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Mechanism of maturational decline of rat intestinal lactase–phlorizin hydrolase

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The maturational decline in lactase–phlorizin hydrolase (LPH) activity was studied in groups of young rats ranging from suckling to early post-weaned states. Associated maturational increases in sucrase–isomaltase (SI) and maltase–glucoamylase (MG) activities were also examined as a comparison. Over this time period changes in cellular concentrations of the three enzymes were observed, reflecting corresponding changes in enzyme activities. Synthesis patterns accompanying these maturational changes in concentration were examined using labelled leucine as a marker. Synthesis of LPH was found to be maintained at constant rates independent of the maturation-associated decline in its concentration, whereas the increases in cellular concentrations of SI and MG were due to accelerated synthesis of the enzyme. Turnover of LPH, based on both the fractional synthesis rate and the disappearance rate of labelled leucine from prelabelled LPH pools, was increased in a quantitatively similar way to the decline in LPH concentration. These findings are consistent with our earlier proposal that the maturational decline of LPH occurs because of accelerated turnover, without a decrease in its rate of synthesis.

INTRODUCTION

The intestine of infant mammals continues development as an adaptation to extra-uterine life. Particularly significant differentiative changes in epithelial structure [1,2], function [2–6] and cytokinetic properties [5,7] occur abruptly during the third week after birth in the rat as temporally related events prior to weaning. Differentiative changes preparatory to a dietary carbohydrate change associated with weaning are especially remarkable, and include the coincident occurrence of a marked decline in lactase–phlorizin hydrolase (LPH; EC 3.2.1.23/62) activity, the appearance of sucrase–isomaltase (SI; EC 3.2.1.10/48) activity and a rapid increase in maltase–glucoamylase (MG; EC 3.2.1.20) activity [4,7,8]. The accompanying cytokinetic changes include increased cell proliferation and migration rates along the villus column, resulting in a decrease in the enterocyte life span from an infant pattern of 7–10 days to an adult pattern of 2–3 days [7,9–12].

Although differentiative changes in intestinal carbohydrase expression have been documented extensively in the weaning mammal, information on the mechanisms involved remains limited. The maturational decline in lactase activity and its recognition as the probable basis of widespread adult hypolactasia in man has evoked particular investigative interest over the years [13], with current research directed towards the molecular events involved. Following initial isolation of the enzyme from rat intestine [14–16] and its identification [15,17] as LPH, current studies have focused on its biosynthesis [9,12,18–22] and the causal mechanism(s) of its maturational decline [22–26].

In previous studies, evidence has been presented in support of a proposal that the maturational decline in LPH is largely a consequence of a more rapid turnover of the enzyme resulting from a decrease in the enterocyte life span [11,12], and occurs without suppression of enzyme synthesis [22,27]. The present study extends these earlier observations and provides further evidence in support of this proposal. Experiments are presented showing a decline in LPH occurring without a decrease in its synthesis rate, but paralleling closely a corresponding decrease in the intestinal half-life of the enzyme. Data relating to the synthesis and turnover of total intestinal protein, SI and MG accompanying their maturational changes are also presented for comparative purposes. The experimental approach involved the administration of labelled leucine at very high doses to groups of young rats (12–30 days) ranging in age from suckling to early post-weaned, and determination of the incorporation and disappearance rates of label from protein and carbohydrase pools. Specific antisera were applied to effect isolation of the enzymes in purity appropriate to their radiochemical analysis [27,28].

MATERIALS AND METHODS

Materials

Wistar strain rats from our breeding colony were utilized in these studies. Infant rats were maintained with their mothers for up to 24 days of age, and were killed by decapitation. L-[3,4-^{3}H]leucine and L-[U-^{14}C]leucine (New England Nuclear, Boston, MA, U.S.A.), glucose oxidase, horseradish peroxidase, L-leucine, papain type II (Sigma Chemical, St. Louis, MO, U.S.A.) and maltase (Calbiochem, San Diego, CA, U.S.A.) were purchased from the indicated sources.

Enzyme and antisera preparations

Rat intestinal LPH, SI and MG were solubilized by papain action and purified to near-homogeneity as assessed by acrylamide-gel electrophoresis and immunodiffusion, to give specific activities equal to or greater than those reported previously [16,29]. Rabbit antisera prepared against each enzyme showed quantitative equivalence in immunotitration curves when comparing the enzymes in pure solution and in crude papain or Triton X-100 extracts [16,29]. The antisera precipitated the respective enzyme antigens in apparent high radiochemical purity from crude papain extracts of radiolabelled intestinal proteins, as indicated by fluorography following SDS/PAGE and by radiochemical analysis of their immunoprecipitates prepared with a wide range of excess antisera additions [28]. The antisera were dialysed against 0.9 NaCl before use.

Abbreviations used: LPH, lactase–phlorizin hydrolase; SI, sucrase–isomaltase; MG, maltase–glucoamylase.

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Intestinal preparation for analysis

The small intestine was removed from the ligament of Treitz to the caecum, and the proximal three-quarters (unless stated otherwise) was rinsed, homogenized and sonicated in 4–9 vol. of medium containing 10 mM-sodium phosphate, 5 mM-Tris, 1 mM-EDTA and 0.002% Triton X-100, pH 6.0. Homogenates were assayed for cellobiase (LPH), SI and MG activities at dilutions free of Tris inhibition [16,29], total protein [30], DNA [31], total radioactivity and radioactivity incorporated into protein. The remaining homogenate was utilized for isolation of LPH, SI and MG by immunoprecipitation techniques. The enzymes were solubilized with papain (6 mg of papain plus 0.12 mg of cysteine hydrochloride per g of intestine), with over 95% release effected after 30 min incubation at 37 °C followed by standing overnight at near 0 °C. The enzymes were also solubilized in certain studies with 1% Triton X-100. The solubilized enzymes were separated from insoluble components by centrifugation for 1 h at 100000 *g*, preparatory to their isolation by immunoprecipitation. We have shown previously that crude enzyme extracts solubilized with either papain or Triton X-100 yield identical immunotitration curves when compared with the respective pure enzymes [16,29]. The different extraction procedures, including proteolysis, were ensured on this basis to have minimal effects on the immunoreactive properties of the enzymes.

Immunoprecipitation

Immunoprecipitations were performed in 1.5 ml centrifuge tubes in 100 mM-NaCl/10 mM-sodium phosphate (pH 6.0)/0.25% Triton X-100 at standardized minimal enzyme and excess antiserum concentrations. The enzymes were precipitated from 1.35 ml solutions containing a minimum of 0.03 unit of cellobiase activity (LPH), 0.25 unit of sucrase activity (SI), 1.0 unit of corrected [28] maltase activity (MG) and constant excess of antiserum (2.5 times the amounts required for quantitative precipitation). Immunoprecipitation of labelled SI from preweaned-rat intestine required addition of unlabelled carrier enzyme (prepared as a crude papain extract) to bring levels to the minimum 0.25 unit of sucrase activity required to ensure quantitative precipitation. Immunoprecipitation was performed on quantities of enzyme exceeding the minimal amounts required for quantitative precipitation in samples of low specific radioactivity (e.g. LPH in pre-weaned-rat intestine). Immunoprecipitates of labelled enzyme samples contained radioactivity in excess of 100 c.p.m. in each case. Immunoprecipitates were allowed to form overnight at near 0 °C and were separated by centrifugation at 12000 *g*. The precipitates were washed three times in precipitating medium, air-dried after washing in 95% ethanol and dissolved in 0.1 M-NaOH for protein and radioactivity assays. Occluded radioactive contaminants of the immunoprecipitates were determined to be minimal [28,32], and have not been corrected for in the present study.

Enzyme assays, units and specific activities

LPH, SI and MG were assayed at 37 °C in a 250 μl reaction volume in 0.05 M-sodium phosphate (pH 6.0)/0.002% Triton X-100 containing 0.015 M-cellobiose, 0.03 M-sucrose and 0.015 M-maltose respectively. Cellobiose and sucrose serve as specific substrates for LPH and SI respectively [28,29]. Since maltose is degraded by both MG and SI, maltase activity values were corrected for the contribution of SI [28]. Reactions were terminated and glucose release was determined by addition of glucose oxidase in the presence of excess Tris [16,29]. Enzyme activity units are expressed in μmol of substrate hydrolysed/min, and catalytic specific activities units/mg of DNA. Conversion of enzyme activity units to enzyme protein concentrations was based on values for units/mg of enzyme of 6.2 (cellobiase), 24 (sucrase) and 58 (maltase) determined for pure LPH, SI and MG respectively [16,28,29]. We have determined previously that the activity/mass ratio of LPH does not change during its maturational activity decline [28].

Administration of labelled leucine and determination of label incorporation into protein and enzyme pools

Rats were injected intraperitoneally with [^{3}H]- or [^{14}C]leucine in either the absence or the presence of a massive dose of leucine (2.2 μmol/g body wt.). Labelled leucine solutions for injection at massive dose were prepared at 150 μmol/ml (340 μCi/ml).

Rats were injected at 09:00 h and killed at the indicated times. Label incorporation into total intestinal proteins was determined using thoroughly washed $HClO_4$ precipitates of the intestinal homogenate. Label incorporation into LPH, SI and MG was determined in their respective immunoprecipitates (see above). Incorporation into total protein was expressed on a cellular basis as c.p.m. in protein/mg of DNA, and was computed as the product of (c.p.m./mg of protein) and (mg of protein/mg of DNA). Radioactivity incorporated in enzyme pools was expressed on a cellular basis as c.p.m. in enzyme/mg of DNA, and computed as the product of (c.p.m./μg of enzyme) and (μg of enzyme/mg of DNA). We have determined previously the number of leucine residues per 100 amino acid residues to be 6.8 for LPH, 7.7 for SI and 7.8 for MG (K. K. Tsuboi, L. K. Kwong, P. Sunshine & R. O. Castillo, unpublished work). Incorporation of labelled leucine into the enzymes was not corrected for these minor differences in leucine content. Radioactivity was measured at 30% counting efficiency in 0.5 ml neutralized protein samples dissolved in 0.1 M-NaOH with 5 ml of Aqueous Counting Scintillant (Amersham) in a liquid scintillation spectrometer (Beckman LS-233). All measurements of radioactivity were carried out over time intervals chosen to ensure a 95% confidence level of counting.

Data analysis

Data values are reported as means ± S.D. Student's *t* test was applied in the evaluation of data, and differences between groups were considered to be significant when the P value was less than 0.05.

RESULTS

Incorporation of labelled leucine into total intestinal proteins of pre- and post-weaned rats

Maturational changes in the rat intestine include a marked increase in the rate of mucosal turnover accompanying weaning. The extent to which accelerated synthesis of intestinal protein is associated with the increased mucosal turnover was examined in intestines from groups of pre-weaned 13-day-old and post-weaned 30-day-old rats. Rats were injected with equivalent (by body wt.) massive doses of [^{3}H]leucine [33], and incorporation of label into total intestinal protein was determined at intervals over a 60 min period (Fig. 1). The massive dose had the purpose of minimizing differences in the specific radioactivity of the precursor amino acid pool over an extended period between the individual rats. As shown in Fig. 1, incorporation of label into intestinal protein was maintained at a near-linear rate over a 30–40-min period, and occurred at a 3-fold greater rate in the post-weaned compared with pre-weaned group of rats. The 3-fold difference in incorporation rates between the two groups continued to be proportionally maintained over the entire 1 h time period examined, indicating a proportional decline in the specific radioactivity of the precursor pool. These data demonstrate an overall acceleration in the synthesis rate of intestinal

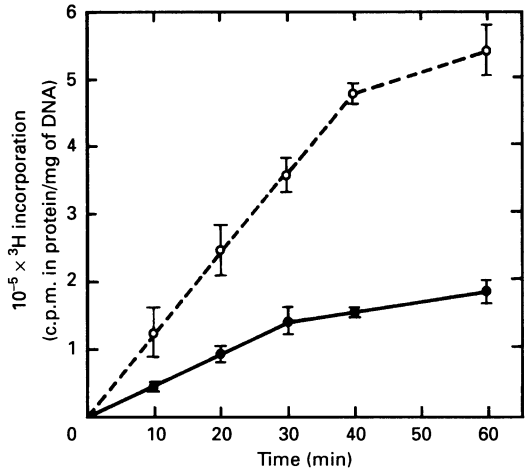

Fig. 1. Rates of incorporation of [^{3}H]leucine into intestinal proteins of 13-day-old (infant, ○) and 30-day-old (post-weaned, ●) rats

Rats were injected with an equivalent (by weight) massive dose of [^{3}H]leucine (2.2 μmol/g; 5 μCi/g). Three rats from each group were killed at each of the indicated time points, the proximal one-third of the small intestine was removed and label incorporated in the intestinal proteins was determined. The data are means ± S.D.

proteins measured as a group, accompanying the well-recognized increased mucosal turnover in the intestines of weaned rats.

Incorporation of labelled leucine into LPH pools in suckling compared with weanling rat intestines

Incorporation of labelled leucine into intestinal LPH pools of 14-day-old suckling and 21-day-old weanling rats was compared (Table 1). The total LPH concentration showed an approx. 7-fold decline in the weanling compared with the suckling group. Each rat was administered an equivalent (by weight) massive dose of [^{3}H]leucine, and label incorporation into LPH pools was determined after 40 and 120 min. LPH pools were extracted by papain or Triton X-100 from separate aliquots of intestinal homogenate. Although equivalent and near-quantitative extraction of LPH from brush border sites by both papain and Triton X-100 occurs [27], it is unlikely that papain has access to intracellular membrane-enclosed pro-LPH forms.

The amounts of labelled leucine incorporated into LPH pools extracted by papain were found to be similar in both the 14- and 21-day-old rats, whether determined at 40 or 120 min. The amounts of radioactivity incorporated into the LPH pools were computed as the product of their concentrations and their specific radioactivities (Table 1). From the computed values shown, it is evident that the specific radioactivities of the LPH pools were about 7 times less in the 14-day-old compared with the 21-day-old rats (in inverse proportions to their approximate 7-fold greater pool concentrations).

Incorporation of label into LPH pools extracted by Triton X-100 was somewhat greater at 40 min in 21-day-old rats compared with 14-day-old rats, but similar at 120 min. LPH pools extracted by Triton X-100 showed greater labelling within the initial 40 min and similar labelling after 120 min compared with papain-extracted pools. Thus LPH pools, whether extracted by papain or Triton X-100, showed either similar or even greater initial labelling rates in intestines from the older rat group, in spite of the considerably lower total LPH concentrations in these animals.

Differential labelling rates of LPH pools extracted by papain or Triton X-100

Differences in labelling rates of LPH pools extracted by papain or Triton X-100 (indicated in Table 1) were examined further.

Table 1. Comparative labelling rates of LPH pools extracted by papain and Triton X-100 from intestines of 14-day- and 21-day-old rats at 40 and 120 min after a massive dose of labelled leucine

Groups of six littermates of 14 (infants) and 21 (weanlings) days of age were administered equivalent (by weight) massive doses of [^{3}H]leucine (2.2 μmol/g; 5 μCi/g), and three rats from each group were killed after both 40 and 120 min. Intestines were separately analysed for LPH content and radioactivity in LPH pools extracted by papain and Triton X-100. LPH concentrations are presented as means ± S.D. (n = 6). Radioactivity in LPH pools is expressed on a cellular basis as c.p.m. in LPH/mg of DNA. Values were obtained as the product of the specific radioactivity and the concentration, i.e. as (c.p.m./mg of LPH) × (mg of LPH/mg of DNA). The presented values are means ± S.D. (n = 3).

Age...	LPH 14 days	LPH 21 days	Ratio (14 days/21 days)
Concentration (mg of LPH/mg of DNA)	93 ± 5	12.6 ± 6.0	7.4
^{3}H incorporation (c.p.m. in LPH/mg of DNA)			
40 min			
Papain	360 ± 40	330 ± 80	1.1
Triton X-100	530 ± 70	740 ± 90	0.7
120 min			
Papain	1270 ± 200	1250 ± 210	1.0
Triton X-100	1140 ± 90	1310 ± 290	0.9

Fig. 2 shows the labelling rates of LPH pools extracted by papain or Triton X-100 from intestines of 14-day-old infant and 25-day-old early post-weaned rats, determined at various times over a 4 h period following administration of a massive dose of labelled leucine. LPH activities were on average 6 times lower in the intestines of the post-weaned compared with the infant group. Labelling of LPH pools extracted by Triton X-100 was significantly greater than in papain extracts within the early time periods (20 and 40 min), and similar after 120 and 240 min in both infant and post-weaned groups. We have previously reported [26] the presence of labelled precursor forms of LPH in Triton X-100 extracts of rat intestine within 30 min of label administration, with subsequent labelling of brush border forms of the enzyme within 120 min. Assuming that the intracellular lipid-enclosed precursor forms are extracted more readily by Triton X-100 than by papain, the rate data suggest a greater rather than a lower synthesis rate of the precursor enzyme in the post-weaned compared with the infant group. The finding of equivalent amounts of incorporated label in LPH pools extracted by either papain or Triton X-100 in intestines from both infant and post-weaned groups after 2 and 4 h indicated that similar amounts of *de novo* synthesized enzyme had arrived at the brush border in both groups, in spite of their marked differences in LPH activity.

Concentration changes and comparative labelling patterns of brush border LPH, SI and MG in the maturing rat intestine

A group of rats ranging from 16–22 days of age, and exhibiting a wide transitional range of declining LPH and increasing SI and MG concentrations, was injected with a massive dose of [^{3}H]leucine. Label incorporation into total intestinal protein and carbohydrase pools was determined after 2 h. Label incorporation into carbohydrase pools when determined at this time was assumed to indicate overall synthesis, processing and transport rates to brush border sites, as indicated in Fig. 2. Labelling patterns of total protein and LPH plotted relative to the LPH

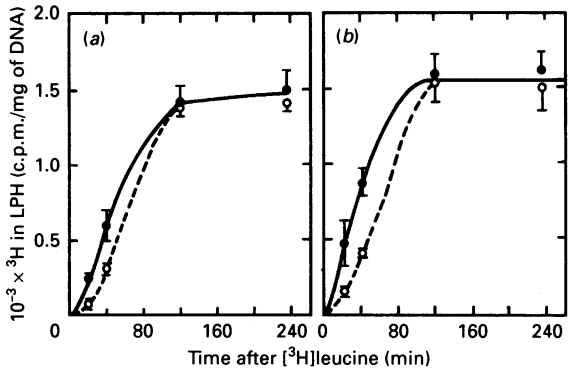

Fig. 2. Rates of incorporation of radioactivity into LPH pools extracted by papain (●) or Triton X-100 (○) from (*a*) 14-day-old infant and (*b*) 25-day-old post-weaned rats

Rats were administered a massive dose of [^{3}H]leucine (2.2 μmol/g; 5 μCi/g) and killed at the indicated times. The proximal three-quarters of small intestine was homogenized and aliquots were extracted with papain or Triton X-100 (see the Materials and methods section). Mean LPH concentrations were at least 6 times less in the intestines from 25-day-old animals. LPH was isolated by immunoprecipitation, and incorporated radioactivity was determined. Plotted values are means $\pm$ S.D. ($n = 3$ for all data points, except $n = 6$ for papain data points obtained for 25-day-old rats). Labelled LPH levels extracted by Triton solubilization were significantly greater ($P < 0.05$) than those obtained by papain extraction at 20 and 40 min in both age groups.

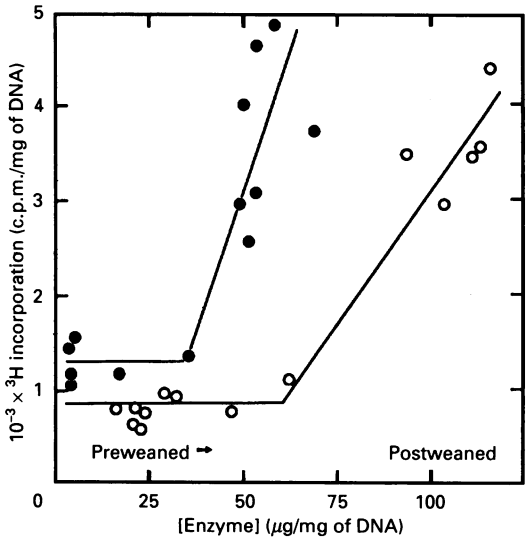

Fig. 4. Labelling of SI and MG at an accelerated rate accompanies their increases in concentration in the maturing rat intestine

Data were obtained on the same group of rats utilized in the study presented in Fig. 3. Label incorporated into SI (●) and MG (○) was determined for each intestine (14 in this study) and the values were plotted relative to the cellular concentrations of the enzymes. Incorporation values into SI and MG have been computed on a cellular (DNA) basis.

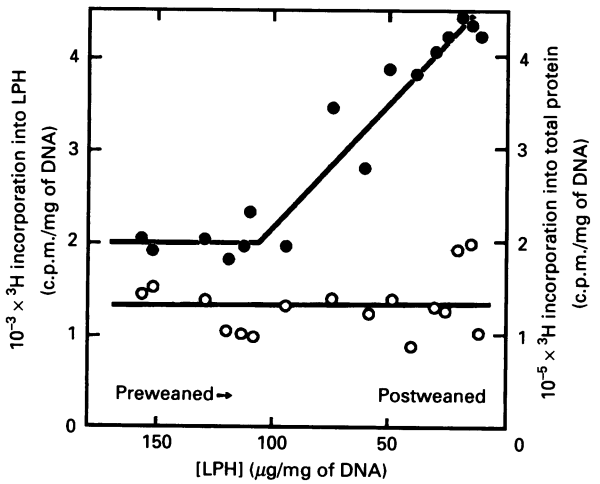

Fig. 3. Labelling of LPH at a constant rate and total protein at an accelerated rate accompany the decline in LPH in the maturing rat intestine

Data were obtained from a group of 16 young rats ranging in age from 16–22 days. Rats received a massive dose of [^{3}H]leucine (2.2 μmol/g; 5 μCi/g) and were killed 2 h later. Label incorporated into total protein (●) and LPH (○) was determined for each intestine and the values were plotted relative to the regular LPH concentration of the particular intestine. Incorporation values have been computed on a cellular (DNA) basis.

concentration of each of the rat intestines are shown in Fig. 3, with intestines from preweaned rats having the highest levels of LPH. Label incorporation into LPH pools (ranging from 15 to 150 μg/mg of DNA among the group of rats studied) did not differ significantly, indicating similar rates of LPH synthesis independent of the LPH concentration. Label incorporation into total protein increased in association with declining LPH concentrations, indicating a higher rate of protein synthesis in post-weaned intestine. Incorporation patterns of label into SI and MG are shown in Fig. 4 relative to the enzyme concentrations present in the individual rat intestines. At a low concentration of less than 50 μg/mg of DNA, characteristic of the immature state of the intestine, incorporation of label into both SI and MG was generally constant and similar to that for LPH (Fig. 3). Subsequent maturational increases in the concentrations of these enzymes are accompanied by a sharp acceleration in their label incorporation. Thus, in contrast to LPH, concentration changes of SI and MG occur in association with corresponding changes in rates of synthesis of these enzymes.

Accelerated rate of LPH loss from the intestines of post-weaned rats

Since a suppressed rate of LPH synthesis was not found, evidence for an accelerated rate of LPH loss was sought as a basis for its decline in concentration in the maturing rat intestine. Groups of 14-day-old infant and 25-day-old post-weaned rats were administered an equivalent (by weight) massive dose of [^{3}H]leucine, and the rate of loss of incorporated label from LPH, SI (in post-weaned intestine only) and total intestinal protein was determined (Fig. 5). Disappearance of label from total intestinal protein occurred at about twice the rate in the post-weaned intestines, with half-lives of 52 and 28 h in 14- and 25-day-old rat intestines respectively. Disappearance of label from the LPH pools occurred at about a 7-fold greater rate in the post-weaned intestines, with half-lives of 74 and 10 h in 14- and 25-day-old intestines respectively. The 7-fold greater disappearance rate of label from the post-weaned LPH pool corresponded generally with the average 5–6-fold decline in pool concentrations found in intestines from post-weaned compared with infant rats (Fig. 5). These data provide correlative evidence of an accelerated rate of loss as a major basis for the maturational decline of LPH in the post-weaned rat intestine. Differences in disappearance rates of label from LPH and SI in post-weaned intestine were not distinguished, and were examined further by a dual-label technique [34].

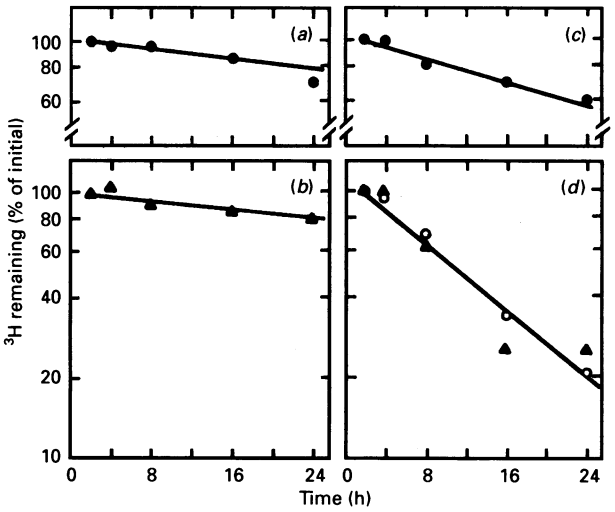

Fig. 5. Disappearance rates of incorporated label from total protein and carbohydrase pools in intestines of infant and post-weaned rats

Infant rats of 14 days of age (*a*, *b*) and post-weaned rats of 25 days of age (*c*, *d*) were administered a massive dose of [^{3}H]leucine (2.2 μmol/g; 5 μCi/g) and the amounts of label incorporated into total protein, LPH and SI (25 day only) were determined at the subsequent indicated times. (*a*), (*c*) Disappearance from total protein had half-lives of (*a*) ~52 h and (*c*) ~28 h. (*b*), (*d*) Disappearance from LPH (▲) and SI (○) showed half-lives of (*b*) ~74 h and (*d*) ~10 h (for both enzymes). Data points represent mean values ($n = 3$ at 2, 4 and 8 h and $n = 2$ at 16 and 24 h). Initial LPH concentrations (means ± S.D.) were 93 ± 6 and 14 ± 7 μg/mg of DNA in the 14- and 25-day-old groups of intestines and the SI concentration was 60 ± 16 μg/mg of DNA in the 25-day-old group.

Table 2. Comparative turnover rates of total protein, LPH, SI and MG in 23-day-old postweaned rat intestines

Four littermates of 23 days of age were each injected intraperitoneally with [^{3}H]leucine at 10 μCi/g, and 21 h later with [^{14}C]leucine at 0.5 μCi/g. Rats were killed 2 h after the second injection. The proximal three-quarters of the small intestine were taken and the mucosa and serosa were separated. Carbohydrases were isolated by immunoprecipitation from papain extracts. ^{14}C/^{3}H ratios are means ± S.D. ($n = 4$). All carbohydrase ratios were significantly different from each other and from that of total protein (by paired t test) at the 95% confidence level.

Intestinal component	Concentration	Relative turnover rates ^{14}C/^{3}H
Protein (mg)		
Mucosa	20.4 ± 0.4	0.34 ± 0.02
Serosa	21.2 ± 1.4	0.37 ± 0.03
Carbohydrase (μg/mg of DNA)		
LPH	11.6 ± 3.0	0.54 ± 0.08
SI	59 ± 7	0.74 ± 0.16
MG	81 ± 8	0.48 ± 0.07

Comparative turnover rates of protein and carbohydrases in post-weaned intestines determined by dual-labelling are shown in Table 2. Rats were injected with tracer doses of [^{3}H]leucine and [^{14}C]leucine at 23 and 2 h respectively before killing the animals. ^{14}C/^{3}H ratios were similar in mucosal and serosal proteins, and significantly lower than in the carbohydrases, indicative of their overall correspondingly slower turnover rate as a group. Among the carbohydrases, turnover of SI was found to be significantly greater than those of LPH and MG, which showed somewhat similar turnover rates.

DISCUSSION

The major focus of the present study was to investigate synthesis and turnover rates of LPH in relation to its maturational decline in the weanling rat. Since a dramatic decrease in the enterocyte life span and sharp increases in SI and MG activity also occur as associated maturational events, synthesis and turnover rates of total intestinal protein as well as of SI and MG were also examined as a part of the study. Rats ranging from 13 to 30 days of age were used in these studies. Within this age group the maturational decline in LPH occurs abruptly, usually after 17 days of age, and approaches low adult levels by 21 days of age. Based on the age of maturational LPH decline, rats of less than 17 days of age have been grouped as 'pre-weaned' and those greater than 20 days as 'post-weaned' for the purposes of the present study.

The measurement of synthesis and turnover of total protein and enzymes was based on relative incorporation and disappearance rates of labelled leucine. In most studies [^{3}H]leucine was administered at an equivalent (by body weight) massive dose in excess of 15–20 times the estimated free leucine pool in the infant (R. O. Castillo, unpublished work) and adult [33] rat. Massive doses of leucine do not appear to affect protein synthesis in rat intestine, and have the purpose of decreasing the effects of label recycling, and of minimizing differences in and maintaining the specific radioactivity of the precursor pool among individual rats [33]. It is evident that, with the massive dose, individual variations in the free leucine pool as great as 2–3-fold would result in only minor differences in the specific radioactivity of the precursor pool (< 10%).

Using the massive dose, [^{3}H]leucine incorporation into total intestinal protein was maintained at a near-linear rate for 30–40 min, and occurred at a significantly greater rate in post-weaning compared with pre-weaning rats (Fig. 1). The rate difference between the two groups continued to be proportionally maintained well beyond the linear rate (beyond 2 h), indicating a similar proportional decline in the specific radioactivities of their precursor pools. The effectiveness of the massive dose in achieving normalization of the specific radioactivity of the precursor pool is indicated from the minimal scatter of the data obtained with groups of three rats at each time point shown. The data presented in Figs. 1 and 3 confirm the expected accelerated rate of protein synthesis in the post-weaned intestine, consistent with the maturational increase in mucosal turnover rate. Little difference in intestinal protein synthesis rates was found by others [35] between rat groups of 18 and 25 days of age. Since rats of 18 days already exhibit a decline in LPH and an associated increase in mucosal turnover rate, the maturational increase in protein synthesis rates similar to those in the 25-day-old rat may have already been achieved.

Based on the incorporation patterns of [^{3}H]leucine into total protein following a massive dose, incorporation into LPH, SI and MG was determined and compared with their maturational concentration changes. Incorporation into LPH was examined in a large group of pre- and post-weaned rats varying in age from 12 to 30 days. Incorporation into LPH was examined during the near-linear 30–40 min incorporation period found for total protein, and during the subsequent non-linear 2–4 h period. It has been found that early labelling of intracellular precursor LPH forms appears to occur within 30 min, with maximal labelling of brush border forms occurring several hours after administration of labelled amino acid [20,26]. On this basis, it was assumed that label incorporation into LPH determined

within 40 min measures the synthesis of immature enzyme forms, whereas that determined after 2 h measures primarily accumulation of newly synthesized LPH in the brush border pools. LPH is thought to be synthesized as a larger precursor of 215–245 kDa with proteolytic processing resulting in a predominant brush-border-bound product of 130–160 kDa on SDS/PAGE [20,21]. The presence of a 300 kDa inactive precursor form of LPH has been described in adult rat intestine [24], but has not been evident in the age group studied here. Label accumulation into LPH pools determined after 40 min may be assumed to occur at diminishing rates, as found for total protein (Fig. 1), presumably reflecting a continuous decline in the specific radioactivity of the precursor pool. The curvilinearity of patterns of label incorporation into LPH pools shown over a 2 h period and the lack of further label incorporation after 4 h (see Fig. 2) is presumed on this basis to reflect a corresponding decrease in the specific radioactivity of the precursor pools.

It was determined in these studies that label incorporation into LPH, whether measured within 40 min or after 120 min, was not decreased in accordance with its maturational concentration decline (Table 1, Figs. 2 and 3). In contrast to LPH, maturational changes (increases) in SI and MG concentrations were found to be accompanied by corresponding changes (increases) in incorporation rates of label into their brush border pools (Fig. 4). Thus it is evident that contrasting mechanisms are involved in effecting concentration changes in LPH compared with SI and MG.

Since the decline in LPH was found to occur without a measurable suppression of synthesis or transport rate to brush border sites, the decline would seem to be the result of an accelerated rate of loss from the maturing intestine. On this basis, turnover rates of LPH were compared in the intestines of groups of pre- and post-weaned rats (Fig. 5). In these studies, disappearance rates of label from LPH pools prelabelled with [^{3}H]leucine were assumed to measure their relative turnover rates. Disappearance of label occurred at a 7-fold greater rate from the LPH pools of post- compared to pre-weaned intestines. The extent to which possible differences in label recycling between the two groups contributed to the apparent 7-fold greater turnover rate of LPH in the post-weaned intestines was not investigated further, and remains to be determined. It is significant, however, that the apparent 7-fold greater turnover rate was in accordance with the 5–6-fold maturational decline in the LPH concentration found in the intestines of the post-weaned group. Although generally similar turnover times for LPH, total protein and SI have been reported in the adult rat intestine [36], as found in the present study on the post-weaned group of rats, the considerably slower turnover of LPH in the immature intestine does not appear to have been reported previously.

For comparative purposes, we have computed from data shown in Table 1 apparent fractional synthesis rates of LPH in the 14-day-old and 21-day-old rats. We have approximated, from the labelling rates of LPH pools extracted by Triton X-100 at 40 min, fractional synthesis rates and corresponding half-lives of 0.14/day and 85 h respectively for the 14-day-old rats, and 1.4/day and 8.5 h for the 21-day-old rats. These values compare generally with half-life values of 74 h and 10 h determined in separate groups of pre-weaned and post-weaned rats of 14 and 25 days of age respectively, in which turnover measurements were based directly on their pool decay rates (see Fig. 5).

Contrary to the findings of the present study, suppression of rates of LPH synthesis has been proposed as a mechanism for the decline in LPH [23]. The proposal was based on the finding of a decline in the LPH/total protein labelling ratio in brush border membranes of adult compared with 15-day-old rat intestines, following administration of labelled leucine. Based on our finding of increased labelling of the total intestinal protein in post- compared with pre-weaned rats (Figs. 1 and 3), the decline in the LPH/protein labelling ratio found in adult intestine could reflect a greater incorporation of label into protein rather than a suppressed rate of label incorporation into LPH. In this regard, increased protein synthesis would be expected due to the well-known greater mucosal turnover rate in adult compared with infant intestine, as demonstrated in the present study (Fig. 1).

The inability to demonstrate a parallel decline of LPH mRNA with the LPH concentration decline in adult compared with infant rat and rabbit intestine has been reported [37]. These findings imply that rates of LPH synthesis do not decline with weaning, as demonstrated by our finding of a constant rate of LPH synthesis accompanying its maturational decline.

Assuming the correctness of our findings of constant synthesis and accelerated turnover accompanying maturational LPH decline, the findings are consistent with an increased loss of LPH due to an accelerated turnover of the enterocyte as a major factor in the LPH decline. On the other hand, our findings do not exclude the possibility of other mechanisms also participating in the maturational decline of LPH, as we have described previously [22].

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