The Morphologic Relationship of Light and Dark Cells of the Collecting Tubule in Potassium-Depleted Rats

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The luminal surface of collecting tubule cells in the inner stripe of the renal medulla in normal and potassium-depleted rats was studied by scanning electron microscopy. In normal rats the luminal surfaces were of two types. One cell type was sparsely covered with small bulbous microvilli and had either a single or double cilium. This type corresponds to the light cell seen in transmission electron microscopy. The second cell type was covered by prominent microplicae and represents the dark cell observed in transmission electron microscopy. In potassium-depleted animals, numerous cells with a morphologic appearance of intermediate forms were identified. By scanning electron microscopy, the luminal surface of these cells was covered by a mixed population of villi and microplicae in different stages of development and often showed cilia, which were previously considered to exist only on light cells. On the basis of these morphologic findings, we conclude that the dark and light cells are not different cell types but rather represent different forms of a single type of cell. (Am J Pathol 84:317–326, 1976)

STUDIES OF CHANGES in the cells of the kidney with potassium depletion have contributed to the understanding of the range of reactive patterns as well as adding insight into the potential of individual cells. Initially, the renal lesion was characterized as a fatty degeneration and hydropic vacuolization in the renal cortex.[1] Improved fixation and better sampling, however, revealed prominent collecting tubule droplet change near the renal papilla.[2] Subsequently, droplets were demonstrated in interstitial cells, capillary endothelial cells, as well as in the covering epithelial cells of the papilla in the kidneys of potassium-depleted animals.[3]

More recent studies of changes in the inner stripe of the outer medulla have revealed proliferation of the collecting tubule cells.[1,4,5] We have extended these studies by using light, transmission, and scanning electron microscopy to clarify the nature of the reactive cell and the distinctive pathologic alteration characteristic of this zonal pathologic lesion.

The concept that the epithelial cells of the collecting tubule are composed of two distinct types of cells with different functions has been the

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object of controversy for many years. The presence of two types of cells was first noted in 1876 by Schachowa, who observed that some cells in the collecting tubules stained darker with hematoxylin and eosin.[6] This finding was later confirmed by the light microscopy findings of others.[7,8] The terms *light cell* (principal) and *dark cell* (intercalated) were based on the studies of transmission electron microscopists who observed that dark cells contained more organelles and possessed a more electron-dense cytoplasm.[9-12] Dark cells, which are less numerous than the light cells, are more frequently found in the renal cortex and decrease in number toward the outer zone of the medulla, disappearing entirely in the papilla.[11] Although the transmission electron microscope (TEM) discerned differences in the surfaces of these cells, it was only with the introduction of the scanning electron microscope (SEM), which permitted the visualization of the entire cellular surface, that the morphology of these cells was more precisely defined. With scanning microscopy the surfaces of the light cells appear to be covered by short sparse microvilli with one, and occasionally two, centrally located cilia. The surface of the dark cells is covered with microplicae or folds,[13-15] or with a dense population of finger-like projections.[14]

The object of the present study is to establish the existence of intermediate forms as evidence that the light and dark cells may represent different forms of the same cell.

Materials and Methods

Fifteen male Sprague-Dawley rats (Sprague-Dawley Farms, Madison, Wisc.) weighing 200 to 300 g were depleted of potassium by the *ad libitum* feeding of a potassium-restricted diet (General Biochemicals, Chagrin Falls, Ohio). Eight normal controls received the same diet supplemented with potassium (1.2 g KCl/100 g diet). All rats were allowed deionized water *ad libitum*.

At 6 weeks after initiation of potassium-free feeding, rats were anesthetized by intraperitoneal injection of 25 mg of sodium thiamylal solution (Parke, Davis and Company, Detroit, Mich.). When anesthesia was attained, the abdominal aorta was cannulated, and the kidneys were fixed *in situ* by retrograde perfusion (Method 1 in Griffith *et al.*[16]). The technique was modified in that a perfusion pressure of 200 mm Hg was used to obtain complete perfusion of the renal medulla. The pressure was controlled with a manometer throughout the procedure. The fixative used was 1.5% gluteraldehyde in 0.08 M sodium cacodylate buffer, and the osmolarity was adjusted to 650 milliOsmoles with sodium chloride. This osmolarity was considered to be optimal for the study of the inner stripe of the outer zone of the renal medulla.[17] After 15 minutes of perfusion, the kidneys were removed and processed. For SEM, specimens were cut into 5-mm slices and left in gluteraldehyde for 12 hours at 4 C. After fixation, each block was cut once and washed overnight in the same buffer used for perfusion containing 0.2 M sucrose. Specimens were postfixed for 1 hour in 1% osmium tetroxide buffered with *s*-collidine (pH 7.4). Samples were dehydrated in graded series of ethanol through absolute alcohol and then in increasing concentrations of amyl acetate (50, 70, 95, and 100%) for 30 minutes. Critical point

drying was done with carbon dioxide, using a Bomar SPC-5 critical point drying apparatus. The specimens were mounted and coated with 300 Å of gold using a Conductivac I microcoater (Seevac Inc., Pittsburgh, Pa.), and examined at 25 kV with a Hitachi HFS-2 scanning electron microscope. For TEM, portions of each kidney were taken after perfusion, fixed for 2 hours in 1.5% gluteraldehyde, postfixed in osmium tetroxide, dehydrated in ethanol, and embedded in Epon epoxy resin.[19] Ultrathin sections were cut with diamond knives on an LKB 2 ultramicrotome, stained with uranyl acetate [20] and lead citrate,[21] and examined at 60 kV with a Siemens 101 electron microscope.

Results

The luminal surface of the collecting tubule in normal rats appears to be lined by two different types of cells. By scanning electron microscopy the light cell has a surface characterized by sparse population of small bulbous microvilli and one or two centrally located cilia. The surface of the dark cell exhibits numerous microplicae, and in some cells microvilli can be seen between the folds. By transmission electron microscopy, the main difference between dark and light cells is the observation that the former contain more cellular organelles and have many supranuclear mitochondria, while the latter exhibit fewer organelles and rarely show mitochondria between the nucleus and the apical surface (Figures 1–3). Cells suggestive of intermediate forms can be observed by transmission and scanning electron microscopy in normal control animals, but these forms become much more numerous in potassium-depleted rats.

The collecting tubules in potassium-depleted animals exhibit marked cellular hyperplasia in the inner stripe of the outer zone of the renal medulla, resulting in polyp-like projections which protude into the tubular lumen. The surfaces observed in these projections may be of several types. Some cell surfaces are nearly or completely devoid of microvilli and may or may not exhibit the usual one or two cilia. Other projections are composed entirely of cells whose surfaces are covered by deep, closely packed microplicae. These folds often appear concentrically arranged as whorl-like figures at the center of the cell. No microvilli or cilia are seen on this surface type (Figures 4–6). Between these two extremes there are several variations of surface morphology. Occasional cells exhibit a microplicae-covered surface similar to that of dark cells in control animals, but contain, in addition, a prominent cilium. In other cells, microvilli longer than those seen in "typical" light cells can be seen between less elaborate microplicae; cilia may or may not be present (Figures 7–10). Other cell surfaces are densely covered with finger-like projections, have no microplicae, and may appear with or without cilia (Figures 11 and 12). Sometimes finger-like projections or very prominent microvilli are seen at the edge of the folds in cells with well-developed microplicae (Figure 13).

Discussion

The existence of intermediate forms of the collecting tubule cells has been suggested by other investigators.[11,22,23,24] In studies with newborn mice, Clark observed that only light cells were present at birth; the first dark cell did not appear until 8 to 24 hours later. Although he could not demonstrate any intermediate forms to show this transition, he suggested that light cells differentiate into dark cells.[9] The observation by Hagège *et al.* that the dark cell index increases as early as 5 hours in bicarbonate-loaded rats support this idea.[24] Previous studies with SEM have shown cell surfaces with both microplicae and villi [13,24] which are suggestive of an intermediate form, but these reports have failed to satisfactorily illustrate the full spectrum of intermediate forms assumed by this cell. In potassium-depleted cells we found two extremes in surface types. One is bald or exhibits very few small bulbous microvilli and contains a cilium, while the other surface shows elaborated folds deeper than those present on the surface of dark cells in control animals (Figure 5). A diversity of intermediate cell surfaces was observed in which microplicae were associated not only with villi, but also with cilia, which were previously considered to be the hallmark of a light cell.[13-15] Cells with a surface of finger-like projections, which were demonstrated by Andrews to have the ultrastructural characteristics of dark cells,[14] were also observed to have cilia. Transmission electron microscopy showed that in potassium-depleted animals, the ultrastructure of most cells was neither distinctly that of a light cell nor that of a dark cell, but rather nearly all cells shared characteristics of both types (Figures 6 and 10).

Recent experiments in rats with bicarbonate loading and respiratory acidosis relate these cellular changes to an increase of bicarbonate absorption in the collecting tubule. The marked dark-cell hyperplasia occurring in potassium-depleted rats, which are chronically alkalotic, supports the theory proposed by Richet and his group [23] that these morphologic changes in the collecting tubule are related in part to acid–base regulation. There is no satisfactory explanation for this short segment of collecting tubule showing the marked zonal hyperplasia with the wide range of dark, intermediate, and light cell proliferation. A detailed study of this interesting aspect of the renal lesion of potassium deficiency has been delayed by the earlier and more impressive lysosomal formation in the papillary tip associated with increased accumulation of phospholipids.

It is now clear that the extension of the lysosomal change through the outer medulla does not involve the collecting tubule cells. The nearly total obstructive proliferation of these collecting tubule cells in the inner stripe of the medulla, with a variety of forms between the light and dark cells is a

distinctive pathologic pattern of reaction, with promise for improved insight into the pathogenetic mechanism of potassium deficiency nephropathy.

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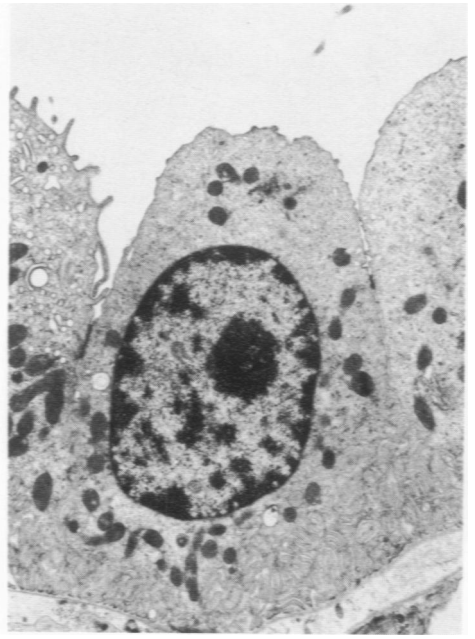
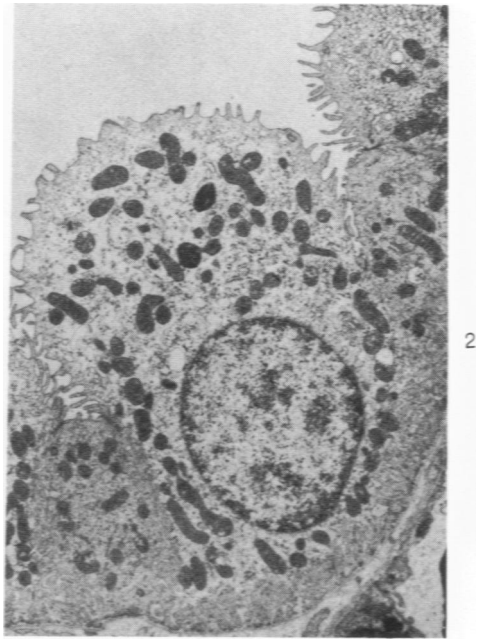




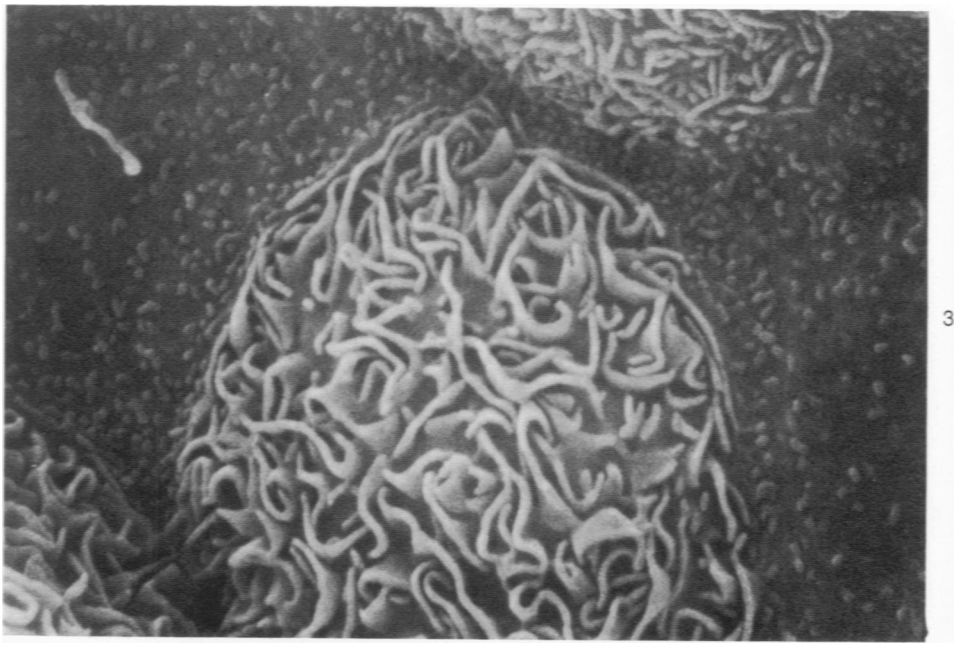
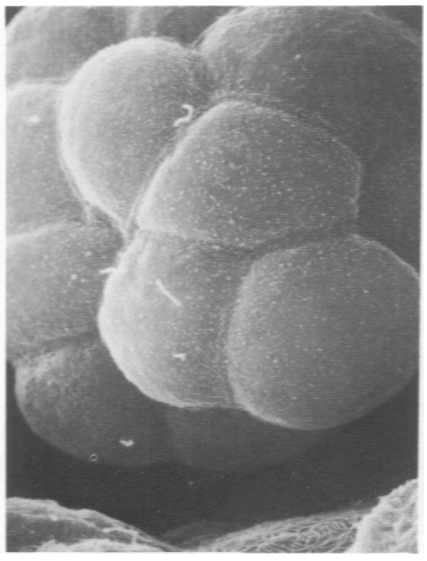
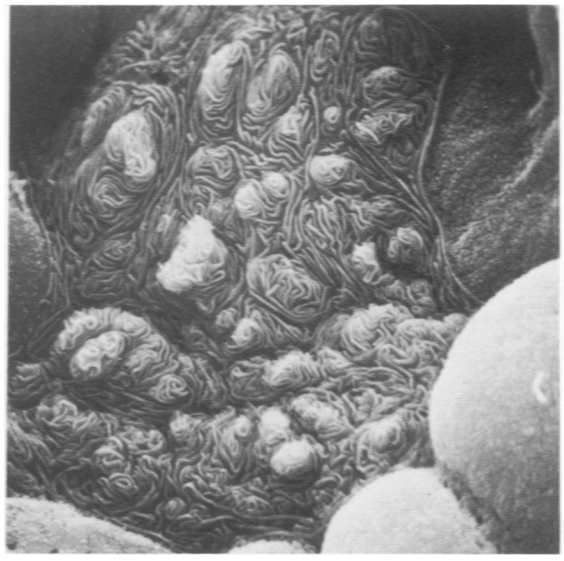


Figure 1—Transmission electron micrograph of a light cell with basal mitochondria and a cilium (× 6750). **Figure 2**—The dark cell contains many organelles including numerous supranuclear mitochondria and long, extensively developed microvilli (× 6750). **Figure 3**—Scanning electron micrograph of the luminal surface of the collecting tubule of a normal rat. The light cell surface exhibits sparse bulbous microvilli and cilia, while the dark cell is covered by microplicae. (× 7500)





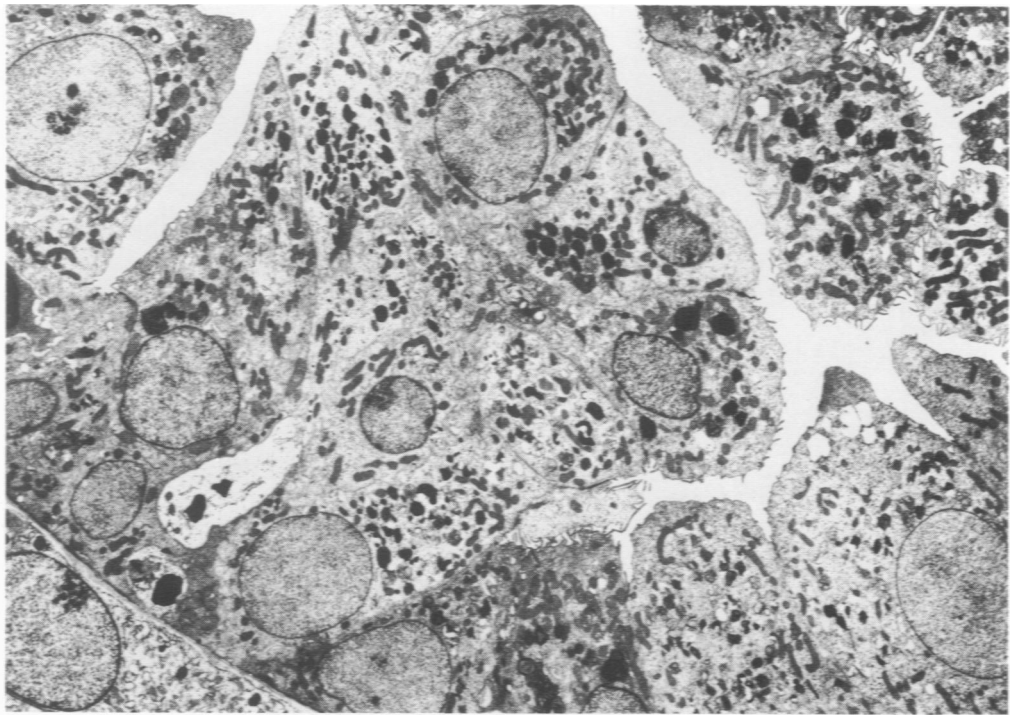
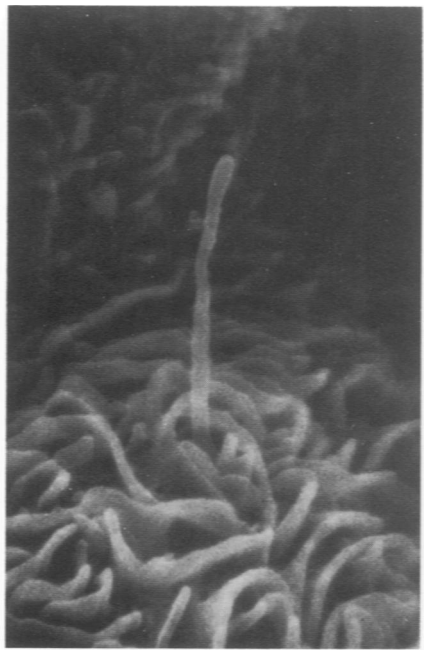
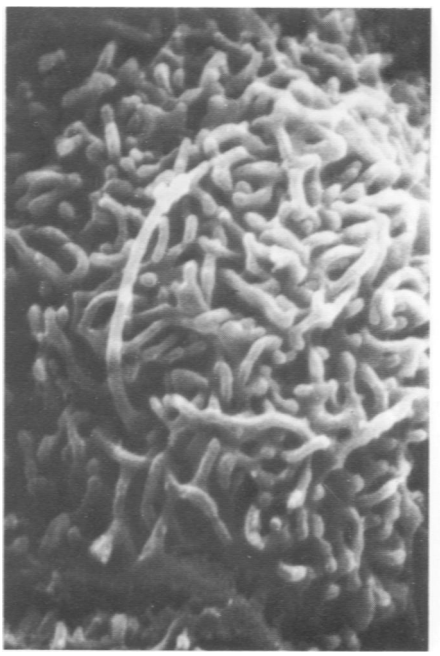
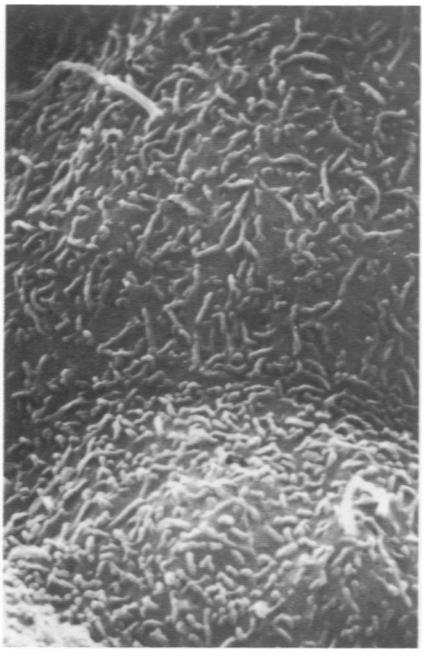


Figure 4—Polyp-like projection in a potassium-depleted rat. The cellular surfaces are smooth and have sparse short bulbous microvilli, Some cells have one or two cilia, while others do not have this structure. (SEM, × 3000) **Figure 5**—Collecting tubule with marked dark cell hyperplasia and very prominent, well-developed microplicae concentrically arranged in the center of each cell. In the upper right corner, a cilium can be seen in conjunction with long, densely packed microvilli. (SEM, × 3000) **Figure 6**—Transmission electron micrograph of a projection composed of cells which share characteristics of light and dark cells. The cell in the upper left corner shows numerous supranuclear mitochondria and a mooth surface, whereas the cells on the upper right corner have prominent branching microvilli. (× 3000)







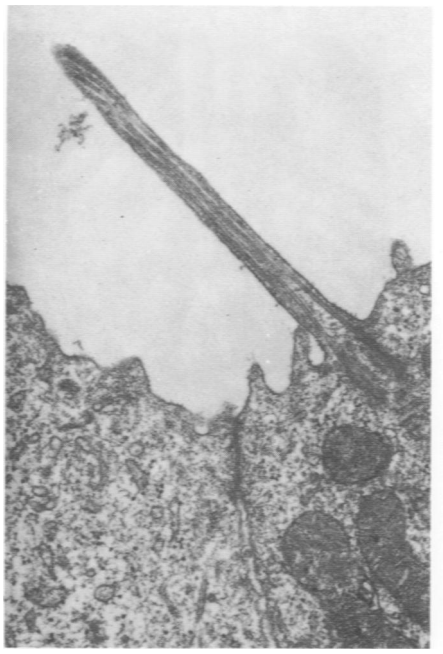
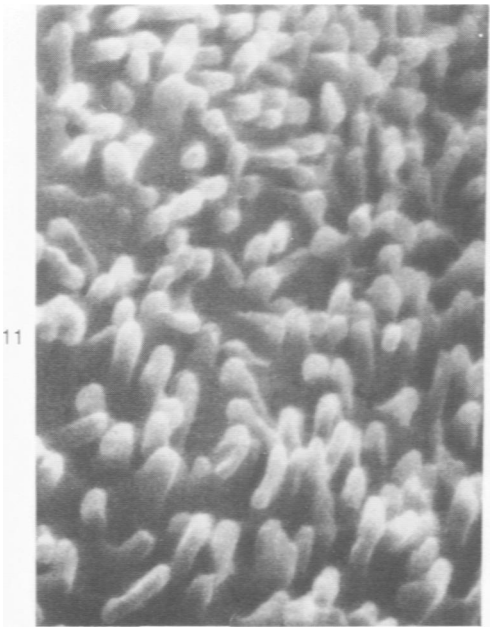


Figure 7—Typical dark cell with microplicae and a cilium in a potassium-depleted rat (SEM, × 23,000). **Figure 8**—Cellular surface with well-developed microplicae, microvilli, and cilium (SEM, × 20,000). **Figure 9**—Cells with rudimentary microplicae, small microvilli, and cilia (SEM, × 9500). **Figure 10**—Transmission electron micrograph of an intermediate form shows the typical dense cytoplasm and supranuclear mitochondria of a dark cell, but displays prominent cilium as well. Note the smooth surface of the adjacent light cell. (× 15,600)



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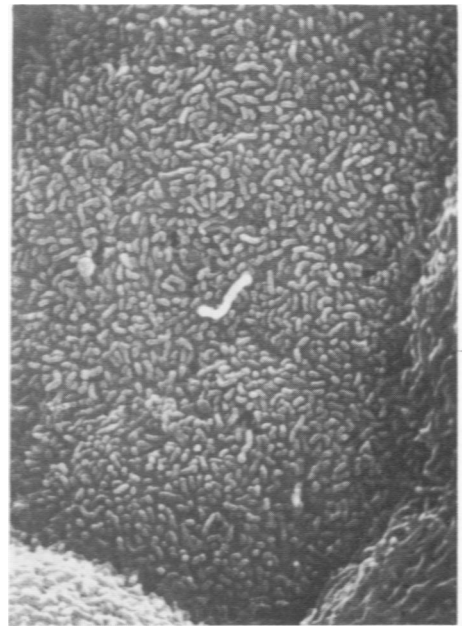

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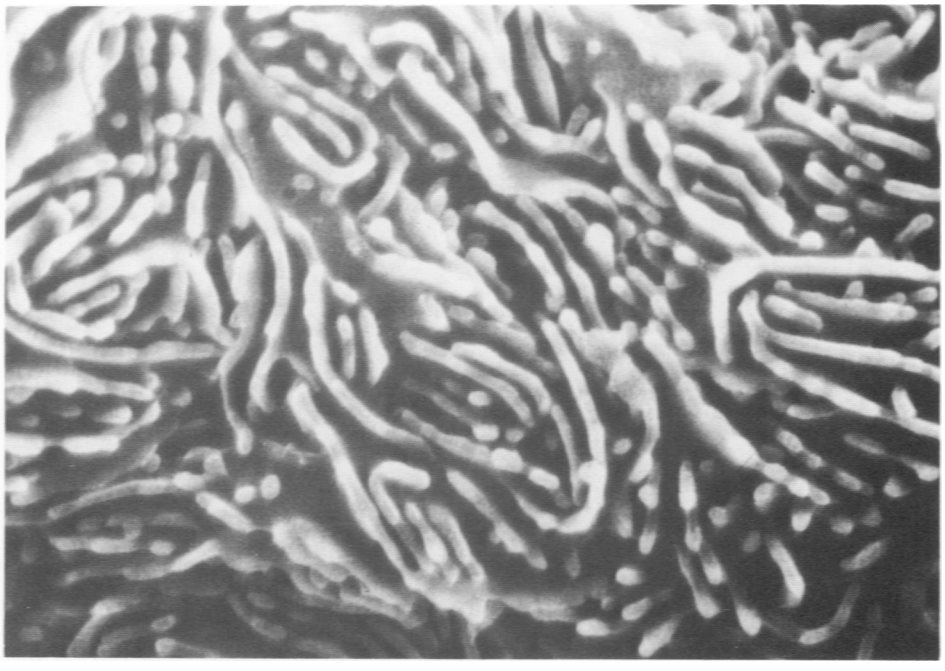

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Figure 11—Dark cell surface of long microvilli with the appearance of finger-like projections (SEM, × 30,000). **Figure 12**—Long microvilli with cilium (SEM, × 9500). **Figure 13**—Dark cell surface with microplicae showing prominent microvilli not only between the folds but also at their edges (SEM, × 25,000).