DISTRIBUTION OF MUCOPOLYSACCHARIDE AND ALKALINE PHOSPHATASE IN TRANSITIONAL EPITHELIA*

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Morphologically little differentiation of cell types has been described in the transitional epithelia of the urinary tract, beyond the occurrence in the outer layer of the epithelium of frequent binucleate cells which possess a cuticular border (15).

During a survey of the distribution of mucopolysaccharides in epithelia, we were interested, therefore, to find prominent periodic acid-Schiff (PAS) positive, diastase-fast granules in all the cells of the outer layer of the epithelium of the frog's urinary bladder, and the absence of these PAS positive granules in the lower layers. The investigation was extended to the transitional epithelia of several mammalian species, in which PAS positive material was found to be similarly distributed (8).

The occurrence of strong alkaline phosphatase activity in the mammalian urinary bladder epithelium was described by Gomori (4). Bourne (1) and Goldi (3) obtained similar findings but noted the absence of this enzyme in the superficial layer of the mammalian urinary bladder epithelium. We have confirmed these findings with respect to the distribution of alkaline phosphatase in the transitional epithelia of several different species of vertebrates.

The data described in this communication reveal that the outermost layer of cells of transitional epithelia differ strikingly in their histochemical properties from the cells of the lower layers.

METHODS

Small portions of the urinary bladder, ureter, and kidney pelvis were obtained from anesthetized frogs, rats, guinea pigs, rabbits and dogs, and from human cadavers 4-8 hours postmortem.

The periodic acid-Schiff reaction according to McManus (7) was applied to 4μ thick sections of tissues which had been fixed in each of the following: Carnoy's fluid, saturated HgCl₂ in 5% acetic acid, and Gendre's fluid (5).

To remove glycogen, sections were incubated 30 to 60 minutes in a solution of 0.2% diastase in 0.02 M phosphate buffer at pH 6.0 and 37°C. (5). Aqueous toluidine blue (0.5%) was used to test for metachromasia (13).

The PAS reaction was also carried out on unsectioned preparations of the urinary bladder of the frog (e.g. Fig. 1). These were prepared by stretching and tying portions of the fresh bladder on small stainless steel rings. Good cellular preservation was obtained by transferring the fresh stretch preparations directly to an approximately isotonic solution containing 0.5% periodic acid and 0.6% NaCl.

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Fig. 1. Surface view of stretch preparation of frog's urinary bladder. X1250, showing PAS positive granules immediately underlying luminal surface of outermost layer of cells.

Alkaline phosphatase was demonstrated according to the method of Gomori (4) using an incubation period of 15 minutes on 4μ thick sections of tissues which had been fixed in cold 80% ethanol and embedded in paraffin at 55°C.

RESULTS

In all the species investigated, a diastase fast periodic acid Schiff (PAS) positive material was found in the cells forming the superficial layer of the transitional epithelia lining the lumen of the urinary tract. Specifically, the following tissues were investigated: the urinary bladder of the frog, rat, dog, rabbit, guinea pig and man, the ureters of the rat, dog and man, and the pelvis of the kidney of the rat, rabbit and man.

In the urinary bladder of the frog the diastase-fast PAS positive substance is located in conspicuous granules (Fig. 1), which are located in a narrow cortical zone just under the luminal surface (Fig. 1). These granules evidently correspond to the prominent refractile granules seen in preparations of the fresh living epithelial cells.

In the mammalian species the diastase-fast PAS positive material is finely granular and diffusely distributed throughout the cytoplasm of the superficial layer of cells (Figs. 7, 10 and 13). Whether or not the granular appearance of the PAS positive material in the mammalian species is due to precipitation by the fixatives remains undetermined.

In all the species examined PAS positive diastase-labile material occurs in the inner layers of the transitional epithelia. Small amounts of diastase-labile material are also present in the surface layer of cells, except in the frog and rat. In these species the amount of diastase-labile material in the superficial layer of cells is negligible. The diastase-labile material may be glycogen, since the PAS reaction of this substance is blocked by prior bromination (5). A peculiar feature, however, is that a material with similar characteristics is present in the lumen of the ureters. Further evidence, therefore, is needed to establish the nature of the diastase-labile material.

Sections treated with toluidine blue showed no metachromasia. However, a positive reaction with the ferric mannitol technique of Lilie and Mowry (5) at pH 6.0 was obtained. The PAS positive material in the urinary bladder of the rat also gave a positive reaction when anhydrous chromyl chloride was used as the oxidizing agent in a new variant of the
Fig. 2, 3 and 4. Sections through epithelium of frog's urinary bladder. X283. Fig. 2 shows sections stained with hematoxylin and eosin. Epithelium several cells thick. Fig. 3 shows distribution of alkaline phosphatase activity. Staining occurs in basal layer of epithelium and subjacent connective tissue. Fig. 4 shows distribution of diastase-fast PAS positive material. This is restricted to a thin layer just under the luminal surface.

Figs. 5, 6 and 7. Sections through epithelium of rat's urinary bladder. X283. Fig. 5 shows section stained with hematoxylin and eosin. Epithelium three to five cells thick. Bordering the lumen is a layer of flattened cells with eosinophilic cytoplasm and deeply basophilic nuclei. Fig. 6 shows distribution of alkaline phosphatase activity. Inappreciable staining in outermost cells bordering the lumen, with maximal activity located in cells immediately below. Note stained walls of minute blood vessels where epithelium rests on underlying connective tissue. Fig. 7 shows distribution of diastase-fast PAS positive substances. Note the PAS positive cytoplasm and negative nuclei of cells bordering the lumen.

oxidation-Schiff reaction (9). This chromyl chloride method has been shown to give a positive reaction principally with glycogen and mucins. The histochemical tests indicate that the PAS positive material is a mucopolysaccharide, but, unlike the mucin of the intestinal tract, it is not metachromatic.

The distribution of alkaline phosphatase was studied in the same tissues on which the PAS reaction had been carried out. The phosphatase reaction is absent in the superficial
Figs. 8, 9 and 10. Sections through epithelium of dog's urinary bladder, X283. Fig. 8 shows section stained with hematoxylin and eosin. Fig. 9 shows distribution of alkaline phosphatase activity. Staining occurs only in basal layers of epithelium and in endothelium of small blood vessel immediately underlying the epithelium. Fig. 10 shows distribution of diastase-fast PAS positive material. This is restricted to the single layer of cells which borders on the lumen. The level where the basal layer of the epithelium rests on the connective tissue is demarcated by the PAS positive walls of small blood vessels.

Figs. 11, 12 and 13. Sections through urinary bladder of rabbit, X283. Fig. 11 shows section stained with hematoxylin and eosin. Epithelium several cells thick. Bordering the lumen is a layer of flattened cells with eosinophilic cytoplasm. Fig. 12 shows distribution of alkaline phosphatase activity. Inappreciable staining in the single layer of cells bordering the lumen. Basal layers of epithelium stain, with maximal activity located in the subepithelial connective tissue. Fig. 13 shows distribution of diastase-fast PAS positive material. This is restricted to the single layer of cells which borders the lumen.

layer of cells of the transitional epithelium, while a strong positive reaction is obtained in the lower layers of the epithelium, or in the subepithelial connective tissue at sites which varied according to the species (Figs. 3, 6, 9 and 12). In the human bladder, no reaction was observed at any of these sites. Gomori (4), however, found alkaline phosphatase present in the urinary bladder epithelium of man. Our failure to demonstrate its presence in the
human bladder epithelium might be due to postmortem changes prior to fixation. The walls of the small blood vessels were positive in the human, and strongly positive in the rat and dog (Figs. 6 and 9). An interesting feature is that, in those species where alkaline phosphatase was observed in the walls of the small blood vessels, the reaction was particularly strong in the immediate proximity of the urinary tract epithelium.

COMMENT

Under normal conditions, it has been generally assumed that the transitional epithelia do not participate in secretory activity, and that mucoid material in human bladder urine originates from a few scattered mucous glands located especially in the trigonal region of the bladder (6).

The presence of a diastase-fast PAS positive material in the superficial cells of the transitional epithelia described in this paper confirms the findings of Göldi (3), and Preto Parvis and Lucarelli (14), and suggests the possibility that one of the normal functions of these epithelia is the secretion of a mucoid material. Since this material does not react metachromatically with toluidine blue, it possesses staining properties which resemble, for example, salivary gland mucoid (13), rather than mucin of the intestinal tract.

Irritative or inflammatory lesions of the urinary tract in the human frequently are accompanied by the appearance of cells in the lining epithelium which morphologically resemble mucous secreting cells (2, 11, 12). This has been ascribed to a metaplastic transformation. An alternative explanation is that the condition represents heightened activity on the part of cells which normally elaborate a mucoid material.

An interesting aspect of our results relates to Moog and Wenger's (10) claim that alkaline phosphatase always occurs at sites where diastase-fast PAS positive material is also present. We observed, however, exceptions to this claim. Alkaline phosphatase activity occurs in the deeper layers of the urinary bladder epithelium of the frog and rat where the PAS reaction is negative (e.g., compare Figs. 3 and 4, 6 and 7). These data indicate that the presence of mucopolysaccharide is not essential for alkaline phosphatase activity.

BIBLIOGRAPHY