CELL ULTRASTRUCTURE IN THE PROXIMAL TUBULE OF THE RAT NEPHRON DURING INTENSIVE GLUCOSE REABSORPTION

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Glucose filtered from the blood plasma by the renal tubules is a constant component of the primary urine, in which its concentration is the same as in plasma. Under ordinary conditions, however, only traces of glucose are found in the urine, because it is reabsorbed practically completely by cells of the proximal portion of the nephron [9]. Glucose is reabsorbed against the concentration gradient and its transport is coupled with the transfer of sodium ions, the gradient of which is regarded as the motive force of the joint transport process [14]. The few investigations of the morphology of the epithelial cells during glucose loading which have so far been undertaken can be reduced to the discovery of intracellular vacuoles which, in the opinion of the authors concerned, are evidence of transport by pinocytosis [12, 15]. However, in these investigations glucose was given in extremely large volumes of fluid and the picture observed corresponded to "osmotic nephrosis." Accordingly an attempt was made to study ultrastructure of the epithelial cells under physiological conditions of maximal glucose reabsorption in order to clarify the role of individual structures in the transport mechanism.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats weighing 180-200 g. Under anesthesia (pentobarbital sodium 50 mg/kg, intraperitoneally) 1 M glucose solution was injected intravenously into the animals at a constant rate of 0.1 ml/min. Rats of another group received an injection of 1 M mannitol solution at the same rate (mannitol, while similar to glucose in structure, being a hexahydric alcohol, unlike glucose which is actively reabsorbed, is not reabsorbed by cells of the renal epithelium). Control animals received an injection of Ringer's solution.

After 30 min of intravenous infusion blood was taken from the animals and glucose determined by the orthotoluidine method. Its concentration after infusion of glucose was 400-500 mg%, which is known to be sufficient to saturate the reabsorption mechanisms in the kidneys completely. After injection of mannitol and Ringer's solution the glucose concentration in the plasma was 110-120 mg%. Without stopping the infusion, laparotomy was performed on the animals, the kidney exposed, and part of its surface was fixed, excised, and transferred to cold fixing solution. The fixative was a solution of OsO_4 in veronal-acetate buffer (Caulfield's fixing solution with tonicity of 320 milliosmoles/liter). The tissue was dehydrated in ethanol and acetone, and then embedded in Araldite by the usual method. Sections were cut on an ultramicrotome of LKB type and stained with uranyl acetate and lead citrate. The sections were examined in the JEM-7 microscope.

EXPERIMENTAL RESULTS

The epithelium of the proximal tubule, where glucose is reabsorbed, in the rat nephron consists of cylindrical cells with an oval nucleus situated basally. A distinguishing feature of the cells is their well-developed brush border, which considerably enlarges the surface of contact of the plasma membrane with the fluid within the tubules and thus enables complate reabsorption of the many component parts of the ultrafiltrate and, in particular, of

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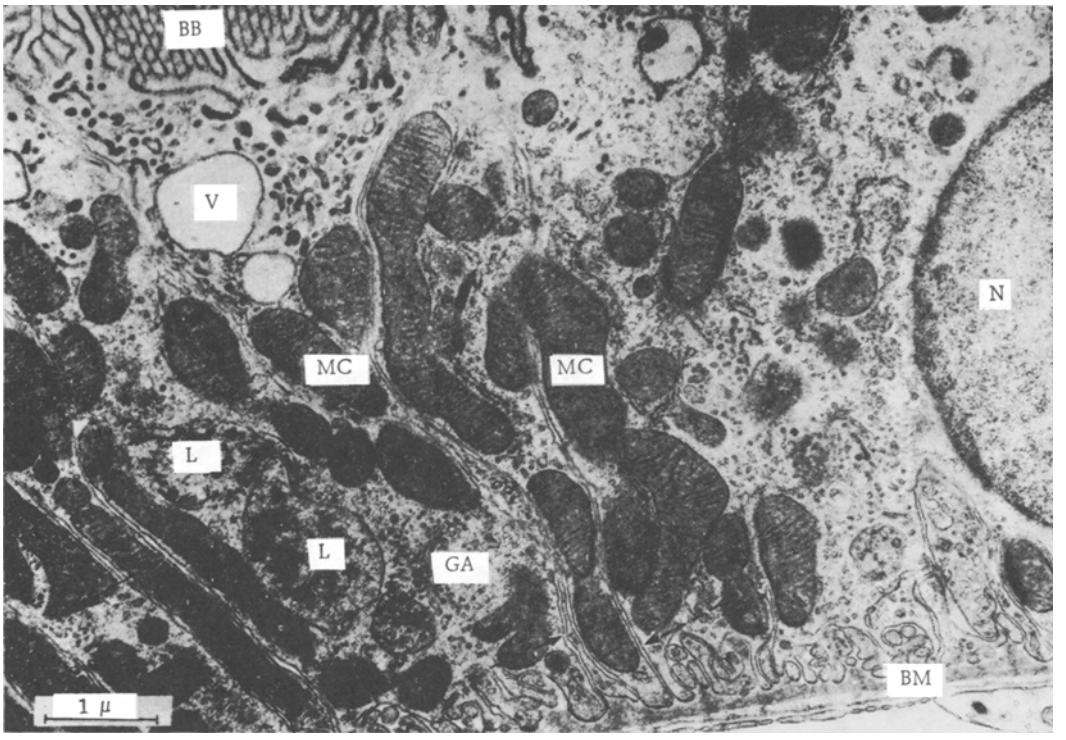

Fig. 1. Epithelial cell of proximal tubule (control). Infusion of Ringer's solution. Magnification 20,000. Here and in Figs. 2-4: GA) Golgi apparatus; BM) basement membrane; V) vacuole; L) lysosomes; MC) mitochondria; MB) microbody; BB) brush border; N) nucleus; arrows — intercellular space.

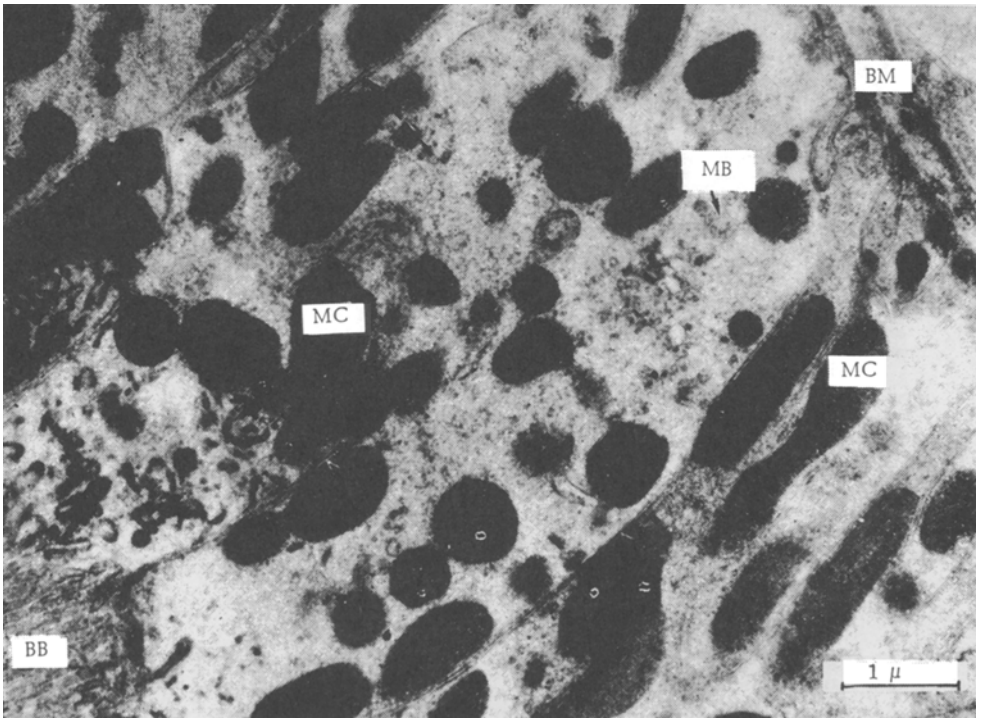

Fig. 2. Epithelial cell of proximal tubule during glucose loading (magnification 20,000).

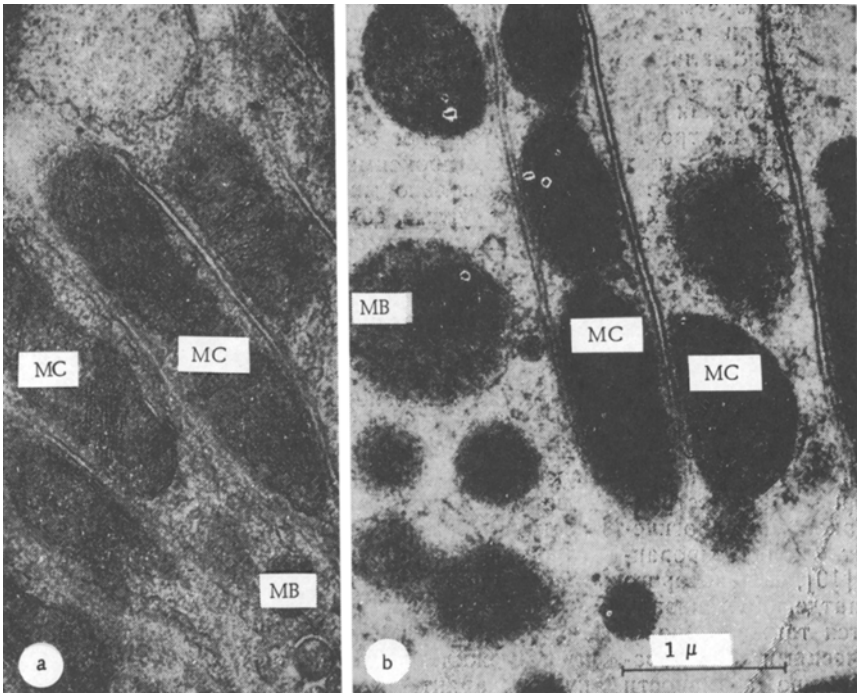

Fig. 3. Part of cell of proximal tubule with mitochondria and microbody (magnification, 33,000). a) Control; b) during glucose loading.

glucose, to take place. Another peculiarity of the cells is the intensive folding of the basolateral plasma membrane, with numerous mitochondria located between the folds (Fig. 1). These features were most marked in epithelial cells of the initial third of the proximal tubule and became less marked toward its end, where the brush border was relatively high, but less dense, and the number and height of the folds and the number of mitochondria between them were considerably reduced. These changes, together with other morphological features, provided a basis for dividing the epithelium of the proximal tubule into three zones, differing both morphologically and functionally [3, 11]. Since the morphological features characteristic of cells of this portion of the nephron differ quantitatively, it can be postulated that the cells also differ in the intensity of the processes taking place in them.

Ultrastructural changes observed in cells of the proximal tubule during maximal glucose reabsorption could be detected in the epithelium along the whole length of the tubule. Translucency of the cell cytoplasm was observed, and compared with the corresponding cells of the control animals the matrix was much paler (Figs. 2 and 3b). It can be tentatively suggested that translucency of the matrix increased as a result of the entry of water into the cytoplasm after glucose. The possibility likewise cannot be ruled out that a primary role in the hydration of the cytoplasm when glucose transport is increased may be played by increased sodium transport, for these two substances are carried through the apical membrane by a joint transport mechanism [13].

Another distinguishing feature of the cells was condensation of the mitochondrial matrix whereas the state of their cristae remained unchanged compared with the control – their number and the width of the intracristal space were the same (Figs. 2 and 3, a, b). This morphological picture corresponds to a "condensed" state of the organoids [10], which characteristically have high succinate dehydrogenase activity [7]. This also agrees with the results of previous biochemical investigations which showed an increase in succinate dehydrogenase activity in the kidney during maximal glucose reabsorption [2].

Glucose loading was accompanied by changes in ultrastructure of the microbodies, possible evidence of their transition into a more active physiological state. Under ordinary conditions these organoids are fairly numerous in cells of the proximal tubules and consist of round bodies, containing a granular matrix and surrounded by a membrane, with a diameter of about 0.3 μ (Fig. 3a). Sometimes the matrix of the microbodies has a condensed region or nucleolus. Intensification of glucose transport was accompanied by an increase in size of the

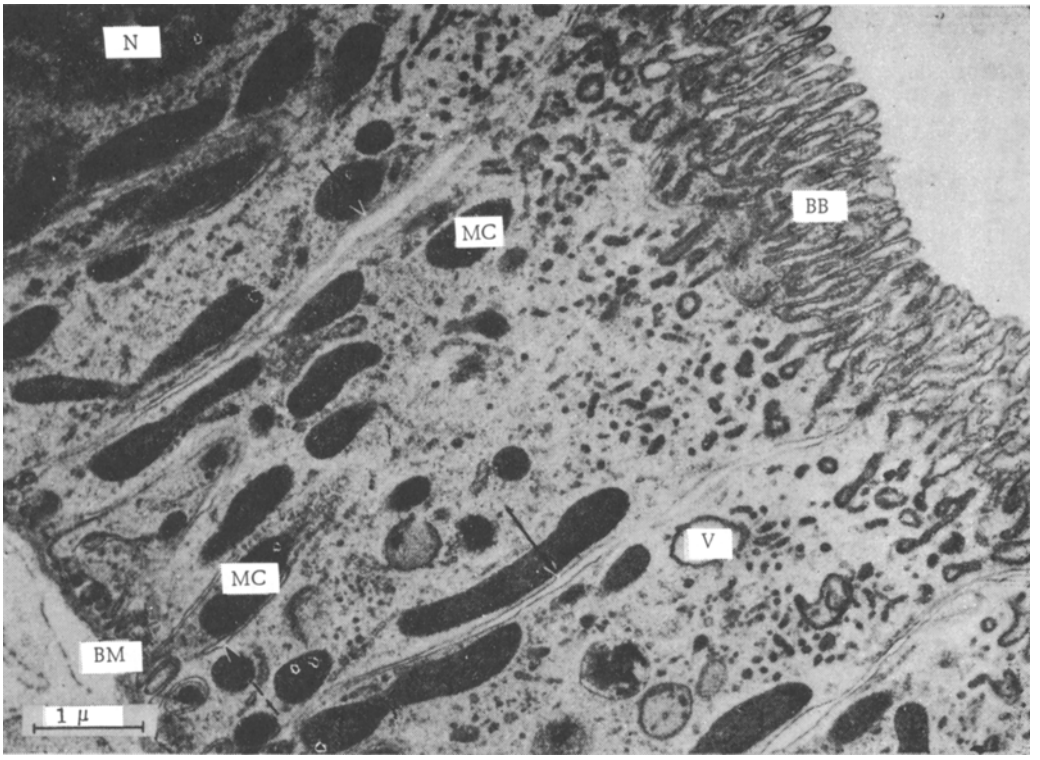

Fig. 4. Epithelial cell of proximal tubule during mannitol loading (magnification 20,000).

microbodies to 1 μ. Nucleoli, which usually are rare, were now found in the overwhelming majority of microbodies (Fig. 3b). Structural changes of this kind in these organoids usually accompanied reorganization of cell metabolism and they have been observed in cells exposed to a very wide variety of influences [1, 4, 6]. The ultrastructure of the other cell organoids remained visibly unchanged. The fine structure of the endoplasmic reticulum and Golgi apparatus of the cells was comparable with the control. No changes in size of the intercellular space of the epithelium were found. Vacuoles characteristic of the apical zone of the cytoplasm in cells of the control animals were present in the usual number during loading, no change was observed in their size, nor did they migrate into the region of the basal cytoplasm.

Injection of the osmotically active sugar, mannitol, which does not undergo reabsorption, caused widening of the intercellular space below the zone of firm contact of the cells, and also some increase in the size of the extracellular space between the basolateral folds (Fig. 4). The cytoplasm of the cells appeared dark and its electron density was higher than that of the cell cytoplasm in the control (Fig. 1), and it exceeded even more the density of the ground substance of the cell cytoplasm after glucose loading (Fig. 2). These changes were perhaps connected with the fact that mannitol, present in the lumen of the tubule, since it does not penetrate inside the cells, interferes with reabsorption of fluid, and this leads to widening of the intercellular spaces. No ultrastructural changes in intracellular organoids were found, confirming previous observations with the osmotic diuretic polyethylene-glycol [5].

Since we know that reabsorbed glucose does not undergo metabolic conversions in cells of the renal epithelium but is transported into the blood unchanged [8], it can be tentatively suggested that the changes observed in cell ultrastructure during glucose loading are in fact associated with the transport of this substance and not with its metabolism. Our observations do not confirm data on the role of intracellular pinocytosis in the mechanism of glucose reabsorption, for no morphological evidence of this phenomenon was found. The divergence between the results of the present experiments and those obtained by other workers [12, 15] may perhaps depend on the volume of fluid in the solutions injected into the animals. By contrast with our own experiments, in previous investigations the injected volume of glucose solution was five to seven times greater, and this probably caused the appearance of vacuoles.

Analysis of the ultrastructure of epithelial cells of the rat nephron during the period of maximal mobilization of the transport mechanism for glucose reabsorption thus showed that under these circumstances changes are observed in the mitochondrial apparatus without participation of the endoplasmic reticulum or of any other morphologically identifiable transport system in the cytoplasm.

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