THE HUMORAL NATURE OF THE OSTEOGENIC ACTIVITY OF TRANSITIONAL EPITHELIUM

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The ability of transitional epithelium to induce osteogenesis was found in ligations of the renal vessels [10] and in grafts of mucous membrane from the urinary bladder [7]. Osteogenesis occurred in points at which there was contact between connective tissue and the deep ingrowths of cells of transitional epithelium at a particular level of differentiation [1]. In the zone of induction the cytoplasmic contents of the epithelial cells were seen to separate [2] and a distinctive secretion of a substance having a histochemically characteristic glycogen was observed [3]. The question now arises as to whether direct contact between the epithelial cells and connective tissue is necessary for induction, and whether the epithelium contains a substance which preserves its active power in the absence of the living epithelial cells. Attempts have been made to induce bone formation by means of cell-free homogenates of urinary bladder mucous membrane extracts, or with a secretion obtained experimentally from transitional epithelium; until now, all such experiments have failed, and nothing but zones of extramedullary myelogenesis developed [4].

In the work reported here, homografts of the epithelium were made in diffusion chambers. The results obtained indicate that transitional epithelium does indeed form and evidently liberates continuously a substance which is capable of inducing bone formation quite independently of any contact with epithelial cells.

EXPERIMENTAL METHODS

Diffusion chambers [5] made up of two filters (Millipore NA) 150 μ thick having pores of 0.45 μ were implanted under the skin of adult guinea pigs of an impure strain. Cells were introduced into the chambers in Hank's solution. The cells were obtained from triturated urinary bladder mucous membrane or from whole bladders with the mucrous membrane turned outwards, by treatment with trypsin. In the first case the suspension consisted of epithelial and connective cells, and in the second almost exclusively of epithelial cells. Each chamber received approximately $2 \cdot 10^4$ cells. By the 30-40th day, the chambers together with the surrounding tissue were fixed in 80% alcohol and embedded in paraffin. Serial sections were stained in Schiff's iodic acid, and counter-stained with hematoxylin and alum, and treated for alkaline phosphatase by Gomori's method.

EXPERIMENTAL RESULTS

Growth of the epithelium was observed in 12 out of the 20 chambers fixed at the 30-40th day. In eight, osteogenesis had occurred in the surrounding connective tissue. No trace of epithelial cells was found outside the chamber. In no one of the eight cases (in which no growth of transitional epithelium was to be found outside the chamber) did any formation of bone occur outside the chamber. In addition, we carried out a histological investigation of the results of implantation of various derivatives of connective tissue into forty-six chambers. The chambers were fixed on the 25th and 50th day; in no single case was there any bone formation.

Within the chamber containing the transitional epithelium there was a growth of epithelial membranes with the 3-layered differentiation typical of transitonal epithelium and the corresponding histochemical features of the cells of the different layers (Fig. 1a, b, c). In some of the chambers in addition to the epithelial layers there was an outgrowth of connective tissue cells.

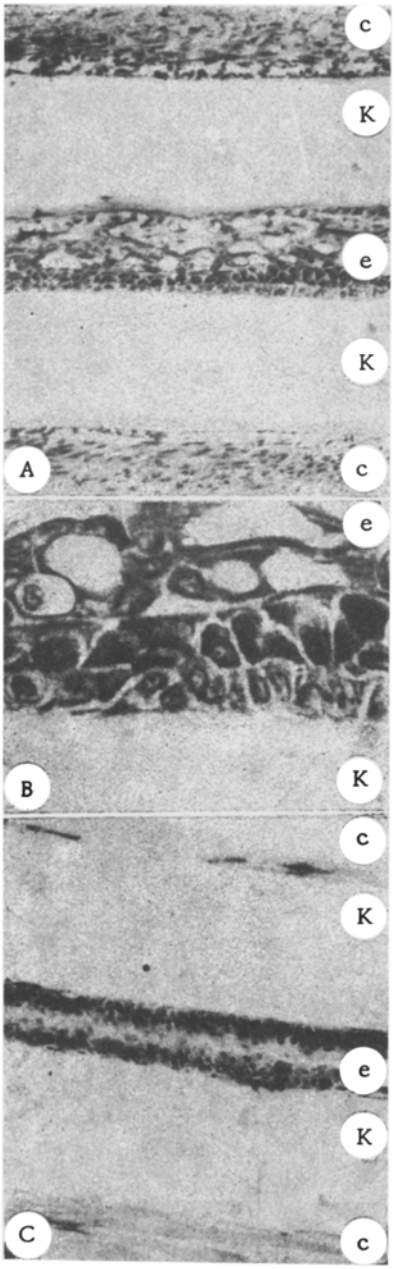

Fig. 1. Diffusion chamber, 30 days after implantation. A) Schiff iodic acid and hematoxylin. Objective 10 ×; B) same. Objective 20 ×; C) Gomori's stain. Objective 10 ×. K) Filter forming wall of chamber; e) transitional epithelium within the chamber; c) connective tissue around chamber.

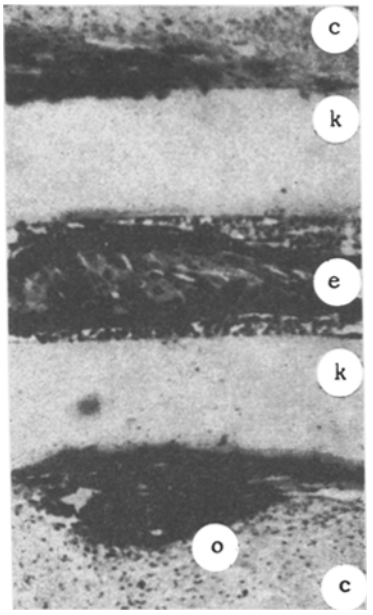

Fig. 2. Zone of osteogenesis outside the chamber on the 35th day after implantation. o) Body tissue induced outside the chamber. Remaining indications as in Fig. 1. Schiff iodic acid and hematoxylin. Objective 20 ×.

No bone formation was ever observed within the chamber.

On the 30th day the proliferative processes in the connective tissue surrounding the chamber were very slight, and the reaction for phosphatase was negative. Against this background zones of typical osteogenesis could be distinguished in which the Gomori reaction was positive (Fig. 2). The zones consisted of osteoblasts and trabeculae of ground substance giving a characteristic Schiff reaction. Some of them were in contact with the surface of the filter, and others lay at a distance of about 50 μ from it (Fig. 3). Within the chamber opposite the induction zones there was an epithelium containing a large amount of glycogen.

Our own experience and that of other workers show that the chamber itself does not induce osteogenesis. Furthermore, even solitary epithelial cells are easily revealed in the connective tissue, especially by use of the Schiff or Gomori reactions. Therefore the absence of epithelium outside the chamber and the integrity of the filters was evidence that the inductive action could be produced only by the epithelium lying within the chamber.

Therefore transitional epithelium is responsible for skeletal induction brought about by liberation from the cells of a substance which passes through the filter and which acts in the same way as has been demonstrated for the activity of the mesonephros [9] and osteogenic tissue [8].

There is reason to suppose that the transitional epithelium of a normal urinary bladder or the epithelium in the diffusion chamber forms and liberates an inductive substance. The results reported show that when the epithelium is separated from the vessels, in the underlying connective tissue a concentration of inductive substance developed which is sufficient to induce osteogensis at a distance of at least 150 μ from the epithelium. We are now required to determine not only the chemical nature but also the physiological role of this inductive substance, and to find whether it consists of a single hormone [6]; we also need to know the reason why the connective tissue which enters the chamber and the mucous membrane of the urinary ducts do not ossify under normal conditions. The reason may be that under normal circumstances the inductive substance is liberated chiefly into the vessels; alternatively it may be that the excessive concentration of this substance makes it ineffectual. We are studying these possibilities at the present time.

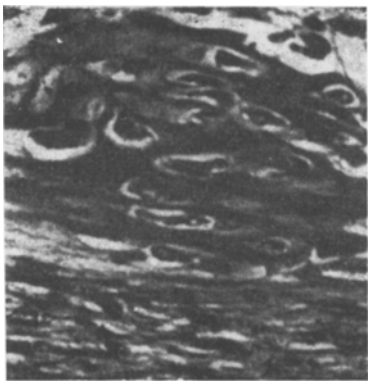

Fig. 3. Zone of osteogenesis outside the chamber on the 30th day after implantation. Stain Schiff iodic acid and hematoxylin.

SUMMARY

In a culture of transitional epithelium in a diffusion chamber, osteogenesis was induced in the connective tissue surrounding the chamber. This result proved that the epithelium produced a substance which penetrated through the filter, and retained inductive properties at a distance of at least 150 μ from the epithelial cells. The humoral nature of the osteogenic activity of transitional epithelium may therefore be considered as proved. To conclude, other related problems are listed.

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