Cortisone-Evoked Decrease of Acid β -Galactosidase, β -Glucuronidase, *N*-Acetyl- β -Glucosaminidase and Arylsulphatase in the Ileum of Suckling Rats

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Changes of activity of intestinal acid β -galactosidase, β -glucuronidase, *N*-acetyl- β -glucosaminidase and arylsulphatase were studied in suckling rats treated with cortisone (5mg/100g body wt. daily, started on day 9 postnatally) and compared with changes in control animals. Specific activities were not changed within the first 72h, but all enzymes decreased similarly 96h after the first injection. Total activities per ileum and animal were not changed within the first 48h, but within 72h a significant decrease was observed. Calculation of the rate of decrease of the hydrolases studied in cortisone-treated animals shows that it proceeds faster than the rate of renewal of enterocytes in this period.

In a previous paper (Koldovský & Sunshine, 1970) it was reported that the high activity of acid β -galactosidase in the mucosa of the small intestine of suckling rats could be precociously decreased by the application of cortisone. Under normal conditions the activity decreases during the third postnatal week (Koldovský & Chytil, 1965). Although the activity of various enzymes of the gastrointestinal tract of suckling rats and mice [duodenal alkaline phosphatase (Moog, 1953); small-intestinal sucrase (Doell & Kretchmer, 1964); pancreatic lipase (Sesso, 1962; Rokos, Hahn, Koldovský & Procházka, 1963); pancreatic amylase (Sesso, 1962; Procházka, Hahn, Koldovský, Nohýnek & Rokos, 1964)] was found to increase after the administration of cortisone, the precocious decrease of acid β -galactosidase activity by cortisone was also considered to be a maturative effect because the final values resembled those found in mature animals.

The question arises as to whether the suppressive effect of cortisone on acid β -galactosidase activity is specific for this enzyme. Danovitch & Laster (1969) found that the activity of arylsulphatase was not influenced by the administration of cortisone to suckling rats, although the biological behaviour of this enzyme resembles that of acid β -galactosidase in several respects (Heringová, Koldovský, Yaffe & Jirsová, 1968). However, they investigated the cortisone effect 24 and 48h after administration, whereas Koldovský & Sunshine (1970) killed their animals after four daily injections of cortisone.

In the present investigation, therefore, the effect of cortisone on acid β -galactosidase activity was compared with the effects on β -glucuronidase, *N*-acetyl- β -glucosaminidase and arylsulphatase. These enzymes possess several characteristics similar to the acid β -galactosidase, namely, developmental curves, distribution along the length of the small intestine in suckling rats, subcellular localization and response to adrenalectomy (Koldovský & Chytil, 1965; Heringová, Jirsová & Koldovský, 1965; Koldovský & Herbst, 1971; Alpers, 1969; Hsu & Tappel, 1965).

MATERIALS AND METHODS

White rats of the Sprague-Dawley strain were used. Litters were decreased to eight animals on day 2 after birth and rats were used regardless of sex. Standard pellet diet (Purina Laboratory Chow) was fed and suckling rats were always kept with the mother. The number of animals per litter never decreased below six. When some of the experimental animals were killed, rats of a similar age from other litters were added to the experimental group.

Preparations of tissue. Fed animals were killed by decapitation and their small intestines excised and rinsed with ice-cold 0.15M-KCl. The duodenum was discarded and the remainder of the small intestine was divided into three equal parts. The distal third (ileum) was dried with filter paper, weighed on a torsion balance and then homogenized (in 2 ml of twice-distilled ice-cold water) in a Potter-Elvehjem homogenizer with a Teflon piston.

Cortisone acetate. This was obtained from Upjohn Co. (Kalamazoo, Mich., U.S.A.) and was diluted in water

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(5 mg/ml) and injected intramuscularly daily at 9–10 a.m. in a dose of 5 mg/24 h per 100 g body wt. Control animals were not injected.

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

Enzyme assays. Acid β -galactosidase, β -glucuronidase and *N*-acetyl- β -glucosaminidase were determined in homogenates with *p*-nitrophenyl- β -galactopyranoside, *p*-nitrophenyl- β -glucuronide and *N*-acetyl-*p*-nitrophenyl-

β -glucosaminide respectively as substrates (all from Calbiochem, Los Angeles, Calif., U.S.A.) (Koldovský, Asp & Dahlqvist, 1969; Koldovský & Herbst, 1971). The reaction mixture consisted of 2 vol. of buffer containing 2 mg of albumin/ml, 1 vol. of substrate solution and 1 vol. of homogenate. The following buffers were used: sodium citrate, pH 3.5 (acid β -galactosidase), sodium acetate, pH 4.0 (β -glucuronidase), and sodium citrate, pH 4.5 (*N*-acetyl- β -glucosaminidase). The final assay concentration of the buffers was 0.05 M and the final concentration

Fig. 1. Changes in activities of β -glycosidases in ileum of 10–18-day-old rats: (a) and (b) acid β -galactosidase; (c) and (d) β -glucuronidase; (e) and (f) *N*-acetyl- β -glucosaminidase. Individual points represent mean values obtained from 7–15 determinations, vertical lines are twice s.e.m. (not given if smaller than symbols used). O, Control animals; ●, animals injected with cortisone.

of substrate was 5 mm for acid β -galactosidase and β -glucuronidase and 2.5 mm for *N*-acetyl- β -glucosaminidase. Incubations were performed at 37°C for 15–60 min; the reaction was proportional to both time and the concentration of enzyme. The reaction was terminated and the colour developed by the addition of 2 vol. of 1 M- K_2CO_3 . Blanks were prepared in a similar manner but the substrate was added at the end of the incubation. The extinction at 420 nm was measured and the amount of *p*-nitrophenol liberated was calculated from a standard curve prepared with free *p*-nitrophenol.

Arylsulphatase activity was determined by a slight modification of the method of Danovitch & Laster (1969). The assay mixture consisted of 2 vol. of sodium acetate buffer, pH 5.5 (0.5 M), 1 vol. of homogenate and 1 vol. of 0.02 M-dipotassium nitrocatechol sulphate (Calbiochem, Los Angeles, Calif., U.S.A.). After 15 min at 37°C the reaction was stopped by the addition of 2 vol. of 5% trichloroacetic acid. The mixture was centrifuged at 1600g for 10 min, and 1 vol. of the supernatant solution was added to 1 vol. of 5 M-NaOH. The extinction at 520 nm was determined, and the amount of nitrocatechol liberated was calculated from a standard curve prepared with free *p*-nitrocatechol. An assay was performed on homogenates of ileum and the concentration of the homogenates used was 5 mg wet wt. of tissue/ml.

All chemicals used were reagent grade.

Expression of results. The activity of all the enzymes studied is given as μ mol of substrates liberated/60 min, either per mg of protein or per total ileum per animal. The half-life of the increase in control animals and of the decrease in cortisone-treated animals of acid- β -galactosidase, β -glucuronidase and *N*-acetyl- β -glucosaminidase was determined as described by Segal & Kim (1963) (see also Schimke & Doyle, 1970).

Statistical evaluation. The *t* test was used.

RESULTS

Changes in specific activities

β -Glycosidases. (a) Intact animals. The activities of all three β -glycosidases in the ileum do not change substantially between day 10 and 16; later the activities fall to the adult values (Figs. 1a, 1c and 1e) (Koldovský & Chytil, 1965; Heringová *et al.* 1965; Koldovský & Herbst, 1971). (b) Cortisone-

treated animals. All three β -glycosidases behaved rather similarly. After injection of cortisone starting on day 9, no significant changes in the activities of acid β -galactosidase and β -glucuronidase were observed 24, 48 or 72 h later; *N*-acetyl- β -glucosaminidase decreased significantly after 48 h. All three β -glycosidase activities decreased substantially after 96 h.

Arylsulphatase. The activity was measured in 9-, 11- and 13-day-old control animals, and in animals injected daily with cortisone from day 9 postnatally. Results are summarized in Table 1, which shows that in agreement with Danovitch & Laster (1969) no effect of cortisone was seen in 11-day-old rats that were injected 48 h before killing. However, 96 h after the first injection a significant decrease was observed, comparable with the decrease observed with the other hydrolases.

To characterize further the rate of increase of the three β -glycosidases in control rats and their rate of decrease in cortisone-treated animals, the half-lives were calculated by the method of Segal & Kim (1963).

Changes in total activities per ileum per animal

Control animals. Total enzyme activities increased from day 10 up to day 16 because of growth of the ileum; after this time the activities decreased (Figs. 1b, 1d and 1f).

Cortisone-injected animals. Activities of all three β -glycosidases were significantly lower 72 h after the first injection both in comparison with values of control animals and with values in animals injected 24 or 48 h before being killed.

Calculation of half-lives and rate constants

Generally the results for all three enzymes were similar, and the values are presented in Table 2. A typical plot of the results obtained for acid β -galactosidase is shown in Fig. 2.

Control animals. The half-life values during the increase range from 2 to 3 days.

Cortisone-treated animals. The rate of decrease is similar for all three enzymes. The half-life is about 0.5–0.6 day.

Table 1. *Effect of cortisone on the arylsulphatase in ileum of 9–13-day-old rats*

Cortisone was injected in daily doses of 5 mg/100 g body wt., started on day 9 postnatally. Treated animals were killed on days 11 and 13, i.e. 48 and 96 h after the injections started. Intact animals (litter mates) were killed on days 9, 11 and 13. Values are expressed as means (μ mol of substrate hydrolysed/60 min per mg of protein) $\pm$ s.e.m. (number of animals in parentheses).

Age (days) ...	Arylsulphatase activity		
	9	11	13
Intact animals	5.0 $\pm$ 0.39 (6)	5.4 $\pm$ 0.78 (3)	5.3 $\pm$ 0.59 (7)
Cortisone-treated animals	—	5.7 $\pm$ 0.48 (6)	2.6 $\pm$ 0.22 (6)*

* Value differs significantly from all other values given ($P < 0.01$).

Table 2. Values of half-lives and rate constants of synthesis and degradation of various β -glycosidases in ileum of intact and cortisone-treated suckling animals

All calculations were performed by the method of Segal & Kim (1963). $t/2$, Half-lives determined from graphical plots (Fig. 2); E_0 , maximum activity in intact animals or minimum activity in animals treated with cortisone, as $\mu\text{mol}/60\text{ min}$ per total ileum; k_2 , rate constant of degradation, as $(\text{days})^{-1}$; k_1 , rate constant of synthesis, as $\text{units}/[\text{ileum} \times (\text{days})^{-1}]$; A , value of k_2 of cortisone-treated animals expressed as percentage of k_2 found in intact animals; B , value of k_1 of intact animals expressed as percentage of k_1 found in cortisone-treated animals.

Animal	Enzyme	$t/2$	E_0	k_2	k_1	A	B
Intact	Acid β -galactosidase	2.5	325	0.276	90		490
Cortisone-treated		0.6	16	1.16	18.5	420	
Intact	β -Glucuronidase	3.0	115	0.230	27		520
Cortisone-treated		0.6	4.4	1.16	5.1	504	
Intact	<i>N</i> -Acetyl- β -glucosaminidase	2.0	1192	0.345	411		674
Cortisone-treated		0.5	44	1.39	61	403

Fig. 2. Logarithmic plot of changes of acid β -galactosidase activity of the ileum in control rats and in rats injected with cortisone. Values on the ordinate are differences between the lowest or highest activity found in the ileum (results taken from Fig. 1) and activity for given age. $\circ$, Control animals; $\bullet$, animals injected with cortisone. Results for 10-day-old rats injected with cortisone are not plotted, since they did not differ significantly from values found in 11-day-old cortisone-treated animals.

Calculation of the rate constants for degradation and synthesis are also given in Table 2.

DISCUSSION

Clearly not only acid β -galactosidase but also β -glucuronidase, *N*-acetyl- β -glucosaminidase and arylsulphatase activities exhibit a precocious decrease in the ileum of suckling rats treated with

cortisone. The results obtained with arylsulphatase resolve further the discrepancy between the results of Danovitch & Laster (1969) and Heringová *et al.* (1968). The latter authors demonstrated the involvement of the adrenal glands in regulation of activity of arylsulphatase in the small intestine of suckling rats, because adrenalectomy delayed the decrease in enzyme activity normally observed in the third postnatal week. The question of why no changes are detected during the first 48 h after the start of injection of cortisone is a problem open to further study. Determinations of the half-lives of the increase in enzyme activity between days 9 and 16, the period subsequent to the first postnatal week, when the plateau of specific activity is achieved (Koldovský & Chytil, 1965; Heringová *et al.* 1965; Koldovský & Herbst, 1971), show values close to 2 or 3 days. On the other hand, during the cortisone-evoked decrease these values are about 0.6 day. Whereas the former value is close to the value of the half-life of the enterocytes in the suckling period the latter is substantially shorter. The life-span of the enterocytes during the suckling period is 96 h, and 72 h of this time is spent by the cells migrating from the top of the crypt to the top of the villus. This was found both in normal rats (Koldovský, Sunshine & Kretchmer, 1966; Herbst & Sunshine, 1969; Clarke & Hardy, 1969) and in rats treated with a dose of cortisone (Herbst & Sunshine, 1969; J. Herbst & O. Koldovský, unpublished work). Our results thus indicate that cortisone might evoke the decrease of enzymes in cells already migrating.

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