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THE DEVELOPMENT OF MEMBRANE SURFACE AREA OF ENTEROCYTES OF RAT DISTAL COLON MUCOSA: STEREOLOGICAL ANALYSIS

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ABSTRACT

The developmental changes of rat distal colon during suckling and weaning periods were studied using electron microscopy. The basolateral membrane surface density of superficial enterocytes was estimated by the stereological method of vertical sections. It was shown that the membrane surface density increases during maturation of rat dital colon.

Key words: basolateral membrane, rat colon, superficial enterocytes, surface density.

INTRODUCTION

The mono-layered epithelia of various organs, such as colon, function as selective barriers between the 'external' and 'internal' (blood, interstitium) compartments with different compositions of solutes and water. During the past two decades, numerous studies have revealed that the quantitative and qualitative ultrastructure of epithelia correlates with their transport capacities. Morphometric and physiological studies performed on mammalian renal epithelia demonstrated that the basolateral surface density is higher if sodium transport rate (Kaissling et al., 1985; Stanton et al., 1985) or Na,K-ATPase activity are increased (Le Hir et al., 1982). Similarly, the increased sodium absorption or potassium secretion positively correlates with the surface density of basolateral membranes of enterocytes in rat distal colon (Kashgarian et al., 1980). In contrast to mammals, the increase of basolateral membrane area was not observed in avian lower intestine (Elbrond et al., 1991).

The nature of the above mentioned structure-function correlation has not been thoroughly investigated in immature intestine at the ultrastructural level. It is known that the immature intestine exhibits developmental changes of ion and water transport. Functional studies indicate that colonic water and sodium absorption increases during suckling and weaning periods and decreases later (Finkel et al., 1985; Pácha et al., 1987, 1995). Furthermore, Na,K-ATPase in colon also rises during early postnatal life (Pácha et al., 1991).

The aim of the present study was (1) to characterize the developmental changes of rat distal colon during suckling and

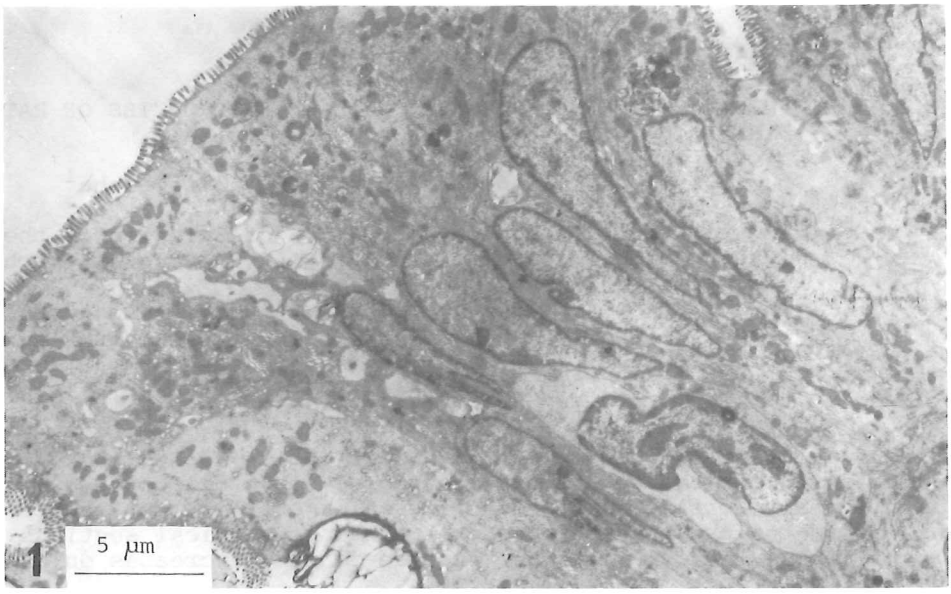

Fig. 1. 30-day-old rat. Epithelium of mucosa of distal colon.

Fig. 2. 30-day-old rat. Apical part of a superficial enterocyte of distal colon with less complicated basolateral membranes.

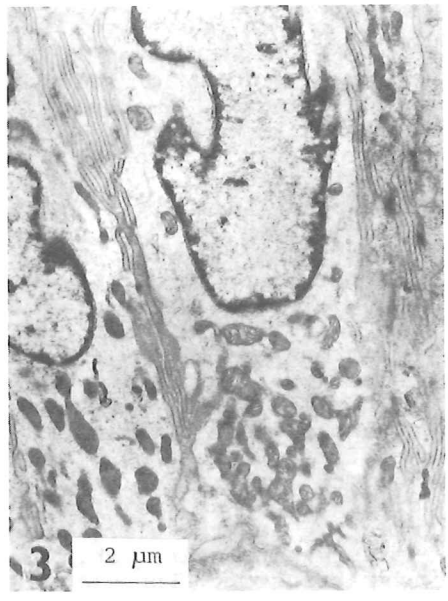

Fig. 3. 30-day-old rat. Basal part of enterocytes with very complicated basolateral membranes.

weaning periods using transmission electron microscopy and stereological methods and (2) to correlate these changes with the previously published functional data. The developmental changes of the basolateral membrane surface densities of superficial enterocytes in rat distal colon during maturation were studied as well as the entire basolateral membrane surface area of enterocytes in the colon and the activity of the sodium pump.

MATERIAL AND METHODS

Two groups of Wistar rats - sucklings (14-day-old) and weanlings (27-day-old) were studied. Weanlings were housed with the dams till the age of 27 days. They were fed regular rat chow and tap water ad libitum. The pups were decapitated, intestinal segment removed, rinsed out (0.9% saline) and its part just above the pulvic brim, i.e. distal colon, was cut off. Superficial enterocytes of the distal colon were studied, i.e. the cells of the mucosa, not the cells inside the crypts of Lieberkühn (Fig.1,2,3).

The distal colon of each rat was weighed. The method of vertical sections (Baddeley et al., 1986) was used for estimation of the basolateral surface area per unit volume of superficial enterocytes (surface density, S_V(bas)). The colon was cut longitudinally, flattened and the vertical axis was chosen to be perpendicular to the flattened colon wall. The colon was cut into five to ten blocks which were embedded in Epon 812. Semi-thin and ultrathin vertical sections were cut from three to five blocks per rat. Several bands encompassing the entire height of cells were evaluated in each block. The cycloidal test system combined with the point test system was superimposed on the electron micrographs of ultrathin sections and the intersections of cycloid arcs with the basolateral membranes of superficial enterocytes were counted as well as the test points lying in cell profiles. The standard formula for surface density estimation (see Baddeley et al., 1986) was used. The volume density of enterocytes in the colon was estimated from semi-thin sections by a point-counting method.

Together with the above structural parameters, a functional parameter of the basolateral membrane was studied, namely the activity of the sodium pump (Na,K-ATPase) enabling the extrusion of Na^+ from the cytosol. It was assessed by measuring hydrolysis of ATP as described in Pácha et al. (1991).

RESULTS

The results (Table 1,2) show an increase in S_V(bas) by 52% during maturation of rat distal colon. The differences, judged by two sample Student's t-test, were significant at 0.01 level.

The volume density of enterocytes in the colon was similar in suckling and weaning periods.

In 14-day-old rats, Na,K-ATPase activity was 3.82±0.20 $\mu molP_i/mg.hr$ (6) and in 27-day-old animals 4.9±0.31 $\mu molP_i/mg.hr$ (6), i.e. it has increased by 29%.

Table 1. Distal colon of 14-day-old rats (sucklings). The weight and basolateral membrane surface density of superficial enterocytes.

SUCKLINGS	colon weight [g]	S_V(bas) [μm^{-1}]
1	0.036	1.60
2	0.028	1.80
3	0.018	1.85
4	0.041	1.95
mean	0.031	1.80
coefficient of variation	0.32	0.08

Table 2. Distal colon of 27-day-old rats (weanlings). The weight and basolateral membrane surface density of superficial enterocytes.

WEANLINGS	colon weight [g]	S_V(bas) [μm^{-1}]
1	0.132	2.58
2	0.073	3.17
3	0.121	2.33
4	0.116	2.83
mean	0.111	2.73
coefficient of variation	0.23	0.13

DISCUSSION

The present study indicates that maturation of rat distal colon represents not only the developmental changes of transport functions but also the alterations of membrane surface densities in superficial enterocytes. Our results demonstrate a significant increase in basolateral membrane surface density between suckling and weanling pups. Taking into account that the colon weight has increased more than twice

(see Table 1,2) and that the volume density of enterocytes in the colon was found to be similar in suckling and weaning periods, it can be concluded that the entire surface area of basolateral membranes of all superficial enterocytes in the colon has increased significantly as well.

Sodium transport (Finkel et al., 1985; Pácha et al., 1995) and Na,K-ATPase follow similar developmental profile as basolateral membrane surface density. It can be concluded that the correlation between structure and function exists already in immature developing colon and not only in mature epithelium as shown by Kashgarian et al. (1980).

The basolateral membrane surface density has increased by 52% between suckling and weaning period, while Na,K-ATPase activity by 29%. In our previous experiment, the electrogenic sodium transport pathway in colon has increased by 110% (Pácha et al., 1995) in the same period. Since Na,K-ATPase is localized in basolateral membranes and sodium ions are extruded from the cell via this enzyme, it seems likely that the accelerated sodium absorption in weanling rats is supported by an increase in basolateral membrane surface density and the entire basolateral membrane surface area of superficial enterocytes in the distal colon.

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