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STUDY OF THE SUBCELLULAR LOCALIZATION OF $^{45}Ca^{++}$ DURING ABSORPTION BY EPITHELIUM OF THE RAT SMALL INTESTINE

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Despite great advances made in recent years in the study of the mechanisms of absorption of Ca^{++} in the intestine, many structural and biochemical aspects of this process still remain unexplained [2, 16]. In particular, the problem of concrete pathways for the transfer of Ca^{++} through the layer of intestinal epithelium still remains unsolved.

The most widely held hypotheses are those based on the assumption that Ca^{++} ions pass through the apical membrane into the cytosol of the enterocyte, and are then expelled from it by the Ca pump on the basal side of the cell [2, 7, 16]. A few electron-microscopic investigations have yielded evidence that Ca^{++} is carried through the enterocyte in a sequestered state, in "packets," evidently not mixed with the cytosol [15]. According to other data, Ca^{++} is carried across a tight junction and then along the lateral membrane, along an intercellular canal [3].

A definite defect of the above investigations is that the methods used did not enable the absorbed Ca^{++} ions to be differentiated from the endogenous cation which is constantly present in the cell and its structures. This difficulty can be overcome by using electron microscopy in conjunction with electron autoradiography, and by the use of radioactive $^{45}Ca^{++}$ as the absorbed ion. The essence of the method is to identify the absorbed radioisotope by means of halides of heavy metals, reduction of which by β-radiation at the site of localization of the isotope leads to deposition of an electron-dense deposit of the reduced metal in these areas [12].

This method was used in the present investigation to study the localization of $^{45}Ca^{++}$ during its absorption in the small intestine of young, growing rats.

EXPERIMENTAL METHOD

Under ether anesthesia, 0.5 ml of 0.25 mM $^{45}CaCl_2$ (2 mCi/mmole) in 0.9% NaCl was injected into a 5-cm segment of the proximal portion of the small intestine of a young (80-100 g) male Wistar rat, isolated in situ

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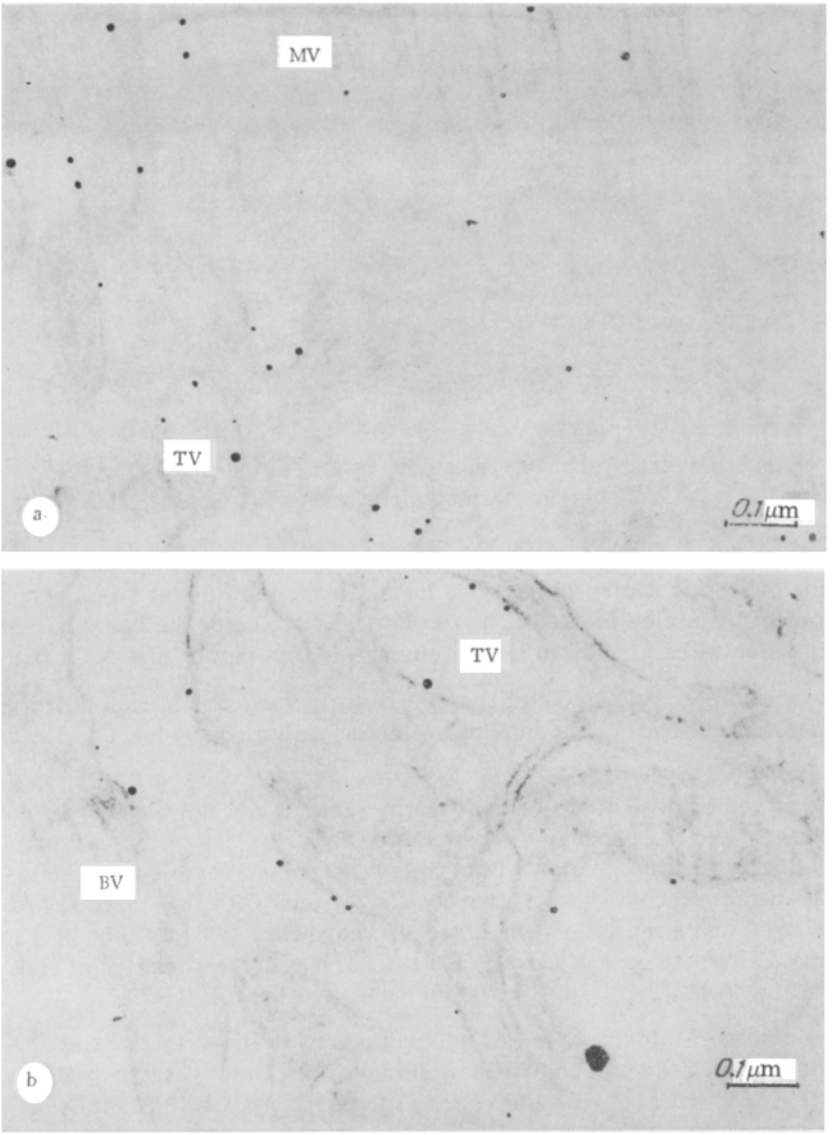

Fig. 1. Localization of radioactive isotope $^{45}Ca^{++}$ in an enterocyte: a) label on microvilli (MV) and in transport vesicles (TV) formed at base of MV; b) label on membranes of basal-lateral villa (BV) of an enterocyte and in TV which have merged with the villi.

by ligatures. The isolated segment of intestine was excised after 10 or 30 min and fixed in 4% paraformaldehyde in Hanks' buffer, postfixed with 1% OsO_4, dehydrated by a rapid method [4], and embedded in a mixture of the epoxide resins Epon and Araldite. Ultrathin sections 80–100 nm thick were mounted on platinum or molybdenum grids. The incubation solution of $AuCl_3$, in a final concentration of 0.000001%, was made up in boiled bidistilled water by repeated dilution. The grids were placed on a drop of incubation solution for 1 min at 20°C and in ordinary illumination. They were then sprinkled successively with 3 drops of bidistilled water for 1 min. Before irradiation in the electron microscope the sections were not stained.

EXPERIMENTAL RESULTS

Crystals of gold (subsequently the label), formed in sites of localization of the radioactive isotope $^{45}Ca^{++}$, were found in the intestinal tissue of animals killed as early as 10 min after injection of the isotope into the lumen of the bowel. The largest number of tags was located on membranes of the microvilli of the apical surface of the enterocytes, evidently in the layer of the glycocalyx. Admittedly, without additional staining, this layer was almost invisible on the microvilli. The label was found intracellularly in vesicles (on the inner surface of the membranes) formed from the hollows between the microvilli (Fig. 1a).

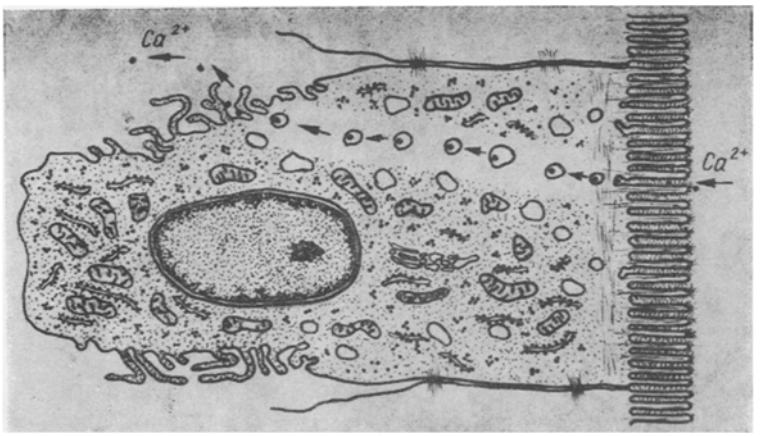

Fig. 2. Diagram showing transport of Ca^{++} ions through cytoplasm of enterocyte (arrow).

In animals killed 30 min after injection of the isotope into the intestinal lumen the label was found not only in the places described above, but also in vesicles located near the lateral membrane of the enterocyte. In addition, $^{45}Ca^{++}$ was found in vesicles located near the lateral surface of the basal part of the cells and on the surface of large villi, which were formed in large numbers in this part of the cells (Fig. 1b).

The distribution of label on the microvilli of the apical surface, in the intracellular vesicles, and on villi of the lateral-basal surface of the enterocytes demonstrates the path along which Ca^{++} is absorbed in the intestine.

No $^{45}Ca^{++}$ was found in the cytosol of the enterocyte in a free form, not bound with membranous vesicles. These observations indicate that in the process of absorption Ca^{++} ions do not mix with the cytosol of the enterocyte, but are carried in sequestered form within the membranous vesicles. Vesicles transporting Ca^{++} are pinched off at the base of the microvilli, retaining the Ca^{++} bound with them, and they carry the calcium through the enterocyte, to open up on the lateral membrane, where they liberate the Ca^{++} ions into the intercellular space. This is the same path as has already been described for absorption of lipids, proteins, and various other substances (Fig. 2) [13].

The results of these electron-microscopic and autoradiographic investigations are in good agreement with data on Ca^{++} transport through the enterocyte in an immobilized state obtained with the aid of less refined methods of investigation [11, 15], and also with the results of recent studies [9], from which it was concluded that lysosome-like structures of the enterocyte participate in Ca^{++} transport.

The intracellular Ca^{++} concentration is known to be maintained at a very low level, of the order of $1 \cdot 10^{-6}$ M, and any increase in it adversely affects many biochemical processes [8, 14]. Vesicular Ca^{++} transport is an important mechanism which prevents any increase in the Ca^{++} concentration in the cytosol during absorption.

The concept of vesicular transport of Ca^{++} through the enterocyte differs from the hypothesis of its extracellular transport, suggested by Bauman et al. [3]. In their investigations, just as in our own, Ca^{++} was shown to be localized on the lateral membrane of the enterocyte in the region of the intercellular spaces. However, whereas our observations showed that Ca^{++} ions appeared on the lateral membrane as the result of opening up of transport vesicles, pinched off the base of the microvilli, on them, according to the hypothesis of Bauman et al. [3], this "cation as it were flows around the cell, penetrating through the tight junction and moving along the lateral membranes, along the intercellular canal." This hypothesis is based on electron-histochemical detection of Ca^{++} in the region of the tight junction. However, as was shown above, the method of isolation of Ca^{++} by means of pyriantimoniate, used in the investigations cited, does not allow differentiation between the absorbed and endogenous Ca^{++}, which is always present in the region of the tight junction as an important structural component, increasing the strength of this formation [5].

Furthermore, in the investigations of Bauman et al. [3], large quantities of Ca^{++} were injected into the intestinal lumen, creating an unphysiologically high concentration of the element of the order of 12-25 mM, 5 to 10 times higher than the Ca^{++} concentration in the blood. Under these conditions Ca^{++} passed through the intestinal wall mainly on account of diffusion processes, in which a definite role may be attributed also to the

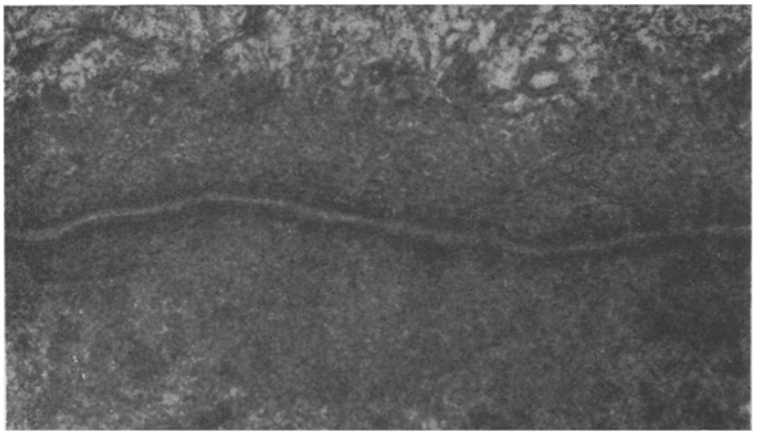

Fig. 3. Transverse section at level of terminal network of linear junction between two enterocytes.

entry of Ca^{++} through tight junctions and intercellular spaces. In the present experiments the initial Ca^{++} concentration in the lumen (0.25 mM) was only one-tenth of its concentration in blood plasma (2.5 mM), so that the picture observed was entirely due to mechanisms of active Ca^{++} transport. In our opinion, under physiological conditions Ca^{++} transport through tight junctions can hardly take place at all, for by contrast with desmosomes, which connect the cells of the gastric mucosa in mosaic fashion, the tight junction between the enterocytes is linear in character. As our investigations showed (Fig. 3) a linear tight junction forms a circular connection of the enterocyte with all neighboring cells of the intestinal epithelium. This firm and stable connection does not separate not only under physiological conditions, but even during homogenization [10].

When studying the subcellular localization of $^{45}Ca^{++}$ during its absorption, no evidence of entry of the label into mitochondria of the enterocyte was found. These findings, together with similar observations by other workers [3, 15], are evidence against any direct participation of mitochondria in the transport or sequestration of Ca^{++} absorbed by the enterocyte. This fact, of course, does not dispute the important role of mitochondria in the provision of energy for the process of Ca^{++} absorption by the mechanism of active transport against the concentration gradient [1].

The question of the mechanism of the coupling of vesicular Ca^{++} transport with the expenditure of metabolic energy, which gives the character of active transport, is a subject for special study [6].

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