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Cell Migration and Cortisone Induction of Sucrase Activity in Jejunum and Ileum

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The increase of sucrase activity in homogenates of jejunum and ileum of suckling rats after cortisone administration has been investigated. Serial tissue sections of villi and crypts were also assayed for sucrase activity and these results were compared with the migration of cells labelled with [^{3}H]thymidine along the villus. By using a low dose of cortisone (0.5mg/day per 100g body wt.) it was found that the sensitivity of the small intestine sucrase-producing system to cortisone stimulation increased during the suckling period. On the other hand, 5mg of cortisone/day per 100g body wt. produced practically the same increase of sucrase during the entire suckling period. Sucrase activity in homogenates of the entire small-intestinal wall was first detected 24h after the first injection of cortisone (5mg/day per 100g body weight) to 9-day-old animals and maximum activity both in the jejunum and ileum was reached by 120h. Jejunal activity was greater than ileal activity, but the rate of the increase was similar. The half-time of the increase was 23–27h, whereas enterocytes migrate from the base to the tip of the villi in approximately 72h. Comparison of sucrase activity in serial tissue sections of villi and crypts at various times after cortisone treatment showed that the leading edge of sucrase activity proceeds toward the tip of the villi at the same rate as the advancing edge of newly formed cells. Sucrase activity increased in the newly induced cells as they migrated to the tip of the villi. It was concluded that the increase of sucrase activity in suckling rats after cortisone stimulation is due to at least three factors: (1) increase of activity in newly differentiating cells, (2) increased percentage of villus cells with sucrase activity and (3) continued production or activation of sucrase activity as the cells migrate along the villi.

During postnatal development, the enzymes of the small intestine undergo substantial changes (Koldovský, 1969). The developmental alterations of sucrase activity (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26), a disaccharidase localized in the microvilli fraction of the enterocytes (Crane, 1966), provide a useful model for the elucidation of events during changes in intestinal enzyme activity. Its activity is negligible in the small intestine of the rat during the suckling period (Doell & Kretchmer, 1964; Rubino *et al.*, 1964; Yezuitova *et al.*, 1964), and after cortisone stimulation, activity increases towards normal adult values (Doell & Kretchmer, 1964). It is unknown if the sensitivity of the sucrase-producing system to cortisone stimulation changes during early development. Recent experiments by Koldovský & Sunshine (1970) have shown that the response of another small-intestinal enzyme, acid β-galactosidase, to cortisone administration does change substantially during the suckling period.

Doell *et al.* (1965) have shown that the precociously induced sucrase does not differ immunologically from the sucrase present in the small intestine of adult rats. Approx. 24h after injection of hydrocortisone into suckling rats the presence of sucrase was demonstrated by the fluorescent antibody technique in cells near the base of the villi; 24h later fluorescence had extended more distally along the villi. No quantitative assay of sucrase and no direct correlation with migration of cells were performed, but comparisons with later published results (Koldovský *et al.*, 1966; Herbst & Sunshine, 1969) suggest a close relationship between the increase of sucrase activity and the rate of cell migration. Sucrase activity also shows a gradient of distribution along the small intestine, being greater in the jejunum than in the ileum of many mammals, including man (Booth, 1968). This gradient is absent in human foetuses of 2 months post-conceptional age (Jirsová *et al.*, 1968), although in older foetuses and newborn infants the activity of the jejunum exceeds that of the ileum.

In light of the previously summarized facts our experiments were designed to answer the following questions. (1) Do changes in sucrase activity of the

intestine evoked by cortisone alter during the postnatal development of the intestine, and does the response to cortisone differ in the jejunum and ileum? (2) What is the quantitative relationship between the increase of sucrase activity and the migration of cells?

Materials and Methods

Materials

White rats of the Sprague–Dawley strain were used. Litters were decreased to eight animals on day 3 after birth and animals were used regardless of sex. They were fed on a standard pellet diet (Purina Laboratory Chow) and were weaned by day 28 after birth.

All chemicals used were of reagent grade.

Preparation of tissue

Fed animals were decapitated and their small intestines were excised and rinsed with ice-cold 15mM-NaCl. The duodenum was discarded and the remainder of the small intestine was divided into three equal parts. The proximal third was used as jejunum and the distal third as ileum. For determination of sucrase in homogenates of the entire jejunum or ileum, the individual thirds were rinsed with ice-cold 15mM-NaCl, blotted with filter paper, weighed on a torsion balance and homogenized in 2ml of ice-cold twice-distilled water in a Potter–Elvehjem homogenizer equipped with a Teflon piston. The mucosa from the jejunum and ileum of 60-day-old rats was scraped off with a spatula and used in place of the whole intestine. In experiments where sucrase was assayed in specimens obtained from different positions along the length of the jejunal villus, samples of jejunum obtained just distal to the ligament of Treitz were serially sectioned at right angles to the long axis of the villi in a cryostat as previously described (Nordström *et al.*, 1967, 1969). The intestine was positioned so that the sequential samples were from tip of villi to base of crypt. Sections were cut at 8μm and every third section was stained with 0.5% Toluidine Blue and examined for the presence of villi and/or crypts. The other two sections were homogenized in 0.2ml of water with a Teflon homogenizer.

Assay of sucrase activity

This was performed as described by Dahlqvist (1964). In each assay the reaction mixture contained 1vol. of homogenate and 1vol. of substrate–buffer mixture (56mM-sucrose in 0.1M-sodium maleate buffer, pH5.8). The reaction was stopped by placing the samples in a boiling-water bath for 2min. The liberated glucose was determined with tris–glucose oxidase reagent prepared as described by Dahlqvist (1964) from Glucostat (Worthington Biochemical Corp., Freehold, N.J., U.S.A.). When low sucrase activities were assayed in concentrated mucosal homogenates a standard glucose curve was run in which the standards contained boiled homogenate of the same concentration as in the assay, since concentrated mucosal homogenates inhibit the glucose oxidase reaction (Asp *et al.*, 1967).

Cell migration

Cell migration along the villus was measured as described earlier (Koldovský *et al.*, 1966; Herbst & Sunshine, 1969). The rats received 2μCi of [^{3}H]-thymidine (>10Ci/mmol)/g body wt. at the time of the first injection of cortisone. Samples of jejunum and ileum were fixed in 10% formalin, processed for radioautography (Kopriwa & Leblond, 1962), and finally counterstained with haematoxylin and eosin. All distances were measured in cell spaces to minimize fixation artifacts as previously noted (Herbst & Sunshine, 1969), and results expressed as the percentage of the length of the villi traversed by the leading edge of labelled cells. Cell migration was determined in 20 villi/rat, and at least three rats were killed at 24, 48, 60, 72 and 96h. Cell migration did not vary more than 10% among rats at each time-interval.

Determination of protein

Protein was determined as described by Lowry *et al.* (1951), by using standards of serum albumin.

Cortisone injection

Cortisone (cortisone acetate, Merck, Sharp & Dohme, Rahway, N.J., U.S.A.) was injected intramuscularly daily in the morning. Litters were randomly divided into different subgroups to insure that compared groups consisted of fairly equal numbers of littermates. In experiments where the effect of various doses of cortisone were compared, control animals were not injected and the cortisone was diluted in water so that each animal received 0.5ml of fluid/100g body wt.

Evaluation of results

Sucrase activity is expressed as μmol of glucose liberated/60min either per mg of protein or per total intestinal section (i.e. jejunum or ileum). Statistical significance of differences was estimated by using the *t* test. The half-time of the increase of sucrase activity after injection of cortisone was determined by the method of Segal & Kim (1963).

Results

Effect of various doses of cortisone and age on increase of sucrase activity in jejunum

In these experiments animals from each litter were divided into four groups, i.e. control animals and animals receiving either 0.5, 2.0 or 5.0mg of cortisone/100g body wt. per 24h. The injections started 5 days before the animals were killed and were repeated daily. The results (Fig. 1) show that sucrase activity spontaneously increases at the end of the third week as previously observed (Doell & Kretchmer, 1964; Rubino *et al.*, 1964; Yezuitova *et al.*, 1964). Application of cortisone increased activity up to 20 days post-natal and as in normal development sucrase activity was always higher in the jejunum than in the ileum. The response of sucrase activity in jejunum and ileum to low doses of cortisone (0.5mg) was greater in 16- and 18-day-old rats as compared with 8- and 12-day-old rats. A 5mg dose produced practically the same response at all ages. In the ileum the response to the 2mg and 5mg dose was the same. In the jejunum of 8- and 12-day-old rats, the response to a 2mg dose was intermediate between a 0.5 and 5.0mg dose, but in 16-day-old rats and older the response to a 2 and 5mg dose was the same. The specific activity of sucrase in intact 30-day-old and 60-day-old rat intestines was close to values found in suckling rats treated with the highest dose of cortisone (5mg/100g body wt.). These experiments thus indicate that the increase of sucrase activity evoked by cortisone differs quantitatively in the jejunum and ileum, and that the sensitivity of the sucrase producing system changed during the suckling

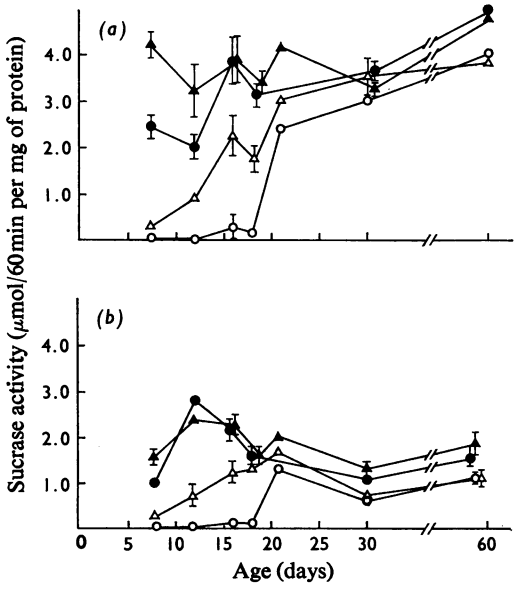

Fig. 1. *Effect of cortisone injection on sucrase activity in jejunum and ileum*

Sucrase activity was measured in (*a*) jejunum and (*b*) ileum of control rats (○), and rats receiving 0.5mg (△), 2mg (●) and 5mg (▲) of cortisone/24h per 100g body weight. The ages of the treated animals are plotted as the age when they were killed, 5 days after the first cortisone injection. Symbols denote mean values from at least seven rats; vertical lines denote ±2 S.E.M. (not given if smaller than symbols used). The values for control rats differed from those injected with either the lowest or highest doses of cortisone in rats 8–18-days-old; 20-day-old rats injected with cortisone have significantly higher values than intact rats. Determinations of the sucrase activity in all animals except 60-day-old rats were performed in homogenates of the entire intestinal wall; in 60-day-old rats only mucosa was used.

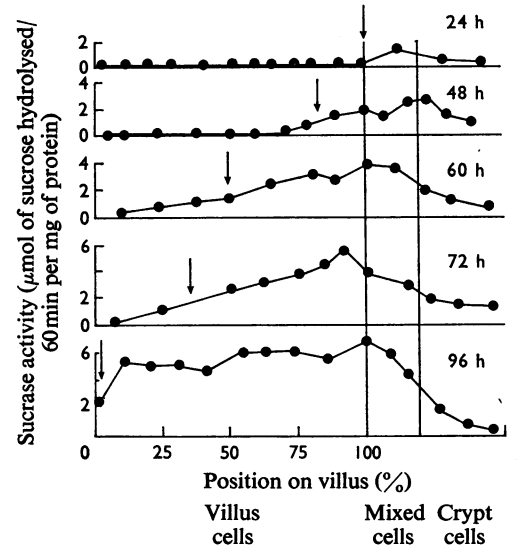

Fig. 2. *Activity of sucrase in serial tissue samples of jejunal villi and crypts*

Sucrase activity was determined at various times after cortisone administration in 12–13-day-old rats. Each point represents a single assay. The arrows indicate the leading edge of thymidine-labelled cells as determined from radioautographs. The values for cell migration also apply to the ileum. [^{3}H]Thymidine was administered with the first dose of cortisone. For comparison purposes, positions along the villi are plotted as a percentage of the length of the entire villus.

period. Since the 5mg dose evoked quantitatively similar responses in different age groups, it was used in further experiments.

Comparison of increase of sucrase activity and rate of cell migration

Cells labelled with thymidine appeared at the base of the villi 24h after simultaneous injections of [^{3}H]-thymidine and cortisone and had migrated to the tip of the villi after 96h. The same rate of migration occurred in the jejunum and ileum; the results from jejunum only are shown (Fig. 2).

Sucrase activity in homogenates of serial tissue sections of villi and crypts

In agreement with the determination of sucrase in whole jejunal homogenate (Table 1), activity was very low at 24h after injection of cortisone, and was detectable only in the crypt–villus junction area (Fig. 2). At successive intervals the leading edge of sucrase activity proceeded toward the tip of the villi at the same rate as the advancing edge of the newly formed cells. At intervals after cortisone administration, not only did a greater proportion of each villus have sucrase activity, but the specific activity of sucrase in the cells at the base of the villi also increased (Fig. 2). This finding was verified in another experiment where only slices close to the tip and base of the villi were assayed in several rats at each time-interval (Table 2). The cells present at the base of the villi 24h after cortisone belong to the same cohort of cells present at the tip of the villi at 96h.

Rate of increase of sucrase in jejunal mucosa

Rats (9 days old) were injected daily with cortisone (5mg/100g body wt.) and killed at different times. The first increase of specific activity both in the jejunum and ileum was seen after 24h and peak values were reached 80h after the first injection (Table 1). To express the rate of increase of sucrase activity in terms of half-time, the total amount of invertase present in the jejunum or ileum per animal was calculated, and Table 1 shows that the maximal values for total activity were attained at 120h. After 120h no significant change of the total invertase activity occurred. These results were then plotted by the method of Segal & Kim (1963) (Fig. 3). Although the quantitative increase in the jejunum and ileum was substantially different, the determined half-time for increase of sucrase activity was similar in the jejunum (23h) and the ileum (27h).

Discussion

Corticosteroids and the adrenal gland have profound maturative effects on the development of the intestine in suckling rats and mice (Koldovský, 1969; Moog, 1971). Injection of corticosteroids into the suckling rats causes a maturation of the morphological appearance of the intestine (Clark, 1959; Overton, 1965; Herbst & Sunshine, 1969) and causes a cessation of absorption of intact antibodies (Clark, 1959; Halliday, 1959). Cortisone injection causes a precocious increase of RNA/DNA ratio in the intestine (Koldovský *et al.*, 1971), an increase in intestinal alkaline phosphatase activity (Moog, 1951), and sucrase activity (Doell & Kretchmer, 1964).

Table 1. *Activity of sucrase in rats injected with cortisone*

Suckling rats were injected daily with 5mg of cortisone/100g body wt. per 24h starting on day 9. Time indicated is hours after the first injection of cortisone. Specific activity is given as μmol of sucrose hydrolysed/60min per mg of protein. Total activity is expressed as μmol of sucrose hydrolysed/60min per total intestinal section. Results are means±S.E.M. of the number of animals per group. There was no significant increase in total or specific activity after 120h.

		Jejunum		Ileum	
Time (h)	No. of animals	Specific activity	Total activity	Specific activity	Total activity
24	3	0.03±0.05	0.7± 0.07	0.01±0.05	0.25±0.06
33	3	0.23±0.03	9.0± 1.6	0.12±0.03	3.2 ±2.00
48	7	1.14±0.03	27 ± 4.4	0.65±0.07	11.5 ±1.5
57	5	1.22±0.29	45 ± 4.4	0.74±0.07	18.2 ±4.1
72	11	2.24±0.25	82 ± 7.5	1.37±0.17	27.5 ±4.7
80	3	3.2 ±0.14	97 ±13.0	2.33±0.17	31.5 ±6.5
95	12	3.1 ±0.28	117 ± 6.5	2.00±0.24	33.7 ±2.2
120	8	3.2 ±0.30	153 ± 8.6	2.25±0.34	44.1 ±3.5
145	7	2.8 ±0.31	148 ± 7.2	2.65±0.37	48.4 ±4.5
168	3	3.0 ±0.35	150 ±10.0	2.80±0.44	48.3 ±6.4

Table 2. *Activity of jejunal sucrase of tip and base of villi after cortisone injection*

Sucrase activity in the 15% of slices closest to the tip and base of the villi was measured at various intervals after injecting 5mg of cortisone/100g body weight. Sucrase activity is expressed as μmol of sucrose hydrolysed/60min per mg of protein. Results are given as means±S.E.M.; six animals were used in each group.

	Sucrase activity	
Time (h)	Tip	Base
24	0.06±0.04	0.54±0.2
48	0.04±0.13	3.9 ±0.2
96	4.8 ±0.3	4.6 ±0.2

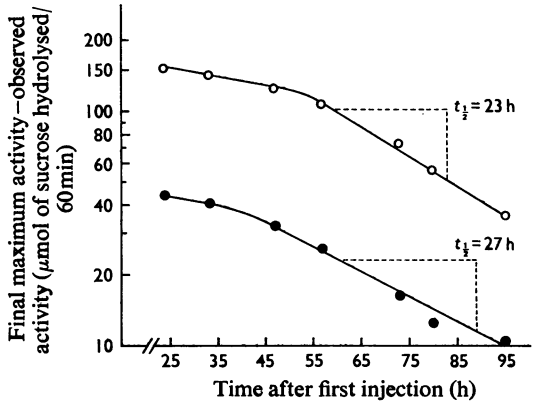

Fig. 3. *Determination of the half-time of the increase of sucrase activity in suckling rats after injection of cortisone*

The results, plotted as described by Segal & Kim (1963), were taken from Table 1. Lines were fitted by eye. ○, Jejunum; ●, ileum.

Cortisone injection will also cause a precocious decrease in acid β-galactosidase activity (Koldovský & Sunshine, 1970) and a decrease of absorption of copper (Mistilis & Mearick, 1970) and vitamin B_{12} (Gallagher, 1969). Conversely, adrenalectomy in the suckling period delays the attainment of adult patterns of various intestinal enzymes (Koldovský, 1969).

The manner in which cortisone effects the maturative changes on suckling rat intestine remains open to investigation. For example, does cortisone affect only crypt or villus cells? Do all of the effects of cortisone stimulation appear simultaneously? What is the effect of various dosages of cortisone at different ages? Does the response vary in jejunum and ileum? In the present study we have investigated the increase of jejunal and ileal sucrase activity after cortisone administration at different ages. We have also compared the rate of increase in enzyme activity with cellular migration along the villi.

The results show that the jejuno-ileal gradient of sucrase activity could be evoked precociously by cortisone (without dietary change), and thus suggest that the appearance of the jejuno-ileal differences described in human foetuses (Jirsová *et al.*, 1968) might be the result of endocrine influences. Although sucrase activity is greater in the jejunum than in the ileum after cortisone administration, the rates of increase of activity are similar in both (Fig. 3), thus suggesting that the different response in the two parts of the intestine is a quantitative rather than a qualitative one. Further, by using low doses of cortisone it was demonstrated that the sensitivity of the small-intestine sucrase-forming system to cortisone increases with age until sucrase appears normally at weaning. On the other hand, the reaction of the intestine to the largest dose of cortisone was similar throughout the suckling period. The large dose is useful for other purposes, but cannot be used to detect changes in sensitivity of cortisone stimulation. A similar phenomenon (increase of sensitivity with age) was observed in previous experiments in which cortisone evoked a precocious decrease of acid β-galactosidase (Koldovský & Sunshine, 1970).

Although sucrase activity was detected only in cells present in the crypts at the time of the first injection of cortisone (Fig. 2) comparison of the cell-renewal results and the rate of increase of sucrase activity after injection of cortisone indicated that the increase cannot be explained by cell renewal alone. Sucrase activity increases exponentially, with a half-time of increase of 23–27h (Fig. 3), whereas cells migrate from the base to tip of the villi in a sequential linear manner in approximately 72h (Fig. 2). The time for cell migration is in close agreement with previously reported results for intact rats at the same age (Koldovský *et al.*, 1966; Herbst & Sunshine, 1969; Clarke & Hardy, 1969). Table 2 and Fig. 2 show that specific sucrase activity at the base of the villi increases substantially from 24 to 48h and increases further by 96h after cortisone administration. Thus there is

increasing sucrase activity in newly differentiating cells with prolonged stimulation. Further, specific activity of sucrase in cells located at the base of the villi 24h after cortisone increases approx. tenfold as these cells migrate to the tip of the villus by 96h, indicating that either enzyme activation or synthesis continues in enterocytes as they migrate along the villi, even though incorporation of radioactive amino acids into the villus cells is much less than in crypt cells (Lipkin & Quastler, 1962). James *et al.* (1971) have found that the half-life of radioactive amino acids incorporated into disaccharidases in adult rats is 11.5h, i.e. shorter than the half-life of radioactive amino acids incorporated into brush borders (18h) or into intestinal homogenates (31h). They demonstrated that migration of cells *per se* cannot account for the turnover of disaccharidases and that control may be at the level of the intestinal villi cell. Thus the increase of sucrase activity after cortisone stimulation is due to at least three factors: (1) increasing activity in newly differentiating cells, (2) increased percentage of villus cells with sucrase activity and (3) continued production or activation of sucrase activity as the cells migrate along the villi.

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