14. J. R. Williamson, J. Ultrastruct. Res., 27, 118 (1969).
15. E. Wisse, G. Gregoriadis, and W. T. Daems, Adv. Exp. Med. Biol., 73-A, 121 (1976).

VESICULAR PEROXIDASE TRANSPORT BY EPITHELIAL CELLS OF THE ADULT RAT SMALL INTESTINE

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The ability of proteins or their fragments to penetrate through the epithelium of the mucosa of the small intestine has been discussed for a long time. There is now no doubt that in the early postnatal period in mammals proteins, in the native form and, in particular, immunoglobulins can pass through the apical membrane of enterocytes in the small intestine, by entering an epithelial cell by pinocytosis and being transported through it in the composition of vesicles [4, 8, 14]. With the development of luminal digestion, on the transition to definitive feeding the transport mechanism is considered to be reorganized. Instead of pinocytosis, a system of transmembrane transfer of nutrients, which are subsequently transported through the cytosol of the enterocyte, comes into action. Meanwhile the mucosa of the small intestine acquires the property of not allowing proteins to pass through in the unsplit form [1].

It is generally considered that in adult individuals whole protein molecules are not absorbed under normal conditions. However, this does not rule out the possibility that an extremely small amount of unsplit protein may enter the bloodstream on account of a disturbance of the digestive system or some other defect of the absorption mechanism. Very often this is accompanied by an allergic reaction as a result of the entry of a foreign protein into the body [9, 11]. Nevertheless, numerous investigations, mainly physiological, have shown that in adult life native food proteins can pass through the epithelial barrier of the small intestine [10, 13]. The mechanism by which they enter the bloodstream is not yet clear, but it has been suggested that vesicular transport may be that mechanism [5]. The hypothesis has been put forward that not only proteins, but also nutrients of other nature (lipids, carbohydrates, pharmacological substances, and so on) are absorbed in the small intestine by pinocytosis [2].

The aim of the present investigation was an electron-microscopic study of protein (peroxidase) transport in the mucosa of different parts of the small intestine in adult rats within comparatively short time intervals after introduction of the protein into the gastrointestinal tract.

EXPERIMENTAL METHOD

Two groups of sexually mature male Wistar rats weighing 250-300 g were used (the animals were kept on the ordinary animal house diet, and before the experiment they were starved for 24 h but allowed water *ad lib.*). Animals of group 1 received 1 mg peroxidase and 20 mg bovine serum albumin in 2 ml of physiological saline by means of a gastric tube (horseradish peroxidase RZ-1.62 was from Sigma, USA). The animals of group 2 received an injection of about 0.5 ml of a solution of peroxidase (0.5 mg/ml) in Hanks' buffer, pH 7.4, into a 5-cm segment of jejunum, isolated between ligatures under superficial ether anesthesia. At intervals of 20 and 40 min after injection of peroxidase into the gastrointestinal tract, in the animals of group 1 segments of the duodenum and proximal part of the ileum were resected (the rats were anesthetized with hexobarbital 10 min before laparotomy), and in the animals of group

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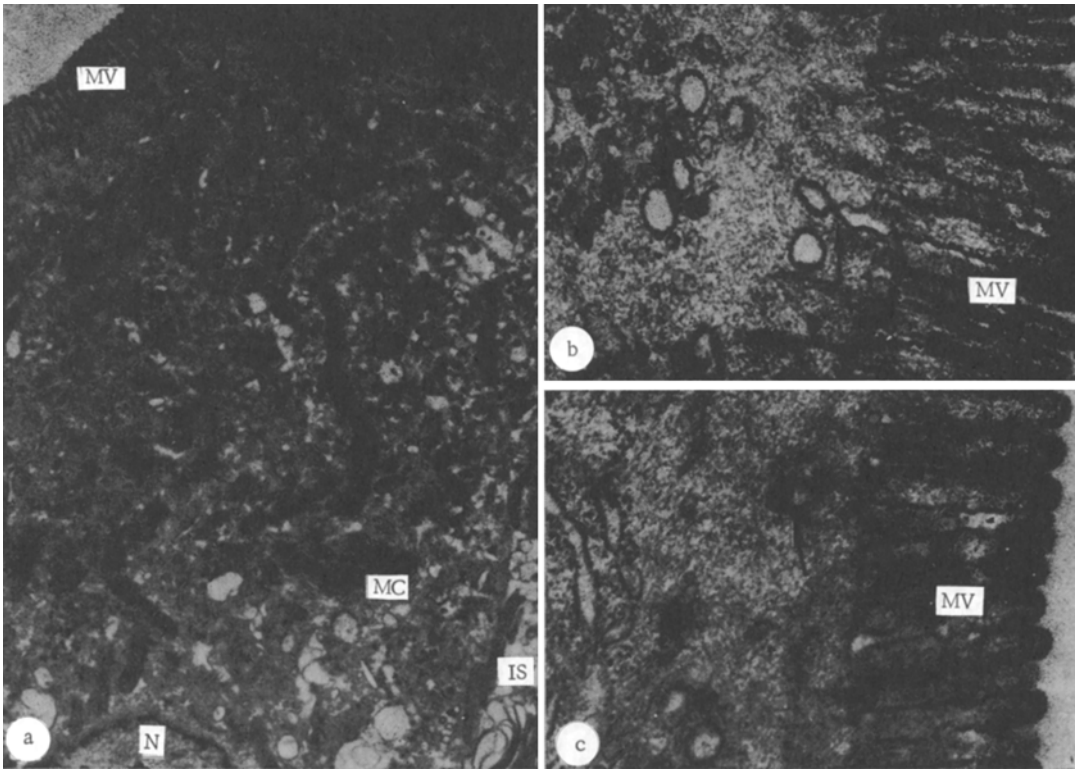

Fig. 1. Ultrastructure of enterocyte 20 min after peroral administration of horseradish peroxidase. a) Peroxidase reaction product localized within vesicles and vacuoles in terminal and supranuclear zones of enterocyte. 10,000 ×; b) pinocytotic vesicle (arrow) being pinched off from the base of microvilli of the apical membrane of the enterocyte. 50,000 ×; c) histochemical reaction product localized (arrow) in pinched off vesicle. 50,000 ×. MV) Microvilli; IS) intercellular space, MC) mitochondrion, N) nucleus.

2 an isolated segment of jejunum was excised. Pieces of tissue taken from electron-microscopic investigation were fixed in a 4% solution of paraformaldehyde in Hanks' buffer for 90 min at 0-4°C. Histochemical determination of peroxidase was then carried out by the method of Graham and Karnovsky [7], using 3,3'-diamino-benzidine (from Merck, West Germany) for 30 min at 20°C. After washing three times with distilled water the tissue was postfixed in 1% osmic acid solution, dehydrated in acetone, and embedded in a mixture of epoxide resins Epon and Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate before examination in the electron microscope.

EXPERIMENTAL RESULTS

The histochemical reaction product for peroxidase (electron-dense precipitate) was found 20 min after injection of peroxidase into the stomach inside vesicles and vacuoles in the terminal and supranuclear zones of duodenal enterocytes at the apex of the villi (Fig. 1a). Pinocytotic vesicles could be seen budding from the base of the microvilli of the apical membrane of the enterocytes (Fig. 1b). Individual pinocytotic vesicles were located in the reticular zone of the cell beneath the apical membrane. In some vesicles electron-dense precipitate was presented (Fig. 1c). A small quantity of peroxidase reaction production was found in the lateral intercellular spaces of the epithelial cells. Fusion of vesicles, some of which contained reaction product, with the lateral membrane of the enterocyte was observed, followed by release of the contents of the vesicles into the intercellular space (Fig. 2a).

Peroxidase was found 40 min after its peroral administration not only in the duodenum, but also in the proximal part of the ileum. There was more peroxidase in the enterocytes than after 20 min. A considerable quantity of peroxidase reaction product was now outside the epithelial cells: in the basal-lateral intercellular space, in the lamina propria of the mucosa (Fig. 2b). Electron-dense precipitate was found also in the region of the capillaries of the villi (Fig. 2c) and in pinocytotic vesicles of capillary endothelial cells (Fig. 2d).

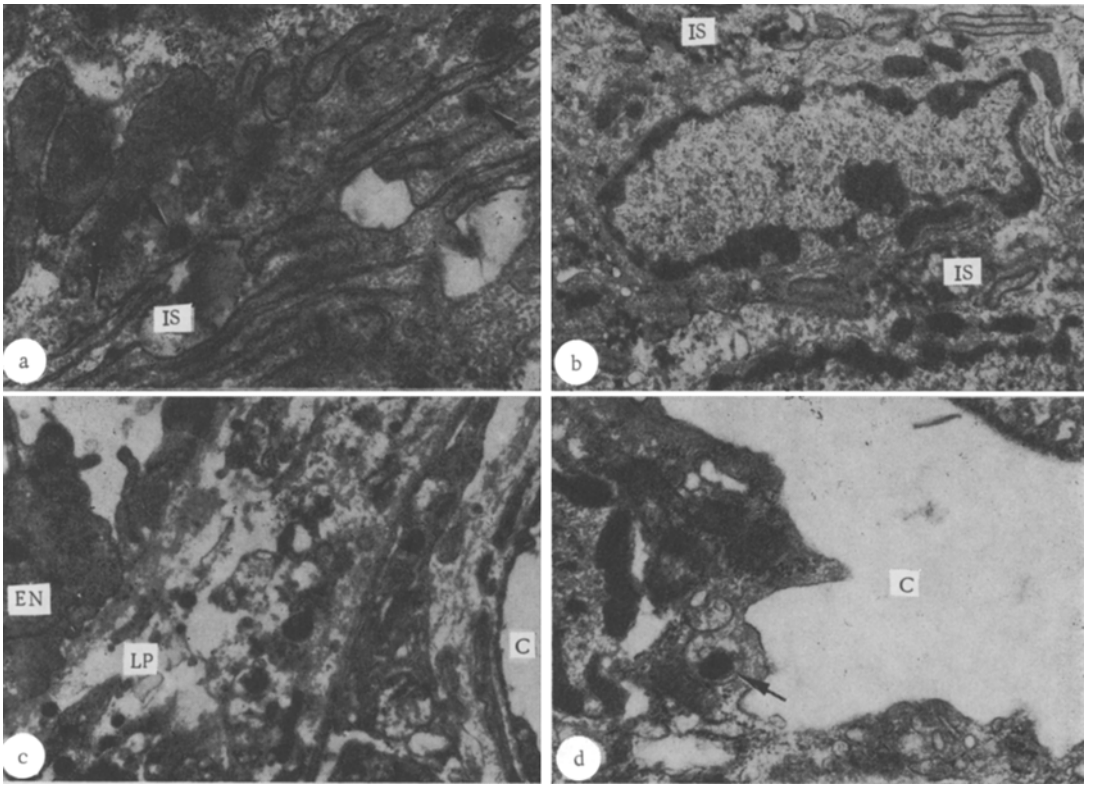

Fig. 2. Ultrastructure of enterocyte 20 and 40 min after peroral administration of peroxidase: a) fusion of transport vesicles (arrow) with lateral membrane of enterocyte and release of contents of vesicles into intercellular space. 30,000 ×; b) histochemical reaction product in intercellular space. 9000 ×; c) peroxidase reaction production in lamina propria of mucosa of small intestine. 15,000 ×; d) electron-dense precipitate (arrow) in vesicle of capillary endothelial cell in villus of small intestine. 35,000 ×. IS) Intercellular space, LP) lamina propria of mucosa, EN) enterocyte, C) capillary.

The peroxidase reaction product in the enterocytes, incidentally, was found only inside the vesicular elements of the cell and was not present in the cytosol or in cisterns of the endoplasmic reticulum. In all probability, vesicles containing peroxidase (or at least, most of them), during transport through the epithelial cell, simply passed through it, avoiding the endoplasmic reticulum. A similar phenomenon has been described in newborn rats during a study of absorption of conjugates of peroxidase with immunoglobulin G [8].

After injection of peroxidase directly into an isolated loop of small intestine it also was actively absorbed by epithelial cells. Peroxidase reaction products also were found inside the vesicular elements of the enterocytes only. In some animals, however, no peroxidase was present in the epithelial cells of the small intestine. This could evidently have been the result of blocking of absorption by the action of the anesthetic or as a result of ischemia arising during the operation.

Analysis of the distribution of the reaction product along villi of the small intestine showed the presence of a protein absorption gradient along the length of the villus. Peroxidase was most abundant in cells and in intercellular spaces in the upper third of the villi and at their apex. Its quantity diminished appreciably from the apex of the villus toward the cryptal zone. The existence of an absorption gradient along the length of the villus can be explained by the accessibility of the substrate passing from the lumen of the intestine to the villi, which under natural conditions are in a closed state and, in addition, are covered with deposits of mucus. It may be noted that if an everted pouch of small intestine is used in experiments *in vivo*, the villi, on the other hand, are in an unfolded state and, in addition, the whole surface of the villus is open to the substrate, a quite unnatural situation of processes taking place *in vitro*.

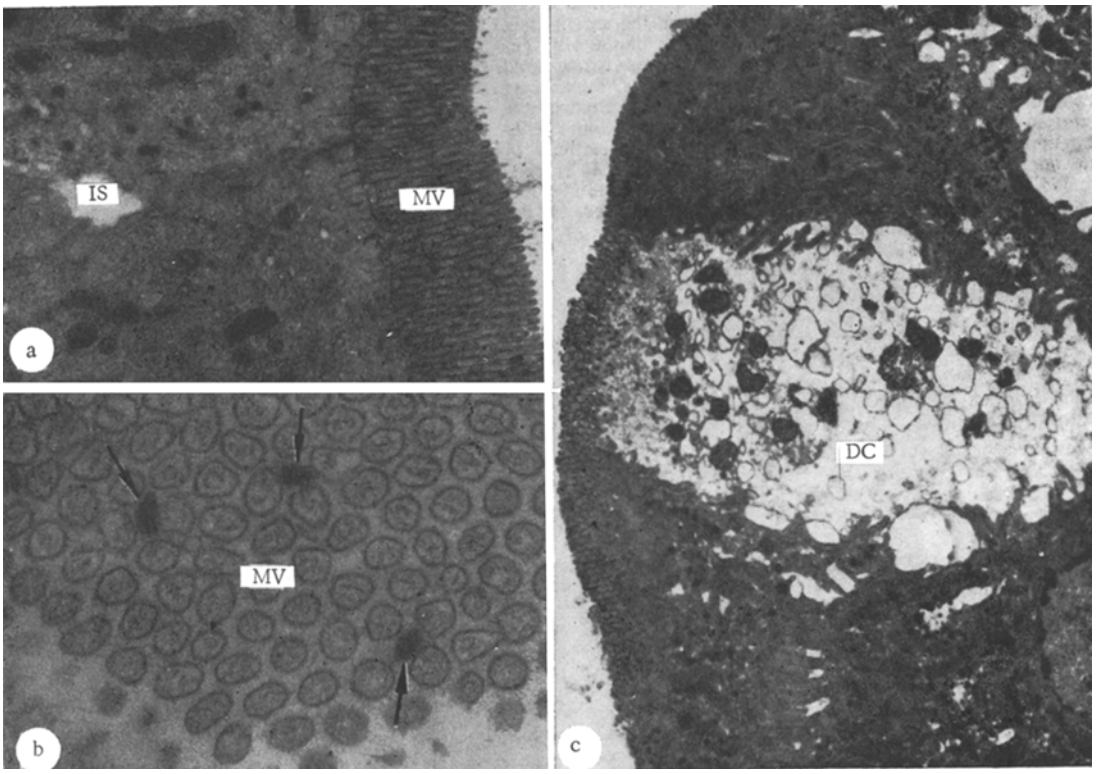

Fig. 3. Ultrastructure of enterocyte 40 min after peroral injection of horseradish peroxidase: a) heterogeneity of functional activity of enterocytes. Peroxidase reaction absent in lower cell. 10,000 ×; b) adsorption of peroxidase reaction product (arrow) on glycocalyx of microvilli of enterocyte (transverse section). 60,000 ×; c) absence of reaction product in dying cell against the background of active absorption in negihboring cells. 6000 ×. MV) Microvilli, DC) dying cell.

During investigation of protein absorption along the villus heterogeneity of functional activity of the epithelial cells was observed. Side by side with actively absorbing cells there were enterocytes in which absorption was much lower or absent altogether. In some cells, although the formation of pinocytotic vesicles was observed, no peroxidase reaction could be found or it was weak (Fig. 3a). Functional heterogeneity of the enterocytes during absorption and differences in the content of various enzymes on the apical membrane of the enterocytes were reported previously [3, 12]. Possibly the heterogeneity of absorption for peroxidase, just as for other nutrients, could be due to difference in the adsorption capacity of the apical enterocyte membrane as a result of its functional selectivity relative to different substrates. Before a given substrate can enter an enterocyte it must be bound to the surface of the microvilli of the apical membrane. Reaction product for peroxidase was found at different levels of microvilli of the apical enterocyte membrane (Fig. 3b).

The question of possible penetration of certain nutrients into the bloodstream through damaged or shed enterocytes has been discussed [6]. However, we were unable to find peroxidase entering cells at the apex of the villus which showed signs of degradation. Conversely, against the background of active absorption, the peroxidase reaction product was absent in dying cells (Fig. 3c).

It can thus be concluded from the results that the pinocytotic mechanism of transport, at least for certain proteins, persists in mammals into adult life. This evidently explains how a certain quantity of foreign protein, capable of inducing an allergic response of the body, can enter the bloodstream, by-passing the intestinal barrier. Absorption of protein is perhaps not so much determined by the actual mechanism of transport itself as dependent on the level of its degradation at different stages of digestion, and also on the adsorption capacity of the apical membrane of the absorbing cells of the small intestine.

LITERATURE CITED

1. R. I. Kushak, in: The Physiology of Absorption [in Russian], Leningrad (1977), p. 285.
2. I. A. Morozov, in: Fundamental Problems in Gastroenterology [in Russian], Kiev (1981), p. 171.
3. V. S. Nesterenko, G. M. Rott, A. V. Piskarev, et al., Byull. Éksp. Biol. Med., No. 9, 267 (1980).
4. A. M. Ugolev, Physiology and Pathology of Contact Digestion [in Russian], Leningrad (1967).
5. S. L. Clark, J. Cell, Biol., 5, 41 (1959).
6. G. Geyer, Acta Histochem., 64, 213 (1979).
7. R. S. Graham and M. J. Karnovsky, J. Histochem. Cytochem., 14, 291 (1966).
8. R. Rodewald, J. Cell Biol., 58, 189 (1973).
9. S. J. Saunders and K. J. Isselbacher, Gastroenterology, 50, 586 (1966).
10. I. Seifert, R. Gancer, and W. Brendel, Z. Gastroent., 17, 1 (1979).
11. T. W. Sheehy and M. H. Floch, The Small Intestine: Its Function and Diseases, New York (1964).
12. K. Smith, T. Mukherjee, and R. Rowland, in: Electron Microscopy, Vol. 11, Melbourne (1980), p. 415.
13. J. N. Udal, K. Pank, L. Fritze, et al., Pediat. Res., 15, 241 (1981).
14. G. Wiseman, Absorption from the Intestine, London (1964).

CHANGES IN EPITHELIUM OF THE NASAL MUCOSA DURING ADAPTATION TO HIGH-ALTITUDE CONDITIONS

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Much attention has recently been paid to the adaptation of man and animals in various extremal climatic zones [2, 3, 8]. One such zone is at high altitudes in the mountains [1]. Although the nose plays an important role in maintaining functional unity of the respiratory system [7] and is the first structure exposed to the action of the atmospheric environment, changes in the nasal mucosa under the influence of high mountain altitudes have not been adequately studied.

To study the time course of adaptive morphological changes in the upper respiratory passage at high altitudes, the nasal mucosa of rabbits was investigated by scanning electron microscopy (SEM) during a stay of 1 month in the Pamir Mountains.

EXPERIMENTAL METHOD

Experiments were carried out on 25 male Chinchilla rabbits weighing 2.8-3.5 kg. The animals were taken up to the Anzob Pass (3375 m above sea level) and killed on the 3rd, 7th, 14th, and 30th day of their stay at a high altitude. The nasal mucosa of rabbits killed at an altitude of 810 m above sea level served as the control. An original method was used for fixation, namely injection of 3-5 ml of 2% glutaraldehyde solution in phosphate buffer (pH 7.3) in a single dose immediately after decapitation. After inspection of the nasal cavity, pieces of mucosa measuring 0.5 × 0.5 cm were excised from the middle third of the nasal septum, fixed in 2% glutaraldehyde solution at 4°C for 24 h, postfixed in 1% OsO_4 in phosphate buffer (pH 7.3) for 1 h, dehydrated in acetone, and dried by the critical point method in liquid CO_2 in the chamber suggested by Murakhovskii and Kholanskii [4]. The pieces of tissue

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