

during life, a view held by OPPEL<sup>1</sup>, and possibly representing an inadequate regenerative capacity in intestinal epithelial cells. Recent studies<sup>2</sup> have shown that epithelial cells of the intestine are involved in the elaboration of a secretory product which may have a role in defence against the inimical environment represented by intestinal contents. A possible role for the thickened basement membrane seen in the platypus as a mechanical glycoprotein-containing barrier merits further investigation, for the areas of gut in which the basement membrane was strikingly prominent and thickened are those exposed to intestinal contents and susceptible to loss of surface epithelium.

**Zusammenfassung.** Es werden die besonderen strukturellen Eigenschaften der Basalmembran beschrieben, die unter dem Mucosaepithel aller Darmregionen (Magen bis Rectum) des Schnabeltiers (*Ornithorhynchus anatinus*) liegt.

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## Bone Induction Evoked in Mouse by Xenogeneic Grafts of the Transitional Epithelium

The aim of this work was to establish facts concerning two problems: a) the problem of interspecies specificity of the inductor of osteogenesis produced and released by the grafts of transitional epithelium; b) the problem of low inductive potency of transitional epithelium of mouse or/and low sensitivity of mouse connective tissue for the inductor.

In numerous previous experiments it was shown that bone tissue develops in the vicinity of the autogeneic graft of urinary bladder mucosa<sup>1-4</sup>. There is considerable evidence that this bone formation depends on the transitional epithelium activity. Allogeneic grafts<sup>5-7</sup> have evidently weaker potency to induce bone tissue; for example, in the dog these grafts do not elicit bone formation<sup>8,9</sup>.

No positive results have been obtained with xenogeneic grafts of transitional epithelium<sup>10-12</sup>. In this case, the grafts were destroyed within relatively short time because of histoincompatibility. Thus it was impossible to establish whether the inductor produced and eventually released by transitional epithelium may exert its activity across species barrier.

On the other hand, it was found<sup>4</sup> that even autogeneic grafts of urinary bladder wall in mouse caused bone induction extremely seldom. The question has been posed that either mouse connective tissue is less sensitive to inductor or mouse transitional epithelium has very weak inductive potency. The latter is suggested by the fact that established epithelial cell lines induce cartilage and bone formation in mice quite frequently<sup>13-15</sup>.

**Material and methods.** Small pieces (1 × 1 mm) of urinary bladder mucosa obtained from dogs, guinea-pig, syrian hamster or mice were grafted intramuscularly into mouse hind legs. The transitional epithelium of the aminocreated species a post from mouse<sup>4</sup> used in these studies has apparently high inductive properties when grafted into autogeneic or allogeneic recipient<sup>3-8</sup>.

In one experiment suspension of trypsin isolated transitional epithelium cells of dog (approx.  $5 \times 10^6$ ) was injected i.m. into hind leg of mouse. The grafts of canine endometrium or hamster seminal vesicle were used as a negative control. Experiments were carried out on mice of both sexes belonging to the following inbred strains: B10D2, B10LP, BN, C57B1/ScSn, CF/W and C3H. At the time of grafting, recipients of graft were treated with 5 mg of cortisone<sup>14-16</sup> and the same dose of the drug was repeated 8 days after grafting. The mice were killed at various time intervals after grafting and the grafts with surrounding tissues were examined histologically.

**Results and discussion.** As can be seen in the Table, grafting of urinary bladder mucosa of dog, guinea-pig or syrian hamster into mice treated with cortisone resulted in cartilage and bone formation in the vicinity of transplant (Figure 1). The sequence of events were similar to those observed after grafting xenogeneic established epithelial cell lines<sup>13-15</sup>. The cartilage which appeared on the 10th day was subsequently substituted by bone tissue. However, as late as on the 36th day after grafting, cartilage was still present. 3-4 weeks after grafting among the bone trabeculae bone marrow-like tissue was found. The grafted transitional epithelium survived nearly 3 weeks and often formed cysts. Grafts of isolated transitional epithelium of dog caused cartilage and bone induction just as often as the transplants of whole urinary bladder mucosa (Figure 2).

The bone induction was obtained only in 1 out of 21 allogeneic grafts of murine urinary bladder mucosa. No induction was observed when xenogeneic endometrium or seminal vesicle were grafted, though the epithelial cells of such grafts survived as long as the grafted transitional epithelium.

The following two conclusions could be formulated on the basis of these results: 1. The data obtained show clearly that osteoinductive properties of the transitional epithelium can be revealed in xenogeneic system, i.e. that inductor does not possess species specificity. Since both urinary bladder mucosa and established epithelial cell lines (WISH, F1, KB, HeLa) evoke bone induction morphologically in a very similar way, it seems that the nature of bone inductor in these two systems is similar if not identical. On the other hand it should be mentioned

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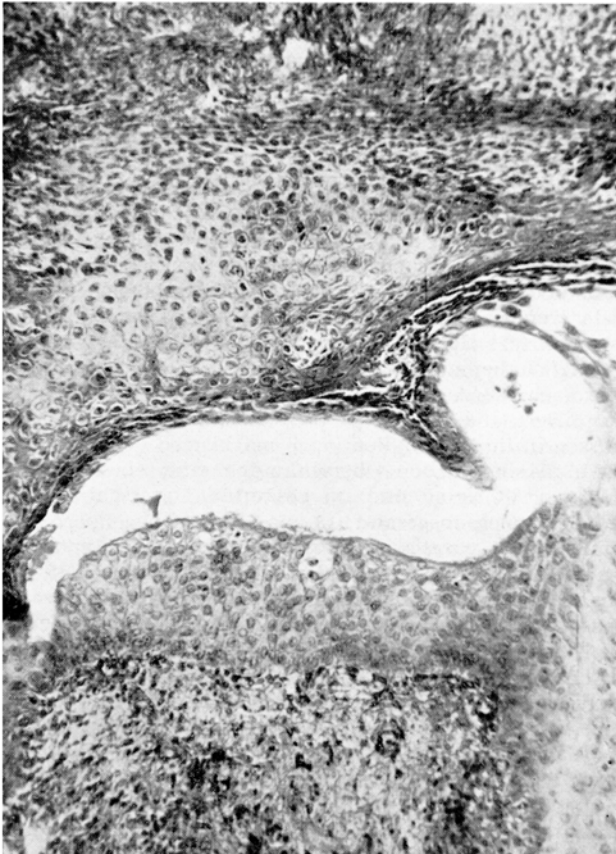


Fig. 1. Cartilage and bone induction in the vicinity of newly formed epithelium covering the cyst. 2 weeks after xenogeneic graft of urinary bladder mucosa of guinea-pig. H. + E. Magn. Obj. 20 Oc. 10.

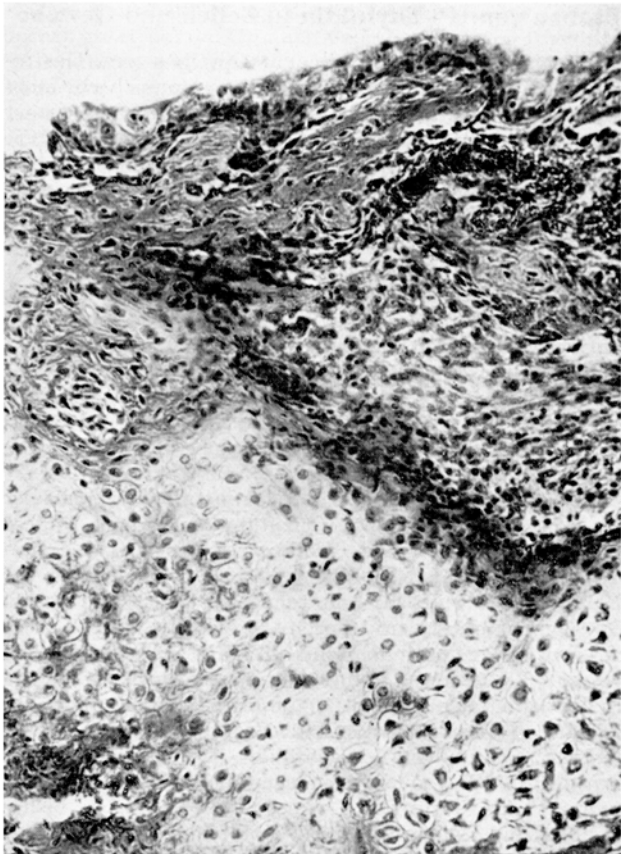


Fig. 2. Skeletal tissues induced on the 14th day after grafting of isolated transitional epithelium of dog. On the left, the isle of epithelium, on the right hypertrophic cartilage which undergo ossification on the periphery. H. + E. Magn. Obj. 20 Oc. 10.

Graft	Days after grafting	No. of grafts	Epi-thelium lage (number of grafts in which present)	Carti-lage	Bone tissue
Urinary bladder mucosa of dog	10-12	9	9	8	0
	13-18	14	13	13	10
	21	9	5	8	8
	24-35	6	4	6	6
Isolated transitional epithelium of dog	10	6	6	6	4
	14	7	6	7	6
	39	8	0	0	8
Urinary bladder mucosa of guinea-pig	14	10	10	3	4
Urinary bladder mucosa of hamster	18	5	4	2	5
Urinary bladder mucosa of mouse	10	3	3	1	1
Urinary bladder mucosa of mouse	14	6	5	2	3
Endometrium of dog	14	10	10	1	1
Seminal vesicles of hamster	28	11	11	0	0
	9	4	4	0	0
	18	4	4	0	0
Seminal vesicles of hamster	26	6	3 (trace)	0	0
	9	4	4	0	0
	14	6	6	0	0

that these two morphological pictures of bone formation observed after auto- or allogeneic grafts of transitional epithelium and after xenogeneic grafts was slightly different. In case of xenogeneic grafts, cartilage is firstly induced and then substituted by bone tissue, while after allogeneic grafts primarily bone tissue is formed<sup>7</sup> and only sporadically cartilage<sup>3</sup>. There are not enough experi-

mental data to offer any explanation for these differences. 2. According to the data presented a weak bone induction after transplantation of auto- or allogeneic transitional epithelium of mice can be explained by low inductive properties of this epithelium. On the same grounds, it seems reasonable that insufficient reactivity of mouse connective tissue can be excluded. Having in mind the results reported above, the opinion that mice are not suitable animals for the studies on bone induction seems to require reconsideration<sup>4</sup>.

*Résumé.* Des épithéliums de muqueuse vésicale de chien, de cobay et de hamster ont été greffés sur des souris préalablement traitées à l'acétate de cortisone. L'induction par ces greffes d'un cartilage et d'un tissu osseux prouve que l'inducteur d'ossification formé et libéré par ces épithéliums manque de spécificité générique. On a montré que le faible pourcentage de l'induction d'ossification est imputable à la faible capacité inductive de cet épithélium et non au manque de sensibilité du tissu conjonctif induit de la souris dans le système allogénique.

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