

# POSSIBLE ROLE OF DARK CELLS OF THE COLLECTING TUBULES IN ACIDIFICATION OF THE URINE

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Comparison of the ultrastructure of the parietal cells of the stomach and cells of the renal collecting tubule of intact animals and also of animals in a state of metabolic acidosis or after prolonged dry feeding revealed a similarity in the structure and distribution of the mitochondria and in the accumulation and liberation of smooth-walled vesicles. During intensified secretion of hydrogen ions an intracellular tubule, a characteristic structure of the parietal cell of the stomach, appears in the cells of the collecting tubules. It is concluded from the ultrastructural analogies obtained that the dark cells of the collecting tubules have the function of acidifying the urine.

Investigations using a micropuncture technique have shown that acidification of the urine takes place in the proximal and distal portions of the nephron and also in the collecting tubules [1, 4, 7].

The presence of two types of cells in the collecting tubules - "dark" and "light" [2, 3] - does not solve the problem of which cells secrete hydrogen ions. The investigation described below was carried out to obtain further information.

## EXPERIMENTAL METHOD

Collecting tubules of the kidney of 15 male rats weighing 100-120 g were investigated in the electron microscope. The animals were divided into three equal groups: 1) control; 2) animals in a state of acute metabolic acidosis produced by a single intraperitoneal injection of 1 ml 1% ammonium chloride solution, sacrificed 2 h later; 3) animals receiving dry food only for 7 days. As ultrastructural reference model of cells secreting  $H^+$ , the parietal cells of the stomach of the control animals were used.

The tissue was prepared for electron microscopy in the usual way by fixation with  $OsO_4$  and embedding in Epon-812.

## EXPERIMENTAL RESULTS

The specific structural features of the parietal cells of the stomach include the presence of many oval mitochondria with an intensively developed system of cristae filling nearly the whole body of the organoid. The mitochondria are uniformly distributed in the cytoplasm and have no definite orientation. Another distinguishing feature of these cells is the periodic filling of the cytoplasm with small vesicles (Fig. 1a) followed by liberation of their contents into the intracellular tubule. The most specific feature of the parietal cell is this intracellular tubule which is compressed in the phase of vesicle formation (Fig. 1c) and dilated in the phase of their expulsion (Fig. 1e). The wall of the intracellular tubule is covered with short microvilli of different shapes, the number of which rises sharply in the state of maximal accumulation and liberation of the vesicles.

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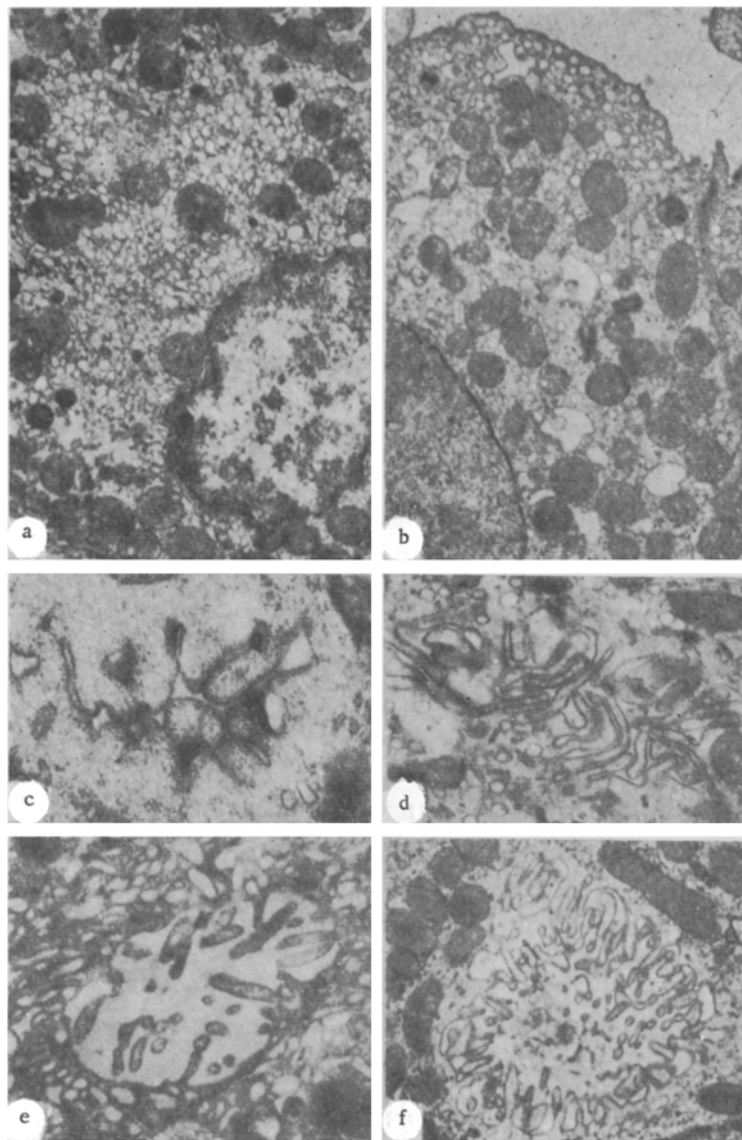


Fig. 1. Ultrastructure of parietal cells of the stomach (a, c, e) and dark cells of the renal collecting tubule (b, d, f): a, b) accumulation of smooth-walled vesicles in cells, 20,000  $\times$ ; c, d) intracellular tubules compressed at the beginning of vesicle formation, 25,000  $\times$ ; e, f) intracellular tubules dilated in the period of maximal accumulation and liberation of vesicles, 25,000  $\times$ .

The light and dark cells of the collecting tubule have different ultrastructural features. The dark cells have a more electron-dense hialoplasm and many more mitochondria, with numerous closely packed cristae. The mitochondria in the dark cells are comparatively uniformly distributed in the cytoplasm and are rather more numerous in the apical part, whereas in the light cells they are concentrated in the basal part. The mitochondria of the light cells have a basal-apical orientation and are located between the folds of the basal plasma membrane. These folds are absent in the dark cells. Characteristically the cytoplasm of the dark cells is filled with smooth-walled vesicles (Fig. 1b) which later move into the apical part and are expelled into the lumen of the collecting tubule. Numerous microvilli of irregular shape are formed on the surface of the cell. Vesicles are not found in the light cells whose surface is always relatively smooth.

In acute metabolic acidosis and after dry feeding the ultrastructural changes in the collecting tubules were identical. In both cases dark cells were more numerous in the tubules. No marked ultrastructural changes were found in the light cells compared with the control, whereas in the dark cells there was a sharp increase in the number of mitochondria and smooth-walled vesicles. The dark cells were covered

with numerous irregularly shaped villi. In addition, an intracellular tubule appeared in some of the dark cells and was compressed in the initial stages of formation of the smooth-walled vesicles (Fig. 1d) and dilated during the period of their maximal accumulation and liberation (Fig. 1f).

Even in the control animals, therefore, a similarity was seen between the ultrastructures of the parietal cells of the stomach and the dark cells of the renal collecting tubules. This similarity was manifested in the uniformity of structure and distribution of the mitochondria and in the existence of analogous periodic accumulations of smooth-walled vesicles. In experimentally induced acidosis and dry feeding the similarity between these two cells was increased, not only on account of an increase in the number of mitochondria and vesicles in the dark cells of the collecting tubule, but also on account of the appearance of an intracellular tubule, a specific feature of the parietal cells of the stomach, in them.

The secretion of  $H^+$  is intimately connected with the bicarbonate system and with the activity of carbonic anhydrase; the activity of this enzyme in the parietal cells of the stomach is higher than in any other organs or tissues [6]. Comparatively recently, with improvements in techniques, high carbonic anhydrase activity has been revealed in the kidney, not only in the proximal and distal portions of the nephron, but also in the collecting tubules [5]. By the localization of carbonic anhydrase in the collecting tubules, a clear distinction can be drawn between the dark (with high activity) and light (with no activity) cells.

The ultrastructural similarity between the parietal cells of the stomach and the dark cells of the collecting tubule described above is thus supported by a common pattern of content of an enzyme intimately connected with hydrogen ion secretion.

The writers consider that this ultrastructural similarity between the parietal cells and the dark cells of the collecting tubule is not accidental but is linked with a similarity between their functions. Of these two types of cells — light and dark — in the collecting tubule, the activity of the dark cells is probably connected with acidification of the urine.

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