Developmental Aspects of Uridine Diphosphate Galactose 4-Epimerase in Rat Intestine

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Kinetic and developmental characteristics of rat intestinal UDP-galactose 4-epimerase activity have been examined. The enzyme in the adult rat had a $V_{\max}$ value 2-3 times higher than that of the newborn animal, but the K_m values for the enzyme in the newborn and adult rat were the same (0.17 mM). No differences in epimerase activity were found along the length of the jejunum-ileum of adult animals, but higher activity was detected in the lower portion of the villi and crypts. The specific activity of the enzyme in the newborn rat began to rise at about 17 days of age, reaching a peak at 29 days of age, and then became constant at adult values. Total epimerase activity in the newborn rat liver was 2-5 times higher than the total activity in the intestine, and total epimerase activity in the adult intestine was 3-4 times higher than the total activity in the liver. Cortisone injection did not enhance the increase of epimerase normally seen during development, but caused a decrease in activity of this enzyme in the jejunum in rats up to 17 days of age. After 17 days, cortisone treatment had no effect on epimerase activity.

UDP-galactose 4-epimerase catalyses the reversible conversion of UDP-galactose into UDP-glucose, thus enabling galactose to enter the metabolic pathways of glucose. It also allows the conversion of glucose into galactose for the formation of polysaccharides. Although the exact role of the intestine in the overall animal metabolism of galactose is not known, it has been shown that the rat intestine possesses all of the enzymes involved in the metabolism of galactose (Cuatrecasas & Segal, 1965; Bertoli & Segal, 1966; Stifel, Herman & Rosensweig, 1968; Cohn & Segal, 1969). A previous paper from this laboratory has indicated that of all of the tissues tested in the adult rat, the intestine has the highest epimerase activity (Cohn & Segal, 1969).

The present paper gives an analysis of kinetic and developmental characteristics of rat small-intestinal UDP-galactose 4-epimerase. Special emphasis has been placed on a comparison of enzyme activity in intestine and liver as part of our investigation of the role of the intestine in total body galactose metabolism.

EXPERIMENTAL

Materials. UDP-galactose, UDP-glucuronic acid, UDP-glucose dehydrogenase and NAD^+ were purchased from Sigma Chemical Co., St Louis, Mo., U.S.A.; UDP-

[U- ^{14}C]galactose (sp. radioactivity 251 mCi/mmol), UDP-[U- ^{14}C]glucuronic acid (sp. radioactivity 233 mCi/mmol) and Liquifluor were purchased from New England Nuclear Corp., Boston, Mass., U.S.A. Thin-layer cellulose plates were obtained from Eastman-Kodak Co., Rochester, N.Y., U.S.A. Polyethyleneimine was obtained from Chemirad Corp., East Brunswick, N.J., U.S.A. Sucrose was obtained from J. T. Baker Co., Phillipsburg, N.J., U.S.A. Cortisone acetate in saline suspension was obtained from Merck, Sharp and Dohme, Rahway, N.J., U.S.A. All other reagents used were AnalaR grade.

Animals. White rats of the Sprague-Dawley strain were obtained from either Charles River Breeding Farms, Wilmington, Mass., U.S.A., or Huntingdon Farms, Conshohocken, Pa., U.S.A. Litters were decreased to eight animals on day 3 after birth and rats were used regardless of sex. They were fed with a Purina rat chow diet and water *ad libitum* until killed, and animals were weaned by day 28 after birth. Animals treated with cortisone received 5 mg/100 g body weight intramuscularly once every 24 h. Adrenalectomies were done on 14-day-old rats via the dorsal route under light ether anesthesia. After adrenalectomy, animals were kept with the mother and the drinking water was replaced by 0.9% NaCl. The survival rate for one week was about 80%.

Tissue homogenates. The rats were killed by decapitation and exsanguination. The intestine was rinsed with ice-cold 0.1 M-KCl, removed, and divided into three equal parts. The proximal third was used as jejunum and the distal third as ileum. The individual thirds were rinsed with ice-cold 0.1 M-KCl, dried with filter paper and

weighed. Liver samples were taken from several lobes, then were rinsed in ice-cold 0.1M-KCl and dried on filter paper and weighed.

Tissue (approx. 100–200mg) was homogenized with ground-glass grinders on ice with 4 vol. of 0.1M-KCl. Gut homogenates were then diluted 1:6 with quartz-distilled water and liver homogenates were diluted 1:8. The tissue preparations were then centrifuged at 30000g for 20 min at 4°C. The clear supernatant was extracted with a pipette and was filtered through glass wool. Care was taken to avoid including any material from the lipid layer, if it was present.

To determine the enzyme activity of the whole organ, the entire small intestine and liver were weighed, homogenized and centrifuged at 30000g, and the specific activity of the supernatant was determined. Total activity was calculated by multiplying the specific activity by the amount of soluble protein in the whole organ. The soluble protein/total homogenate protein ratio was the same at all ages of tissue studied.

To establish that the gut epimerase was a soluble enzyme, as for the liver enzyme (Cohn & Segal, 1969), differential centrifugation was done. Enzyme assays indicated that nearly all of the epimerase activity was recovered in the 100000g supernatant, with less than 7% of the original activity remaining in the pellet. The analysis of the 30000g fraction was essentially the same with less than 3% of the original homogenate activity remaining in the pellet. Therefore the 30000g supernatant was used in further experiments. The approximate protein concentrations in 20μl of the clear supernatants were 20–40μg. Proteins were determined by the method of Lowry, Rosebrough, Farr & Randall (1951) with crystalline bovine albumin as standard.

In some experiments the distribution of epimerase in different layers of the intestinal wall was determined. As UDP-galactose 5-epimerase loses activity during freezing and thawing it was not possible to use the microtome sectioning technique of Nordström, Dahlqvist & Josefsson (1967). Instead, an approach similar to that described by Dietschy & Siperstein (1965) was used. The rinsed intestine (approx. 2cm in length) was placed on a filter paper and opened with scissors. The mucosa was patted gently with wet tissue paper and was scraped off lightly by hand with a small metal spatula. After removal of the first layer, which was shown histologically to contain mostly villi, a second deeper scraping was made to remove almost all of the remaining mucosa. This second layer contained some villi and almost all of the crypt cells. The third and last layer contained very few villi, some crypts and mainly the serosal part of the small-intestinal wall. These three layers were called the upper villi, lower villi with crypts, and muscle. They were homogenized as described above and samples were taken for sucrose determination before centrifugation. Sucrase was determined by the method of Dahlqvist (1964).

Determination of UDP-galactose 4-epimerase. The incubation mixture, containing 20nmol of UDP-[¹⁴C]-galactose (0.04μCi), 0.4μmol of NAD⁺, 40μl of 1M-glycine-NaOH buffer (pH9.1), and 20μl of the enzyme preparation, was made up to a final volume of 0.2ml with water. The reaction was started by addition of the enzyme preparation. Incubation was done at 37°C for 6 min and the reaction was terminated by placing the tube in boiling

water for 2 min. The reaction mixture could be frozen for 1 week at this stage without affecting the results obtained after the remaining steps of the assay.

The assay procedure, based on anion-exchange t.l.c. separation of sugar nucleotides, involves the formation of UDP-[¹⁴C]glucose during incubation of the epimerase enzyme with UDP-[¹⁴C]galactose. Since UDP-glucose and UDP-galactose both have *R_F* 0.34 with the 0.2M-LiCl solvent, the UDP-[¹⁴C]glucose was converted into UDP-[¹⁴C]glucuronic acid (*R_F* 0.04) by addition of UDP-glucose dehydrogenase.

After cooling the solution, 0.3μmol of NAD⁺ was added as coenzyme for the UDP-glucose dehydrogenase reaction. The reaction was initiated by the addition of 100 EC units of UDP-glucose dehydrogenase and was done at room temperature. After 15 min, a 20μl portion (about 8000 c.p.m.) was streaked at the origin of the polyethyleneimine-impregnated cellulose t.l.c. plates, which were prepared as described by Cohn & Segal (1969). After the spots had dried, the cellulose plate was placed in the chromatographic tank containing 0.2M-LiCl solution (1 cm deep). The plates were aligned in the same direction as during the impregnation procedures. The chromatogram was removed from the tank when the solvent front had reached the top of the sheet; the plate was then dried at room temperature and scored with a pencil into four sections of 4, 3, 6 and 3.5 cm each, as described by Cohn & Segal (1969). These sections were then counted for radioactivity separately in a liquid-scintillation spectrometer in a Liquifluor-toluene system at a dilution of 1:20 (v/v).

The product of the reaction, after being converted into UDP-glucuronic acid, was represented by the radioactivity in the first section from the origin, and the unchanged UDP-galactose was represented by the radioactivity in the third section. Commercially prepared UDP-[¹⁴C]-galactose and UDP-[¹⁴C]glucuronic acid were run as markers on each plate and zero-time or boiled-enzyme reaction mixtures were used as blanks. The amount of radioactivity in each section was the number of counts recorded minus the number of counts in the blank. Percentage conversion was calculated by dividing the number of counts in the first section (the UDP-glucuronic acid section) by the sum of the counts in the first and third sections. To determine the number of mol of UDP-galactose converted, the percentage conversion was multiplied by the number of mol of UDP-galactose present in the assay tube at the start of the reaction. Specific activity was defined as pmol of UDP-glucose formed/min per μg of tissue protein. As previously reported for the epimerase reaction (Kaplan, 1955), in the absence of NAD⁺ no epimerase activity could be detected.

Although liver enzyme preparations are stable for more than one month at -45°C, the intestine preparations lost 50–75% of their activity after being frozen and thawed once. Therefore, all gut preparations were prepared fresh before each assay.

The amount of UDP-glucuronic acid formed by the intestinal preparation was proportional to the incubation time for 7 min. The incubations were therefore done for 6 min. The measured epimerase activity was proportional to the amount of material for up to 50μg of gut protein. The incubation mixture contained 20–40μg of protein in 10 or 20μl of the supernatant preparation.

RESULTS

Relationship between UDP-galactose concentration and epimerase activity in the jejunum. A Lineweaver-Burk analysis of the relationship between UDP-galactose concentration and epimerase activity in the jejunum of adult and newborn rats is shown in Fig. 1. Whereas K_m values for enzyme preparations from adult and newborn animals were found to be the same (0.17 mM) and close to values given for rat liver (Cohn & Segal, 1969), the V_{max} values for the adult was 2–3 times higher than that of the newborn animal (60 and 24 pmol/min per μ g of protein respectively).

Localization of epimerase activity along the length of the small intestine and in mucosal scrapings of the jejunum. Comparisons of epimerase specific activities in different sections of the adult small intestine showed that the duodenum was the least active site (Table 1). All other sections checked throughout the jejunum and ileum had uniform activity. In suckling rats the activity was generally lower (see below). Unlike that in adult animals, in suckling rats the activity appeared not to be uniformly distributed along the length of the gut. The 30% lower activity in the ileum compared with that in the jejunum had a barely significant statistical difference ($P < 0.05$). The activity in the duodenum was lower compared with the other sections.

When the jejunal mucosa of the adult small intestine was scraped in two separate layers (Table 2), the first containing the villi and the second containing predominantly crypts, there was a consistently higher specific activity in the latter portion than in the former. The muscle layer that remained

after the mucosa had been scraped contained a lower epimerase activity than either of the two mucosal layers. Analysis of the sucrase activity in these different scrapings was used to characterize further the incidence of the relative proportions of villi and crypts. The highest activity was found in the portion termed 'villi'. The activity of the second so-called 'crypt layer' was about 50% of the first layer, and no activity was detected in the last layer.

Epimerase activity change in the developing rat. Cohn & Segal (1969) showed that the specific activity of UDP-galactose 4-epimerase in rat liver is highest at birth and slowly decreases to adult values, and the activity in the intestine is much higher in the adult rat than at birth. UDP-galactose 4-epimerase was investigated in jejunum to obtain a more detailed pattern of enzyme-activity changes with age. Also the work of Cohn & Segal (1969) on the liver developmental pattern was repeated with tissue from the animals undergoing jejunum analysis.

Fig. 2 shows the epimerase specific activities in the developing rat jejunum and liver. At birth, the epimerase specific activity in the rat jejunum was 10 ± 0.5 pmol/min per μ g of protein, which was only 50% of that in the liver. This value was relatively constant in the jejunum until the age of 16 days at which time the specific activity began to rise gradually to 40 ± 2.7 pmol/min per μ g of protein by 30 days and then declined to the adult value, of about 20 ± 1.3 pmol/min per μ g of protein, twice that in the newborn. Mixing the enzyme preparations from newborn and adult jejunum resulted in an enzyme activity that was the mean value of the two. Results with liver enzymes were consistent with the work of Cohn & Segal (1969), showing that epimerase specific activity in the liver was highest at the time of birth (21 ± 1.3 pmol/min per μ g of protein) and slowly decreased to less than 30% of its original value in the adult animal, where the specific activity was 6.0 ± 1.3 pmol/min per μ g of protein.

Fig. 1. Lineweaver-Burk plot of epimerase activity with UDP-galactose as the varied substrate. Velocities (v) are expressed as pmol of UDP-glucose formed/min per μ g of newborn and adult rat jejunal protein. ●, Adult enzyme; ○, newborn enzyme.

Table 1. Epimerase activity along length of the gut of adult and 10-day-old rats

Values represent mean epimerase specific activity $\pm$ s.e.m. (for the number of animals in parentheses).

Section of gut	UDP-glucose formed (pmol/min per μ g of protein)	
	10-day-old rats	Adult rats
Duodenum	6.0 ± 1.1 (5)	4.0 ± 0.8 (4)
Jejunum	11.5 ± 1.2 (4)	23.7 ± 2.9 (3)
Ileum	8.2 ± 0.7 (5)	27.8 ± 1.9 (4)

Table 2. *Localization of epimerase activity in mucosal scrapings of the jejunum of adult rat*

Experiments A and B each represent a single animal. Epimerase specific activities determined in duplicate are expressed as pmol of UDP-glucose formed/min per μg of protein and sucrase units are expressed as μmol of sucrose split/h per mg of protein. The first third of the small intestine was rinsed with 0.1 M-KCl, then slit longitudinally, and two scrapings were made free-hand. The first scraping contained the upper villus, the second scraping primarily contained the crypts although some portion of the villus was present, and the remainder was principally the muscle layer.

Section of gut	Specific activity			
	Expt. A		Expt. B	
	Epimerase	Sucrase	Epimerase	Sucrase
Villus	13.1	6.2	14.9	6.0
Crypts	17.5	3.3	22.4	3.28
Muscle	10.3	0.0	10.0	0.0

Fig. 2. Epimerase specific activity in rat jejunum (●) and liver (Δ) as a function of age. Each point represents the mean value obtained from 3 to 13 animals. Activities are expressed as pmol of UDP-glucose formed/min per μg of protein.

For comparative purposes the total activity of epimerase in liver and small intestine was assessed in rats of various ages. Fig. 3 shows results of these experiments. Whereas in suckling rats the amount of enzyme in the liver exceeds that in the gut (2–5 times), in adult animals this ratio is reversed, with the gut having approx. 3–4 times more activity.

Effect of corticoids. The increase in epimerase activity occurred during the third postnatal week, at a time when an increase occurs in several enzymes of the gastrointestinal tract (Koldovský, 1969) the activities of which could be precociously induced by treatment with cortisone, or delayed by adrenalectomy (Moog, 1953; Doell & Kretchmer, 1964; Koldovský & Chytil, 1965; Koldovský,

Heringová & Jirsová, 1965). It was therefore decided to see if UDP-galactose 4-epimerase is under similar control. For comparative purposes the activity of epimerase in the liver was also determined. A treatment was used similar to that described in experiments where an effect of cortisone was found on sucrase (J. Herbst & O. Koldovský, unpublished work) and acid β -galactosidase (Koldovský & Sunshine, 1970). Cortisone (5 mg/100 g body weight) was injected daily for four days and animals were killed on the fifth day after the first injection. Table 3 summarizes the results. Unexpectedly, cortisone did not enhance the increase of epimerase activity normally seen during development, but it caused a decrease of

Fig. 3. Comparison of total rat jejunal (●) and liver (Δ) epimerase activity as a function of age. Each point represents the mean value of three or more animals. Activities are expressed as μmol of UDP-glucose formed/min.

Table 3. *Effect of cortisone on jejunal and liver epimerase activity*

Values represent mean jejunal or liver epimerase specific activity (for the number of animals in parentheses). Litter mates either were untreated or received cortisone ultramuscularly (5mg/100g body wt.) once every 24h for 4 days. Control values followed the normal developmental pattern (Fig. 2). Activities are expressed as pmol of UDP-glucose formed/min per μg of protein.

Age at assay (days)	Treatment ...	Intestine		Liver	
		Control	Cortisone	Control	Cortisone
13		12.7 (2)	4.0 (3)	14.2 (2)	10.8 (3)
14		15.7 (2)	3.4 (3)	14.8 (2)	12.5 (3)
16		13.9 (2)	5.4 (2)	17.3 (2)	14.5 (2)
17		19.9 (1)	3.5 (2)	16.1 (1)	15.5 (2)
20		22.4 (2)	27.7 (3)	11.1 (3)	10.0 (3)
Adult		22.8 (3)	24.9 (3)	5.7 (3)	6.8 (3)

activity of this enzyme in the jejunum. This effect was observed only in rats up to 17 days of age, after which cortisone did not cause a decrease in epimerase activity. Further, treatment with cortisone of the oldest animals used in the study did not prevent the increase in epimerase activity normally found during development and had no effect at all in adult animals. Epimerase activity in the liver was the same in intact and cortisone-treated animals.

To characterize the effect of cortisone on intestinal epimerase activity in suckling rats, changes in enzyme activity were measured at daily intervals

for 4 days. Rats that had received an injection of cortisone once every 24h beginning on day 9 showed no substantial change in activity after 24, 48 or 72h. Only after 96h was the decrease in epimerase activity observed. Mixing the enzyme preparation from an intact animal with the preparation from an animal treated for 96h with cortisone gave the calculated mean enzyme activity.

Adrenalectomy done on 14-day-old rats did not prevent the developmental increase in intestinal epimerase activity normally observed at 21 days of age.

DISCUSSION

Results for UDP-galactose 4-epimerase activity in various rat tissue (Cohn & Segal, 1969) showed essentially no epimerase activity in the intestine of newborn rats and extremely high activity in the jejunum of adult rats. These observations prompted the present more detailed analysis of this enzyme in rat intestine during development. Small-intestinal enzyme activity found in these experiments in the newborn animals is much higher than that reported by Cohn & Segal (1969). This could most probably be explained by our increased knowledge of the stability characteristics of the enzyme, namely that this enzyme in the small intestine is unstable to freezing and thawing (unlike the relatively good stability of the liver enzyme). Although the stability of this enzyme is different in the small intestine and liver, the similarity of the K_m values indicates that the UDP-galactose 4-epimerase of the liver and small intestine are similar, and further, that they do not differ in newborn and adult animals.

The localization of the epimerase enzyme in the small intestine, namely the lack of a gradient of activity along the length of the jejuno-ileum of adult animals and the presence of higher activity in the lower portion of the villi and crypts, differs from the distribution of enzymes directly related to digestive functions, such as disaccharidases (Nordström, Koldovský & Dahlqvist, 1969; Nordström *et al.* 1967), enterokinase (Nordström & Dahlqvist, 1970), or as far as has been proved for absorption of monosaccharides (Booth, 1968). The functional meaning of this distribution remains an open question and might be related to other metabolic activities of the enzyme in gut (see below).

The developmental pattern of epimerase in the small intestine delineated here is similar to that observed for various functions (Koldovský, 1969) that increase in activity during the third postnatal week. The rise in specific activity of epimerase beginning at 19 days to a peak at about 29 days of age may be related to various factors, such as qualitative and quantitative changes in food composition, occurring during the weaning period (Hahn & Koldovský, 1966), and to maturational and growth changes in the mucosa as well as the effect of hormonal factors. The supernatant enzyme from rats of various ages was stable at 4°C thus obviating stability as a factor in the developmental pattern.

Glucocorticoids were injected at various ages before the anticipated rise in intestinal epimerase to determine if adrenal steroids are influential, as was observed with several other intestinal enzymes. Cortisone did not cause an increase in enzyme activity, but after 4 days of treatment, lowered the activity. This unusual effect on the developmental

pattern was restricted only to the suckling period, before the increase of activity. At the present time no explanation can be given, but it seems possible to relate the effect to a 'step up' in inactivation of the enzyme, a mechanism postulated by Boctor & Grossman (1970) to be associated with steroid alteration of tyrosine aminotransferase activity.

A comparison of the developmental patterns of liver and gut enzymes (Fig. 2) reveals striking differences. While the specific activity is increasing in the gut it is slowly decreasing in the liver. Cortisone does not affect the enzyme activity in liver. Thus, both the different developmental changes and the different effect of cortisone on epimerase in both organs studied indicate that there are obviously different factors that regulate the activity observed in these organs.

Finally, the question of why total epimerase activity in adult rats is so much higher in intestine than liver when the liver is thought to be the principal organ of galactose disposition (Tygstrup & Winkler, 1958) deserves some comment. Comparison of results on rat liver and intestinal galactokinase and galactose 1-phosphate uridylyltransferase (Cuatrecasas & Segal, 1965; Bertoli & Segal, 1966) indicates that UDP-galactose 4-epimerase activity is the lowest of the galactose-metabolizing enzymes in liver but the highest in intestine. It may well be that the high epimerase activity in gut is not related to the conversion of galactose into glucose, but more to the reverse. The intestine is known to have a surface coat of mucopolysaccharide (the glycocalyx) over the brush border (Bennett, 1963). Such a substance would require UDP-galactose as a constituent and necessitate the presence of epimerase for converting UDP-glucose. Such a need may be partly responsible for the increase in epimerase activity during the weaning period, when galactose supply in the diet is decreased, and thus does not provide a sufficient source of UDP-galactose via galactose 1-phosphate uridylyltransferase activity.

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