ACTIVITY OF LYSOSOMAL PROTEINASES AND LYSOSOMES OF THE SMALL INTESTINE DURING POSTNATAL DEVELOPMENT

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Various opinions have been expressed on mechanisms whereby the utilization of milk proteins in early postnatal development attains such a high efficiency. It has been suggested that the main form of digestion of milk proteins in infancy is by pinocytosis and intracellular digestion [1]. Data have been obtained to show the predominant role of membrane digestion in the postnatal period of development [2]. The view is also expressed in the literature that the proteolytic system of milk protein digestion and on the existence of well-developed intraluminal digestion in early postnatal ontogeny [3].

The aim of this investigation was to study activity of lysosomal proteinases in the small intestine at different stages of postnatal development and also to undertake a morphological study of epithelial cells of the rat small intestine.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats aged 3-15 days and on adult animals. Milk-fed animals were killed after natural milk feeding for 1 h. Adult animals also were killed after feeding for 1 h. To prepare homogenates the small intestine was removed from the animals soon after sacrifice, washed with physiological saline to remove the contents, and the mucosa was separated. The mucosa was minced and homogenized for 2 min in a homogenizer at a speed of 1000 rpm. The lysosomal fraction was isolated from mucosal homogenates of the ileum and jejunum by differential centrifugation by De Duve's method. Activity of lysosomal marker enzymes – acid phosphatase (AP) [7] and cathepsins [8] – was determined in the lysosomal fraction. Tissue for electron-microscopic investigation was taken from the proximal and distal portions of the small intestine of rats aged 15 days and 1 month, and fixed in 2.5% glutaraldehyde solution in phosphate buffer, pH 7.2. After rinsing in buffer the tissue was postfixed in 1% OsO_4 solution, dehydrated in alcohols of increasing concentration, saturated, and embedded in a mixture of Epon and Araldite.

EXPERIMENTAL RESULTS

AP activity in the lysosomal fraction of the jejunal mucosa of 3-day-old rats was almost two-thirds lower than in the ileal mucosa. In rats aged 15 days AP activity in the lysosomal fraction of the ileal mucosa also was almost three times higher than in the jejunal mucosa (Fig. 1A). AP activity in the lysosomal fraction of the jejunum and ileum of adult rats was about equal.

Activity of cathepsins in the lysosomal fraction of the ileal mucosa of 3-day-old rats was more than 2.5 times higher than in the jejunal mucosa. The same shift of the maximum of cathepsin activity toward the distal part of the small intestine was observed in animals aged 15 days. Cathepsin activity of the lysosomal fraction of the mucosa of the jejunum and ileum of adult rats was equal (Fig. 1B).

The data described above, indicating that in animals during postnatal development activity of lysosomal proteinases in the mucosa of the distal part of the small intestine is three times higher than in the mucosa of the proximal part, are evidence that lysosomes in the distal portion of the small intestine play a special role in the assimilation of milk proteins at an early age. To confirm these data an electron-microscopic study was made of cells of the proximal and distal portions of the small intestine of rats during postnatal development (aged 15 days) and in rats aged 1 month, after the transition to definitive feeding. Enterocytes of the ileum of rats aged 15 days differed in the location of giant lysosomes (phagosomes) in them, namely close to

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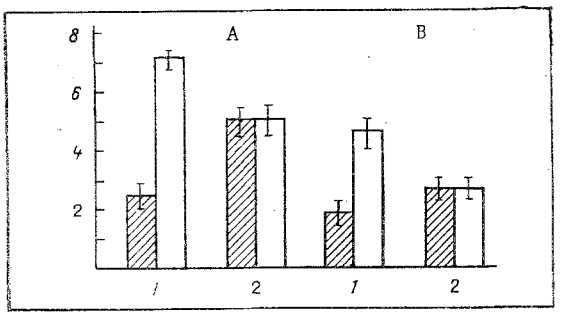

Fig. 1. Activity of AP (A) and cathepsins (B) in milk-fed animals (1) and adult rats (2). Shaded columns indicate jejunum, unshaded columns – ileum. Ordinate, activity (in units/mg protein).

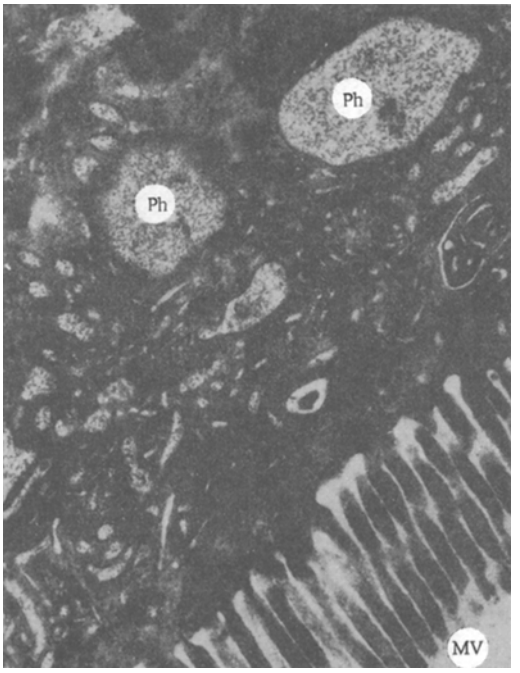

Fig. 2. Phagosomes (Ph) in apical part of enterocytes in ileum of naturally fed 15-day-old rat. MV) Microvilli. 15,000 ×.

the microvilli (Fig. 2). These data agree with the results of biochemical investigations, demonstrating higher (threefold) activity of lysosomal proteinases in the mucosa of the ileum compared with the jejunum in animals aged 3-15 days. It will be clear from Fig. 2 that the membrane of the microvilli is folded to form pinocytotic vesicles. In adult rats, after the transition to a definitive diet, phagosomes are no longer present and ordinary lysosomes are no longer located close to the microvilli, as at an early age. Many mitochondria are observed in the prenuclear zone of adult rats (Fig. 3).

The writers showed previously that during postnatal development of rats intraluminal digestion of milk proteins takes place to a marked degree, and is completed in the distal part of the small intestine – in the ileum. In adult animals intraluminal digestion of milk proteins is completed in the proximal part of the small intestine – in the jejunum [3-6]. Since intraluminal digestion of milk proteins in milk-fed animals is completed in the distal part of the small intestine, lysosomal proteinases in the distal part of the small intesting participate in these concluding stages of protein assimilation. The process of protein assimilation – absorption of proteins in accordance with the principle of pinocytosis – also plays a special role at an early age. Histological confirmation of the biochemical data on the localization of giant lysosomes (phagosomes) in enterocytes of the ileum agreed with data obtained previously. It is also stated in the literature that large primary lysosomes

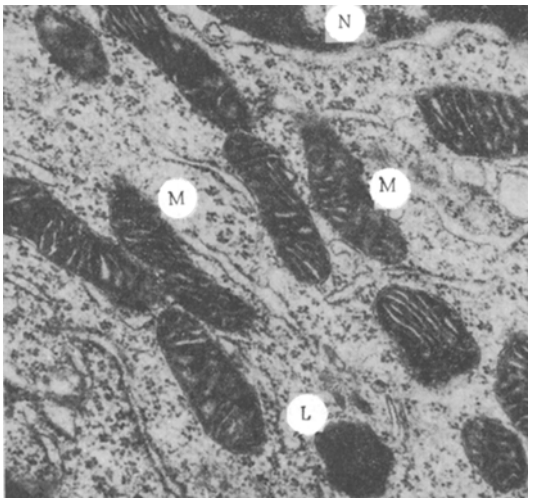

Fig. 3. Lysosomes (L) in prenuclear zone of enterocytes in small intesting of adult rat on definitive feeding. N) Nucleus, M) mitochondria. 15,000 ×.

about 2 μ in diameter [12], which is more than three times larger than the small lysosomes, are observed in most cells in all parts of the small intestine.

According to other workers [2, 7], activity of lysosomal proteinases is reduced in the proximal part of the small intestine, and this facilitates absorption of immunoglobulins present in the maternal milk [13].

Previous observations [2, 7-9] are evidence of a proximal-distal gradient of intraluminal digestion of milk proteins in the postnatal period of development.

On the basis of the results of these biochemical and histological investigations it can be concluded that a proximal-distal gradient of distribution of lysosomal proteinases is present in the small intestine and that giant lysosomes are located in enterocytes of the ileum of milk-fed animals. The transition from milk to definitive feeding is accompanied by equalization of the distribution of activity of lysosomal proteinases between the proximal and distal portions of the small intestine, with disappearance of giant lysosomes in its distal portion. The distal localization of lysosomal proteinases at an early age increases the reliability of small intestinal function and the efficiency of protein assimilation.

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