ULTRASTRUCTURE OF THE JUXTAMURAL MUCOSAL LAYER OF THE SMALL INTESTINE

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The surface of the mucosa of the small intestine is covered with a hydrated layer, the principal structural and binding component of which is mucus. For a long period of time the juxtamural mucosal layer has been regarded as a protective or diffusion barrier, whose properties are determined by the effect both of the mucinous gel and of the nonmiscible aqueous layer lying next to the surface of the epithelium [14]. Attention has now returned to it. This is largely due to the fact that a large quantity of hydrolytic enzymes and of substrate-binding proteins has been found in the juxtamural zone of the mucous membrane [1, 2, 7]. These and other facts give grounds for the suggestion that the juxtamural layer performs not only a barrier, but also a digestive and a transport function. It will be evident that the properties of the juxtamural layer will largely depend on the presence of particular structural and functional components in it. The aim of the present investigation was to study the structure and composition of the juxtamural mucosal layer.

EXPERIMENTAL METHOD

The structure of the juxtamural layer was studied by using samples of mucous membrane of the small intestine of rats and chickens weighing 150-250 g, kept on a standard animal house diet, and also on biopsy specimens taken from the human small intestine. Preparations of mucus obtained from the surface of the small intestine of animals by the method of shaking from an everted intestinal pouch [7]. To ensure maximal preservation of the juxtamural layer chemical fixation was used and was combined with methods of mechanical stabilization of the mucu. It was found that the juxtamural layer is better preserved if it is covered with chyme or with a layer of agar gel. One series of specimens of intestine was therefore fixed together with its contents, or before fixation melted aga, made up in 4% paraformaldehyde, was applied to the surface of the mucosa. In some animals, under anesthesia, molten agar was introduced into a segment of small intestine isolated by ligatures, in situ and, after polymerization of the agar, the intestine was fixed and embedded in resin together with the agar. Samples of small intestine and preparations of mucus sedimented by centrifugation at 3000 rpm, were fixed in a 4% solution of paraformaldehyde, made up in Hanks' buffer, for 3 h. This was followed by postfixation for 3 h in 1% OsO_4. The stability of the juxtamural mucosal layer was appreciably improved by the addition of 0.2% OsO_4 to the aldehyde fixative, and the use of the method of OTO-contrasting [5]. The material was dehydrated in acetone and embedded in a mixture of Epoxide resins (Epon and Araldite). Semi-thin sections for light microscopy were stained with toluidine blue, and ultrathin sections for electron-microscopic study were stained successively with uranyl acetate and lead citrate.

EXPERIMENTAL RESULTS

When the structure of the juxtamural mucosal layer is examined and its extent estimated, the structure of the surface of the small intestinal mucosa must be taken into consideration, distinguishing the juxtamural layer, lying between the intestinal villi and above their apices. Since the villi are in the closed state and the distance between them does not exceed a few microns, the thickness of the intervillous layer was approximately the same. This region of the juxtamural layer also is isolated from the external medium. Above the apices of the villi the mucosal layer is not in any way limited and may be much more extensive. It makes direct contact with the intestinal contents. In our view the concept of "thickness of the mucosal layer" (usually the thickness of the layer is judged in relation to its luminal part) is somewhat conventional. The study of the juxtamural layer in animals in whose intestinal

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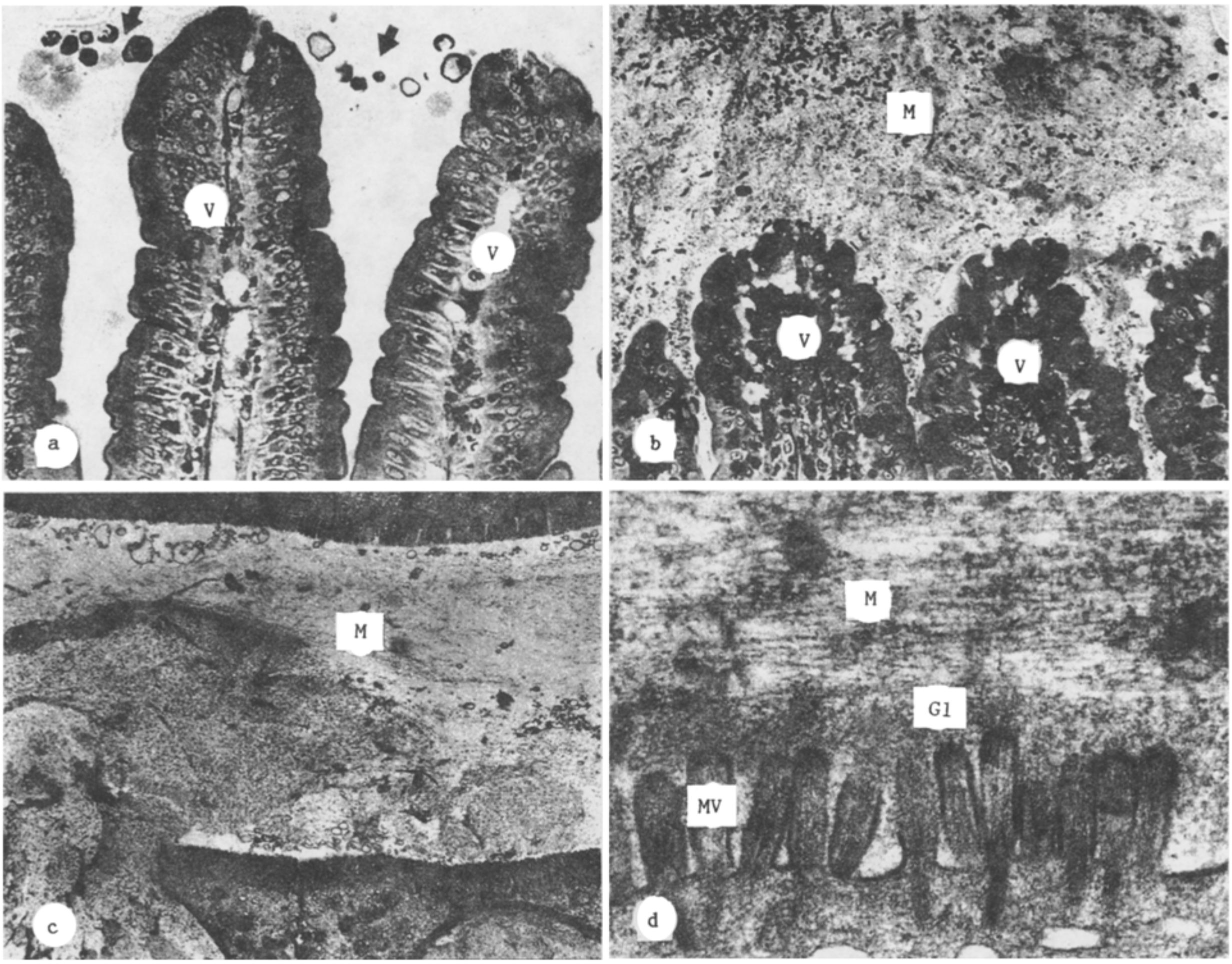

Fig. 1. Structure of juxtamural mucosal layer of small intestine. a) Location of food particles (arrow) along boundary of juxtamural layer of rat small intestine. 350×; b) absence of outer border of juxtamural mucosal layer in rat small intestine. 350×; c) regions with different density of mucus in intervillous space of rat small intestine. 6000×; d) relations between glycoproteins of mucus and glycocalyx of microvilli of an enterocyte. Human jejunum. 50,000×. V) Villi; Gl) glycocalyx; Mv) microvilli; M) mucus.

lumen no chyme was present showed that it had a visible outer border, along which there could be food particles (Fig. 1a). The mucinous gel probably prevented the penetration of large food food particles to the surface of the epithelium. However, on fixation of the small intestine together with its contents, it was found that the juxtamural layer does not have a clear outer boundary and changes directly into chyme (Fig. 1b).

The principal structural component of the juxtamural layer is mucus, which is continuously secreted by the goblet cells of the intestinal epithelium. The juxtamural mucus forms a viscoelastic water-soluble gel, of which 95% is water [9]. The study of the ultrastructure of the juxtamural layer revealed that in different parts of it the density of the mucinous gel may vary. During secretion by goblet cells the mucus has a high density, but later, when distributed along the surface of the epithelium, it may form less dense reticular structures (Fig. 1c). Under these circumstances the mucins of the mucus, when in contact with the glycocalyx of the microvilli of the enterocytes, always form a visible border (Fig. 1d). In individual regions of the juxtamural zone of the epithelium areas may be found in which the concentration of mucins of the mucus is very high (Fig. 2a). These concentrations of mucus evidently possess internal stability and may be preserved, or may even become components of the cyme. The heterogeneity of the mucosal layer can exert a significant influence on digestive and transport processes in the juxtamural zone, for the glycoproteins of the mucus possess marked adsorptive properties and can bind with both food substrates [12] and hydrolytic enzymes [7].

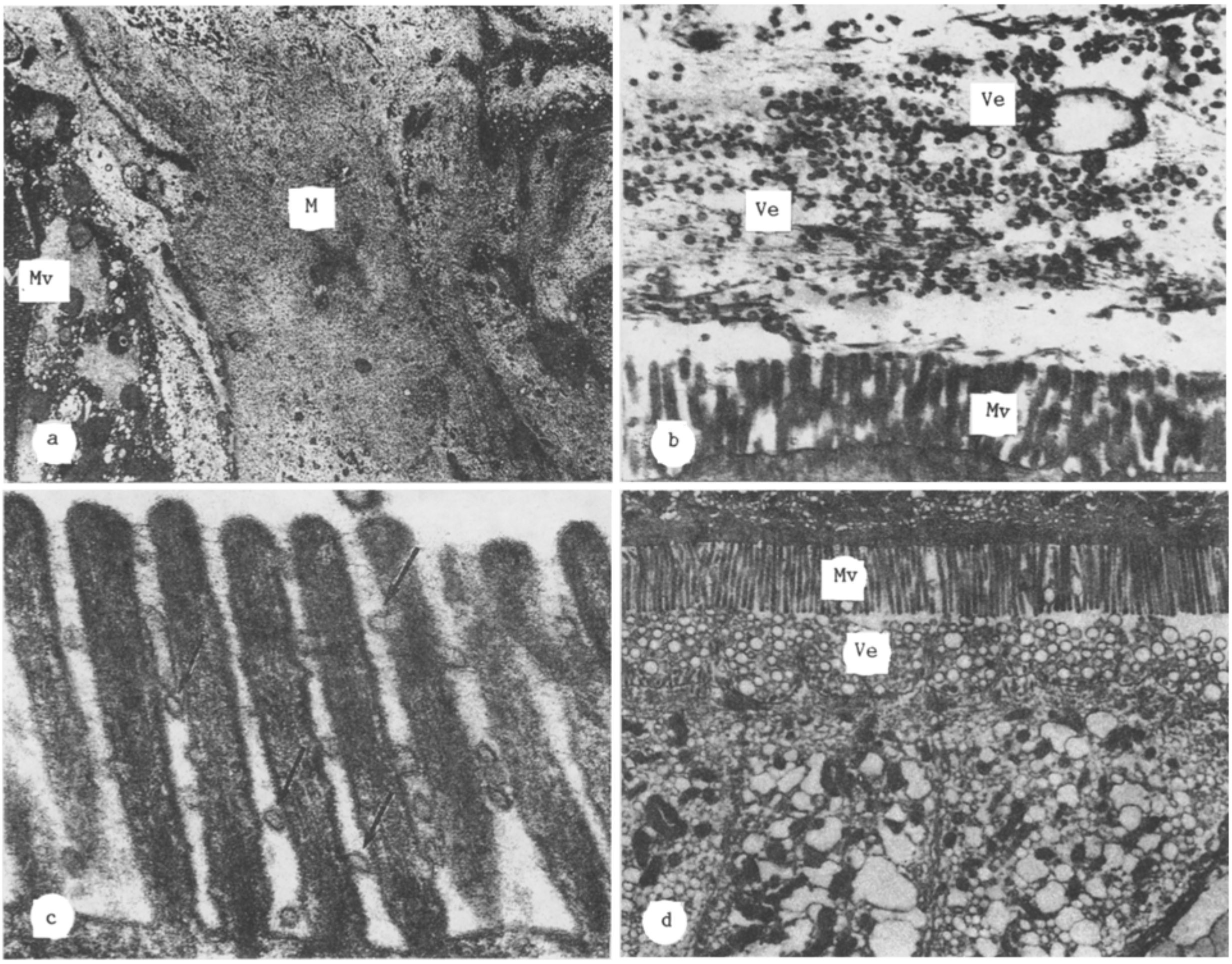

Fig. 2. Composition of juxtamural layer and mechanisms of its formation. a) Concentrations of dense mucus in juxtamural zone of rat small intestine. 3500×; b) membrane vesicles in juxtamural layer of rat small intestine. 15,000×; c) budding of secretory vesicles (arrow) from lateral surface of microvilli of a rat enterocyte. 60,000×; d) fragmentation of microvilli of enterocytes of human jejunum. 6000×. Ve) Vesicles; Mv) microvilli; M) mucus.

Besides glycoproteins of mucus, the juxtamural layer also has in its composition other structural components, which could be divided into endogeneous and exogenous. The endogenous may include desquamated epithelial cells or their fragments, and membrane-like and vesicular structures, some of which are probably remains of the same cells, and also lymphocytes and other cells, entering the juxtamural layer from the epithelium. Among the number of exogenous components must be included various food particles and fibrous structures, bacteria and microorganisms,s such as Lamblia, which possess their own motor apparatus. Each of these components may also affect the general and local properties of the juxtamural layer.

A large number of membranous vesicles may also be found in the juxtamural zone, especially close to the surface of the enterocytes (Fig. 2b). It has been shown that such vesicles are formed from membranes of microvilli of the enterocytes, as a result of microapocrine secretion (Fig. 2c). Similar vesicles also are formed on destruction of the enterocytes due to partial or total fragmentation of the microvilli (Fig. 2d).

As regards the presence of desquamated epithelial cells in the composition of the juxtamural layer, it has to be said that their number may differ in different animals. According to our data, a very small number of such cells can be identified in the juxtamural zone of the rat and chicken small intestine. A study of the content of nucleic acids and various intracellular enzymes in the juxtamural mucus showed that it amounted to not more than 3% compared with the mucosal homogenate [7]. Meanwhile, during a study of biopsy material from the human small intestine, we very often found numerous single desquamated cells and whole cell sheets in the juxtamural zone of the epithelium (Fig. 3a). It was found that they can

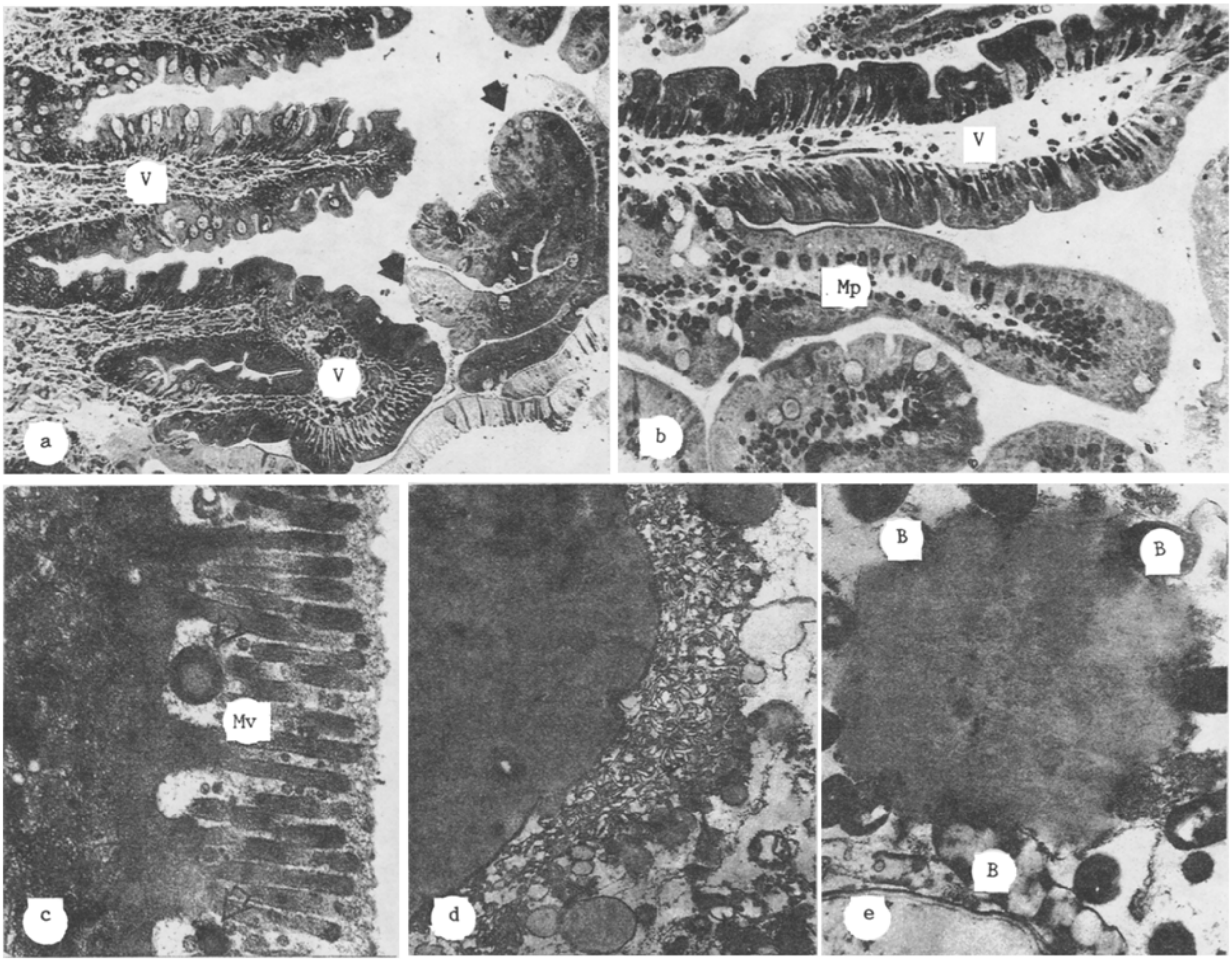

Fig. 3. Epithelial cells and food particles in juxtamural zone of epithelium of small intestine: a) fragments of epithelium (arrow) in juxtamural zone of huma jejunum. 290×; b) micropolyp-like formations in mucosa of human jujunum. 320×; c) particles of food substrates (arrow) in space between microvilli of an enterocyte. Heman jejunum. 30,000×; d) lamellar structures on surface of a lipid drop, lying in juxtamural zone of rat small intestine. 10,000×; e) bacteria on surface of food particle in juxtamural layer of rat small intestine. 15,000×. B) Bacteria; V) villi; Mv) microvilli; Mp) micropolyp.

appear as a result of destruction of micropolyp-like formations, formed in the mucosa (Fig. 3b). According to data in the literature, single epithelial cells and fragments of villi may also be found in the contents of the intestinal lumen and in the juxtamural mucosal layer in dogs. It is claimed that villi enter the juxtamural layer as a result of intravital desquamation [8]. Besides desquamated enterocytes, lymphocytes and certain other cells of the immune system also are found in the supraepithelial mucosal layer. They penetrate into the lumen of the intestine most frequently in the zone of rejection of enterocytes, along the intercellular spaces. It is considered that these cells perform a protective function in the layer of mucus and in the lumen of the intestine [10].

Although the juxtamural mucosal layer is also a diffusion barrier, during digestion many food particles may be found in its different parts. Even comparatively large particles 10 μm or more in diameter penetrate as far as the surface of the enterocytes in the upper third of the intestinal villi. Under these cirumstances, smaller particles of food substances measuring from 10 to 150 nm may also be found in the space between the microvilli of enterocytes (Fig. 3c). The presence of food substances in the juxtamural zone is coupled with their active hydrolysis, individual morphological featuresof which can be observed (Fig. 3d). As has been shown, not only pancreatic enzymes adsorbed in mucus can participate in contact digestion [3], but also enterocytic enzymes of the final stages of hydrolysis [2, 7]. The number of the latter in the juxtamural layer varies (depending on species) from 2 to 20%

[6, 7]. Bacteria also take part in the hydrolysis of food substrates. This can be judged by the formation of erosions on the boundary of contact between bacteria and food particles (Fig. 3e).

Thus when the structure and composition of the juxtamural mucosal layer are examined, it can be concluded that it is a complex, multiple component, and dynamic system. Its luminal region, in contact with the intestinal contents and subjected to continuous mechanical and chemical action, is destroyed and rejected from the surface of the mucosa. On the other hand, continuous growth of the mucosal layer takes place on account both of the secretion of mucus itself and of other secretory processes, taking place in the epithelium of the small intestine. Attention must also be paid to the fact that powerful transport flows exist in the juxtamural zone of the epithelium. One of them is directed to the surface of the epithelium. By means of it food substrates may reach the enterocytes. Another flow runs in the opposite direction and is linked with intestinal secretion. Meanwhile, the juxtamural zone of the epithelium is characterized by definite species-specific constancy of a whole range of parameters (pH, composition of trace ions, etc.) [11, 13]. It is evident that all the factors listed above ultimately enable the creation of optimal conditions in the juxtamural layer not only for the maintenance of the barrier function of the juxtamural mucus, but also regulating hydrolysis of food substrates and their delivery to the transport systems of the epithelial cells.

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