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# COLCHICINE SELECTIVELY INHIBITS LACTASE EXPRESSION BY RAT ENTEROCYTES

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**Abstract**—One early aspect of enterocyte differentiation involves the appearance of digestive enzymes in the brush border membrane during cell migration from intestinal crypts onto villi. Present experiments describe how small amounts of colchicine selectively affect this particular aspect of enterocyte development. Oral ingestion of approximately 50 μg cochicine per day halves lactase activity in intestinal homogenates without affecting sucrase, maltase or alkaline phosphatase activities. This inhibition, which is completely reversible, takes about 48 hr to become complete. Further analysis of this effect by quantitative cytochemistry shows colchicine to reduce the maximal rate at which lactase activity appears in the brush border membrane. This reduction takes place without substantially affecting enterocyte migration rate or the time taken to fully complete lactase development. The possibility is discussed that small amounts of colchicine can selectively inhibit lactase biosynthesis in both crypt and mature villus enterocytes.

Hydrolase enzymes appear as brush border components in enterocytes at distinct stages of differentiation as cells migrate from the crypts of Lieberkühn towards the villus tips. By combining kinetic data on cell proliferation and migration with quantitative cytochemical techniques it is now possible to describe this normal pattern of development in detail and determine the way enzyme activities can become altered by age, diet, hormones and environmental temperature [1–5]. It is also possible to apply these analytical techniques to identify the stage of differentiation and the manner in which different drugs induce changes in this particular aspect of enterocyte development.

Long term clinical use of colchicine has been shown previously to cause malabsorption as well as inhibition of disaccharidase activities in man [6]. Similar inhibition of intestinal disaccharidase activities takes place in rats given small amounts of colchicine to drink for a period of two weeks [7], but the possible mechanism and the cellular site at which this drug acts remain unknown. The present work uses quantitative cytochemistry to investigate at the cellular level how colchicine affects lactase expression during enterocyte migration along intestinal villi.

## METHODS

*Animals.* Male and female Wistar rats purchased from A. Tuck & Son Ltd., Battlesbridge, Kent, U.K. weighing 100–120 g, were housed under controlled conditions of temperature and lighting (12 hr light; 12 hr dark) and allowed free access to water and food (CRM-X diet, K & K Greef Ltd., Croydon, Surrey, U.K.) prior to experiment.

*Experimental.* Rats kept as controls or given colchicine in their drinking water (2.5 mg/dm$^3$) for a period of 12 days were weighed regularly and their fluid intake measured before being killed by stunning and cervical dislocation for experiment. The approximate daily intake of colchicine during this pre-experimental period was 0.4 mg/kg body wt. Portions of proximal jejunal tissue dissected from these animals were then either (a) scraped free from muscle and the scrapings sonicated (Probe Sonicator, Heat Systems-Ultrasonics, New York) in 5 parts (w/v) cold 0.9% saline for the biochemical determination of lactase, sucrase, maltase and alkaline phosphatase activities; (b) frozen between liver slices in isopentane cooled in liquid $N_2$ for the cytochemical determination of lactase activity or (c) fixed in 4% (v/v) glutaraldehyde, 2% (w/v) sucrose, 0.1 M phosphate buffer, pH 7.2, for subsequent measurement of intestinal structure.

*Analytical.* Lactase activity was determined biochemically by incubating aliquots of intestinal sonicates for 30 min at 37° with 90 mM lactose in 100 mM sodium citrate buffer, pH 6.0, containing 0.1 mM *p*-chloromercuribenzoate [8]. Glucose release was then measured using the glucose oxidase method (kit from Boehringer GmbH, Mannheim, F.R.G.). Similar assays for sucrase and maltase activities were carried out using 90 mM sucrose or maltose in 90 mM NaCl 4 mM disodium succinate buffer, pH 6.0, intestinal sonicates being heated at 55° for 60 min previously when estimating maltase to destroy the ability of sucrase-isomaltase to hydrolyse maltose [9]. Alkaline phosphatase activity was determined by incubating samples of intestinal sonicates with 10 mM *p*-nitrophenyl phosphate for 10 min at 37° in 50 mM Tris buffer, pH 10.1, containing 50 mM $MgCl_2$. *p*-Nitrophenol release was then measured, in 0.5 N NaOH

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solution, by spectrophotometry at 410 nm (modification of [10]). All enzyme activities were finally related to protein content estimated according to the method of Markwell *et al.* [11].

Lactase activity in enterocyte brush border membranes was also determined cytochemically by incubating frozen sections with 4-Cl-5-Br-3-indolyl-β-D-fucoside at 37° for 20–60 min. The enzyme product formed in this reaction was then converted to indigo for measurement by microdensitometry at 640 nm [12]. This method for estimating lactase activity involved taking sequential measurements of optical density along the brush border membranes of enterocytes, starting at the crypt–villus junction and finishing at the villus tip. The increase in activity recorded on moving up the villus could then be computer-fitted by a logistic growth curve having the form

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

where $a$ is the smallest amount of lactase considered to be present in newly formed enterocytes, $c$ is the predicted maximal lactase activity found in mid-villus enterocytes when $a$ is assumed to be zero, $m$ is the location on the crypt–villus axis where lactase activity can be shown to increase at maximal rate and $b$ is the exponential coefficient of the change in activity related to distance. The constants $m$ and $b$ can also be expressed in terms of enterocyte ages, calculated from separate thymidine labelling experiments assuming that the last division of daughter cells marked the time at which migrating enterocytes were born. Identical curve-fitting routines have been used previously to describe a number of different aspects of enterocyte development [3].

Care was taken to ensure that only initial rates of hydrolysis were being measured at saturating concentrations of substrate when carrying out both biochemical and cytochemical estimates of all hydrolase activities.

*Measurement of enterocyte migration rate.* Estimates of enterocyte migration were obtained by injecting tritiated thymidine (approximately 1 μCi/g body wt) intraperitoneally into control and 12 day colchicine-treated rats. These animals were then killed at known times afterwards and pieces of jejunum removed for autoradiographic analysis as described previously [1]. The leading edge of migrating thymidine-labelled enterocytes was determined subsequently in haematoxylin and eosin stained autoradiographs of longitudinally sectioned villi to calculate enterocyte migration rates for control and colchicine-treated tissue. These values were then used to calculate the age of enterocytes at different points along the crypt–villus axis. Additional measurements of crypt depth and villus height were made on haematoxylin and eosin stained sections of glycolmethacrylate-embedded tissue using a certain eyepiece micrometer.

*Materials.* [Methyl-$^3$H]thymidine (40–60 Ci/mmol) was obtained from Amersham International p.l.c., Amersham, Bucks, U.K. Sucrose came from Fisons Scientific Apparatus, Loughborough, Leics, U.K. Lactose came from BDH Chemicals Ltd. Poole, Dorset; and maltase, *p*-nitrophenyl phosphate, colchicine and 5-bromo-4-chloro-3-indolyl-β-D-fucoside were all purchased from the Sigma Chemical Company, Poole, Dorset, U.K. All other reagents used were of AR grade.

## RESULTS

### *Control experiments*

It was initially observed that groups of rats given 2.5 mg/dm$^3$ colchicine in their drinking water consumed less fluid than controls during the first two days contact with the drug (13.7 ± 1.5 compared with 22.8 ± 1.4 ml/day, respectively; means ± SEM; values determined per rat for four groups of three rats). Rats ingesting colchicine during this period also failed to increase in weight (mean body weight of 117 ± 5 and 118 ± 5 g for control and colchicine-treated rats measured initially; body weight 131 ± 4 and 118 ± 7 g measured two days later; means ± SEM; nine rats in each group). Both the growth rate and the fluid intake of rats measured 2–12 days after the initial administration of colchicine became similar to that seen in control animals (5.5 ± 0.2 and 4.2 ± 0.5 g/day; 25 ± 2 and 21 ± 3 ml/day for control and colchicine-treated rats, respectively). Mean body weights of control and colchicine-treated rats were also found to be similar after a period of 12 days contact with the drug (173 ± 10 and 157 ± 9 g for control and colchicine-treated animals; $P > 0.1$). Subsequent experiments were carried out on 12 day colchicine-treated rats except when studying the time course for onset of colchicine effects on disaccharidase activities.

### *Hydrolase activities in intestinal homogenates*

Homogenates of jejunum taken from control and 12 day colchicine-treated rats were assayed biochemically for lactase, sucrase, maltase and alkaline phosphatase to determine the selectivity of colchicine action on brush border enzyme activities. The results obtained from these experiments are shown in Table 1.

Lactase activity is decreased by colchicine treatment. This action is highly selective, there being no statistically significant effect of the drug on sucrase, maltase or alkaline phosphatase activities. Thyroxine injected into rats also inhibits lactase activity in a way superficially similar to that seen for colchicine [5]. Experiments were therefore undertaken to test whether there was any interaction between colchicine and thyroxine in their ability to affect lactase activity. The results obtained from these experiments are summarized in Table 2.

Daily injection of a high dose of thyroxine (1 μg/g body wt) produced a maximal 50% inhibition of lactase activity after a period of 48 hr. Treating rats with colchicine for 12 days also produced a similar 50% reduction in enzyme activity. Injecting colchicine-treated rats with thyroxine caused no further inhibition of lactase activity. It is concluded from these results that thyroxine and colchicine act at the same or at different points along a common pathway to inhibit the expression of lactase activity.

### *Cellular development of lactase activity*

The net appearance of lactase in brush border

Table 1. Hydrolase activities in rat jejunum

| Enzyme | Hydrolase activity (μmol substrate hydrolysed/mg protein/hr) | | P |
|---|---|---|---|
| | Control | Colchicine-treated | |
| Lactase | 0.79 ± 0.07 | 0.40 ± 0.07 | <0.001 |
| Sucrase | 6.71 ± 0.40 | 5.76 ± 0.58 | n.s. |
| Maltase | 17.41 ± 1.19 | 14.55 ± 1.25 | n.s. |
| Alkaline phosphatase | 20.05 ± 2.78 | 24.23 ± 1.72 | n.s. |

Each value gives the mean ± SEM of 14 estimates carried out on jejunal homogenates prepared from control and 12-day colchicine-treated rats.

membranes of enterocytes located at different points along the intestinal villus is shown in Fig. 1.

Enterocytes coming from intestinal crypts in control rats already possess lactase activities greater than those found in 12 day colchicine-treated animals (30.0 ± 3.6 and 8.4 ± 1.3 absorbance units respectively; means ± SEM; 21 rats in each group; $P < 0.001$). This difference increases as enterocytes begin to migrate over the lower part of the villus. There is then no further increase in enzyme activity during subsequent migration over the upper part of the villus towards the villus tip. Logistic curve analysis gives calculated values for maximal lactase activities of 145 ± 3 and 62 ± 2 absorbance units for control and colchine-treated rats, respectively. The distance travelled in order to half-complete this particular aspect of development also appears to be greater in tissue taken from colchine-treated rats (125 ± 8 and 76 ± 4 μm for colchicine-treated and control rats, respectively). These effects of colchicine are not associated with any change in villus length measured in haematoxylin and eosin stained serial sections (429 ± 10 and 455 ± 13 μm for control and colchicine-treated animals; $P > 0.2$), although there are significant changes in crypt depths measured in the same pieces of tissue (169 ± 6 and 214 ± 8 μm for control and colchicine-treated rats). A more useful description of how colchicine works at the cellular level can be obtained by relating the results obtained to the actual age of enterocytes.

Table 2. Additive effects of thyroxine ($T_4$) and colchicine on rat jejunal lactase activity.

| Treatment | Lactase activity | P |
|---|---|---|
| Control | 0.92 ± 0.12 (7) | |
| Colchicine-treated | 0.40 ± 0.04 (8) | <0.001† |
| $T_4$-injected | 0.58 ± 0.09 (6) | <0.05† |
| $T_4$-injected and colchicine-treated | 0.49 ± 0.10 (7) | <0.002†; n.s.* |

Lactase activities (μmol lactose/hydrolysed mg protein/hr), determined in homogenates of mucosal scrapings prepared from rats treated with colchicine for 12 days and/or injected with 1 μg $T_4$/g body wt daily for 2 days, were compared with that found in control rats (†). Lactase activity in $T_4$-injected, colchicine-treated rats was also compared with activities determined after giving either drug singly (*). All values give means ± SEM (No. of rats).

*Temporal aspects of enterocyte development*

Fifteen control and 10 colchicine-treated rats were injected with tritiated thymidine (1 μCi/g body weight) to determine enterocyte migration rates as described previously. The results obtained from these experiments are summarized in Fig. 2. Regression analysis showed enterocyte migration to be linearly related to time up to 30 hr after the injection of isotope (correlation coefficients of 0.99 for both colchicine-treated and control animals). The calculated enterocyte migration rate for colchicine-treated tissue was about 25% greater than that found in control rats, but this difference was found not to be statistically significant (13.2 ± 1.8 and 16.7 ± 1.2 μm/hr for control and test animals; $P > 0.1$). It was, nevertheless, decided to use separately determined migration rates to calculate time profiles for lactase development. Fitting logistic curves to these profiles then showed the main effect of colchicine to be to reduce, by a factor of three, the maximal rate at which lactase activity appeared in the brush border membranes of maturing enterocytes. This reduction took place without any marked change in the time taken to express this enzyme at maximal rate (11.6 ± 0.3 and 13.1 ± 0.5 hr for control and colchicine-treated enterocytes).

*Time course for colchicine effects on lactase activity*

Further information on the possible mechanism of colchicine action was sought by examining the rapidity of onset of inhibition and recovery of lactase activity after addition or removal of colchicine from the rat's drinking water. The results obtained from these experiments are summarized in Fig. 3.

Significant inhibition of lactase activity occurred 24 hr after adding colchicine to drinking water ($P < 0.05$). Further inhibition of lactase activity then took place reaching a maximum one day later. Full recovery of lactase activity took place 48 hr after giving rats normal tap water to drink. Colchicine had no significant effect on sucrase activity measured under these conditions. This time course for lactase inhibition in the presence of colchicine is too long to involve an immediate effect on enzyme already present in the brush border membrane and too short to coincide with the time needed to completely renew the epithelium. In this case it became interesting to determine the cellular origin of colchicine action. Rats given colchicine in their drinking water were therefore killed 24 hr later and lactase activities com-

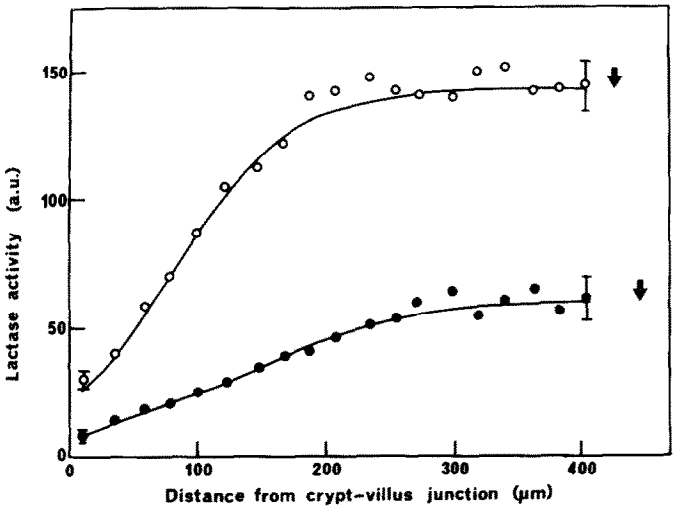


Fig. 1. Positional distribution of lactase activity along the rat jejunal villus. Frozen sections of rat jejunum prepared from animals given normal or colchicine-containing tap water to drink for a period of 12 days (○ and ●, respectively) were incubated for lactase and the amount of enzyme reaction product determined cytochemically as described in the text. Arrows mark the positions of the villus tips. Each value gives the mean (±SEM in some cases) of estimates carried out on tissue taken from 14 rats (three villi per rat). Curves fitting these points were constructed as described in the text.

pared cytochemically with those found in control animals. The results obtained from these experiments are shown in Fig. 4.

Lactase activity in a 10-hr-old enterocyte leaving the crypt is already significantly reduced after short-term contact with colchicine ($P < 0.001$). This difference is maintained as enterocytes migrate towards the villus tip ($P < 0.001$ and $<0.01$ for 18- and 39-hr-old cells, respectively). These results again demonstrate that colchicine affects crypt enterocytes. Some idea of the quantitative changes taking place during the 24 hr following colchicine ingestion can also be obtained from the present results.

An enterocyte at A leaving the zone of proliferation in the crypt will have virtually completed its ability to increase brush border lactase content at B 24 hr later. The corresponding value for lactase measured at C, after 24 hr contact with colchicine,

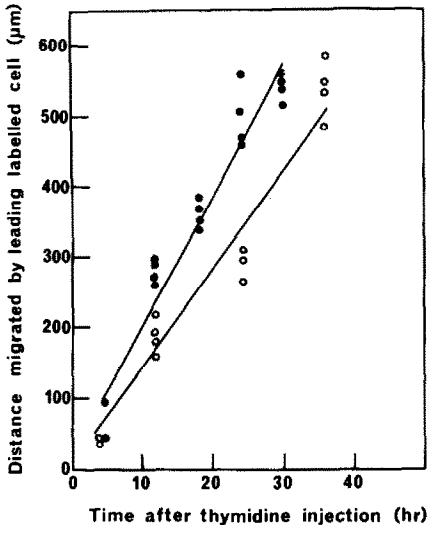


Fig. 2. Enterocyte migration along rat jejunal villi. Rats given normal or colchicine-containing tap water to drink (○ and ●, respectively) were killed at known times after the intraperitoneal injection of tritiated thymidine (1 μCi/g body wt). Pieces of jejunum were then removed and processed for autoradiography as described in the text. Values show the highest positions reached by migrating labelled enterocytes measured from the bottom of each crypt. Each value gives the mean of 3–6 measurements carried out in a single rat jejunum.

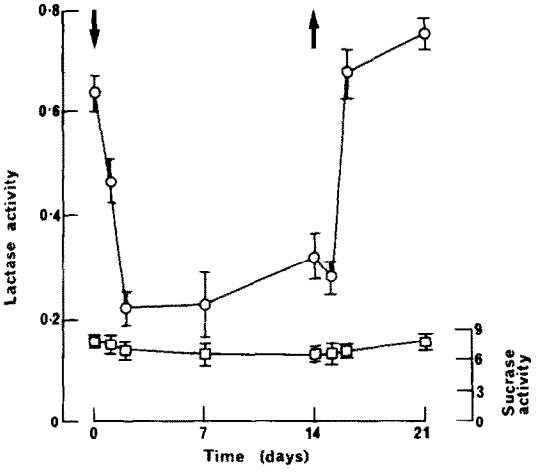


Fig. 3. Short-term effects of colchicine on rat jejunal disaccharidase activities. Jejunal homogenates, prepared from rats killed at known times after adding (↓) or removing (↑) colchicine (2.5 mg/dm$^3$) from the drinking water, were assayed biochemically for lactase (○) or sucrase (□) activities (μmol substrate hydrolysed/mg protein/hr) as described in the text. Values give means ± SEM of estimates carried out on 3–8 rats.

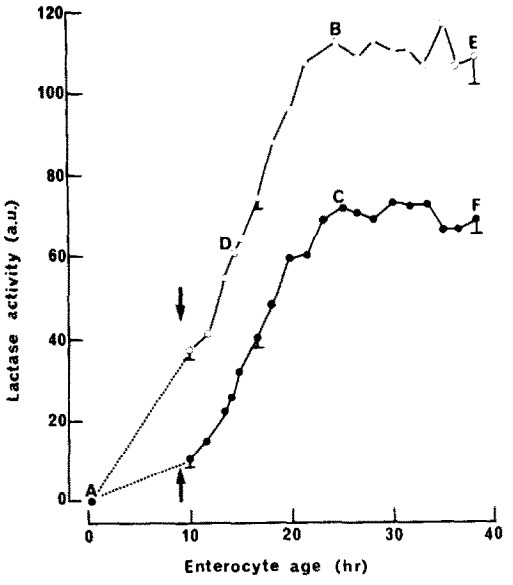


Fig. 4. Short-term effect of colchicine on lactase distribution along rat jejunal villi. Frozen sections of rat jejunum prepared from 4 control animals (○) and from seven animals given colchicine-containing water to drink for 24 hr (●) were incubated for lactase and the amount of enzyme reaction product related to the age of enterocyte as described in the text. Each value gives the mean (±SEM in some cases) of estimates carried out on 12 or 21 villi (○ and ● respectively). Arrows mark the position of the crypt–villus junction. Broken lines indicate changes in lactase assumed to take place within the crypt. A–F have meanings referred to in the text.

is reduced by 40 units. This difference represents a cumulative inhibition induced by colchicine acting initially on crypt enterocytes. Suppose however that the enterocyte was already 16 hr old at D, when the rat first began to ingest colchicine. Such a cell would be approaching the villus tip 24 hr later and in this case lactase activity would have shown no further increase (cf. D and F). A control 16-hr-old enterocyte, on the other hand, would have been able to increase further its lactase activity during subsequent migration, final activity being some 40 units higher than that seen in colchicine-treated animals (cf. E and F). It is concluded from these results that colchicine inhibits lactase expression by acting on both villus and crypt enterocytes.

## DISCUSSION

Intestinal disaccharidases are initially synthesized in a high mannose form by ribosomes present in the rough endoplasmic reticulum, later trimming and terminal glycosylation taking place in the Golgi complex [13–15]. There then follows an intracellular proteolytic cleavage of the primary translation product for lactase [15], subsequent cleavage of sucrase–isomaltase and maltase–glucoamylase only occurring after final insertion into the brush border membrane [16]. Additional proteolysis of brush border hydrolases is also thought to take place during intracellular transport following fusion of some disaccharidase-containing vesicles with lysosomes present in the apical cytoplasm [17, 18]. This general description of hydrolase biosynthesis has been taken previously to apply to all disaccharidases. Present work suggests that lactase biosynthesis might be somewhat different and that colchicine could prove a useful drug to distinguish this difference in detail.

High concentrations of colchicine have already been shown to block mitosis in the intestine and cause structural alterations in enterocytes leading to Golgi displacement, redistribution of disaccharidase transport vesicles and the appearance of brush border like structures in the basolateral membrane (see [18] for references). The rate of appearance of both sucrase–isomaltase and maltase–glucoamylase in the brush border is also reduced considerably in intestinal explants cultured in the presence of colchicine [19]. Non-selective disruption of microtubules could account for all of these findings. It is less easy, however, to imagine how such an effect could selectively inhibit the synthesis of one particular enzyme.

Colchicine used at low concentration has, on the other hand, been shown to inhibit glycosylating enzymes selectively in rat hepatocytes [20], to inhibit selectively the expression of some hydrolase enzymes in rat brush border membranes [7], and to stimulate DNA synthesis in other cell types [21]. These results raise further questions concerning the dose dependency and possible site of action of colchicine in producing selective effects on lactase biosynthesis and cell proliferation in intestinal crypts.

Present experience suggests that the degree of selectivity achieved in inhibiting different hydrolases is critically dependent upon the condition of the animal and/or the precise amount of colchicine ingested in the drinking water. All animals were kept under identical conditions and yet rats obtained from an alternative source were found, in unpublished work, to grow less readily and show greater inhibition of a wider variety of hydrolases following colchicine treatment. Although very susceptible to external factors, present results do nevertheless show that voluntary ingestion of colchicine can be used to produce highly consistent effects on lactase expression suitable for further biochemical investigations.

One possible intracellular site for this type of selective effect would be in the Golgi, through inhibition of membrane-associated glycosylases in a way similar to that described previously for rat hepatocytes [20]. An alternative site would be on separate transport receptor systems for hydrolases if these were to show variable sensitivity to inhibition by colchicine. A similar possible diversity in hydrolase transport receptor systems has been proposed recently to account for discrepancies noted in the times needed to transport different hydrolases to the Golgi under normal conditions [22]. Further information on these two possibilities could now be obtained by combining pulse chase experiments *in vivo* with a study of the endoglycosidase H sensitivity of the synthesized products.

Finally it is becoming of increasing interest to specify exactly which cell is being affected before entering into any speculation concerning the possible mechanism of drug action on enterocyte function. A

previous suggestion, based on homogenate data, that colchicine inhibits lactase expression by increasing the rate of cell proliferation [23], is not, for instance, borne out by direct observations carried out at the cellular level. The general usefulness of quantitative cytochemistry in obtaining this precise type of information is particularly well exemplified in the present work, where the specific enzyme reaction product for lactase is located in the immediate vicinity of the brush border membrane. Combining this technique with conventional measurement of enterocyte migration rates then allows one to state, with certainty, that colchicine inhibits lactase activity in both mature villus and immature crypt enterocytes. This effect could, of course, be produced by activation of degradative pathways as well as by inhibition of enzyme synthesis, since there is known to be considerable turnover of all disaccharidases throughout the life of an enterocyte [24]. Separate experiments are needed to distinguish between these two possibilities.

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