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RAT JEJUNAL DISACCHARIDASE ACTIVITY INCREASES BIPHASICALLY DURING EARLY POST-NATAL DEVELOPMENT

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SUMMARY

1. Injection of hydrocortisone into 9-day-old rats induces the early appearance of sucrase in jejunal homogenates, the time course of the subsequent increase and magnitude of the final effect being similar to that seen to start on day 16 during normal development.

2. Cytochemical comparison of the effect of hydrocortisone and normal development on the appearance of a mixture of sucrase, maltase, isomaltase and trehalase disaccharidases (α-glucosidase activity) shows this enzyme to appear first in enterocytes at the base of the villus. Enzyme activity then increases and spreads along the whole villus during the next 96 h.

3. The rate at which enterocytes migrate along the villus after hydrocortisone injection is not significantly different from that measured during the early phase of normal development. The later phase of normal development is associated with a threefold increase in cell migration rate and a twofold increase in crypt depth.

4. The rate at which α-glucosidase activity increases in enterocytes at the base of the villus during early normal development is similar to that determined after hydrocortisone injection into younger animals. This rate of appearance increases eight to tenfold during normal development, shortly after the appearance of solid food in the stomach of normal control animals.

5. Injection of steroid hormones into young rats is generally supposed to mimic events taking place normally at weaning. Present results show α-glucosidase induction during normal development to be under more complicated control than had been previously suspected.

INTRODUCTION

Intestinal enzymes undergo substantial changes post-natally which later enable the animal to digest a post-weaning diet rich in carbohydrates (Rubino, Zimbalatti & Auricchio, 1964; Reddy & Wostmann, 1966). In spite of much subsequent work on this subject, however, it is still not clear exactly which one of a number of dietary and hormonal factors are of prime importance in initiating these events (Henning, 1981). Recently it has been found that the timing of a post-natal increase in sucrase activity remains unaffected when grafting fetal intestine into syngeneic hosts (Ferguson, Gerskowitch & Russell, 1973; Kendall, Jumawan & Koldovsky, 1979; Mont-

gomery, Sybicki & Grand, 1981; Yeh & Holt, 1986). The present conclusion is that a local timing mechanism triggers these changes but with hormones still playing a role in determining the overall pattern of enzyme development (Henning, 1985).

Present interest in this subject arose from the finding that an immune reaction mounted by foreign lymphocytes in mice produced an early appearance of sucrase activity in crypt and villus enterocytes (Lund, Bruce, Smith & Ferguson, 1986) and that this change closely followed an increase in crypt cell proliferation brought about through a reduction in cell cycle time (Lund & Smith, 1987). Increased levels of endogenous steroids could facilitate this increase in sucrase activity in a way similar to that reported previously in steroid-injected rats (Herbst & Koldovsky, 1972; Henning, Helman & Kretchmer, 1975). Whether or not any of these circumstances reflect events happening during normal development remains to be tested.

The object of the present work was to investigate these questions further by making a detailed comparison of hydrocortisone-induced and naturally occurring changes in rat jejunal disaccharidase activity. These comparisons were carried out at the cellular level using quantitative cytochemistry to obtain an improved localization of α-glucosidase activity in individual enterocytes (King, Paterson, Peacock, Smith & Syme, 1983; Smith, 1985; Chaves, Smith & Williamson, 1987). Changes in enzyme expression probably take place as a two-stage process under normal conditions.

METHODS

Animals

Neonatal Wistar rats bred at Babraham, housed under controlled conditions of temperature and lighting (daily cycle of 12 h light and 12 h darkness), were injected intramuscularly at 9 days of age with 50 mg/kg body wt. of hydrocortisone acetate (25 mg/ml). Litter-mate controls were injected with an equal volume of normal saline. After injection all animals were returned to their mothers before being killed 1–5 days later. Another group of uninjected control rats were left with their mothers under the same conditions of heating and lighting for a period of 16–23 days of age before being used for experiment.

Experimental

Neonatal rats taken from their mothers were stunned and killed by cervical dislocation, the small intestine then being removed, flushed through with saline and the mid-third used for analysis. Biochemical determinations of sucrase activity were carried out on mucosal scrapings of this tissue suspended in normal saline after disruption in a Probe sonicator (Heat Systems – Ultrasonics, New York).

Cytochemical analysis of rat intestine involved dividing adjacent tissue samples into two, the proximal part being cut open, placed between two slices of liver, frozen in isopentane pre-chilled in liquid N_2 and stored at -20 °C for subsequent cytochemical determination of α-glucosidase activity. The distal part was cut open, spread on card and fixed in 0·1 M-phosphate buffer, pH 7·2, containing 4% (v/v) glutaraldehyde and 2% (w/v) sucrose for a period of 2 h. This tissue was later dehydrated and embedded in glycolmethacrylate, 3 μm sections then being cut for autoradiography and histological analysis. One of these sections was stained routinely with haematoxylin and eosin for measurement of villus height and crypt depth. Serial sections were coated with Ilford K2 nuclear track emulsion, diluted 1:1 with distilled water to determine enterocyte migration rates in eosin-stained autoradiographs.

Analytical

Biochemical assays for sucrase were performed by incubating aliquots of intestinal homogenates at 37 °C with 90 mM-sucrose in 90 mM-NaCl, 4 mM-disodium succinate buffer, pH 6·0, glucose

release being measured subsequently using the glucose oxidase assay kit of Boehringer GmbH, Mannheim, F.R.G. Final activities were related to protein content estimated according to the method of Markwell, Haas, Bieber & Tolbert (1978).

Cytochemical estimates of α-glucosidase activity (a mixture of sucrase–isomaltase, maltase and trehalase activities) were determined in 10 μm cryostat sections by incubation for 7 min at 37 °C with 6 mM-2-naphthyl-α-D-glucopyranoside in citrate phosphate buffer, pH 6·0, after preliminary fixation in Baker's formal–calcium medium for 10 min at 4 °C. Colour development using hexazonium-*p*-rosaniline was as described previously (Gutschmidt, Kaul & Riecken, 1979). The amount of enzyme reaction product present in enterocyte brush-border membranes was later measured by microdensitometry at a wavelength of 530 nm. Care was taken to ensure that only initial rates of hydrolysis of colour-producing substrate were measured in these experiments.

Enterocyte migration rates in control and hydrocortisone-injected rats were measured by injecting tritiated thymidine (1 μCi/g body wt.) intraperitoneally into rats which were killed at different times up to 48 h afterwards. The leading edge of migrating thymidine-labelled cells was later measured in eosin-stained autoradiographs as described previously (Smith, Jarvis & King, 1980). Regression analysis showed enterocyte movement to be linearly related to time after thymidine injection.

Statistical analysis of results was by unpaired Student's *t* test. Linear regression analysis was carried out by the method of least squares. Other results given in Table 1 are presented as means ± S.E. of means with the number of observations given in parentheses. The sample size chosen to study different stages of development depended on what stage enzyme induction was predicted to have reached at the time rats were killed for experiment.

Materials

[*Methyl* ^{3}H]thymidine (40–60 Ci/mmol) was obtained from Amersham International plc, Amersham, Bucks. 2-Naphthyl-α-D-glucopyranoside came from Koch–Light Laboratories, Colnbrook, Bucks. Sucrose was purchased from Fisons Scientific Apparatus, Loughborough, Leics. Hydrocortisone acetate was purchased as Hydrocortistab from Boots Co. Ltd, Nottingham, Notts. All other reagents used were of AR grade.

RESULTS

Control experiments

Previous studies have shown sucrase activity to increase post-natally in rat jejunum, both at weaning and following glucocorticoid treatment of suckling animals (Henning *et al.* 1975). It was decided to repeat this work initially to test whether these effects could be confirmed, both during normal development and after the injection of hydrocortisone. Both the timing and magnitude of naturally occurring and steroid-induced changes in sucrase activity were found to closely resemble those reported previously by Henning *et al.* (1975). Having demonstrated consistency between one set of data and another it then became interesting to make a further comparison of the cellular origin for these two apparently similar types of effect.

Quantitative expression of α-glucosidase activity by rat jejunal enterocytes

α-Glucosidase activity was determined in frozen sections of jejunal tissue taken from rats during normal post-natal development and after hydrocortisone injection. Results obtained from control animals killed 16–23 days after birth are shown in Fig. 1.

Only trace amounts of α-glucosidase activity could be detected, at the base of villi, in 16-day-old animals. Activity increased gradually between days 16 and 18, but more noticeably between days 19 and 20 and hardly at all between days 20 and 23.

α-Glucosidase activity also spread along the villus during development, with enzyme activities at the villus tip always being less than those found in the lower regions of the villus. Similar developmental profiles for α-glucosidase distribution along jejunal villi of hydrocortisone-injected rats are shown in Fig. 2.

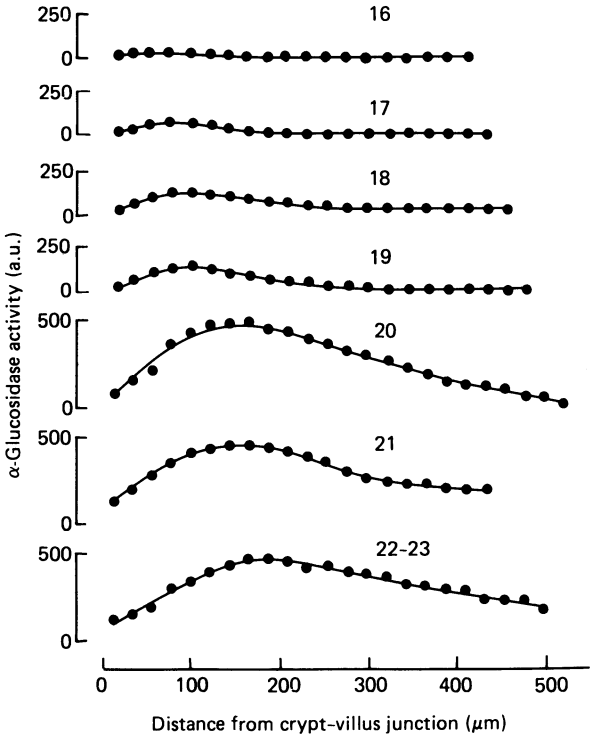

Fig. 1. Effect of age on the positional distribution of α-glucosidase activity in rat villus enterocytes. Rats aged 16–23 days were killed and α-glucosidase activity determined in frozen sections in arbitrary units (a.u.) as described in the text. The number of villi analysed to provide mean estimates of enzyme activity is given, for each stage of development, in Table 1. The standard error associated with each mean estimate of enzyme activity never exceeded 10% of the recorded value. Each point gives the mean of determinations carried out on fifteen to thirty villi obtained from three to six rats. Numbers in the Figure refer to the age of the rats in days.

Hydrocortisone induces the appearance of α-glucosidase activity in the basal half of rat jejunal villi within 24 h, highest activity occurring in 10-day-old rats about 50 μm from the crypt–villus junction. This activity spreads towards the villus tip during the next 4 days without any marked increase in the maximum activity being recorded in the lower part of the villus. There is a fall in α-glucosidase activity as enterocytes near the tips of villi but this is not as great as that seen during normal development. No activity is detected in tissue taken from control 9- to 14-day-old rats. These results show some similarity, both in the time course and changing shape of distribution profiles, for α-glucosidase appearance in tissue taken from 16- to 23-day-old control and 10- to 14-day-old hydrocortisone-injected rats.

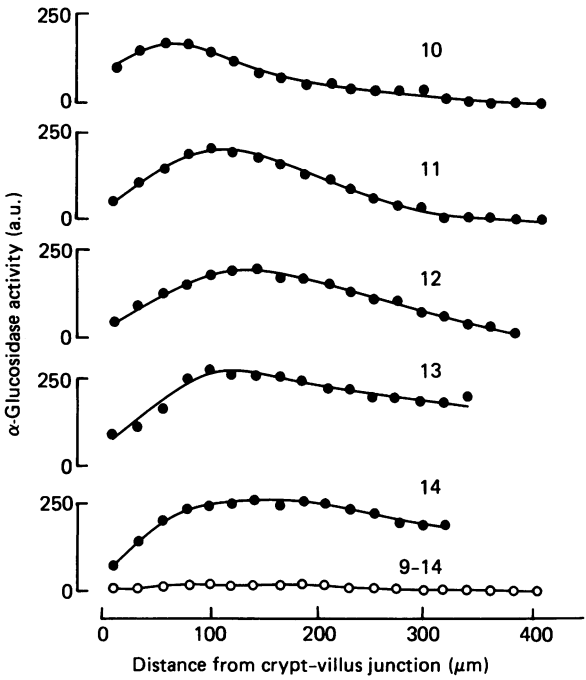

Fig. 2. Effect of hydrocortisone on the positional distribution of α-glucosidase activity in rat villus enterocytes. Rats at 9 days of age injected intramuscularly with 50 mg/kg body wt. of hydrocortisone (●) or an equal volume of saline (○) were killed for the cytochemical determination of α-glucosidase activity in frozen sections of jejunal tissue as described in the text. The number of villi analysed to provide mean estimates of enzyme activity is given, for each stage of development, in Table 1. The standard error associated with each mean estimate of enzyme activity never exceeded 10% of the recorded value. Each point for controls gives the mean of determinations carried out on one hundred and eight villi obtained from thirty-six 9- to 14-day-old rats. Numbers in the Figure refer to the age of the rats in days.

Kinetic aspects of enterocyte development

A more useful description of how enterocytes change their properties during development is obtained by determining the rate of cell migration along the crypt–villus axis. This rate can then be used to calculate absolute rates of enzyme expression. This procedure has been carried out in the present work by injecting control and hydrocortisone-injected rats with tritiated thymidine at different times prior to killing and then determining the position of the leading edge of labelled cells on the villus in prepared autoradiographs. The results obtained from these experiments are shown in Fig. 3.

Enterocyte migration along the crypt–villus axis is linearly related to time after injection of thymidine with the rate of cell movement depending upon the age of the rat. Linear regression of data obtained for 10- to 18-day-old controls and 10- to 14-day-old hydrocortisone-injected rats produced rates of migration which were not significantly different (2·65 ± 0·40 and 2·45 ± 0·42 μm/h, respectively; means ± S.E. of mean) while that for 19–23-day-old control rats was about three times greater

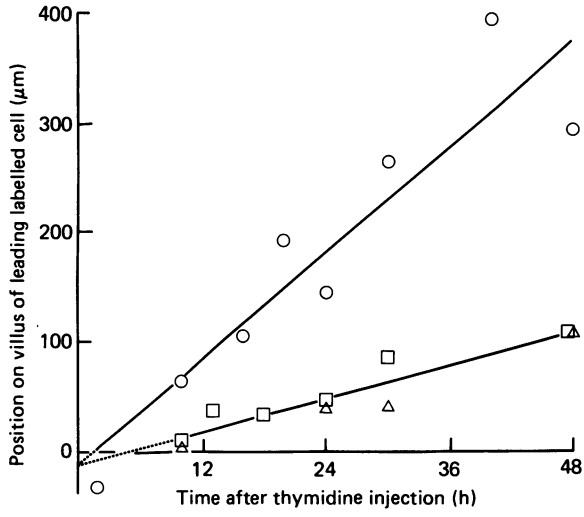

Fig. 3. Enterocyte migration along neonatal rat villi. Control rats aged 10–23 days and 10- to 14-day-old rats injected with 50 mg/kg body wt. hydrocortisone on day 9 were killed at known times after the intraperitoneal injection of 1 μCi/g body wt. of tritiated thymidine. Pieces of jejunum were then removed and processed for autoradiography as described in the text. Values show the highest positions reached by migrating labelled enterocytes measured from the crypt–villus junction. Each value gives the mean of measurements carried out on three to twenty-nine 10- to 18-day-old control, four to seven 19- to 23-day-old control, and three to sixteen 10- to 14-day-old hydrocortisone-injected rats (△, ○ and □, respectively).

(8·32 ± 1·45 μm/h). These results were used to calculate a mean migration rate for the first two groups of animals and then time-dependent development profiles for α-glucosidase appearance as described previously (King *et al.* 1983). The results obtained from carrying out these manipulations are summarized in Fig. 4.

Time-dependent profiles for hydrocortisone-induced α-glucosidase activity (Fig. 4*A*) show enzyme activity to appear in enterocytes which were both in the crypt and on the villus at the time of steroid injection (filled and open circles, respectively). There was also a graded ability of villus enterocytes to express α-glucosidase in response to hydrocortisone; basal villus cells 10 h old gave a maximum response after hydrocortisone injection whereas mid-villus 10 h old cells gave a minimal response and upper villus cells failed to respond to steroid injection. It took approximately 5 days for cell replacement to take place in hydrocortisone-treated rats. This time would have been longer had not hydrocortisone also caused a significant shortening of villi during the 5 days after injection (444 ± 21 and 324 ± 17 μm for 9-day-old control and 14-day-old hydrocortisone-treated rats ($P < 0{\cdot}001$), the corresponding length for villi in control 14-day-old animals being 422 ± 15 μm). Crypt depth also increased significantly as rats became older ($P < 0{\cdot}01$), but this was not dependent upon the previous injection of hydrocortisone (crypt depths of 47·4 ± 3·2 and 42·1 ± 2·1 μm for control and hydrocortisone-injected 10-day-old rats; 60·9 ± 3·0 and 55·2 ± 2·5 μm for control and hydrocortisone-injected 14-day-old animals).

The sequence of events leading up to the early phase of α-glucosidase appearance

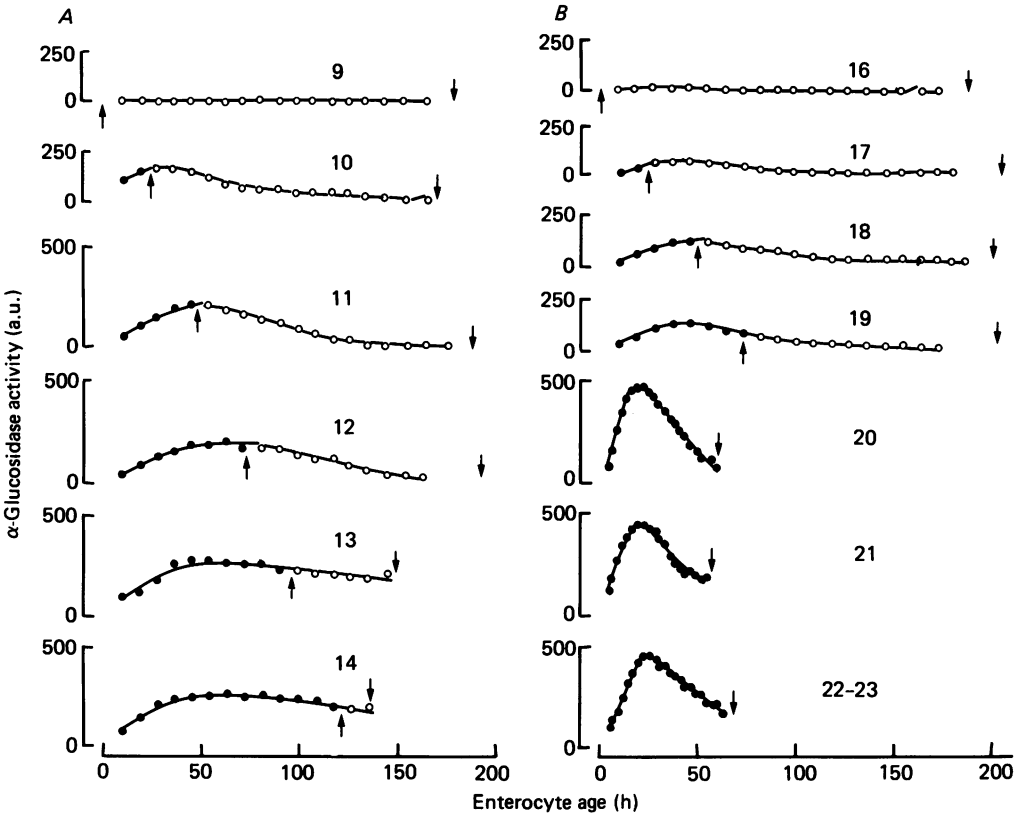

Fig. 4. Time dependence of α-glucosidase appearance in rat enterocytes. Values showing the positional distribution of α-glucosidase in Figs 1 and 2 have been related to enterocyte age using migration rates calculated from results shown in Fig. 3. *A*, rats injected with hydrocortisone on day 9; *B*, rats taken during normal development. Enterocytes in the crypts in 16-day-old control and 9-day-old hydrocortisone-injected rats (●) are separated by an upward arrow from enterocytes originally on the villus in these animals (○). The second, downward, arrow shows the calculated age of villus tip enterocytes. Numbers in the Figure refer to the age of rats in days.

in jejunal enterocytes during normal development (Fig. 4*B*) appeared to be very similar to that seen in younger animals following injection of hydrocortisone. Both villus and crypt cells begin to express α-glucosidase activity in their brush-border membranes in the 17- to 19-day-old animal with older cells on the villus showing little or no response. The rate at which these changes took place changed dramatically on day 20. This change coincided with an approximate doubling of crypt depth (63·9 ± 7·4 to 110·1 ± 8·5 μm for 19- and 20-day-old rat jejunum) with no change in villus length (484 ± 28 and 450 ± 16 μm, respectively). This more adult pattern of α-glucosidase expression then continued unchanged up to 23 days after birth.

A quantitative comparison between the effects of hydrocortisone and natural development on enzyme appearance can be made by calculating the rate at which α-glucosidase appears in enterocytes during early migration over the basal part of the villus. Regression analysis of data obtained from enterocytes during the first 20 h of their lifespan gives rates of appearance which are summarized in Table 1. There is a

close correspondence between the initial rate at which enzyme activity appears following steroid injection and that shown to occur naturally 17–19 days after birth. This is followed by a 8- to 10-fold increase in the rate at which α-glucosidase appears in enterocytes of 20- to 23-day-old control animals. This switch from slow to fast expression of α-glucosidase coincides with the first detection of solid food in the stomach contents.

TABLE 1. Increase in α-glucosidase activity measured in enterocytes during early migration over the lower villus

Hydrocortisone-injected rat		Normal development	
Age (days)	Rate of α-glucosidase activity increase (arbitrary units/h)	Age (days)	Rate of α-glucosidase activity increase (arbitrary units/h)
10	3·9±0·6 (4/20)	17	2·8±0·1 (5/25)
11	5·3±0·5 (5/25)	18	3·5±0·2 (5/25)
12	3·7±0·4 (5/25)	19	3·4±0·2 (6/30)
13	5·8±0·7 (11/55)	20	32·2±2·5 (8/40)
14	6·1±0·7 (11/55)	21	20·2±1·1 (5/23)
		22	25·7±1·7 (3/15)
		23	25·7±1·7 (3/15)

Rats were either injected with 50 mg/kg body wt. hydrocortisone on day 9 or left to develop normally up to 23 days of age. Values give calculated slopes±S.E. of mean of regression lines calculated 0–20 h after the birth of enterocytes using data taken from Fig. 4. Numbers inside parentheses give first the number of rats used and secondly the number of villi analysed for each stage of development.

DISCUSSION

It has long been known that sucrase–isomaltase, maltase and trehalase activities increase dramatically in rat intestine during neonatal development (Rubino *et al.* 1964; Reddy & Wostmann, 1966) and that all of these changes can be induced prematurely through the injection of glucocorticoids (Doell & Kretchmer, 1964; Galand & Forstner, 1974). At the same time it was shown in the rat that immunologically identified sucrase first appeared at the base of villi both during normal development and after hydrocortisone injection (Doell, Rosen & Kretchmer, 1965). The conclusions drawn from this work, i.e. that hydrocortisone only affected cells deep in the crypt and glucocorticoid injection mimicked similar changes taking place during normal development, have not been confirmed in the present work. It is, however, only through the combined use of an improved technique to localize the cellular site of hormone action and the simultaneous measurement of cell migration rate that one can begin to appreciate an alternative view of events taking place during normal development.

Present results show α-glucosidase activity to appear quite suddenly in young villus enterocytes during both normal development and after injection of hydrocortisone. Similar changes on α-glucosidase activity also take place in villus enterocytes during normal development in the mouse intestine (Lund & Smith, 1986). This ability of villus as well as crypt cells to respond to hormones is not, however, confined to hydrocortisone. Vitamin D_3, thyroxine and epidermal growth factor, are all now known to increase the capacity of young villus cells to express different proteins

(Smith, Bruns & Lawson, 1985; Hewitt & Smith, 1986; James, Smith, Tivey & Wilson, 1987). The consequences of this general finding have yet to be assimilated into present interpretations of how timing or glucocorticoids affect neonatal development.

Changes in cell cycle time, crypt cell production and enterocyte migration rates, all take place normally during neonatal development, but none of these appear to be directly connected either with the early phase of normal development 16–19 days after birth or with the ability of hydrocortisone to induce changes in disaccharidase activities. Cell migration takes place slowly in pre-weaned rats and this remains true after the injection of hydrocortisone. Later, rapid increases in α-glucosidase activity taking place during normal post-natal development are accompanied by large changes in crypt structure and enterocyte migration rate. Ingestion of solid food containing increased amounts of carbohydrates is probably responsible for initiating all these later effects.

Evidence accumulated over the past few years strongly suggests that an intrinsic timing mechanism plays an important part in preparing the intestine for an adult-type diet (Ferguson *et al.* 1973; Kendall *et al.* 1979; Montgomery *et al.* 1981). Recent work further suggests that this programme might be mediated through the number of cell divisions taking place in the crypt (Yeh & Holt, 1986). This latter idea, although attractive, is not supported by experiments carried out under conditions where crypt cell proliferation and α-glucosidase expression are both being artificially stimulated (Lund & Smith, 1987). Neither does it explain the sudden appearance of α-glucosidase activity in villus enterocytes or the ability of glucocorticoids to induce these enzymes without affecting cell division. Perhaps the mistake lies in trying to portray timing and/or glucocorticoid control of development as all-or-nothing events occurring independently at a single point in time. Evidence already exists to show an increasing sensitivity of rat intestine to hydrocortisone during the first and second weeks of post-natal life (Henning *et al.* 1975) and an apparent alteration of the timing mechanism in mice undergoing a Graft-*versus*-Host Reaction (Lund & Smith, 1987). It may also be significant that the total amount of sucrase appearing in intestinal implants is less than that found under normal conditions even though the timing of the initial event remains comparable (Kendall *et al.* 1979).

We suggest that the precise timing and degree to which α-glucosidase becomes expressed prior to weaning is likely to reflect an increasing sensitivity of villus as well as crypt cells to low concentrations of endogenous steroids and to the possible gradual removal of factors which are capable of inhibiting gene expression of adult-type enzymes. Either of these process would, according to this theory, eventually produce the desired effect, but both would be needed to achieve optimal results. High doses of glucocorticoid would then be expected to swing the product of this equation towards early expression of adult-type enzymes as would an acceleration of the removal of putative inhibitors. Once the system has been primed, however, there seems to be little doubt that it is the actual change in diet which exerts the major effect on enzyme composition. Present results show that it is now possible to separate primary from diet-induced effects on enzyme expression during normal post-natal development. Future work should concentrate on a further separation of different processes likely to affect cell priming in the neonate.

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