INDUCTIVE PROPERTIES OF TRANSPLANTED TRANSITIONAL RAT EPITHELIUM

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Previous reports [1-4] have described the morphological and histochemical changes associated with the induction of bone formation induced by transplantation of mucous membrane from the urinary bladder of the guinea pig. In these animals the induction takes place extraordinarily easily in almost every case, whether the transplantation is an auto- or a homograft. The bone is formed after as little as 10 days.

In other mammals the effect is quite different. In the first place, in rats bone formation around the autograft of transitional epithelium is a rarity, and no bone is ever formed around a homograft. A comparative study has been required for a further analysis of the nature of the inductive properties of transitional epithelium.

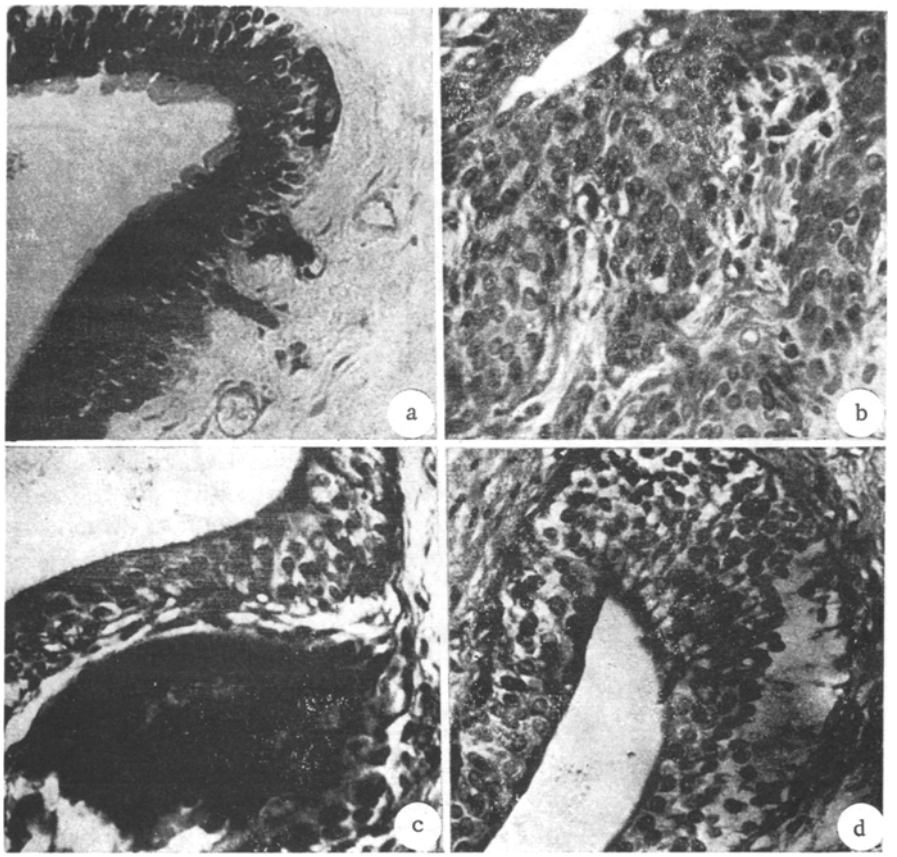
EXPERIMENTAL METHOD

White rats of the Wistar strain received grafts of pieces of urinary bladder introduced beneath the fascia of the anterior abdominal wall. After 14-27 days, pieces of the surrounding tissue were fixed in acetone or in alcohol and formalin. Serial sections were stained for nonspecific alkaline phosphatase in Gomori, and tested by the Schiff iodic acid reaction for polysaccharides, and stained with hematoxylineosin. Autografts were made on 44 rats and 3 were fixed on the 14th day, 6 on the 15th, 3 on the 18th, 12 on the 20th, 7 on the 23rd, and 13 on the 27th day. Homografts were made on 11 rats, and 2 were fixed on the 13th, 2 on the 17th, and 7 on the 22nd day after the operation.

EXPERIMENTAL RESULTS

The transitional epithelium of the urinary bladder of the rat consists of three layers of cells of a structure usual for this tissue. The cells of the basal layer have no glycogen and zero alkaline phosphatase activity; in this respect they differ from the interstitial cells. The reaction for phosphatase is even more intense in the surface layer. Here, in addition to glycogen, a polysaccharide resistant to amylase is also present. The transitional epithelium is connected with a thick plexus of capillaries lying directly beneath the epithelium and penetrating into it as far as the surface layer.

In the autografts the transitional epithelium grows out from the grafts and forms vesicles with fluid contents. By the 14-18th day after grafting, the epithelium lining most of the vesicles consists of three clearly-defined layers of cells. By comparison with the mucous membrane of a normal urinary bladder the epithelium of a vesicle is thickened, but normally differentiated. The Gomori reaction is positive throughout the whole of the epithelium except for the cells of the basal layer (see Figure, a), and it is strongly positive in the numerous subepithelial capillaries. It is characteristic that at a distance from the epithelium the capillaries show no phosphatase activity, the line of demarcation being sharp. The basement membrane beneath the epithelium is clearly shown. The connective tissue surrounding the vesicles contains numerous collagen fibers; at this time the reaction in it for alkaline phosphatase is already negative. In the grafts there are also buried outgrowths of epithelium. In most cases at these points there is a clearly defined boundary between the epithelium and the connective tissue, and a basement membrane is apparent, though beneath the epithelium the capillary plexus is less thick. In addition, in such regions the alkaline phosphatase is active throughout the whole supraepithelial connective tissue around the epithelial outgrowths. Finally, in some parts the growing epithelium is atypical (see Figure, b). Here it is separated neither from the connective tissue nor from the basement membrane nor from the capillaries. Occasional epithelial cells grow out into the depth of the connective tissue and become separated from the epithelium. In the regions of atypical growth the connective tissue consists of proliferating basophil cells surrounded by a ground substance and a few fibers, and it contains a highly active alkaline phosphatase.



Urinary bladder autograft. a) 14 days after grafting. Acetone; Gomori stain; objective 10x; b) after 23 days. Alcohol-formol; hematoxylin-eosin; objective 20x; c) after 27 days. Alcohol-formol; stain Schiff iodic acid and hematoxylin; objective 20x; d) after 20 days. Alcohol-formol; stain Schiff iodic acid and hematoxylin; objective 10x.

In the region of the atypical epithelial growths into the connective tissue sometimes many lumps of glycogen are found; in most cases they lie between the connecting cells in a layer about 80 μ thick beneath the epithelium. However, as a rule the glycogen in the basal parts of the layer of transitional epithelium and in the surrounding connective tissue is either very sparse or not present at all.

In general, a similar appearance is found 20-23 days after transplantation. Also, in one of the 19 grafts, bony tissue was induced (see Figure, c). The bone was formed in the wall of the vesicle, and the epithelial lining was incompletely differentiated consisting of 3-5 layers of cells which were not separated into zones. This epithelium had a reduced alkaline phosphatase activity. At the site of induction there was a buried growth of epithelial cells in the adjacent reactive connective tissue, and in it there were defects in the epithelial layer. The alkaline phosphatase reaction was positive over a wide area beneath the epithelium. The osteogenic tissue beneath the epithelium consisted of typical osteoblasts with well developed anastomosing processes in the cytoplasm. In the center of the fragment there was an osteoid enclosing osteocytes.

In two autografts out of 13 bony tissue was found 27 days after the operation. The morphological picture corresponded to the description we have given. In most cases, however, the epithelium in the wall of the vesicle lay on the basement membrane and thick capillary plexus, as it did in previous stages, and no bone was formed. Typically, at this time there was a particularly close contact between the epithelium and the capillaries. As part of the basal layer, superimposed on the remaining epithelial growth a group of strongly vacuolized epithelial cells lying up against the greatly dilated capillaries could be made out (see Figure, d). These cells gave negative Schiff iodic acid and Gomori reactions and resembled cells which had liberated a secretion. The remaining cells of the layer contained polysaccharides, gave a Gomori reaction, and actively liberated secretion into the cavity of the vesicle.

In the first 10 days the homografts did not differ from the autografts. However, by the 14-22nd day a lymphoid infiltration and resorption of epithelium could be observed in them. In none of the nine grafts fixed after the 17th day was any boney tissue present.

The mucous membrane of a normal urinary bladder in rats shows three important features which distinguish it from that of the guinea pig: 1) in the transitional epithelium of the rat there is a gradient in the distribution of glycogen resembling that of the guinea pig, but the alkaline phosphatase gradient is in the reverse direction; 2) the connective tissue of the basement membrane of the urinary bladder of rats has absolutely no alkaline phosphatase activity; 3) in rats the capillaries penetrate deeper into the epithelium than they do in guinea pigs and make direct contact with the cells of the middle and upper layers. Here the subepithelial capillaries give a strongly positive Gomori reaction, unlike the other vessels of the mucous membrane which do not come in contact with the epithelium.

The results obtained on rats differ firstly in that the induction of osteogenesis does not occur before the 18-20th day, whereas in guinea pigs it occurs around the 10th day. Evidently it is for this reason that homografting in rats does not cause induction. Indeed bone begins to develop in rats at a time when the inductive tissue is unable to survive on account of developing immunity to the grafts. Secondly, in rats the development of boney tissue on homografting transitional epithelium occurs only in 10% of the cases. Nevertheless, both morphologically and histochemically the inductive process itself shows many common features in guinea pigs and rats. As in guinea pigs, in the rat induction occurs only in the regions of atypical ingrowths of epithelium into the underlying young connective tissue, where there is a separation of the cytoplasmic contents of the incompletely differentiated epithelial cells. Then in some cases the liberation from the epithelium of polysaccharides with the histochemical properties of glycogen may be observed.

In the rat, usually the epithelium of the graft undergoes complete differentiation and produces no buried growth, and at the same time no induction occurs. The mucous membrane of the urinary bladder then reproduces a normal structure: there is a transitional epithelium with a basal layer showing no active alkaline phosphatase and it lies under a thick plexus of capillaries which penetrate into the epithelial layer right up to the surface. Unlike the surrounding vessels the capillaries, which make contact with the outgrowths of the transplanted epithelium, show a high phosphatase activity. Exceptions to this commonly observed arrangement are seen in portions of epithelial outgrowths beneath which the vessels form a less compact plexus and do not penetrate deep into the epithelium. In such places, beneath the epithelium there is a more or less broad zone of highly active phosphatase in the connective tissue itself. It is easy to see that here we have the arrangement typical of the normal guinea pig urinary bladder [5].

Finally, at points where osteogenesis is induced, in rat grafts there is a greater difference from normal in the relationship of the transitional epithelium to the underlying tissue; beneath the epithelial outgrowths there are no vessels, and among the connective tissue giving a positive phosphatase reaction zones of osteogenesis develop beneath the epithelium. This arrangement is common to inductive areas in both guinea pigs and rats; in pig grafts it is the usual arrangement, but in rats it occurs only occasionally. Evidently these features explain the fact that the incidence of induced osteogenesis differs in the two rodent species.

At times soon after grafting diffuse alkaline phosphatase activity may develop in the connective tissue surrounding the graft, and it is associated with a post-traumatic proliferation of the connective tissue [5]. However, the prolonged maintenance of alkaline phosphatase activity in the subcutaneous connective tissue (including the capillary walls) around the grafted transitional epithelium is a consequence of the action of the latter. This change represents the first stage of induction of osteogenesis in grafts where induction proceeds to completion [3]. As we have described earlier, the inductive process is associated with the liberation into the connective tissue of certain substances present in the cytoplasm of the epithelial cells. Presumably the ultimate effect of the action of the transitional epithelium is determined by whether these substances enter the underlying connective tissue, and whether they attain a sufficient concentration there.

The results we have reported show that the effect of transplantation of transitional epithelium does indeed depend directly on the degree of contact established between the epithelium and the capillaries. With very close contact, when substances liberated by the epithelium are able to enter the bloodstream directly, avoiding the connective tissue, the Gomori reaction occurs only in the wall of the vessels in contact with the epithelium; when the vessels accompany the epithelium lying not within, but beneath it, in the basement membrane, and substances entering the vessels from the epithelium have to pass through the layer of connective tissue, alkaline phosphatase activity develops in this layer; finally, in the absence of a vascular plexus the epithelium grows into the connective tissue and its secretory products are able to accumulate in it inducing not only alkaline phosphatase activity but also osteogenesis.

Thus the results we have obtained on the transplantion of transitional epithelium in the rat reinforce the view proposed previously of the relationship between its histogenic activity and secretory (possibly incretory) function.

SUMMARY

In transplanting the mucous membrane of the urinary bladder to rats under the fascia of the anterior abdominal wall osteogenesis was induced not earlier than in 18-20 days, whereas in guinea pigs – in 10 days; the bone tissue development occurs in about 10% of the cases. Morphology and histochemistry of osteogenesis induction are very much alike in guinea pigs and rats. Low inducing activity of the rat transitional epithelium may be explained by the close contact of transitional epithelium and the vascular network in these animals. Such relationship is easily explained if to accept that the production of the inducing substance-factor from the epithelium is constant.

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