The ultrastructure of the epithelial cells of the toad urinary bladder has been described by several laboratories (Choi, 1963; DiBona et al., 1969a; Peachey and Rasmussen, 1961). This hormone-sensitive cell layer consists of four distinct cell types: (a) basal cells; (b) goblet cells; (c) granular cells; and (d) mitochondria-rich cells. The granular cells are the most numerous, comprising 80–85% of the total. The mitochondria-rich cells represent 10–15% of the total, with the remainder represented by the other two types. All cells, except the basal cells, have direct access to the bladder lumen.

Ultrastructural studies have also revealed changes in cell structure after the addition of the polypeptide hormone, arginine vasopressin (AVP), to the bathing medium. This hormone is known to increase transcellular sodium transport in this tissue and to increase its permeability to water as well as bulk water flow in the presence of a mucosal to serosal osmotic gradient (Leaf, 1967).

The effects of vasopressin on the epithelial cell layer have included the appearance of intracellular lakes during hormone-induced bulk H₂O flow (Grantham et al., 1971), cell swelling (Peachey and Rasmussen, 1961), intracellular granules migration, and increased glycocalyx deposition (Masur et al., 1972), as well as the appearance of an increased number of small intracellular vesicles in both the granular and basal cells.¹

In spite of considerable speculation concerning the functional roles of the different cell types and particularly the possible relationship between the granular and mitochondria-rich cells, no specific evidence has heretofore been reported that links, no specific evidence has heretofore been reported that links these cell types in either an anatomical or functional relationship.

¹ Davis, W. L., D. B. P. Goodman, and H. Rasmussen. 1974. The effect of cytochalasin B on the response of the toad bladder to vasopressin. J. Cell Biol. Submitted for publication.

Previous ultrastructural studies have employed standard transmission electron microscopy. The present report deals with studies of the ultrastructural architecture of the luminal (mucosal) surface of this tissue as determined by scanning electron microscopy. This investigation has revealed a repeating geometric arrangement of the surface architecture with units consisting of four or five flattened, polygonal cells surrounding a single smaller ovoid cell with distinctly different surface characteristics. In addition, scanning microscopy has revealed that the luminal processes on the granular cells, which appear as microvilli in transmission electron microscope sections, are in actuality a series of surface ridges. Finally, vasopressin, in addition to causing cell swelling, leads to a morphological alteration in these surface ridges.

MATERIALS AND METHODS

Female toads, *Bufo marinus* (National Reagents, Inc., Bridgeport, Conn.), were kept on moist bedding at room temperature. Hemibladders from doubly pithed toads were mounted on glass cannulae; the serosal surface was bathed in an aerated Ling-Ringer phosphate buffer of the following composition (millimeters/liter): NaCl, 92.7; KCl, 2.5; CaCl₂, 1.0; MgSO₄, 1.2; NaHCO₃, 6.4; Na₂HPO₄, 1.2; NaH₂PO₄, 2.0 (pH 7.4); 210 mosmol/kg H₂O. The mucosal medium consisted of one-fifth-strength buffer.

After a 1–2-h preincubation period, osmotic water flow was determined gravimetrically (Bentley, 1958). Vasopressin-AVP (Pitressin, Parke, Davis & Company, Detroit, Mich.), 65 nm/ml, was added to the serosa, and the weight loss, i.e., osmotic flow, was determined over the subsequent 30-min period.

For scanning electron microscopy, tissues were fixed 30 min after the addition of AVP by the simultaneous addition of concentrated glutaraldehyde (50%, Fisher Scientific Co., Pittsburgh, Pa.) to both the mucosal and serosal media to give a final glutaraldehyde concentration of 2%. Within 1 h, the tissues were removed from the annulare and placed in 2% glutaraldehyde, 0.1 M cacodylate buffer, pH 7.4, for 12–18 h. After three buffer washes, speci-
mens were postosmicated (90 min) in 1% osmium tetroxide, 0.1 M cacodylate buffer containing 5 mM CaCl₂. After washing, tissues were dehydrated in graded acetones and prepared for scanning electron microscopy by the critical-point CO₂ method (Anderson, 1951). Specimens were coated with gold-palladium and examined in an AMR scanning electron microscope operating at 20 kV.

RESULTS

In control tissue, numerous flattened polygonal cells were identified on the urinary surface (Fig. 1). The surface of these cells was covered by numerous branching, arborizing ridges separated by flattened zones or channels. Such structures coursed for great distances and often had a globular appearance. Also identified were small globular structures; these were often found in clumps or dispersed as globular chains. The globular entities were particularly prevalent at cell boundaries, and probably represent small stubby microvilli.

A second cell type was also identified (Fig. 1). This cell, much less numerous than the aforementioned flattened cells, was small and ovoid in shape. Its surface was characterized by the presence of numerous globules, i.e., microvilli.

FIGURE 1 Control preparation of toad bladder mucosal surface. Two cell types are clearly demonstrated. The central ovoid cell (O), characterized by distinct microvilli, is surrounded by four cells (1-4), each showing a more expansive lumenal surface area. In addition, the surface morphology of the latter cells consists of a complex system of arborizing ridges and channels. Junctional ridges (arrows) denote boundaries of adjacent cells. × 11,200.
A unique geometric relationship between the two described cell types was apparent. As indicated, the smaller cell was less numerous. However, when present, this cell was surrounded by four or five flattened polygonal cells. Such a pattern was consistently repeated across the epithelial surface.

After the addition of AVP several prominent structural changes occurred in the flattened polygonal cells (Fig. 2). The cells were now rounded or elongated; most appeared swollen. Deep crevices delineated the cell boundaries. Conspicuous changes in the cell surfaces were also apparent. The channels and ridges seen in hormone-free tissues were absent; instead, numerous microprojections were now seen. These structures appeared to be true microvilli. In addition, spiculated projections and longer attenuated processes were also seen. No changes were observed, however, in the smaller ovoid cell type. The changes in the cell surface were not a secondary consequence of cell swelling since morphological alterations similar to those shown in Fig. 2 were observed in tissue treated with AVP in the absence of an osmotic gradient.

**DISCUSSION**

Two major points reported in the present study should be emphasized: (a) the relationship of the cell types revealed by scanning electron
microscopy observation of the bladder surface; and (b) the changes in surface architecture induced by vasopressin.

As is clearly seen, there are two predominant cell types with projections onto the luminal surface. The first and most numerous is the large flat polygonal cell with extensive ridgelike surface processes. The second is the small ovoid cell, much less extensive in total surface, and possessing on its surface individual projections or microvilli. On the basis of previous (Choi, 1963; DiBona et al., 1969 a; Peachey and Rasmussen, 1961) and present data, the large polygonal cells almost certainly represent the granular cells described in transmission electron micrographs, and the ovoid cells represent the mitochondria-rich cells. The latter conclusion is in keeping both with the relative numbers of these cells seen in ordinary transmission electron micrographs and with the fact that the mitochondria-rich cells have the characteristic appearance, in transverse section, of an inverted flask with a narrow neck of tissue projecting to the surface.

The most impressive feature of this surface architecture is that each ovoid or mitochondria-rich cell is surrounded by four or five polygonal or granular cells, and that this type of multicellular unit is consistently repeated over the entire surface. This very striking anatomical relationship argues for a distinct functional relationship between these two cell types. Further experimental work, however, is needed to define their functional interaction.

The other notable feature of the surface structure is the ridgelike network on the surface of the granular cells. This surface view gives a distinctly different notion of the extent of surface processes on these cells than that obtained from viewing transmission micrographs. This structural feature is of interest because it has been a widely accepted view that one of the consequences of vasopressin action is a distinct change in the permeability of the luminal cell surface to both Na+ and H2O (Leaf, 1967). It is, therefore, of considerable interest that after vasopressin addition there were both a distinct swelling of the granular cells and a striking change in their surface architecture characterized by a transformation of the extensive ridgelike network into distinct processes resembling microvilli.

As far as is possible to determine, the present study indicates that the mitochondria-rich cells do not undergo any significant structural alteration after vasopressin addition. Thus, it remains a distinct possibility, as previously proposed (DiBona et al., 1969 b), that the granular cells are the major site of vasopressin action in this tissue. Clearly, in the light of the newly discovered anatomical relationship between these two cell types, it is necessary to reexamine the effects of this hormone upon each specific cell type.

It is of interest that thyrotropin induces changes in the shape and length of the surface processes of thyroid acinar cells (Ketelbant-Balasse et al., 1973). It is, therefore, possible that activation of a variety of transporting tissues by specific hormonal or humoral stimuli may lead to significant changes in cell surface architecture.

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