Postnatal Development of β -Galactosidase Activity in the Small Intestine of the Rat

EFFECT OF ADRENALECTOMY AND DIET

BY O. KOLDOVSKÝ AND F. CHYTIL

Institutes of Physiology and Microbiology, Czechoslovak Academy of Sciences, Prague 6, Czechoslovakia

(Received 24 April 1964)

1. β -Galactosidase activity was studied in homogenates of the proximal and distal thirds of the small intestine from adult and infant rats. *o*-Nitrophenyl β -D-galactoside served as the substrate. 2. Activity in suckling rats is highest in the distal part of the small intestine. 3. The pH optimum was 3.5 in the distal third of the small intestine in rats aged 5 and 15 days, whereas in the proximal third the maximum was not clearly defined. 4. Activity was higher in both thirds in newborn than in adult rats, expressed per wet wt. or per wt. of protein. In the proximal third activity continually decreases with age, whereas in the distal part there is a rise up to day 15 and then a sudden decrease. Total β -galactosidase activity changes very little in the proximal third during postnatal development; the greatest changes occur in the distal third. 5. Adrenalectomy performed on day 15 postnatally slows down the decrease in β -galactosidase activity, particularly in the distal part. 6. Feeding a lactose diet to infant rats from day 14 postnatally in the presence of the mother rat also slows down the decrease in β -galactosidase activity and this is not found with a diet containing glucose and galactose instead of lactose.

It has been repeatedly found that the activity of β -galactosidase is high in the small intestine of infant mammals (for review of older literature see Herzenberg & Herzenberg, 1959; Maio & Rickenberg, 1960; Huber, Jacobson, Allen & Hartman, 1961; Alvarez & Sas, 1961; Doell & Kretchmer, 1962). The activity of this enzyme decreases at the time of weaning to values found in adult animals. This decrease occurs at a time when the quality of the carbohydrate component of the food changes. In milk, lactose is the sole carbohydrate present, whereas solid food contains a considerable amount of polysaccharides (Spector, 1956).

Investigators have not succeeded in preventing the fall in β -galactosidase activity in the small intestine during the weaning period by increasing the lactose content of the diet (Heilskov, 1952; Alvarez & Sas, 1961) or by applying lactose intraperitoneally (Doell & Kretchmer, 1962).

In the present paper changes in distribution of β -galactosidase (EC 3.2.1.23, β -D-galactoside galactohydrolase) along the small intestine during development were investigated, since previous work has shown that the activity of some intestinal enzymes is distributed differently in suckling and adult rats (Verne & Hébert, 1949; Anisimova, Vacek, Hahn & Koldovský, 1959; Koldovský,

Faltová, Hahn & Vacek, 1960; Yezuitova, Timofeeva, Koldovský, Nurx & Ugolev, 1964) and mice (Moog, 1961).

In addition, an attempt was also made to influence developmental changes by interfering with adrenal function and by changing the diet at the time of weaning. Previous work has shown that other intestinal functions could be altered during development by changes in diet and adrenal function [glucose absorption (Koldovský, Hahn & Jiránek, 1958; Faltová, Hahn & Koldovský, 1961, 1962); pancreatic lipase (Rokos, Hahn, Koldovský & Procházka, 1963); pancreatic amylase (Procházka, Hahn, Koldovský & Rokos, 1964)]. A preliminary account of this work has been given (Koldovský, Chytil & Muzyčenkova, 1964).

EXPERIMENTAL

Animals. White rats of the Wistar strain were used. Litters were reduced to eight animals on the third day after birth and were used when aged 1 to 22 days regardless of sex. Female rats weighing about 200 g. and aged 6–7 months served as adult animals.

Diets. The following diets were used. Standard diet (Fábry, 1959): 11% casein, 7.4% dried milk, 2.3% lucerne, 41.5% wholemeal wheat, 3.1% margarine, 0.5% cod-liver oil, 0.16% NaCl, 1.1% CaCO₃ and 33% water.

In special experiments animals (in the presence of the mother animals) were divided into two groups on the fourteenth postnatal day. One group received a lactose diet, the other was fed with a diet containing galactose plus glucose. The composition of these experimental diets was: margarine 500 g., casein 250 g., cod-liver oil 10 g., CaCO_3 2 g., Na_2HPO_4 2.7 g., MgCl_2 0.1 g., thiamine 4 mg., riboflavin 6 mg., pyridoxine 5 mg., calcium pantothenate 40 mg., nicotinamide 40 mg., inositol 200 mg., biotin 20 mg., folic acid 2 mg. The whole mixture was divided into two equal parts (w/w). The first was supplemented with 75 g. of lactose, and the other with 37.5 g. of glucose and 37.5 g. of galactose.

Adrenalectomy. This was performed by the dorsal route under ether anaesthesia. Control animals were sham-operated. Operated animals were offered 0.9% NaCl instead of water. They remained together with the mother animal.

Preparation of tissue. Fed animals were decapitated. The abdomen was opened and a polythene tube attached to a syringe was inserted into the duodenum and the intestine was rinsed with 10 ml. of ice-cold 0.15 M-KCl. The small intestine was then freed from all surrounding tissue 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. The individual thirds were everted with the use of a polyethylene tube, rinsed with ice-cold 0.15 M-KCl and dried with filter paper. In adult animals only a length 2 cm. from the middle of each third was used. The thirds were weighed (± 1 mg.) on a torsion balance and then homogenized in 2 ml. of ice-cold 0.15 M-KCl with a Potter-Elvehjem homogenizer having a Teflon piston. After about 20 sec. of homogenization a further 2 ml. of 0.15 M-KCl was added and homogenization was repeated. Then further KCl was added to give a final concentration of 5–20 mg. of wet tissue/ml.

Buffers. All buffers were 0.3 M solutions. For pH 1–2.5 sodium citrate was used. Sodium acetate served as buffer for pH 3.0–5.5 and potassium phosphate for pH 6–7.

Determination of protein. The method of Lowry, Rosebrough, Farr & Randall (1951) was used. A fresh serum albumin solution served as standard.

Enzymic assay. β -Galactosidase activity was estimated according to Lederberg (1950). Test tubes in a water bath at 37° were filled with 0.5 ml. of buffer and 0.5 ml. of homogenate was added. Two minutes later 0.3 ml. of 0.003 M-*o*-nitrophenyl- β -D-galactoside (product of California Corp. for Biochemical Research) was added (1.5 μ moles). After 20 min. of incubation the reaction was terminated by adding 2 ml. of 1 M- K_2CO_3 . Activity was expressed in μ moles of *o*-nitrophenol/60 min./1 mg. of wet tissue or 1 mg. of protein. Activity was also calculated for the whole portion of the intestine that was used.

Statistical evaluation. The *t*-test was used.

RESULTS

β -Galactosidase activity in different parts of the small intestine from rats aged 5 and 15 days. In preliminary experiments, activity was determined at pH 3.5 and 5.5, because Alvarez & Sas (1961) determined activity at pH 3.5 whereas Doell & Kretchmer (1962) used pH 5.5. It was found that activity was higher at pH 3.5, in agreement with

Maio & Rickenberg (1960), and that the reaction was linear for the 30 min. of incubation and directly proportional to the amount of homogenate added.

In 5- and 15-day-old rats activity rises distally from the duodenum towards the ileum (Table 1). Since the difference was greatest between the distal and proximal thirds of the small intestine only those two thirds were compared in further work.

pH optimum of β -galactosidase activity in the proximal and distal parts of the small intestine from rats aged 5 and 15 days. β -Galactosidase activity was maximal in both age groups at pH 3.5 in the distal part of the small intestine (Fig. 1). The pH optimum for the proximal part of the small intestine was found to be less well defined.

Postnatal development of β -galactosidase activity in the proximal and distal parts of the small intestine. In newborn rats that have not yet consumed milk

Table 1. β -Galactosidase activity in homogenates of different parts of the small intestine of infant rats

Homogenates of thirds were incubated for 20 min. in acetate buffer, pH 3.5, with *o*-nitrophenyl- β -D-galactoside as substrate. Activities are given as means $\pm$ s.e. (number of animals).

	$10^3 \times$ <i>o</i> -Nitrophenol liberated (μ moles/hr./mg. wet wt.)	
	5-day-old rats	15-day-old rats
Duodenum	100.1 $\pm$ 11.3 (3)	135.0 $\pm$ 12.2 (4)
Proximal third	110.0 $\pm$ 13.2 (3)	133.0 $\pm$ 11.7 (4)
Central third	384.1 $\pm$ 28.0 (3)	455.2 $\pm$ 31.2 (4)
Distal third	455.0 $\pm$ 23.0 (3)	685.4 $\pm$ 47.0 (4)

Fig. 1. pH-optimum curves for β -galactosidase in homogenates of the proximal and distal thirds of the small intestine in young rats. The homogenates were diluted to give approximately the same activity. Activity is expressed as % of the maximum value. (a) Proximal third: Δ , 5-day-old rats; $\blacktriangle$, 15-day-old rats; (b) distal third: $\circ$, 5-day-old rats; $\bullet$, 15-day-old rats.

Fig. 2. Postnatal development of β -galactosidase activity. $\circ$, Proximal third of the small intestine; $\blacktriangle$, distal third. Symbols denote the mean value, vertical lines denote $2 \times$ S.E.M. (in the curve for the proximal third S.E.M. is not given). Activity was estimated at pH 3.5. Numbers in parentheses indicate numbers of animals of the same age. Differences are statistically significant in the proximal third: for $P < 0.05$, 6-day-old against 15-day-old, 10-day-old against 16-day-old; for $P < 0.02$, 15-day-old against 16-day-old; for $P < 0.001$, 16-day-old against 18-day-old. In the distal third: for $P < 0.01$, 0-day-old against all except 1-day-old; 1-day-old against all except 0-day-old and 2-day-old; 2-day-old against all except 1-day-old and 4-day-old; 4-day-old against all except 2-day-old and 6-day-old; 6-day-old against all except 4-day-old; 10-day-old against all except 15-day-old; 15-day-old against all except 16-day-old; 16-day-old against all except 15-day-old; 18-day-old against all; 20-day-old against all except adults; adults against all except 20-day-old.

activity is the same in both parts of the small intestine (Fig. 2), yet 24 hr. later activity rises in the distal parts. During further development, activity continues to rise in the distal part and a slight fall is seen proximally. On day 16, activity suddenly significantly decreases and on day 20 low adult values are found in both parts of the small intestine. (Figs. 1 and 2 show results for pH 3.5. Determinations at pH 5.5 gave basically the same developmental changes.)

Since the morphological structure of the small intestine and the ratio of mucosa to muscularis change during postnatal development, an attempt was made to express the developmental changes as changes of total β -galactosidase activity in the two

Table 2. Total β -galactosidase activity in the proximal and distal thirds of the small intestine during postnatal development

Homogenates of thirds were incubated for 20 min. in acetate buffer, pH 3.5, with *o*-nitrophenyl β -D-galactoside as substrate. Activities are given as means $\pm$ S.E. (number of animals). (a) Statistical significances between one age group and the following age group for distal thirds; (b) statistical significances between both thirds of the same age group. In the proximal third the following differences are significant for $P < 0.02$: 0-day-old against 6-day-old; for $P < 0.05$: 2-day-old against 6-day-old; for $P < 0.001$: 1-day-old against 10-day-old. n.s., Not significant.

Age (days)	o-Nitrophenol liberated (m-moles/hr.)		Statistical significance	
	Proximal third	Distal third	(a)	(b)
0	11.0 $\pm$ 1.2 (4)	7.5 $\pm$ 2.1 (4)	0.05	n.s.
1	11.6 $\pm$ 1.7 (7)	13.0 $\pm$ 1.98 (7)		n.s.
2	14.3 $\pm$ 1.6 (7)	18.7 $\pm$ 1.59 (7)	0.001	n.s.
4	15.0 $\pm$ 1.9 (6)	34.8 $\pm$ 2.70 (6)		0.001
6	24.3 $\pm$ 4.2 (6)	51.3 $\pm$ 6.90 (6)	0.001	0.01
10	21.6 $\pm$ 2.3 (5)	97.8 $\pm$ 9.0 (5)		0.001
15	24.6 $\pm$ 1.7 (13)	120.0 $\pm$ 11.4 (13)	n.s.	0.001
16	19.2 $\pm$ 1.7 (4)	123.0 $\pm$ 5.4 (4)		0.001
18	15.0 $\pm$ 0.7 (3)	112.6 $\pm$ 11.1 (3)	n.s.	0.001
19	18.0 $\pm$ 0.7 (7)	20.1 $\pm$ 0.7 (7)		n.s.
20	15.9 $\pm$ 0.4 (5)	9.0 $\pm$ 0.7 (5)	0.001	0.001

parts of the small intestine. Table 2 shows that activity changes only slightly in the proximal part whereas the greatest changes occur in the distal one.

In newborn and 20-day-old rats values are very close to each other. In addition activity is greater proximally than distally only in those two age groups.

Effect of adrenalectomy. Adrenalectomy performed on day 15 slows down the decrease in activity normally observed on day 19 (Table 3) in the distal part. A similar effect was observed if activity was determined at pH 5.5, but differences were not quite so pronounced.

Effect of a lactose-containing diet. As in adrenalectomy, feeding with a diet containing lactose from day 14 slows down the normal fall in β -galactosidase activity distally and proximally (Table 4). The proximal-distal difference in activity was also

Table 3. *Effect of adrenalectomy on β -galactosidase activity in the proximal and distal thirds of the small intestine of young rats*

Rats were adrenalectomized on day 15 postnatally. The animals lived with their mother, having free access to physiological saline; enzymic activity was estimated on day 19 postnatally. Control animals were sham-operated on day 15 postnatally and treated as the adrenalectomized group. Means $\pm$ S.E.M. (number of animals) are given. n.s., Not significant; **, value differs from the corresponding value in proximal third for $P < 0.001$.

Proximal third			Distal third		
μ moles liberated/ mg. wet wt./ 60 min.	μ moles liberated/ mg. of protein/ 60 min.	m-moles liberated/ total third/ 60 min.	μ moles liberated/ mg. wet wt./ 60 min.	μ moles liberated/ mg. of protein/ 60 min.	m-moles liberated/ total third/ 60 min.
Adrenalectomized group					
72.0 $\pm$ 6.0 (7)	556.0 $\pm$ 45.0 (7)	25.9 $\pm$ 2.0 (7)	383.0 $\pm$ 30.0** (7)	2583 $\pm$ 229** (7)	116 $\pm$ 8.6** (7)
Control group					
39.3 $\pm$ 16.0 (7)	415.5 $\pm$ 125.0 (7)	15.5 $\pm$ 4.3 (7)	62.0 $\pm$ 8.2 (7)	514 $\pm$ 215 (7)	37 $\pm$ 10.0 (7)
Statistical significance between adrenalectomized and control groups					
n.s.	$P < 0.05$	n.s.	$P < 0.001$	$P < 0.001$	$P < 0.001$

Table 4. *Effect of diets containing lactose or glucose and galactose on the β -galactosidase activity in the proximal and distal thirds of the small intestine of young rats*

From day 14 postnatally the animals, in the presence of the mother, were divided into two groups. One received a lactose diet, the second was fed with a diet containing glucose and galactose. In Expt. 1 the animals were killed on day 19 postnatally, in Expt. 2 on day 20 postnatally. Means $\pm$ S.E.M. (number of animals) are given. n.s., Not significant; **, value differs from corresponding value in proximal third for $P < 0.001$; *, for $P < 0.01$.

	Proximal third			Distal third		
	μ moles liberated/ mg. wet wt./ 60 min.	μ moles liberated/ mg. of protein/ 60 min.	m-moles liberated/ total third/ 60 min.	μ moles liberated/ mg. wet wt./ 60 min.	μ moles liberated/ mg. of protein/ 60 min.	m-moles liberated/ total third/ 60 min.
Expt. 1 (19-day-old rats)						
Lactose group	88.5 $\pm$ 6.9 (6)		23.1 $\pm$ 1.0 (6)	291.6 $\pm$ 26.5** (6)		62.6 $\pm$ 3.7* (6)
Glucose + galactose group	74.7 $\pm$ 4.4 (6)		21.8 $\pm$ 2.7 (6)	132.0 $\pm$ 12.0* (6)		33.5 $\pm$ 2.1* (6)
Statistical significance between both groups						
	n.s.		n.s.	$P < 0.001$		$P < 0.001$
Expt. 2 (20-day-old rats)						
Lactose group	86.3 $\pm$ 7.1 (6)	477.0 $\pm$ 41.0 (6)	13.3 $\pm$ 1.0 (6)	197.0 $\pm$ 29.0* (6)	1113 $\pm$ 143** (6)	29.5 $\pm$ 15.2* (6)
Glucose + galactose group	68.0 $\pm$ 4.0 (6)	362.0 $\pm$ 23.0 (6)	10.9 $\pm$ 0.6 (6)	68.0 $\pm$ 9.8 (6)	346 $\pm$ 43 (6)	10.1 $\pm$ 1.3 (6)
Statistical significance between both groups						
	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.002$	$P < 0.002$	$P < 0.001$

preserved. This could not be achieved when feeding with a diet containing glucose and galactose instead of lactose (20-day-old rats). A similar effect was observed again if activity was determined at pH 5.5, but differences were not quite so pronounced.

DISCUSSION

It has been shown that β -galactosidase activity is not evenly distributed along the small intestine of

infant rats and that postnatal changes in activity are mainly due to changes in the distal portion. In the proximal part, activity per unit wet wt. or wt. of protein decreases during development, whereas total activity remains practically unchanged. Thus it appears that activity in the proximal part is diluted during growth.

This difference between the distal and proximal parts of the small intestine during development might be due to the presence of at least two enzymes,

one of which develops predominantly in the distal part. If this is so, the enzyme isolated from the proximal part should behave differently from that found distally. Differences in the pH optima for activity seem to support this idea. Doell & Kretchmer (1962, 1963) also demonstrated the presence of two β -galactosidases: one was soluble with a low pH optimum; the other was a particulate enzyme with a pH optimum about 6. It is also possible to assume that β -galactosidase activity is also localized differently in cells from the proximal and distal parts. The results show that β -galactosidase in the small intestine of the rat cannot be studied in any detail unless the part of the small intestine in which it is determined is also considered.

The present findings pertain to the rat. They need not be the same in other species. Huber *et al.* (1961) demonstrated that β -galactosidase activity in the distal part of the small intestine in the calf, a species that is born very mature, decreases 20-fold during postnatal development, whereas the decrease in activity in the proximal part was only 10%. This shows that the proximal-distal difference exists also in other species.

Previous attempts to show an effect of the composition of the diet on β -galactosidase activity in rats have been unsuccessful (Alvarez & Sas, 1961; Herzenberg & Herzenberg, 1959). The exact experimental conditions used by these workers are not stated. In view of the fact that infant rats first start to consume food on day 15 (Murphy & Dunn, 1949; Křeček, Křečková & Dlouhá, 1956) it is possible that the absence of any effect of a lactose diet was due to the fact that the animals had already consumed the normal laboratory diet, which had led to a fall in β -galactosidase activity of the small intestine. Apparently once activity has decreased it can no longer be raised without an increase in the weight of the small intestine, as follows from experiments with adult rats (Fischer, 1957). This consideration probably also applies to the results of Doell & Kretchmer (1962) with young rats and those of Heilskov (1952) with young rabbits. In both experiments animals were weaned to normal laboratory diets and lactose was only added.

Nevertheless, even though in the present experiments the fall in β -galactosidase activity could be slowed down by feeding on lactose it could not be prevented completely. Thus the diet is not the only regulator. It can, however, be stated that a regulatory mechanism, perhaps similar to those found in microbes, does exist for β -galactosidase in the rat, although this has been denied (Herzenberg & Herzenberg, 1959; Alvarez & Sas, 1961).

Two mechanisms have been shown to participate in the regulation of the development of β -galactosidase in the rat. One is the change in diet; the other,

not found in microbes, is the development of adrenal function. In contrast with most other enzyme activities of the gastrointestinal tract (Moog, 1953; Moog & Thomas, 1955; Rokos *et al.* 1963; Procházka *et al.* 1964) the adrenal cortex suppresses the decrease of β -galactosidase activity. There is, however, one common factor: in all cases the application of cortisone speeds up normal development, whereas adrenalectomy slows it down.

REFERENCES

- Alvarez, A. & Sas, J. (1961). *Nature, Lond.*, **190**, 826.
 Anisimova, E., Vacek, Z., Hahn, P. & Koldovský, O. (1959). *Čsl. Fysiol.* **8**, 392.
 Doell, R. G. & Kretchmer, N. (1962). *Biochim. biophys. Acta*, **62**, 353.
 Doell, R. G. & Kretchmer, N. (1963). *Biochim. biophys. Acta*, **66**, 516.
 Fábry, P. (1959). *Čsl. Fysiol.* **8**, 529.
 Faltová, E., Hahn, P. & Koldovský, O. (1961). *Proc. 5th nat. Congr. Czechoslovak Physiol. Soc., K. Vary*, p. 128. Ed. by Poupá, O. Prague: Czechoslovak Academy of Sciences.
 Faltová, E., Hahn, P. & Koldovský, O. (1962). *Fiziol. Zh., Mosk.*, **48**, 1392. Engl. Transl.: *Fed. Proc.* (1963). **22**, T941.
 Fischer, J. E. (1957). *Amer. J. Physiol.* **188**, 49.
 Heilskov, N. S. C. (1952). *Acta physiol. scand.* **24**, 84.
 Herzenberg, L. A. & Herzenberg, L. A. (1959). *Nutr. Rev.* **17**, 65.
 Huber, J. T., Jacobson, N. L., Allen, R. S. & Hartman, P. A. (1961). *J. Dairy Sci.* **44**, 1944.
 Koldovský, O., Chytil, F. & Muzyčenkova, H. (1964). *Experientia*, **20**, 87.
 Koldovský, O., Faltová, E., Hahn, P. & Vacek, Z. (1960). *Symp.: The Development of Homeostasis*, p. 155. Prague: Czechoslovak Academy of Sciences; New York: Academic Press Inc.
 Koldovský, O., Hahn, P. & Jiránek, J. (1958). *Čsl. Fysiol.* **7**, 491.
 Křeček, J., Křečková, J. & Dlouhá, H. (1956). *Physiol. bohemoslov.* **5** (suppl.), 33.
 Lederberg, J. (1950). *J. Bact.* **60**, 1381.
 Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
 Maio, J. J. & Rickenberg, H. V. (1960). *Biochim. biophys. Acta*, **37**, 101.
 Moog, F. (1953). *J. exp. Zool.* **124**, 329.
 Moog, F. (1961). *Development Biol.* **3**, 153.
 Moog, F. & Thomas, E. R. (1955). *Endocrinology*, **56**, 187.
 Murphy, E. A. & Dunn, M. S. (1949). *Proc. Soc. exp. Biol., N.Y.*, **71**, 241.
 Procházka, P., Hahn, P., Koldovský, O. & Rokos, J. (1964). *Čsl. Fysiol.* **13**, 223.
 Rokos, J., Hahn, P., Koldovský, O. & Procházka, P. (1963). *Physiol. bohemoslov.* **12**, 213.
 Spector, W. S. (1956). *Handbook of Biological Data*. Philadelphia: Saunders Co.
 Verne, J. & Hébert, S. (1949). *Ann. Endocr., Paris*, **10**, 456.
 Yezuitova, N. N., Timofeeva, N. M., Koldovský, O. K., Nurx, Ya. Ya. & Ugolev, A. M. (1964). *Dokl. Akad. Nauk S.S.S.R.* **154**, 990.