POSTNATAL CHANGES IN THE LOCALIZATION OF SMALL INTESTINAL NAD(P)H-DEPENDENT H_2O_2 -GENERATING ENZYME ACTIVITY IN THE RAT

YOSHIMARO ISHIKAWA, KEI-ICHI HIRAI* AND KAZUO OGAWA*

*Department of Pathology, Kanazawa Medical University, Uchinada, Kahoku-gun,
Ishikawa 920-20, and Department of Anatomy*, Faculty of Medicine,
Kyoto University, Sakyo-ku, Kyoto 606*

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A glutaraldehyde-insensitive, NAD(P)H-dependent H_2O_2 -generating enzyme activity in the rat small intestine and its regional differences in the small intestine of the adult rat have been described previously by us (5, 6). In the present investigation the postnatal changes of this enzyme activity in the small intestine were studied using 1-day to 6-months old male Kyo-Wistar rats.

It was found in this study that the NAD(P)H-dependent H_2O_2 -generating enzyme activity showed a marked postnatal change. The enzyme activity was significantly higher in the apical membrane of absorptive cells of both duodeno-jejunum and ileum in newborn rats, however the activity in the apical membrane of the ileum was decreased from the 7th postnatal day on, reaching eventually to an extremely weak activity in the adult. The activity in the apical membrane of the duodeno-jejunum remained the same throughout the course of growth. These findings are discussed in connection with the postnatal changes in absorptive function.

Our previous papers (5, 6) have described that a glutaraldehyde-insensitive NAD(P)H-dependent H_2O_2 -generating enzyme activity is localized in the rat duodeno-jejunal mucosa. The localization of the activity is limited on the apical membranes of differentiated absorptive cells and goblet cells when generated H_2O_2 is electron-cytochemically demonstrated by means of Briggs' cerium method (1). The ileum also showed an H_2O_2 -generating activity, but the intensity was much weaker than that of the duodeno-jejunum (6).

In the present paper, we have investigated the postnatal changes of the H_2O_2 -generating enzyme activity which is dependent on NADH in the rat small intestine.

MATERIALS AND METHODS

Male Kyo-Wistar rats, 1-day old newborn to 6-months old, were fasted 24 hr and killed by bleeding. Duodeno-jejunum from the portion of 3 cm anal to the pyloric ring, and ileum from the portion of 3 cm oral to the ileum end were dissected out. The tissues were cut into fragments of 0.5 mm squares and fixed for 10 min

in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, with 7% sucrose at room temperature. After fixation, the fragments were rinsed 5 times in the same buffer. The washed fragments were then preincubated for 10 min at 37°C in 0.1 M tris-maleate with 7% sucrose, pH 7.5, containing 1 mM 3-amino-1,2,4-triazole. The final incubation medium consisted of 0.1 M tris-maleate, pH 7.5, with 7% sucrose, 10 mM 3-amino-1,2,4-triazole, 1 mM $CeCl_3$, and 0.8 mM NADH. The fragments were incubated in this medium for 1 min at 37°C. After incubation the fragments were rinsed 5 times with 0.1 M cacodylate buffer, pH 6, and stored overnight in cold storage. The incubated fragments were post-fixed for 60 min in cold 1% OsO_4 in cacodylate buffer, pH 7.2, dehydrated by alcohols and embedded in Spurr's resin by a conventional method. The ultrathin sections were prepared on an LKB ultratome. Unstained sections were observed under a Hitachi H-500 electron microscope.

FIG. 1. A duodeno-jejunal villus of a one-day old newborn rat. Reaction products showing an NADH-dependent H_2O_2 -generating enzyme activity are seen on the apical membrane covering microvilli. $\times 2,600$

FIG. 2. A duodeno-jejunal absorptive cell of a 3-day old newborn rat. Many intervillous pits which are positive for the activity can be seen. $\times 4,000$

FIG. 3. 3-day old rat duodeno-jejenum. Reaction products can be seen in an apical spherical membrane of a degenerating absorptive cell which is being extruded. $\times 4,000$

RESULTS

Deposits of cerium perhydroxide were seen at the surface of microvilli of absorptive cells at the apical portion of villus (Fig. 1). In 3-day old duodeno-jejunal mucosa, the microvilli are not so complete as that in adults and many intervillous pits or caveolae with reaction products were seen (Fig. 2). However, the absorptive cells which are in the process of being extruded were already seen in 3-day olds and reaction products were seen on the apical spherical membrane of these degenerating cells (Fig. 3). A few vacuoles with some reaction products were observed in the subapical cytoplasm of duodeno-jejunal absorptive cells (Fig. 4). It seemed that the reaction activity was unchanged throughout the growth from the newborn to the adult (Figs. 5 to 7).

Many vacuoles were depicted in the apical cytoplasm of ileal absorptive cells of the one-day old rat. Reaction products were seen in these vacuoles (Figs. 8, 9). The reaction products seen in the ileal absorptive cells of 3-day old rats were similar to these of the duodeno-jejunal cells (Figs. 10, 11). In 7-day old rats, the reaction

FIG. 4. Distribution of the reaction products on the absorptive cell in the 3-days old rat duodeno-jejunum. $\times 17,000$

FIGS. 5-7. Distribution of the reaction products on the duodeno-jejunal absorptive cells. Note no changes throughout the course.

FIG. 5. A 7-day old rat. $\times 15,000$

FIG. 6. A 14-day old rat. $\times 16,000$

FIG. 7. A 27-day old rat. $\times 18,000$

FIGS. 8 and 9. The ileum of a one-day old rat. Reaction products are seen on the apical plasma membrane and on the vacuolar membrane of absorptive cells. Fig. 8. $\times 18,000$, Fig. 9. $\times 8,600$

FIGS. 10 and 11. Distribution of the reaction products on the apical and vacuolar membranes in the 3-day old rat ileum. Fig. 10. A longitudinal section. $\times 14,000$ Fig. 11. An oblique section. $\times 14,000$

FIG. 12. The ileum of a 7-day old rat. Amounts of the reaction products were decreased in the absorptive cells (A). A goblet cell (G) represents comparatively higher activity than the absorptive cells. $\times 5,800$

FIG. 13. The ileum of an adult rat. The reaction products are extremely sparse in the absorptive cells (A). G, Goblet cell. $\times 8,000$

TABLE 1. Postnatal changes of an NADH-dependent H_2O_2 -generating enzyme activity in the small intestine of rats

Tissue	Activity* (Acceptor; $CeCl_3$)				
	1	3	7	14	27 (days after birth)
Doudeno-jejenum	++	++	++	++	++
Ileum	++	++	+	+	$\pm$

* Activity expressed by the amount of deposits.

++, heavy; +, visible; $\pm$, little or sparse

products were decreased in the ileal portion of absorptive cells (Fig. 12). The adult ileum exhibited markedly weaker activity (Fig. 13) as compared with that of the newborn. Ileal goblet cells showed reaction products (Figs. 12, 13), similar to that in the duodeno-jejenum (see reference 6). Results obtained in the present investigation are summarized in Table 1.

DISCUSSION

The present electron-cytochemical investigation revealed postnatal changes in an NAD(P)H-dependent H₂O₂-generating enzyme activity in the rat small intestine. The intensity of the activity in the duodeno-jejunum was unchanged throughout, from the newborn to the adult, whereas obvious alteration was observed in the ileum. The ileal activity was as strong as that in the duodeno-jejunum in newborn rats, but was weakened during the growth and became almost undetectable around 27 days after birth. The difference of the H₂O₂-generating enzyme activity in between the duodeno-jejunum and the ileum in adult animals has been reported (6). In many adult animals, an absorption of intact protein is limited to the small intestinal absorptive cells (3, 11, 12). However, in the newborn, high molecular peptides are absorbed by the cells of both the jejunum and ileum by endocytosis, but in the duodenum. This phenomenon in a period of the suckling is extremely important in receiving passive immunity provided by antibodies from the colostrum (7). This endocytotic capacity is altered during maturation of animals (2, 4, 8), which indicates changes of the properties of the absorptive cell membranes during growth. In the rats 10 days after birth, the absorptive capacity of extracellular horseradish peroxidase was significantly lower in the absorptive cells of the ileal segments than that of the proximal, duodeno-jejunal segments (9). These findings strongly support the idea that the postnatal changes of the NAD(P)H-dependent H₂O₂-generating enzyme activity investigated in the small intestine may represent changes in an energy-dependent absorptive function of absorptive cells during the growth.

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