOCHEMICAL CHARACTERISTICS OF OSTEOGENIC ACTIVITY OF TRANSITIONAL EPITHELIUM IN GRAFTS

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The morphological changes arising during bone formation as a result of the interaction between transitional epithelium and connective tissue during homografting in guinea pigs have been described in previous commnications [1, 2].

It has been shown that induction is brought about by atypical growth of the transitional epithelium and in several other processes, as a result of which the contents of the cytoplasm of the epithelial cells at a definite level of differentiation are secreted into the underlying connective tissue.

This is particularly the case with a substance of polysaccharide nature, which passes from the epithelium to the underlying tissue under the conditions of transplantation [3, 4].

It is also known that induction of bone tissue is brought about by high alkaline phospatase activity in the transitional epithelium [5, 6], which certain authors [6] are inclined to associate with the mechanism of induction, bearing in mind the important role of this enzyme in osteogenesis.

In view of these findings, it appears desirable to compare the distribution of polysaccharides and alkaline phosphatase in the transitional epithelium when osteogenetic induction is present, or when it is absent.

EXPERIMENTAL METHOD

Three series of experiments were carried out:

I. Homotransplantation of finely cut mucous membrane of the urinary bladder of guinea pigs into the anterior abdominal wall by means of a grooved probe (i.e. with trauma to the connective tissue).

II. The same transplantation, but using fragments suspended in physiological saline, implanted with a syringe (i.e. with minimal trauma to the connective tissue).

III. Homotransplantation of finely cut mucous membrane of the urinary bladder of rabbits into the anterior abdominal wall by means of a grooved probe.

On the 7th-15th day the grafts were fixed by the Shabadash method and also with acetone. As well as the ordinary histological methods which were used in the previous research [1, 2], the Gomori test for alkaline phosphatase and for calcium phosphate, the Koos test for calcium salts and the Schiff periodic acid test for polysaccharides were performed.

EXPERIMENTAL RESULTS

As a result of the proliferation of the transitional epithelium in the transplantates in the guinea pigs on the 7th-10th day, cysts of a dual nature were formed. During the growth of the epithelium along early sclerosing or adult connective tissue cysts developed with a normal, fully differentiated transitional epithelium and with the formation of a basal membrane.

On the 12th-15th day the epithelial lining acquired the structure of ordinary transitional epithelium, consisting of basal, intermediate and large integumentary cells. Such cysts always appeared in the course of transplatation with the syringe, and in some areas of the grafts made with the grooved probe. Induction of bone tissue never occured around these cysts.

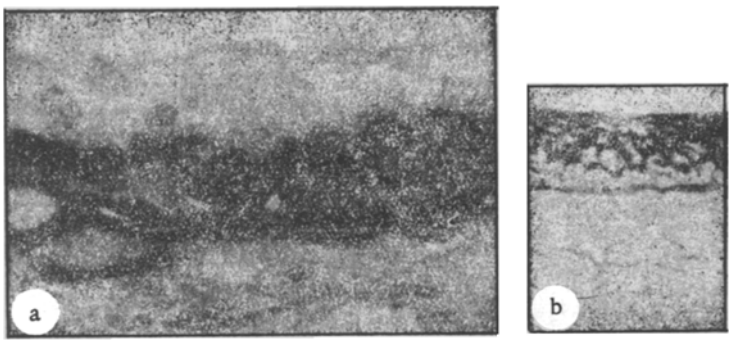

Fig. 1. Normal differentiation of the transitional epithelium in the transplantate; 12 days. Guinea pig.
a)Distribution of alkaline phosphatase. Acetone. Gomori. Objective 40 ×. b)Distribution of glycogen. Shabadash – Schiff periodic acid. Objective 20×.

The second type of cyst was characteristic only of those transplantates made by means of a grooved probe, and it appeared during growth of the epithelial layer over the reactive, young connective tissue. Under these circumstances the transitional epithelium remained incompletely differentiated, integumentary cells were not formed in the epithelial layer, no basal membrane was developed and atypical infiltration of the epithelium into the underlying connective tissue was observed. At the same time osteogenesis was induced there [1, 2].

The distribution of alkaline phosphatase and glycogen in the wall of the cysts with completely differentiated transitional epithelium was very characteristic. A strongly positive reaction for phosphatase was given by the basal layer of epithelial cells, the layer of intermediate cells contained less phosphatase or did not react at all, and the superficial cells were always without phosphatase (Fig. 1, a).

Glycogen showed the opposite gradient in its distribution. In the basal cells it could be found only in the form of isolated, separate droplets, or not at all; in the intermediate cells the quantity of glycogen increased sharply, and during the transition to the surface of the layer the entire cytoplasm of the epithelial cells became filled with polysaccharide in the form of droplets of glycogen and of a homogeneous substance which was not fermented by amylase (Fig. 1, b). Under these circumstances it was evident that glycogen was used up in the formation of this mucin-like substance.

The cysts of transitional epithelium with normal differentiation were surrounded by connective tissue consisting of fibroblastic cells, which gave a positive reaction for phosphatase until the 6th-7th day; on the 10th-17th day a reaction for phosphatase was given by only a narrow border of connective tissue directly in contact with the epithelial lining of the cyst; all the remaining connective tissue around the cyst gave a negative reaction.

Where differentiation was incomplete and atypical areas of infiltration were present, the transitional epithelium had different histochemical features. The usual distribution gradients of alkaline phosphatase and glycogen in the epithelial layer were disturbed: the basal cells of the epithelium, including those invading the depths of the connective tissue, contained not only phosphatase but also a large amount of glycogen, which was concentrated mainly in the most basal areas of their cytoplasm.

The cells thus corresponded to the cells of the intermediate layer and the phosphatase activity in them was less than in the basal cells of the transitional epithelium with normal differentiation.

After the 10th day this epithelium began to secrete into the underlying connective tissue substances from its cytoplasm, including glycogen. This resulted from destruction of the epithelial cells buried in the connective tissue, expulsion of fragments of the cytoplasm of the basal cells (Fig. 2, a), and also from a peculiar form of secretory process, directed both into the depths of the connective tissue and through the surface of the epithelial layer into the cavity of the cysts.

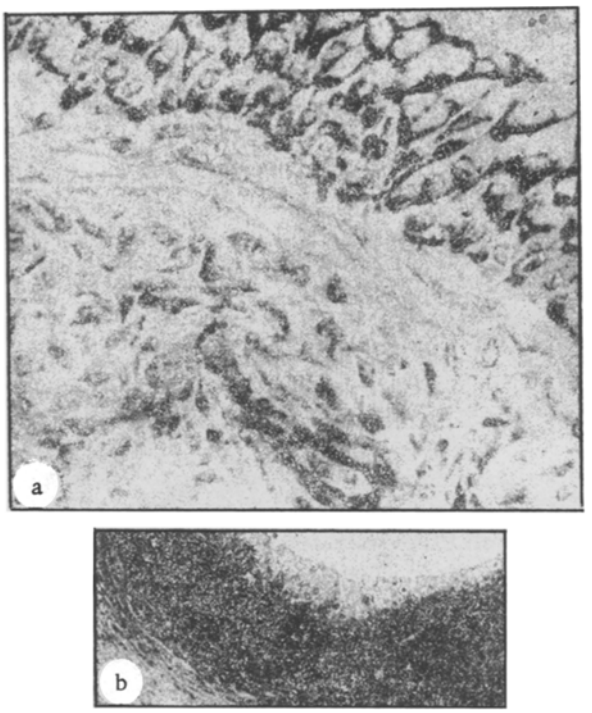

Fig. 2. Epithelium above a focus of induction of osteogenesis. Transplantate; 11 days. Guinea pig. a) Distribution of glycogen. Shabadash – Schiff periodic acid with counterstaining with hematoxylin. Objective 40 × b) Distribution of alkaline phosphatase. Acetone. Objective 20 ×.

The details of these secretory processes in transplantates in guinea pigs have been described previously [1-5]. As a result, after the 10th day osteogenic tissue appeared and bone formation began in the connective tissue underlying the inducing areas of epithelium.

Although the connective tissue surrounding the whole of the transplantate gave a negative reaction for phosphatase on the 10th-12th day and appeared to be adult, a wide zone of tissue lying next to these cysts showed a strongly positive reaction for alkaline phosphatase, characteristic of osteogenic tissue (Fig. 2, b). This reaction was more intensive than in the transitional epithelium situated over it and responsible for the induction.

The formed bone tissue lying in the center of the rudimentary bone gave an intensive Schiff periodic acid reaction and a negative reaction for alkaline phosphatase, and contained calcium salts in the form of confluent granules; droplets of glycogen were contained in the cytoplasm of the immured osteoblasts and in the preosteoblasts.

The osteoblasts, fibers and basic substance of the osteogenic tissue at the periphery of the rudimentary bone gave an intensive reaction for alkaline phosphatase, but contained no calcium salts nor glycogen. Layers of osteoid, giving a weak Schiff reaction, appeared between the cells.

Transplantation in rabbits in all cases gave negative results – no induction of bone tissue was obtained.

In the cysts which arose in the transplantates, complete differentiation of the epithelium with the formation of large superficial cells was observed. Both at the moment of completion of differentiation and in the period of growth of the epithelial layers, they were clearly delineated from the underlying connective tissue; no signs of invasive growth of the epithelium were observed in rabbits, and the differentiated layers had a clear basal membrane. Glycogen was present only in the cells of the intermediate layer and the integumentary cells, and droplets of glycogen could be secreted into the cavity of the cysts from the superficial cells of the epithelium.

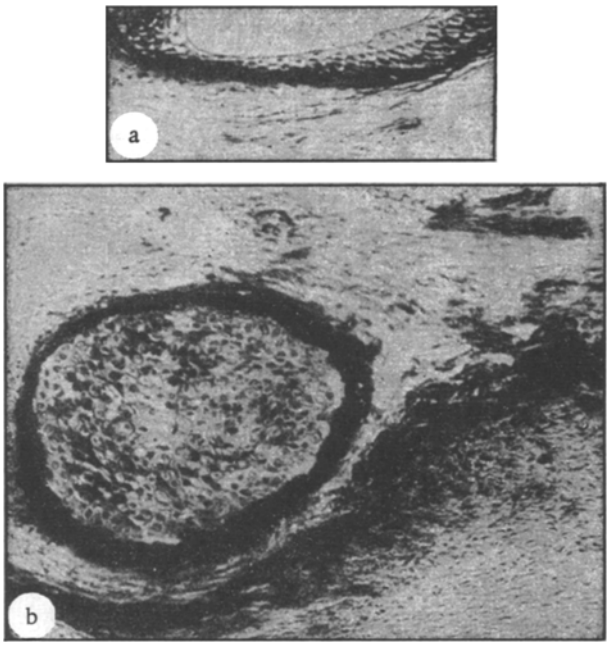

Fig. 3. Transplantate in a rabbit 15 days after grafting. Distribution of alkaline phosphatase. a) In the cyst wall; b) around the transplantate (viewed from above). Acetone. Gomori. Objective 20 ×.

In addition to glycogen, the cytoplasm of the superficial cells and the cysts themselves in the rabbits, as in the guinea pigs, contained polysaccharides in the form of pale pink drops and flocculi which were not fermented by amylase. In some cysts the majority of the superficial cells also contained polysaccharides, probably of mucous origin, in homogeneous form in the superficial areas of their cytoplasm. Polysaccharides of the glycogen type were evidently utilized in the formation of these substances.

Attention was drawn to the large number of capillaries in the cyst walls in rabbits and to their intimate contact with the epithelium, just as occurs in the normal transitional epithelium of the urinary bladder.

Phosphatase was detected in the epithelium only in the basal layer of cells. In the underlying connective tissue a positive reaction for phosphatase was present in a narrow zone (Fig. 3, a) lying immediately beneath the epithelium and containing a large number of collagenous fibers, but not rich in cells, and moreover the reaction for phosphatase was more intensive than in the basal layer of epithelium situated above it. In the tunica propia of the transplantates in the rabbits a feebly positive reaction for phosphatase was often preserved. Furthermore, around the whole of the transplantate there was usually a narrow ring in which alkaline phosphatase activity was observed (Fig. 3, b). On the 15th day this outer ring disappeared.

Thus although the transitional epithelium contained an osteogenic factor, it was evidently able to display its activity only if differentiation of the epithelium had taken place to such an extent that this factor was secreted into the underlying connective tissue. For this to be so it was necessary for the epithelium and connective tissue to be correlated in a particular manner, differing from normal and expressed morphologically by the reactive

state of the connective tissue, the incomplete differentiation of the epithelial layer and the atypical infiltration of the epithelium into the connective tissue.

The trauma of transplantation was sufficient to create these relationships in guinea pigs, in which induction of osteogenesis took place; in rabbits these reactive changes were feebly shown and did not interfere with the normal differentiation of the epithelium – osteogenesis did not arise in the transplantates, as was the case in guinea pigs after implantation through a syringe (with minimal trauma). At the same time growth of the transplanted epithelium took place in both cases.

The histochemical characteristics of normally differentiated transitional epithelium and of epithelium bringing about osteogenic induction were also sufficiently well defined. During induction of osteogenesis, in intimate contact with the connective tissue was a layer of epithelial cells whose cytoplasm contained a large amount of glycogen which could be seen to be secreted from the epithelium into the underlying connective tissue where osteogenesis was being induced. Where induction was absent, as in the normal urinary bladder, the basal cells of the epithelium were without glycogen. During induction of osteogenesis, the epithelial cells next to the connective tissue gave a less intensive reaction for phosphatase than the basal cells of normally differentiated transitional epithelium. Taken as a whole, the inducing cells corresponded in their characteristics to the intermediate cells of the transitional epithelium. Glycogen formation in the layer of transitional epithelium was evidently carried out through hexose monophosphate by the action of phosphorylase, which was found to be present in transitional epithelium [9].

In those transitional epithelial cells in which the alkaline phosphatase activity was high, this glycogen synthesis must have been inhibited on account of dephosphorylation of the Cori ester. In this way the absence of glycogen from the basal cells of the transitional epithelium and its presence in the cells of the overlying layers were presumably explained. The importance of glycogen in the transitional epithelium and the manner of its utilization are unknown [8]. It is obvious in any case that during osteogenic induction, when the phosphatase activity in the layer of transitional epithelium was lowered, a more intensive formation of glycogen in this structure was possible, and this was in fact observed. In certain conditions, this substance, with the histochemical signs of glycogen, obtained access to the underlying connective tissue, where it induced osteogenesis.

The observation was thus confirmed that induction was associated with changes in the gradient of metabolic processes in the epithelial layer, with secretion of intermediate products of polysaccharide conversion into the connective tissue. These active substances were also present in the transitional epithelium of the rabbit, as shown by bone formation in the renal pelvis after ligation of the vessels in experiments in which pelvin was drained away through the ureters [4]. During the conditions of transplantation in the rabbit, however, they were not secreted into the connective tissue.

It must also be emphasized that the same alkaline phosphatase activity was shown in the connective tissue underlying the transitional epithelium in the transplantates as in the tunica propria of the urinary tract [7].

This was the case during both the presence and absence of osteogenesis. In these circumstances, in the transplantates in the rabbit just as in the tunica propria of the normal urinary bladder, a higher phosphatase activity was observed in the underlying connective tissue than in the epithelium itself, and the inducing cells of the epithelium in guinea pigs were characterized by a relatively low phosphatase activity.

These findings were contrary to the view that the phosphatase of the transitional epithelium plays a leading role in its osteogenic activity.

SUMMARY

Comparative histological and histochemical (alkaline phosphatase, polysaccharides) study of transitional epithelium transplants was conducted in guinea pigs and rabbits. It was demonstrated that the osteogenetic effect of transitional epithelium on the connective tissue is associated with the penetration into it of the polysaccharide metabolism intermediate products from the epithelium. High activity of alkaline phosphatase is revealed in the subepithelial connective tissue independent of the presence or absence of osteogenesis. A definite level of epithelial differentiation is necessary for the secretion of the osteogenetic factors into the underlying tissue, this level depending on the condition of connective tissue around the transplant.

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