# AGE DIFFERENCES IN TRANSPORT ATP-ASE ACTIVITY IN PLASMA MEMBRANES OF SMALL INTESTINAL ENTEROCYTES

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The conditions of neonatal feeding in the first days after birth and particular features of digestion, characterized by the intensive absorption of nutrients in the small intestine, imply a special state of the functional regions of the enterocyte plasma membrane: the apical (brush border — BB) and the basolateral membranes (BM). Also during this period of neonatal development acute disorders of digestion of noninfectious nature are observed, with the occurrence of diarrhea and dehydration, with loss of electrolytes [5, 7, 11]. It has been shown [9] that under these circumstances significant changes in lipid composition take place in both BB and BM, and these may be reflected in the passive permeability of these membranes. The aim of the present investigation was to study activity of enzymes involved in the active transport of ions, namely the transport adenosine triphosphatases: Na,K-ATPase, Ca,Mg-ATPase, and Mg-ATPase [6].

## EXPERIMENTAL METHOD

Membrane preparations of BB and BM were obtained from a homogenate of the mucosa of the proximal part of the jejunum of an adult cow (age 1.5-3 years), or of newborn healthy calves, or calves suffering from noninfectious diarrhea (age 3-5 days), by the method of differential centrifugation [8]. The protein content in the samples was determined by the method of Lowry et al. [12].

Hydrolysis of ATP by the test membranes was studied by measuring the change in pH of the incubation medium [1]. Specific ATPase activity was assessed by inhibiting alkaline phosphatase with theophiline (20-40 mM) and allowing for the degree of vesiculation (0.5 mg alamethicin/mg protein) of the membranes, as described previously [6].

## EXPERIMENTAL RESULTS

Na,K-ATPase was found to be located only in BM of the small intestinal enterocytes [6]. Its activity was the same in the adult and healthy newborn animals (Fig. 1a), whereas in calves with diarrhea it was reduced by 5.3 times. Considering the functional role of Na,K-ATPase [4], it can be tentatively suggested that weakening of its activity in pathology leads to loss of sodium from the body.

Those well-known diarrhea-inducing factors — laxatives [14] and cholera toxin [13] — also depress Na,K-ATPase activity in the mucous epithelium of the intestine. It can therefore be tentatively suggested that biochemical changes connected with inhibition of Na,K-ATPase may be one explanation of the loss of Na by the body during diarrhea. In particular, in noninfectious diarrhea this may be due to a change in the lipid composition of BM: an extremely low concentration of phosphatidylcholine, on the presence of which Na,K-ATPase activity depends [4], in BM of the sick animals, the appearance of lyso-forms of phospholipids, lowering of the cholesterol/phospholipid ratio, and a change in the fatty-acid composition of the membranes [9], which may ultimately change the conformation of the enzyme [3, 10].

The functional role of Ca,Mg-ATPase in the membranes of BB and BM differs and was discussed in [6]: in BB it takes part in the work of the contractile apparatus of the microvilli of the enterocytes, whereas in BM it is involved in active $Ca^{2+}$ transport.

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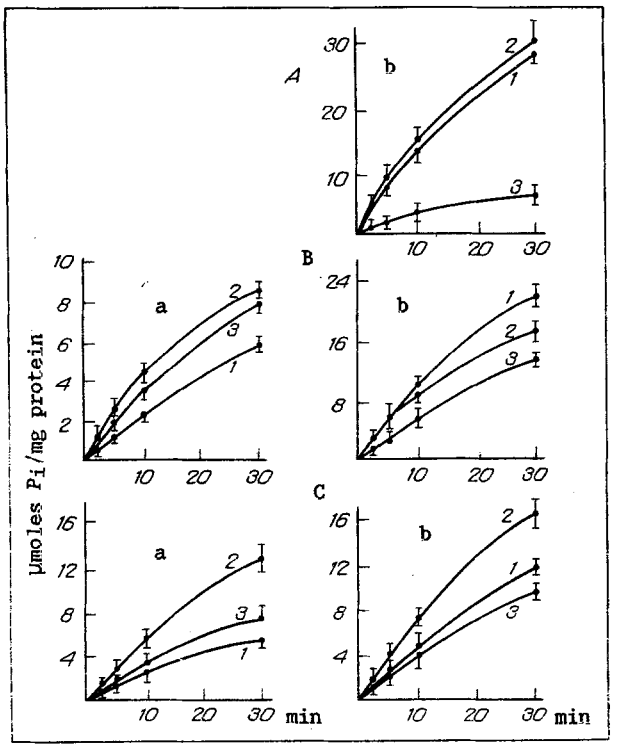


Fig. 1. Activity of Na,K-ATPase (A), Ca,Mg-ATPase (B), and Mg-ATPase (C) in brush border (a) and basolateral membranes (b). **Legend:** 1) adult animals; 2) healthy neonates; 3) neonates with diarrhea.

Activity of Ca,Mg-ATPase in BB did not differ in the healthy newborn animals, but was lower in adults (Fig. 1b). A special functional state of this part of the enterocyte in the colostrum period, connected with intensive absorption of nutrients, can be postulated.

In BM, Ca,Mg-ATPase activity in the adult animals was higher than in neonates, confirming the necessity for activity of this particular enzyme in lactating animals. The sufficiently high Ca,Mg-ATPase activity in healthy neonates is in harmony with the intensive utilization of calcium to provide for growth of bone tissue [2]. Meanwhile, in calves with diarrhea, Ca,Mg-ATPase activity was lower than in healthy neonates. In calves with diarrhea the total serum calcium level is depressed [2]. This has been explained on the basis of data showing increased excretion of calcium phosphates and vitamin D with the feces. Another possibility is that limited intake of Ca by the sick animals is linked with a decrease in Ca,Mg-ATPase activity which we found in the basolateral membranes.

Mg-ATPase activity in BM of all groups of the experimental animals was higher than in BB. Moreover, in healthy neonates it was appreciably higher than in adults, whereas in the sick animals the converse was the case. If a role of this enzyme of the surface membrane of the enterocyte in regulation of cell pH is accepted [15], the pinocytotic pathway of entry of nutrients into the body in the postnatal period demands a more powerful pH-regulating system. The change in Mg-ATPase activity in diarrhea is in agreement with disturbance of the acid—base balance of the sick animal [5].

In our opinion, if noninfectious diarrhea is regarded as a compensatory and adaptive response of the body aimed at flushing out the potentially harmful contents of the alimentary canal (excess food, casein clots, poor quality food, etc.), the need for abundant secretion of $H_2O$ and sodium — the main cellular electrolyte in the intestinal cavity, becomes evident. In this connection weakening of Na,K-ATPase activity can be classed as a compensatory adaptive change, whereas a decrease in activity of the other ATPases can be regarded as an undesirable phenomenon.

The changes in ATPase activity discovered in the membrane fractions thus, on the one hand (the brush border and basolateral membranes), reflect age differences in the functional state of the plasma membrane of the enterocyte, and on the other hand they are evidence of the important role of ATPases in active transport of ions (Na, K, Ca) in the intestine during diarrhea.

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