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Cellular origin of lactase decline in postweaned rats

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Intestinal lactase activity falls at weaning reaching low levels in the adult rat. Present work measures cellular migration rates and lactase activity along villi of intestines taken from rats of different ages to determine the cellular basis for this developmentally regulated fall in enzyme expression. An early fall in lactase activity taking place 15 to 23 days after birth was found to be associated with a shortening of the time available for lactase expression in brush-border membranes. A later fall in lactase activity occurring 23 to 46 days after birth was caused by an additional reduction in the rate at which lactase activity appeared in the brush-border membrane. These results are discussed in relation to previous work describing lactase biosynthesis in post-weaned rats.

Intestinal lactase activity in the newborn rat declines gradually to reach low levels in the adult [1,2]. Present controversy about how this fall comes about centres around the measurement of lactase biosynthesis, one group of workers claiming that reduced lactase synthesis is largely responsible for the post weaning decline in enzyme activity [3], while another insists that lactase synthesis takes place at a constant rate throughout development, the net fall in activity resulting from a more rapid rate of enterocyte replacement in the adult [4]. Interest in knowing which of these explanations might be correct is increased by the suggestion that a similar regulatory mechanism is probably responsible for causing milk intolerance in adult humans [5]. Recently it has become possible to use quantitative cytochemistry to determine changes in lactase activity occurring as enterocytes migrate along the crypt-villus axis of intestinal villi [6,7]. The present work uses this technique in a new attempt to find out which of the above two mechanisms might be responsible for affecting lactase expression in neonatal rat intestine.

Jejunal tissue removed from rats killed by cervical dislocation at known times after the intraperitoneal injection of 1 μCi/g body weight of [^{3}H]thymidine was flushed with ice-cold saline and a portion frozen in liquid N_2-cooled isopentane for cytochemical determination of lactase activity [8]. A second part of the tissue was fixed in glutaraldehyde-sucrose buffer to prepare autoradiographs for the subsequent determination of enterocyte migration rate [6,7]. A third part was taken to prepare mucosal scrapings which were then homogenised and later assayed for lactase activity and protein content [9,10]. Separate measurements of villus length and crypt depth were carried out on sections of tissue stained with haematoxylin and eosin.

Cytochemical estimates of lactase activity took place in the presence of saturating concentrations of artificial substrate at incubation times chosen to ensure that only initial rates of hydrolysis were being measured. The amount of enzyme reaction

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product formed in tissue sections under these conditions was recorded sequentially in absorbance units (log I_o/I referred to a.u.) along the crypt-villus axis by microdensitometry. Logistic growth curves used to fit this data had the general formula

$$y = a + c/[1 + \exp(-b(x - m))]$$

where a is the initial amount of lactase activity present in a newly formed enterocyte, m is the time at which lactase activity increases at maximal rate in the brush-border membrane, c is the maximal lactase activity expressed by an enterocyte and b is the exponential coefficient describing the rate of increase in lactase activity during development. Further details of this general curve fitting procedure have been published previously [11].

Experiments carried out on tissue taken from 15-, 23- and 46-day-old rats showed villus height to remain constant (515 ± 15 (23); 477 ± 13 (22) and 523 ± 11 (24) μm; means $\pm$ S.E. (number of animals)) and crypt depth to increase markedly (50 ± 2 (23), 119 ± 4 (22) and 175 ± 6 (24) μm) during neonatal development. Lactase activity in mucosal homogenates also declined with age in these three groups of rats (5.9 ± 0.5, 1.3 ± 0.1 and 0.7 ± 0.1 μmol lactose hydrolysed/mg protein per h). This fall was accompanied by a corresponding increase in enterocyte migration rate (2.6 ± 0.4, 10.9 ± 0.3 and 12.5 ± 2.7 μm/h for jejunal tissue taken from 15-, 23- and 46-day-old rats). All of these results confirm earlier published findings [1,2,12,13]. What is interesting in the present context however is the finding that the crypt depth continues to increase significantly and lactase activity to fall significantly between 23 and 46 days after birth ($P < 0.001$ in both cases). These two groups have been previously considered to represent a single population of adult rats up till now [3,4] and this is clearly not the case for lactase development.

Lactase activity present in enterocytes located at different positions along the villus are shown, for tissue obtained from 15-, 23- and 46-day-old animals, in Fig. 1. Similarities between these three developmental profiles include an increase in activity occurring as enterocytes migrate over the basal region of the villus, a period during which

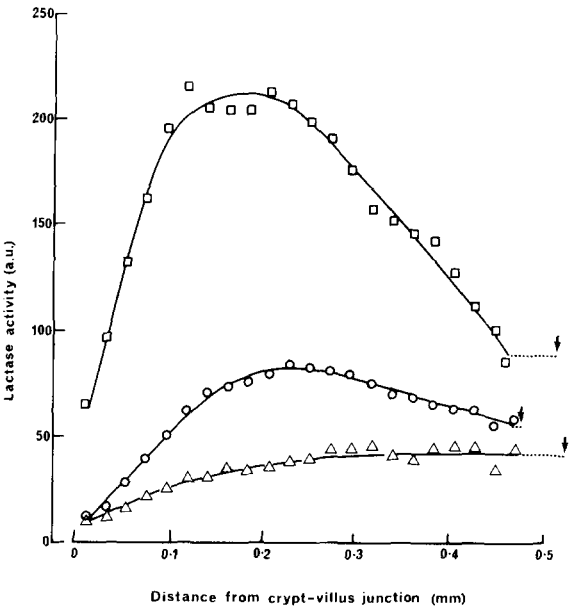

Fig. 1. Developmental profiles for lactase appearance in rat jejunum measured during neonatal development. Results show lactase activities determined cytochemically as absorbance (arbitrary units) along villi obtained from 15-, 23- and 46-day-old rats (□, ○ and △, respectively). Each value gives the mean estimate obtained from 12 rats (36 villi). ↓, marks the position of the villus tips.

activity remains constant in the mid-villus region and a period during which lactase activity tends to fall as enterocytes approach the villus tip. The amount of activity present in mid-villus enterocytes changes with the age of the rat in a way similar to that measured in homogenates. This graded difference is not however present in enterocytes crossing the crypt-villus junction (where 23- and 46-day-old rats have similar lactase activities) or in villus tip enterocytes (where all activities appear similar). Further insight into how these apparently complicated changes in lactase expression come about is obtained by relating these different developmental profiles to enterocyte migration rates. Results obtained from carrying out this transformation are summarized in Fig. 2.

Enterocytes migrating onto the base of villi in 15-day-old rats have a higher lactase activity and are about twice as old as those studied in 23-day-old animals (Fig. 2). It is this difference in age which largely accounts for the initial discrepancy in enzyme activity. The time during which lactase

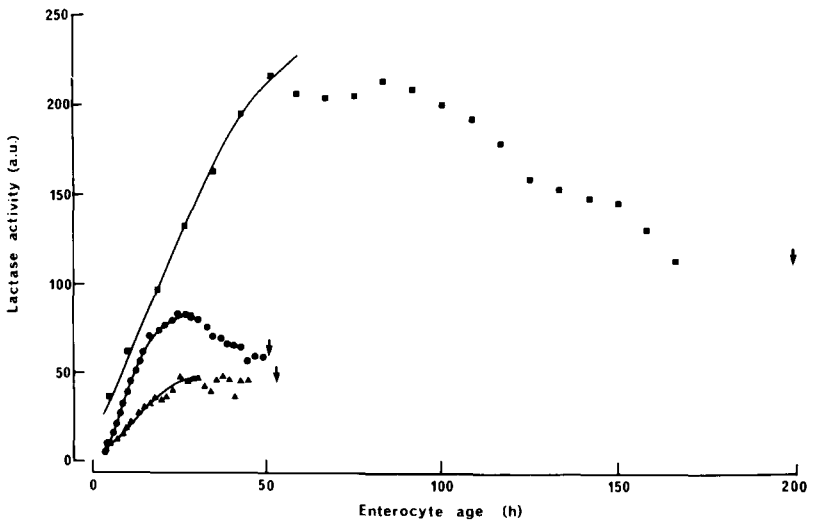

Fig. 2. Time dependence of lactase appearance in brush-border membranes of rat enterocytes. Values obtained for lactase activities in Fig. 1 have been plotted here in relation to enterocyte age calculated using estimated migration rates for 15-, 23- and 46-day-old rats of 2.6, 10.9 and 12.5 μm/h (■, ● and ▲, respectively). Logistic curves fitted to the rising phase of lactase expression are shown as solid lines. ↓, predicted age of enterocytes at villus tips.

activity continues to increase in 23-day-old rat enterocytes is considerably shorter than that found in 15-day-old animals. This effect largely accounts for the final reduction in enzyme activity seen to take place in the 23-day-old animal. Constants obtained from fitting logistic curves to these two sets of data are shown in Table I.

The calculated time for an enterocyte to half complete maximal expression of lactase activity is reduced from 20 to 11 h during this early period of postnatal development. The maximal rate at which lactase activity can be expressed in these cells is however somewhat greater in the 23-day-old rat ($bc/4$ values of 4.3 and 6.3 a.u./h for 15- and 23-day-old rats, respectively). These adaptive changes in lactase expression are very similar to those found previously in neonatal mice when stimulating crypt cell proliferation through the induction of a Graft-versus-Host reaction [6].

A further interesting comparison can be made between the way lactase is expressed in the two older groups of rats. Enterocytes in 46-day-old rat intestine show a time dependency for lactase expression essentially similar to that found in the 23-day-old animal, but with the maximal rate of enzyme expression reduced to about half that determined in the younger adults. Logistic curve analysis of these two sets of results confirms that identical times are taken to half complete this aspect of development (Table I; m values of 10.8 $\pm$ 0.2 and 11.9 $\pm$ 0.7 h for 23- and 46-day-old rat intestines) with the maximal rate of enzyme expression in the 46-day-old animal being markedly reduced ($bc/4$ value of 2.3 compared with 6.3 a.u./h for the 23-day-old rat). The overall reduction in lactase activity occurring between

TABLE I

CALCULATED CONSTANTS OBTAINED FROM LOGISTIC CURVE ANALYSIS OF EXPERIMENTAL DATA DESCRIBING THE FALL IN LACTASE ACTIVITY DURING NEONATAL DEVELOPMENT

Logistic constants used to fit data shown in Fig. 2 give the maximal lactase activity expressed by an enterocyte, (c), the time at which lactase activity appears to increase at maximal rate, (m), and the coefficient describing the time dependency of lactase expression (b). Standard errors to these constants reflect the goodness of fit to experimental data.

Rate age (days)	c (absorbance)	m (h)	b (h^{-1})
15	214.1 $\pm$ 4.7	20.3 $\pm$ 0.9	0.08 $\pm$ 0.01
23	82.0 $\pm$ 1.2	10.8 $\pm$ 0.2	0.31 $\pm$ 0.01
46	46.1 $\pm$ 2.2	11.9 $\pm$ 0.7	0.20 $\pm$ 0.02

these two groups of animals is largely accounted for by this inhibition in the rate at which enterocytes express lactase. Similar selective effects on lactase expression have been reported for tissue taken from children suffering from coeliac disease [14].

Cytochemically detected lactase activity reflects only the balance between enzyme synthesis and degradation and a change in either will affect the result obtained. Direct measurement of lactase biosynthesis has, on the other hand, only been carried out on mixed populations of cells making it impossible to determine what is happening in single cells at different stages of development. In the present work it has been assumed that lactase biosynthesis predominates in young enterocytes and that the use of quantitative cytochemistry in this situation allows one to reconcile previously contrary views concerning the way lactase biosynthesis is regulated in the post-weaned rat [3,4].

What is now suggested on the basis of these results is that regulation of lactase expression takes place in two stages during neonatal development. The first stage involves a shortening of the time allowed for enzyme expression with no inhibition of the inherent capacity of cells to express lactase at maximal rate. Such an effect supports the findings of Tsuboi et al. [4]. The second stage of regulation involves an inhibition in the rate at which lactase activity is expressed by young enterocytes. Such an effect supports the work carried out by Jonas et al. on 46-day-old animals [3]. The mechanism responsible for the first stage of regulation bears some resemblance to that commonly seen to occur in response to increased crypt cell proliferation [15]. The second stage could reflect changes in the immunological status of the gut [14]. It should now be possible to test this latter hypothesis by administering suitable antibiotics to this older group of rats.

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