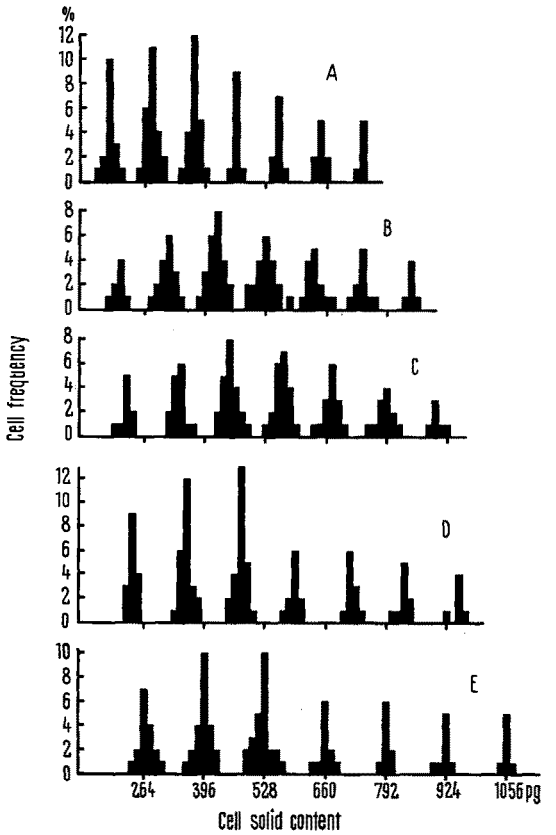

Fig. 2. Hepatocytes of 120-day-old golden hamster. As in Figure 1.

(1 pg = 1 g × 10^{-12}). On the contrary, it is 118.8 pg, 112.2 pg, 105.6 pg and 92.4 pg when the liver tissue was incubated in PCM supplemented with 0.4105 *M*, 0.2736 *M*, 0.1368 *M* and no glycerol, respectively.

4. Consequently, it is apparent that the hepatocytes of the same class weigh differently according to the isolation method (dispersion in PCM, PCM + G, anhydrous glycerol).

5. Thus weight differences are as higher as the glycerol concentration in the incubation medium is lower: under our experimental conditions, −30% when no glycerol was added, −20.4%, −14.8% and −10% when 0.1368 *M*, 0.2736 *M* and 0.4105 *M* glycerol was supplemented, respectively.

6. On the basis of the Student's *t*-test, the weight differences observed are highly significant ($P < 0.001$).

We can therefore conclude that the addition of glycerol to the PCM reduces the loss of protoplasmic substances in the incubation fluid. This effect is the same for both, rat and hamster hepatocytes.

Riassunto. Epatociti isolati mediante dispersione meccanica dopo incubazione del tessuto epatico in un mezzo acquoso contenente tetrafenilborato di Na, un agente chelante il K, perdono circa il 30% del loro contenuto in sostanze solide. L'aggiunta al mezzo di incubazione di glicerolo riduce tale perdita.

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An Unusual Basement Membrane Underlying Intestinal Epithelium of the Platypus (*Ornithorhynchus anatinus*)

The functional role or roles of the basement membrane which separates epithelial and connective tissue components of the gastrointestinal mucous membrane is far from clear. In mammalian gut 2 relatively distinct components can be distinguished, a narrow layer of collagen fibres surmounted by a thin basal lamina which is demonstrable only at high resolution, and these apparently represent contributions from non-epithelial and epithelial tissues respectively.

In the platypus (*Ornithorhynchus anatinus*), the basement membrane subjacent to the surface epithelium of the intestines is especially prominent[1]. Histochemical and ultrastructural studies reported here show that it presents a number of unusual features.

Specimens of stomach, duodenum, mid-jejunum, terminal ileum, colon and rectum were removed under anaesthesia from 1 adult female and 3 adult male animals, all of which appeared free of disease. For light microscopy tissues were fixed in cold 10% neutral formalin or Bouin's solution, or were freeze-dried. Following routine processing and embedding in paraffin, sections were stained by each of the following: haematoxylin and eosin; PAS, with and without prior digestion with diastase; Alcian blue (pH 1.0 and 2.5) either alone or combined with PAS; toluidine blue (pH 3.5, 7.0 and 9.0); aldehyde fuchsin, with or without prior oxidation with potassium monpersulphate; Verhoeff's elastin stain; Van Gieson stain; and Masson's original trichrome. In addition, unstained sections were examined microscopically under polarized light, and sections of freeze-dried tissues were examined for the presence of amine fluorophores using the formaldehyde-induced fluorescence histochemical technique[2]. For ultrastructural study small blocks of mucosa were fixed in 4% phosphate-buffered glutaraldehyde and subsequently treated in osmium tetroxide[3]. Thin sections were stained with uranyl acetate and lead citrate[4] and examined with a Siemens Elmiskop 1 A electron microscope.

The basement membrane situated beneath the surface epithelium was found to be uniformly thickened throughout the intestines. It was continuous with a much less prominent basement membrane enveloping the intestinal glands. In respect of structure and histochemical reactions the glandular basement membrane was indistinguishable from that found in the stomach of the platypus and in the gastrointestinal tract generally of other mammals. The basement membrane associated with the

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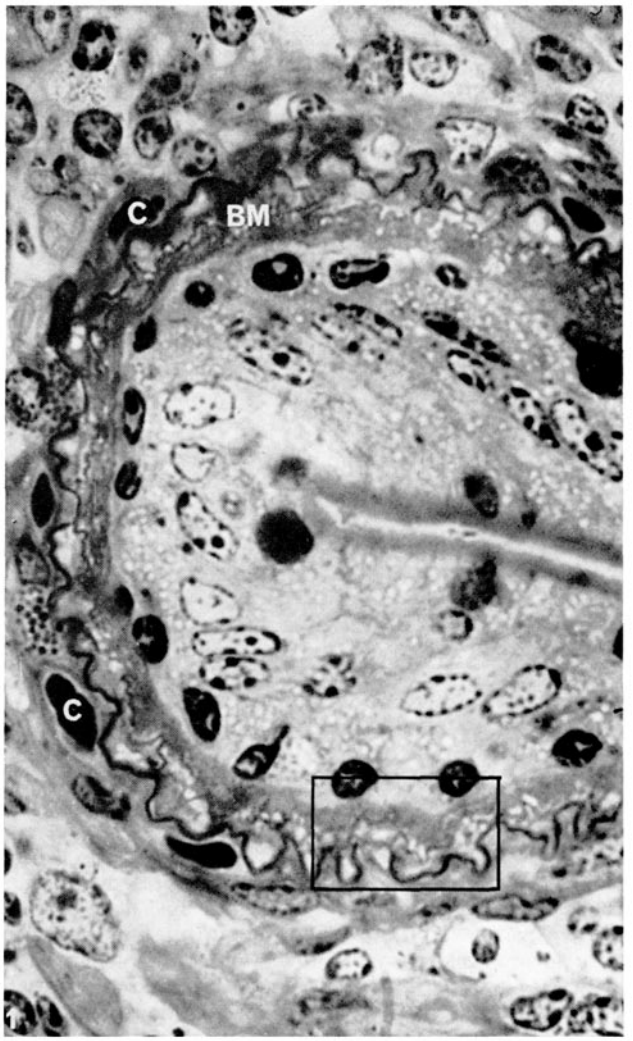

Fig. 1. An indentation in the surface epithelium of a duodenal villous fold illustrating the prominence of the basement membrane (BM) and the adjacent plexus of capillary vessels (C). The epithelium is pseudo-stratified in type. Toluidine blue. ×1200.

surface epithelium was 10–20 μm thick and contained several undulating strands within an apparently acellular matrix (Figure 1). Staining of the basement membrane could not be obtained with the use of Alcian blue, toluidine blue, or Verhoeff's elastin stain. A light staining reaction was obtained with aldehyde fuchsin following oxidation, and fibre-like components were seen within the membrane after the use of either Van Gieson or PAS stains; a less marked PAS-reactivity was also present throughout the remainder of the membrane. Bright green-yellow formaldehyde-induced fluorescence, representing enteric nerve fibres including those accompanying blood vessels, was seen in the lamina propria but was not found either within or immediately adjacent to the membrane. A dense network of capillary vessels and numerous polymorphonuclear leucocytes, lymphoid cells and mast cells were located in loose connective tissue immediately beneath the membrane.

Ultrastructurally, a continuous basal lamina was situated immediately subjacent to the plasma membrane of the epithelial cells; it was of uniform thickness but irregular density. Several undulating and irregularly fenestrated lamellae, of which the most basal was generally the most prominent, were seen between the basal lamina and the adjacent lamina propria; they comprised homogeneous electron-dense material (Figure 3) and were located within a broad zone containing a filamentous ground substance, some collagen fibres and attenuated cell processes.

There appear to be no reports in other mammals of an intestinal basement membrane comparable to that seen in the platypus. In one species of opossum, *Didelphis virginiana*, a thickened basement membrane is seen at the fundus of intestinal glands in the upper part mainly of the intestinal tract and ultrastructurally has the appearance of amorphous ground substance[5].

In the intestines of the platypus, despite careful fixation of fresh tissues, there is often widespread absence of surface epithelium so that the basement membrane abuts on the lumen. Thus in the platypus the possibility exists of an incomplete epithelial lining to the intestine

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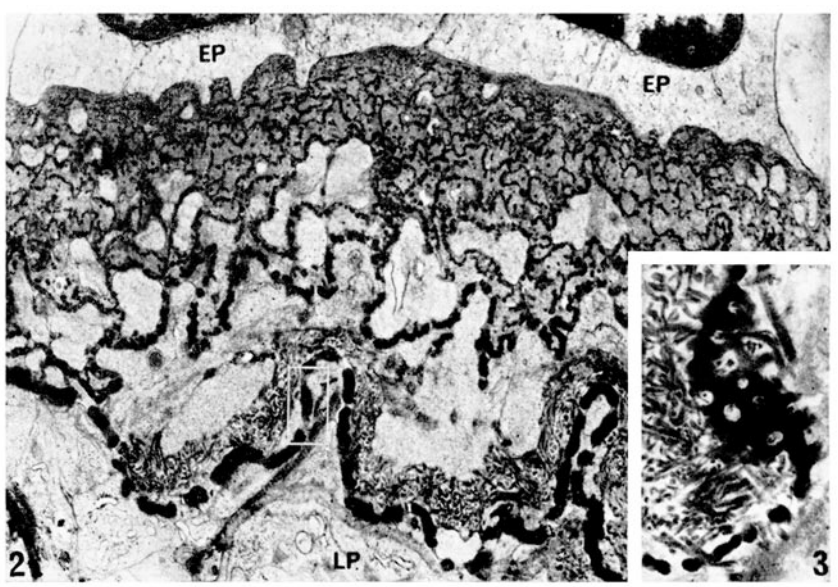

Fig. 2. An electron micrograph from an area similar to that outlined in Figure 1, illustrating components of the lamina propria (LP) and of the membrane underlying two basal epithelial cells (EP). ×11,500.

Fig. 3. A tangential view of a constituent lamella from an area similar to that outlined in Figure 2, showing numerous fenestrae traversed by collagen fibres. ×9,600.

during life, a view held by OPPEL[1], and possibly representing an inadequate regenerative capacity in intestinal epithelial cells. Recent studies[6] 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 oseogenens 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[1–4]. There is considerable evidence that this bone formation depends on the transitional epithelium activity. Allogeneic grafts[5–7] have evidently weaker potency to induce bone tissue; for example, in the dog these grafts do not elicite bone formation[8,9].

No positive results have been obtained with xenogeneic grafts of transitional epithelium[10–12]. 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[4] 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[13–15].

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

In one experiment suspension of trypsin isolated transitional epithelium cells of dog (approx. 5×10^6) 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[14–16] 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[13–15]. 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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