Proteolytic and Peptidase Activities of the Jejunum and Ileum of the Rat during Postnatal Development

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1. Proteolytic (substrate nitrocasein), tripeptidase (substrate glycylglycylglycine) and aminopeptidase (substrate leucyl- β -naphthylamide) activities were studied in homogenates of jejunal and ileal mucosa of 7-, 10-, 14-, 21-, 35- and 60-day-old rats. 2. Proteolytic activity was practically the same in jejunum of 7-, 10-, 14- and 21-day-old rats, but after day 21 a significant increase was observed. The activity of the ileum changed very little during postnatal development and was always higher than that of the jejunum. 3. Tripeptidase activity was low in the jejunum of 7- and 14-day-old rats, an increase was observed between day 14 and 21, but later no substantial changes were found. There were no changes in the ileum. The activity in the jejunum of 7- and 14-day-old rats was lower than in the ileum, but later the jejunum was more active than the ileum. 4. Aminopeptidase activity had a similar developmental pattern to tripeptidase activity. A low activity was found in the jejunum of 7- and 14-day-old rats, the maximum was in 21-day-old rats and then a decrease was observed, though values for 60-day-old rats were still higher than for 7- and 10-day-old rats. The activity in the ileum was practically the same in all age groups studied except in 14- and 21-day-old rats, where a transient peak was observed.

The enzymic activities of the small intestine during postnatal development of the rat have attracted the attention of several Laboratories during recent years. From these studies it can be concluded that the enzymic equipment of the small intestine of the infant rat differs substantially from that of the adult. The activity of some enzymes is lower [invertase (Doell & Kretchmer, 1962; Yezuitova, Timofeyeva, Koldovský, Nurx & Ugolev, 1964); other α -glucosidases (Rubino, Zimbalatti & Auricchio, 1964); lipase (Rokos, Hahn, Koldovský & Procházka, 1963); non-specific esterase (Koldovský, Hahn, Faltová & Vacek, 1960)] and that of others higher in this period than in adult animals [β -galactosidase (Alvarez & Sas, 1961; Doell & Kretchmer, 1962; Koldovský & Chytil, 1965); β -glucosidase (Rubino *et al.* 1964; Koldovský, Heringová & Jirsová, 1965); β -glucuronidase (Heringová, Jirsová & Koldovský, 1965)]. It is surprising that very little attention has been paid to enzymes connected with the hydrolysis of proteins, especially since it is known that proteins such as antibodies (cf. Halliday 1955*a,b*) or insulin (Mosinger, Placer & Koldovský, 1960) pass intact

through the wall of the gastrointestinal tract of suckling rats. This suggests that the processes of protein digestion in the suckling animals could be different. It is only known that proteolytic activity in the stomach is practically negligible during the suckling period (Hill, 1956; Mosinger *et al.* 1960). However, Mosinger *et al.* (1960) found that in 2- and 7-day-old animals proteolytic activity in the combined homogenate of the pancreas and small intestine is present in a considerable amount. Further, Yezuitova *et al.* (1964), studying the activity of the small-intestinal surface, showed that in 10-day-old rats the glycyl-leucine-splitting activity is approx. 50% of that of 30-day-old rats.

This all suggested that the proteolytic and peptidase activities of the small intestine might develop differently from the proteolytic activity of the stomach, and it was therefore decided to study this problem in more detail. Since in previous papers it was shown that the activities of different enzymes develop differently in the jejunum and ileum of rats (e.g. Koldovský & Chytil, 1965; Heringová *et al.* 1965; Koldovský *et al.* 1966) it was decided to study both parts of the small intestine separately.

The postnatal changes of the following enzymes are reported below: tripeptidase (substrate glycylglycylglycine; EC 3.4.1.3), aminopeptidase (substrate leucyl- β -naphthylamide; EC 3.4.1.1) and proteolytic activity (substrate nitrocasein). A preliminary report of part of the present work has been published (Koldovský *et al.* 1966).

EXPERIMENTAL

Animals. White rats of the Wistar strain were used. Litters were reduced to eight animals in the third day after birth and were used regardless of sex. They were weaned on day 30 postnatally.

Diets. The standard pelleted diet was used, consisting of 14% of casein, 10% of dried milk, 56% of ground wheat, 3.7% of dehydrated lucerne hay, 4.6% of margarine, 3.7% of wheat germ, 0.9% of wheat-germ oil, 0.5% of cod-liver oil, 1.9% of yeast, 1.9% of ground soya beans, 0.75% of NaCl, 1.49% of CaCO_3 , 0.93% of vitamin mixture (Farmakon, Olomouc, Czechoslovakia), 0.75% of mineral mixture (Plastin; Farmakon).

Preparation of tissue. Fed animals were decapitated. The abdomen was opened and the small intestine was freed from all surrounding tissues by hand and placed on an ice-cold glass dish. The duodenum was discarded and the rest of the small intestine was divided into three equal parts by length. A polythene tube attached to a syringe was inserted into the proximal and distal thirds and these were separately rinsed with 10–20 ml. of ice-cold 0.15M-KCl. The intestinal thirds were opened and dried gently with filter paper. The mucosa was scraped off and weighed (± 1 mg.) on a torsion balance. Then it was homogenized with 10 ml. of ice-cold twice-distilled water/g. wet wt. in a Potter-Elvehjem homogenizer with a Teflon piston. Further water was added to give the concentrations used in the enzymic assays.

The proximal and distal thirds are referred to below as jejunum and ileum respectively.

Assay of proteolytic activity. The activity was measured with nitrocasein as substrate. The nitrocasein was prepared by the method of Pechman (1950). The final concentrations in the assay system were: nitrocasein, 0.5% (w/v); phosphate buffer, pH 7.8, 50 mM; mucosa, 16–18 mg. wet wt./2 ml. final volume. After 60 min. incubation at 37° the reaction was stopped with 3.0 ml. of 10% (w/v) trichloroacetic acid, and 30 min. later the material was filtered through Whatman no. 2 paper and $E_{360\text{ m}\mu}$ of the filtrate was measured. Two blanks were used: one blank with water instead of substrate and one with water instead of homogenate. After subtraction of the blank values the activity is given as $\Delta E_{360\text{ m}\mu}^1/\text{cm.}/\text{g. wet wt.}/60\text{ min.}$ The SF4 photometer with Zeiss cuvettes (1 cm. deep) was used for measurement of $E_{360\text{ m}\mu}$.

Assay of tripeptidase activity. Glycylglycylglycine (Reanal, Budapest, Hungary) served as substrate. The activity was measured by using the biuret reaction according to the method of Haschen (1962). The final concentrations in the assay system were: glycylglycylglycine, 25 mM; phosphate buffer, pH 7.75, 50 mM; CoCl_2 , 50 mM; mucosa, 1.0–1.5 mg. wet wt./0.4 ml. final volume. Blanks were run with the test samples, but the homogenate was added after stopping the reaction. This was done after 60 min. incubation

at 37° by adding 0.5 ml. of 20% (w/v) trichloroacetic acid-methanol (1:1, v/v) mixture. Colour was developed by adding 3.0 ml. of borate buffer and 1.0 ml. of copper phosphate gel. After centrifugation the $E_{540\text{ m}\mu}$ of the clear supernatant was measured. The activity is given as $\mu\text{moles of substrate split/mg. wet wt.}/60\text{ min.}$

Assay of aminopeptidase activity. The substrate was leucyl- β -naphthylamide (VEB Berlin Chemie, Berlin, Germany). The liberated β -naphthylamide was coupled with Fast Blue B (diazonium salt) (E. Merck A.-G., Darmstadt, Germany). The final concentrations were: leucyl- β -naphthylamide, 10 mM; phosphate buffer, pH 7.8, 33 mM; CoCl_2 , 5 mM; mucosa, 0.6 mg. wet wt./1.5 ml. final volume. After 30 min. incubation at 37°, 1 ml. of Fast Blue B solution (4 mg./ml. of water) was added and after exactly 1 min. the reaction was stopped with 0.5 ml. of 7% (v/v) HClO_4 . Then 5 ml. of ethanol was added and without further handling $E_{546\text{ m}\mu}$ was measured. This method is a modification of the procedure described by Green, Tsoü, Bressler & Seligman (1955). Two blanks were used: in one water replaced the substrate and in the other water replaced the homogenate. The activity is expressed as $\mu\text{moles of substrate split/mg. wet wt.}/60\text{ min.}$

Expression of results. The activities are given per mg. wet wt., since it has been found previously (Koldovský & Chytil, 1965; Heringová *et al.* 1965) that the wet wt./protein ratio in the small-intestinal homogenates does not change substantially during postnatal development. Thus the expression of results on a wet-weight basis is sufficient. Proteins were determined by the method of Lowry, Rosebrough, Farr & Randall (1951). A fresh serum albumin solution served as standard.

Measurement of pH optimum. For all three enzymes the pH optimum was measured in the proximal and distal thirds of 10-day-old rats and found to be the same as in adult rats (Friedrich, Noack & Schenk, 1966).

Statistical evaluation. The *t* test was used.

RESULTS

Proteolytic activity (Fig. 1). The activity in the jejunum was practically the same in 7-, 10-, 14- and 21-day-old rats. After day 21 a significant increase was observed. The highest values were found in 60-day-old rats. The activities of the ileum were the same in all age groups except for the transient increase in 15-day-old rats. A comparison of activities of both intestinal parts showed that the activity of the jejunum was always lower, except in 60-day-old rats, where the jejuno-ileal difference was not statistically significant. During postnatal development the difference between both parts became smaller, owing to the increase of jejunal activity.

Peptidase activities. In general the developmental curves of tripeptidase and aminopeptidase activities had the same pattern (Figs. 2 and 3). First, the maximal changes occurred in the proximal third, and, secondly, the activity of the jejunum in suckling animals was always lower than that of the ileum. This changed during the beginning of the weaning period (between days 15 and 20), when

Fig. 1. Postnatal development of proteolytic activity (substrate nitrocasein). ○, Proximal third of the small intestine; ●, distal third. Symbols denote the mean value; vertical lines denote $2 \times \text{s.e.m.}$ (not given if the symbols are greater). Each point represents the mean from at least five determinations each made on pooled samples from eight to 24 animals. Differences are statistically significant as follows. In the proximal third: 7-day-old against 35- and 60-day-old, 10-day-old against 35- and 60-day-old, 14-day-old against 60-day-old, 35-day-old against 60-day-old, 21-day-old against 60-day-old, all $P < 0.01$; 14-day-old against 35-day-old, $P < 0.02$. In the distal third: 14-day-old against 7-day-old, $P < 0.05$; 14-day-old against 21- and 60-day-old, $P < 0.02$; 14-day-old against 10- and 35-day-old, $P < 0.01$. The following proximo-distal differences are significant: 7-, 10-, 14-, 21- and 35-day-old, all $P < 0.01$.

the activity of the jejunum started to become higher than that of the ileum. The changes in the ileum were negligible: only the aminopeptidase showed a transient peak between days 14 and 21. The leucyl- β -naphthylamide-splitting activity of the jejunum was low in suckling animals, the maximum was in 21-day-old rats and then a decrease was observed, though values for 60-day-old rats were still higher than for 7- and 10-day-old rats. The tripeptidase activity of the jejunum was also low in the suckling period, an increase was observed between day 14 and 21 and later no substantial changes were found.

DISCUSSION

From the experiments described above it can be concluded that the development of the proteolytic activity in the small intestine develops differently from that in the stomach. Whereas in the stomach the activity is very low during the newborn and suckling period (Hill, 1956; Mosinger *et al.* 1960) and increases during the third week of postnatal life, the proteolytic activity of the small intestine of suckling animals does not differ substantially from that of adult animals. This suggests that for the absorption of unsplit proteins during the suckling period the lower digestion of proteins in the stomach could be the critical factor.

Also, if we compare the aminopeptidase (substrate leucyl- β -naphthylamide) and tripeptidase

Fig. 2. Postnatal development of tripeptidase activity (substrate glycylglycylglycine). Details are as given in Fig. 1. Differences are statistically different as follows. In the proximal third: 7-day-old rats against 35- and 60-day-old, 10-day-old against 35- and 60-day-old, 14-day-old against 35- and 60-day-old, all $P < 0.001$. The following proximo-distal differences are significant: 7- and 60-day-old, $P < 0.01$; 10-day-old, $P < 0.02$; 35-day-old, $P < 0.001$.

Fig. 3. Postnatal development of aminopeptidase activity (substrate leucyl- β -naphthylamide). Details are as given in Fig. 1. Differences are statistically different as follows. In the proximal third: 7-day-old against 21-, 35- and 60-day-old, 10-day-old against 21-, 35- and 60-day-old, 14-day-old against 21-day-old, 21-day-old against 35-day-old, all $P < 0.01$. In the distal third: 14-day-old against 25- and 60-day-old, 21-day-old against 35- and 60-day-old, all $P < 0.01$. The following proximo-distal differences are significant: 7-, 10-, 21-, 35- and 60-day-old, $P < 0.01$.

(substrate glycylglycylglycine) activities of suckling rats with the corresponding activities of weanling rats the differences are relatively small in comparison with changes of stomach proteolytic activity.

All three enzymes studied show further differences in developmental pattern in the jejunum and ileum and this is most obvious for the peptidase activities. It can be concluded, as has been done

for example in previous papers dealing with β -galactosidase (Koldovský & Chytil, 1965) and β -glucuronidase (Heringová *et al.* 1965) in rat small intestine, that the ileum might have a different role during the suckling period from that in adult animals.

The distribution of dipeptidases along the length of the small intestine in the adult rat was studied by Robinson & Shaw (1960). They showed that the activity of the distal part of the small intestine is always higher than that of the proximal part, but they did not use leucyl- β -naphthylamide as substrate. Thus our results, showing that leucyl- β -naphthylamide-splitting activity is higher in the jejunum than in the ileum, may indicate a different distribution of different peptidase activities along the small intestine of adult rats. Finally, the distribution of the glycylglycine-splitting and leucyl- β -naphthylamide-splitting activities differs also in the intestinal cell itself. Whereas the former is not associated with the microvilli fraction the latter is (Friedrich *et al.* 1965).

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