In Vivo Sucrase-Isomaltase and Lactase-Phlorizin Hydrolase Turnover in the Fed Adult Rat*

(Received for publication, October 22, 1992, and in revised form, February 23, 1993)

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We estimated in vivo turnover rates of sucrase-isomaltase and lactase-phlorizin hydrolase in adult rats. Fed animals received a primed continuous infusion of phenylalanine (300 μCi, 150 μmol Phe/100 g of body weight for 30 s, then 7.5 μCi, 3.75 μmol Phe/min for 10 to 140 min). Sucrase-isomaltase and lactase-phlorizin hydrolase were immunoprecipitated from jejunal mucosal membranes; isoforms were separated by SDS-polyacrylamide gel electrophoresis. Endoglucosidase H digestions and (for lactase-phlorizin hydrolase) N-terminal amino acid sequencing were performed on all isoforms. Specific radioactivity of pro-sucrase-isomaltase and prolactase-phlorizin hydrolase isoforms reached isotopic equilibrium by 60 and 90 min, respectively. Specific radioactivity of brush border sucrase and lactase did not reach steady state. The isotope kinetic, N-terminal amino acid sequencing, and endoglucosidase H digestion data suggested that one of the high molecular weight lactase isoforms is a dimer of mature lactase. Compartamental modeling of specific radioactivity demonstrated that mean intracellular residence time is 59 min for pro-sucrase-isomaltase isoforms and 68 min for prolactase-phlorizin hydrolase isoforms. Mean residence time in the brush border was 5.8 h for sucrase and 7.8 h for lactase. Fractional synthesis rates were 41.4%/day for sucrase and 307%/day for lactase. Thus, in vivo brush border sucrase and lactase turn over at similar rates in the adult rat.

Disaccharidases such as sucrase-isomaltase (SI) and lactase-phlorizin hydrolase (LPH) are integral membrane glycoproteins located on the brush border of villous enterocytes and are responsible for the final stages of carbohydrate digestion. Although the initiation of SI and LPH synthesis is genetically regulated during development, it has been shown that expression of SI and LPH is also regulated by posttranslational events associated with age, pregnancy, and diet (1, 2). Further, defects in synthesis and intracellular processing of SI and LPH have been reported in some enzyme-deficient persons (3).

Both SI and LPH have been studied extensively, and their physical and chemical properties and biosynthesis have been described (4–8). Because both enzymes are glycoproteins found in the brush border membrane, several steps occur during intracellular processing throughout which the steady state levels of precursor and mature isoforms may be controlled. As the pre-pro forms of the enzymes pass through the endoplasmic reticulum and into the Golgi apparatus, they are glycosylated first to high mannose intermediates, then to complex glycosylated polypeptides (4–6). The complex glycosylated isoforms are then translocated to the brush border membrane. In the case of SI, sucrase is immediately cleaved from the membrane-bound portion of the molecule (isomaltase) by lumenal proteases but remains attached by noncovalent interaction (4, 5). LPH is also proteolytically cleaved to form mature lactase, either in the trans-Golgi or brush border, but the cleaved portion of the molecule does not remain attached to the membrane-bound moiety (6). Although the pathways of SI and LPH synthesis are very similar, the two enzymes are attached to the brush border membrane differently: SI is anchored at the N-terminal region of the molecule, LPH at the C-terminal region (9).

The turnover rates of brush border disaccharidases have been determined, with a few exceptions, from the study of intestinal explants or tissue culture cell lines (10). Such studies have shown that 60–90 min are required for the migration of a pre-proisoform through the endoplasmic reticulum/Golgi to the brush border membrane (10). No information exists concerning the synthesis rates of precursor isoforms. Thus, these studies were undertaken to measure in vivo the turnover rates (fractional catabolic and/or fractional synthesis rates) of SI and LPH isoforms and mucosal protein in adult rats.

EXPERIMENTAL PROCEDURES

Materials

14C-phenylalanine was purchased from Amersham Corp. and Dulbecco's phosphate-buffered saline from Gibco. Leupeptin, aprotinin, and the glucose oxidase kit were purchased from Sigma. Ultrapure hydrochloric acid (12 M) was purchased from J. T. Baker Chemical Co. and endoglucosidase H from Du Pont-New England Nuclear. Pickering diluent was obtained from Pickering Laboratories (Mountain View, CA), and polyvinylidene

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The abbreviations used are: SI, sucrase-isomaltase; LPH, lactase-phlorizin hydrolase; Endo H, Endoglucosidase H; FCR, fractional catabolic rate; FTR, fractional transfer rate; Ks, fractional synthesis rate; SR, specific radioactivity; T,, mean residence time; dpm, disintegrations/minute.

*This work is a publication of the United States Department of Agriculture (USDA)/Agricultural Research Service (ARS) Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, TX. This project has been funded in part with federal funds from the USDA/ARS under Cooperative Agreement number 58-6250-1-003. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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Difluoride/ProBlott membranes were obtained from Applied Biosystems (Foster City, CA). All other chemicals were of analytical grade.

**Infusion and Tissue Preparation**

Male Sprague-Dawley rats (175–200 g) were purchased from Harlan (Houston, TX) and were fed a nonpurified diet (Chow No. 5008, Ralston Purina, Richmond, IN). The animals were maintained for at least 1 week in a room with alternating 12-h periods of light and darkness (lights on at 0700). Rats were given free access to food and water. Protein synthesis was measured by a modification of the flooding-dose technique described by Garlick et al. (11). The animals were lightly anesthetized with intraperitoneal injections of nembutal (50 mg/ml), and a 26-gauge needle was inserted into a lateral tail vein. The animals received 0.1–0.15 μCi of [3H]Phe (50 mCi/ml) and 80 mCi cold Phe per g body weight during the first 30 s of infusion. Thereafter, each animal received 7.5 μCi of [3H]Phe and 1.5 μmol of Phe·min⁻¹·g⁻¹ body weight for the remainder of the infusion period (10, 20, 30, 40, 60, 90, and 140 min). Before the animals were sacrificed, they were briefly anesthetized and then killed by decapitation. The terminal abdomen contained 10 cm distal to the ligament of Treitz. Blood samples were taken from the portal vein and the jejunal contents were flushed with 30 ml ice-cold NaClO₃ (154 mM), and the mucosae were scraped. The scraped mucosa was dispersed in 5 ml of Dulbecco's phosphate-buffered saline containing 2 mM phenylmethylsulfonyl fluoride, 52 μM leupeptin, 29 TIU/liter aprotinin, 35 μM antipain, and 2.6 μM benzamidine. The gel was homogenized for 2 min with a tissue homogenizer (Tekna Co, Cincinnati, OH) at a 70% setting, and frozen at −70 °C.

**Preparation of Free Amino Acid Pools from Blood and Mucosa**

The preparation of free amino acid pools from blood and mucosa has been previously described (12). Briefly, protein was precipitated from samples of blood and scraped mucosa with 1 M HClO₃. Precipitated protein was removed by centrifugation at 12,000 × g. The precipitated mucosal protein was used to measure the SR of total mucosal protein. The supernatant solutions were neutralized with KOH. KCΙ was removed by centrifugation, and the supernatant fraction was dried by centrifugation under vacuum. The samples were dissolved in purified water (Milli-Q Water System, Millipore, Bedford, MA), centrifuged to remove precipitate, and the supernatant solutions were neutralized with 2 ml of a 20 mM phosphate buffer, pH 8.0, containing 100 mM NaCl. The precipitated mucosal protein was suspended in purified water, and the supernatant solutions were neutralized with 2 ml of a 2% SDS sample buffer containing 5% β-mercaptoethanol. The

**Hydrolysis of Mucosal Protein**

The precipitated mucosal protein was suspended in purified water, sonicated with a microprobe (HeatSystems, Farmingdale, NY) to disperse the pellet, and centrifuged at 12,000 × g for 30 min. This procedure was repeated three times, and the final pellet was suspended in 12 m HCl. Hydrolysis was performed as described below for SI and LPH isoforms.

**Purification and Immobilization of Monoclonal Antibody**

Ascites cells were prepared by Charles River Laboratories (Wilmington, MA) using the hydrazones, BBE1/55, and PBE3/4. The preparation and characterization of these antibodies has been previously described (12). The ascites fluid was partially purified by (NH₄)₂SO₄ precipitation (to 40% saturation) and applied to a 3 × 10-cm column of protein A-Sepharose. The IgG fraction was eluted with 0.1 M glycine, pH 2.6, and centrifuged at 12,000 × g for 90 min. This procedure was repeated three times, and the final pellet was suspended in 12 m HCl. Hydrolysis was performed as described below for SI and LPH isoforms.

**Immunooisolation of SI and LPH Isoforms**

SI and LPH isoforms were immunoprecipitated from homogenized scraped mucosa and solubilized in 1% Triton X-100 as described elsewhere (2). SI and LPH were sequentially immunoprecipitated with 2 ml of a 50% suspension of Sepharose 6B to which anti-SI or anti-LPH antibody had been bound (2). The mixtures were rocked at 22 °C for 1 h or 4 °C for 16 h. The precipitates were separated by centrifugation at 3000 × g for 15 min, washed, and eluted with 1 ml of 2% SDS sample buffer containing 5% β-mercaptoethanol. The eluted sample was heated at 95 °C for 4 min, and the beads were removed by centrifugation. The solution was stored at −70 °C.

*In one study 100-μg animals were infused for 3 h to assure that the precursor isoforms were at isotopic steady state. The disaccharidases were isolated as usual, and the immunoprecipitate-bead complex was used for endoglucosidase H (Endo H) digestions as described by Le Bivic et al. (13).*

**Separation and Hydrolysis of SI and LPH Isoforms**

The separation of SI and LPH isoforms obtained with this technique has been described by us and others (2, 8, 14–16). SI and LPH isoforms were separated by SDS-polyacrylamide gel electrophoresis, stained with Coomassie Blue, destained in 7% acetic acid, and washed with purified water (2, 16). All gels were scanned in an LKB Ultrascan XL Laser densitometer using GelScan XL software (LKB/Broma, Uppsala, Sweden) to determine the steady state mass of each isofrom (16). Some gels were dried and used for fluorography (2); most were used for the hydrolysis of SI and LPH isoforms (2). Four of the five SI isoforms (those with molecular masses of 140, 220, 230, and 240 kDa) and all visible LPH isoforms were cut from the gels. The gel segments were dried, hydrolyzed in 12 m HCl (500 μl/slice) for 24 h at 110 °C, and prepared for amino acid analysis (2).

**Amino Acid Analysis**

The Phe in each specimen was separated from other amino acids by high pressure liquid chromatography on an anion-exchange column (AminoPack PA1, Dionex, Sunnyvale, CA). Samples were eluted with 20 m sodium hydroxide and 80 m sodium acetate at a flow rate of 1 ml/min. As the amino acids emerged from the column, they were reacted with 0.65% O-phthaldialdehyde in Fickert's diluent containing 0.06% Brij 35 and 2% β-mercaptoethanol and detected by an on-line fluorimeter. Quantitative measurements were obtained by comparison with a Phe standard of known concentration. Separated amino acids were collected on a Gilson 201 fraction collector (Gilson Instruments, Middleton, WI), and the appropriate fractions were counted on a Beckman LS3801 scintillation counter (Beckman Instruments, Fullerton, CA). SR was measured in dpm·mg⁻¹·Phe.

**Estimation of Enzyme Mass for Compartmental Analysis**

The disaccharidase activity (μmol·min⁻¹·mg⁻¹), the protein concentration (μg/ml), and the volume of all fractions at each stage of purification were determined in six animals. Disaccharidases were assayed using 0.3 m sucrose or 0.6 m lactose and quantified with glutamic acid (16). Protein concentration was measured using bichinonic acid reagent. Recovery of enzyme activity after immunopurification was 88 ± 3% for sucrose and lactase, and recovery of total protein was 92 ± 2%. The quantity of immunopurified enzyme was then estimated as previously described (16, 17). Briefly, known amounts of immunopurified SI or LPH and albumin standards (0.5, 1, 1.5, 2, 2.5, and 5 μg) were electrophoresed on the same gel and stained with Coomassie Blue. After destaining, the gel was scanned to determine the relative mass of each isoform and the albumin standard. Linear plots of albumin-staining intensity versus albumin concentration were prepared (R = 0.99 in every case), and the quantities of SI and LPH isoforms in the sample were calculated from the curve and extrapolated to grams of disaccharidase/grams of protein or grams of scraped mucosa. These values were then used for compartmental modeling.

**N-terminal Amino Acid Sequence Analysis of LPH Isoforms**

LPH isoforms were immunopurified and separated by gel electrophoresis. Gels were equilibrated in chilled transfer buffer (25 mM Tris·HCl, 192 mM glycine, 0.01% SDS, 5% methanol, pH 8.3) for 10 min. A polyvinylidene difluoride membrane, wetted with methanol, was equilibrated in transfer buffer for 10 min. The gels were then electroblotted for 3 h at 75 V at 4 °C in a Bio-Rad Transblott Cell (Bio-Rad). After the transfer, the membranes were washed in two changes of purified water, rinsed briefly in methanol, and stained for 2 min in 0.1% Coomassie Blue R-250 in 40% methanol, 1% acetic acid. The stained membranes were washed in purified water, destained in 50% methanol, and air-dried. Individual protein bands were cut from the membrane and stored at −20 °C. The N-terminal amino acid sequences of a portion of each band were analyzed by automated Edman degradation on a model 473A pulsed liquid sequencer (Applied Biosystems, Foster City, CA).
Mucosal Protein Fractional Synthesis Rates (K) — The K, of mucosal protein (defined as the percentage of protein mass synthesized in a day) was calculated as described previously (18):

\[
K, = \frac{S,}{S,} \times \frac{1440}{T} \times 100 \quad \text{(Eq. 1)}
\]

where \(S,\) is the SR of protein-bound Phe and \(S,\) is the SR of tissue-free Phe. \(T\) is the time of labeling in min.

Fractional Catabolic Rates of SI and LPH Isoforms and \(K,\) of Brush Border Sucrase and Lactase — The fractional catabolic rates (FCR), mean residence times (T), and production and conversion rates were calculated by compartmental analyses using a linear, time-invariant, steady state compartmental model with the Conversational Simulation Analysis and Modeling V30.1 computer program supplied by Dr. Loren A. Zech (National Institutes of Health, Bethesda, MD). Modeling terms and concepts have been defined elsewhere (19, 20). Fractional transfer rate (FTR) constants are reported using the standard nomenclature, thus \(k_{\text{1,3}}\) denotes the transfer to compartment 0 (the irreversible system loss compartment, C-0) from compartment 13 (C-13). As a starting point, the sequence of protein labeling was suggested by pulse-chase experiments (14, 21). Parsimony was used to select the simplest model to fit the data; thus, one protein constitutes a single compartment. The adjustment of the FTR parameters (\(k_{\text{1,3}}\)) by the program was considered complete when the values for the sums of squares of the residuals and the fractional error on the fitted parameters could not be improved by further iteration.

The FCR of the free amino acid pool in cardiac blood (mean ± S.D. from three rats at each time) was fitted to a single exponential function, and the shape of the fitted line was used as a forcing function (C-1) to drive the model. The mean steady state mass (sum of all animals, \(n = 21\)) of each SI or LPH isoform, determined densitometrically, was employed in the model, and the SR of each SI and LPH isoform (mean ± S.D. from three rats at each time) defined one compartment. Tracer could be lost from a compartment either by conversion to the next protein in the synthetic pathway, or by irreversible proteolysis. A delay compartment (C-3) was introduced between the SR of the free amino acid pool compartment in cardiac blood and the SR of the first protein compartment in the pathway to account for the time required for transit of the tracer through intracellular free amino acid pools and tRNA. A second delay compartment (C-20) was introduced between the last intracellular compartment and the SR of the mature enzyme compartment to account for translocation time to the brush border. Although the delay times were adjustable to permit the best fit of the data, ultimately they were fixed to finally determine FTR.

All microscopic parameters were adjustable, with three exceptions: 1) as described above, the number of elements in the delay compartment, the delay times and the \(T,\) from the delay compartments were set manually, 2) because the SR of brush border sucrase and lactase were low, the conversion of their respective complex glycosylated isoforms to mature enzymes could not be determined accurately by Conversational Simulation Analysis and Modeling and were therefore determined manually by a visual best fit of the tracer data, and 3) the FCR of both mature sucrase and lactase were computed manually from Equation 2, and the \(K,\) was computed manually from Equation 3.

\[
k_{\text{1,3}} = \frac{M_{\text{prec}}}{M_{\text{m}}} \quad \text{(Eq. 2)}
\]

where \(k_{\text{1,3}}\) is the FCR of C-13, \(k_{\text{1,3}}\) is the FTR of the precursor isoform \((M_{\text{prec}})\) to the mature enzyme \((M_{\text{m}})\) via a delay compartment (C-20)

\[
K, = \frac{24}{T,} \times 100 \quad \text{(Eq. 3)}
\]

**Statistics**

Unless otherwise indicated, data are presented as means ± S.E. Results were subjected to one-way analysis of variance, and differences between means were assessed by Student’s t test.

### RESULTS

**Free Amino Acid Pools in Blood and Mucosa**

The SR of the free amino acid pools in cardiac and portal blood were not significantly different at any time during the infusions (portal blood data not shown). Despite the large dose of Phe initially administered to these animals and the subsequent infusion of small amounts of Phe, the SR of the free amino acid pool of both cardiac and portal blood decreased an average of 11%/h (1292 ± 61 dpm-nmol\(^{-1}\) Phe at 10 min to 954 ± 16 dpm-nmol\(^{-1}\) Phe at 140 min in cardiac blood). The SR of the free amino acid pool in the mucosa reached 70% of the level measured in the blood during the first 20 min of infusion (Table I) but fell, on average, to 54% of the blood value during the last 2 h (data not shown).

### Fractional Synthesis Rates of Mucosal Protein

SR and the \(K,\) of mucosal protein measured during the first 20 min of infusion are shown in Table I. On average the \(K,\) for mucosal proteins was 139%/day (coefficient of variation 6%).

### Disaccharidase Identification and Labeling

**SI** = Electrophoresis separated the immunoprecipitated protein into five bands (with molecular masses of 140, 160, 220, 230, and 240 kDa) with Coomassie Blue staining or fluorography (Fig. 1). As previously described, the 140-kDa polypeptide represents brush border sucrase and the 160-kDa polypeptide represents brush border isomaltase (2, 15). The 220-, 230-, and 240-kDa polypeptides are precursor isoforms previously described as proSIh, proSIi, and proSIr, respectively (8). By fluorography, radiolabel was first detected in the proSIh (Fig. 1). Thereafter, isotope appeared sequentially in proSIi, then in proSIr, and finally in sucrase and isomaltase (Fig. 1). ProSIr and proSIi were Endo H sensitive (Fig. 2), but proSIh was not. The SR of proSIr isoforms and sucrase are shown in Fig. 3. The SR of proSIr rose rapidly during the first 30 min of infusion. By 40 min this isoform appeared to have reached an isotopic steady state that was maintained for the remainder of the infusion. The mean SR was 490 ± 9 dpm-nmol\(^{-1}\) Phe between 40 and 140 min (coefficient of

| Infusion time | SR, amino acid pool mucosa | SR, mucosal protein | \(K,\) |
|--------------|-----------------------------|---------------------|------|
| min          | dpm·nmol\(^{-1}\) Phe       | %/day               |      |
| 10           | 928 ± 142                   | 9 ± 0.7             | 145 ± 25 |
| 20           | 859 ± 65                    | 16 ± 1.0            | 133 ± 16 |

**Table I**

**Fig. 1.** Fluorogram of \(^{14}\)Cphenylalanine incorporation into sucrase-isomaltase (SI) isoforms over time.
variation 4.0%), which was significantly ($p < 0.05$) lower than the mean SR of the free amino acid pool in mucosa (576 ± 15 dpm·nmol$^{-1}$ Phe for the sample period).

The SR of proSI$_{h}$ and proSI$_{i}$ rose more slowly than proSI$_{k}$, but by 60 min both isoforms were at an isotopic steady state that was not significantly different from proSI$_{k}$. From 60 to 140 min, the mean value for proSI$_{k}$ was 428 ± 24 dpm·nmol$^{-1}$ Phe (coefficient of variation 10%) and for proSI$_{i}$ was 463 ± 42 dpm·nmol$^{-1}$ Phe (coefficient of variation 16%). The SR of sucrase rose very slowly during the infusion: at 90 min it had reached 20 ± 2 dpm·nmol$^{-1}$ Phe. Between 90 and 140 min the SR of sucrase rose more rapidly (SR = 104 ± 10 dpm·nmol$^{-1}$ Phe at 140 min) but never reached steady state.

**FIG. 2.** **Fluorogram of sucrase-isomaltase (SI) (A) and lactase-phlorizin hydrolase (LPH) (B) isoforms before and after treatment with endoglycosidase H (Endo H).**

Endo H treatment of immunoprecipitated LPH from animals in which the SR of the 200 kDa band was at isotopic steady state (i.e. infused 3 h) demonstrated that the 200 kDa band was composed of two isoforms (Fig. 2). As determined by densitometric measurements of fluorograms, 75% of the 200 kDa band was Endo H-sensitive; the remainder of the protein was Endo H-insensitive. Even using 5% SDS-polyacrylamide gels, we were unable to separate the two isoforms sufficiently to permit cutting of separate bands. Thus the entire 200 kDa band was cut and analyzed as one.

**FIG. 3.** The specific radioactivity of cardiac blood phenylalanine (M, panel A) and sucrase-isomaltase isoforms (panel B): proSI$_{h}$, □; proSI$_{i}$, ○; proSI$_{k}$, △; mature sucrase, ●. Symbols represent the mean (± S.D.) values for three rats determined at each time point. Error bars for proSI$_{i}$ and proSI$_{k}$ were omitted for clarity, but they were similar in magnitude to those for proSI$_{h}$ (shown).

**FIG. 4.** **Fluorogram of $[^{3}H]$phenylalanine incorporation in lactase-phlorizin hydrolase (LPH) over time.**

Endo H treatment of immunoprecipitated LPH from animals in which the SR of the 200 kDa band was at isotopic steady state (i.e. infused 3 h) demonstrated that the 200 kDa band was composed of two isoforms (Fig. 2). As determined by densitometric measurements of fluorograms, 75% of the 200 kDa band was Endo H-sensitive; the remainder of the protein was Endo H-insensitive. Even using 5% SDS-polyacrylamide gels, we were unable to separate the two isoforms sufficiently to permit cutting of separate bands. Thus the entire 200 kDa band was cut and analyzed as one.

The results of N-terminal amino acid sequencing of the 140, 200, and 220 kDa bands are shown in Table II. Of the first 20 N-terminal amino acids, 19 were identified for the 140-kDa band, 12 for the 200-kDa band, and 11 for the 220-kDa band. All signals appeared to be homogeneous, i.e. uncontaminated by minor proteins. The N-terminal amino acids of the 200-kDa polypeptide shared no homology and little identity with the N-terminal amino acids
of the 140 or the 220 kDa bands. We conclude on the basis of these and the Endo H data that the 200 kDa band probably represented a mixture of the high mannose (proLPHh) and complex glycosylated (proLPHh+) precursors of brush border lactase as previously described (22); we therefore have called the gel band proLPHh+. The 140-kDa isoform, which has been identified repeatedly as brush border lactase (1) and the 220-kDa proteins appeared to have identical N termini.

The SR of proLPHh+ and lactase are shown in Fig. 5. The SR of proLPHh+ rose slowly during the infusion and reached isotopic steady state only after 90 min (the mean SR was 485 ± 21 dpm·nmol⁻¹ Phe between 90 and 140 min, coefficient of variation 10%). The steady state SR of proLPHh+ was not significantly different from the steady state SR of the pros1 isoforms. The SR of lactase (like that of sucrase) rose very slowly during the first 90 min of infusion and rapidly thereafter. By 140 min the SR of lactase was 92 ± 2 dpm·nmol⁻¹ Phe. The SR of the 220-kDa polypeptide paralleled but never exceeded the SR of 140-kDa polypeptide (data not shown).

**FCR and Kₜ of SI Isoforms**

The compartmental model of SI synthesis is shown in Fig. 6, and the system parameters, including relative mass and quantity of each isoform, are described in Table III. The model in Fig. 6 is a graphic representation of the mass of each compartment and the patterns of tracer flow from which numerical values were computed by the CONSAM program using the experimentally determined SR of each compartment. The SR of the free amino acid pool in the cardiac blood compartment (C-1) was used as a forcing function to drive protein synthesis via a brief delay compartment (C-3) of 2.5 min. Since the SR of proSIh rose more rapidly than that of proSIi, we did not determine the SR of isomaltase, assigning the mass of isomaltase to the 220-kDa isoform, 97.5% (140-kDa isoform), 97.7% (220-kDa isoform).

**TABLE II**

N-terminal amino acid sequence of lactase phlorizin hydrolase isoforms

| Amino acid no. | 200-kDa isoform | 140-kDa isoform | 220-kDa isoform |
|---------------|-----------------|-----------------|-----------------|
| Rat*          | Rabbit*         | Human*          | Rat*            |
| 1             | Ser             | Ser             | Val             | Arg             | Arg             | Val             |
| 2             | Asp             | Asp             | Thr             | Ala             | Ala             |
| 3             | Trp             | Trp             | Trp             | Asp             | Ser             | Phe             | Asp             |
| 4             | Gly             | Gly             | Gly             | Gly             | Lys             | Lys             | Lys             |
| 5             | Ser             | Ser             | Leu             | Leu             | Phe             | Leu             |
| 6             | Asp             | Asp             | Pro             | Pro             | Pro             | Pro             |
| 7             | Arg             | Ser             | Pro             | Pro             | Pro             | Pro             |
| 8             | Asn             | Asn             | Glu             | Glu             | Glu             | Glu             |
| 9             | Phe             | Phe             | Val             | Val             | Val             | Val             |
| 10            | Phe             | Phe             | Val             | Val             | Val             | Val             |
| 11            | Ser             | Ser             | Ser             | Ser             | Ser             |
| 12            | Ala             | Ala             | Thr             | Lys             | Lys             | Lys             |
| 13            | Ala             | Ala             | Ala             | Ala             | Ala             | Ala             |
| 14            | Gly             | Gly             | Gly             | Lys             | Lys             | Lys             |
| 15            | Pro             | Pro             | Pro             | Val             | Val             | Val             | Val             |
| 16            | Pro             | Pro             | Val             | Val             | Val             | Val             | Val             |
| 17            | (Thr)           | Thr             | Thr             | Thr             | Thr             | Thr             | Thr             |
| 18            | Asn             | Thr             | Asn             | Glu             | Glu             | Glu             | Glu             |
| 19            | Asp             | Asp             | Asp             | Lys             | Lys             | Lys             | Lys             |
| 20            | Lys             | Lys             | Leu             | Leu             | Phe             | Lys             | Phe             | Phe             | Phe             |

* Initial yield: 26.7 pmol (200-kDa isoform), 56.4 pmol (140-kDa isoform), 39.3 pmol (220-kDa isoform); repetitive yield: 97% (200-kDa isoform), 97.5% (140-kDa isoform), 97.7% (220-kDa isoform).

**Fig. 5. The specific radioactivity of cardiac blood phenylalanine (A, panel A) and lactase-phlorizin hydrolase isoforms (panel B): proLPHh+; L lactase. Symbols represent the mean (± S.D.) values for three rats determined at each time point.**

**DISCUSSION**

Measurements of the rate of brush border hydrolase synthesis have generally been made *in vitro* using either intestinal explants or Caco-2 cells and pulse-chase labeling (23). In pulse-chase studies using human intestinal biopsies, for example, synthesis rates have been estimated by fluorography...
Fig. 6. The compartmental model of sucrase-isomaltase (SI). Numbers within the circles and rectangles identify compartment numbers mentioned in the text. Above each compartment is the identity of the protein. Fractional transfer rate constants (min^-1) are above and beside the arrows noting the transfer direction. The fractional standard deviations of the fitted values are given as percentages in parentheses (%), and the letters F and D denote whether the value was fixed (F) or determined (D) by an equation involving other model parameters.

Table III
System parameters for the SI compartmental model

| Parameter                  | proSI_h | proSI_l | proSI_c | Sucrase |
|----------------------------|---------|---------|---------|---------|
| Mass (kDa)                 | 220     | 230     | 240     | 140     |
| Mass (% of total)          | 18.1 ± 3.1 | 5.2 ± 0.8 | 18.4 ± 3.0 | 148.3 ± 26.0 |
| Production rate (µg·g tissue^-1·h^-1) | 44.6 ± 18.9 | 51.4 ± 9.6 | 39.4    | 25.4    |
| FCR (h^-1)                 | 2.46 ± 10.2 | 6.07 ± 1.04 | 2.14    | 0.17    |
| Mean residence (h)         | 0.41 ± 0.17 | 0.10 ± 0.03 | 0.47    | 5.84    |
| Conversion (%)             | 100     | 100     | 65      | 100     |

* Total mass: 323 ± 57 µg/g tissue, including isomaltase.
* Fractional catabolic rate.

Fig. 7. The compartmental model of lactase-phlorizin hydrolase (LPH). Numbers within the circles and rectangles identify compartment numbers mentioned in the text. Above each compartment is the identity of the protein. Fractional transfer rate constants (min^-1) are above and beside the arrows noting the transfer direction. The fractional standard deviations of the fitted values are given as percentages in parentheses (%), and the letters F and D denote whether the value was fixed (F) or determined (D) by an equation involving other model parameters.

Table IV
System parameters for the LPH compartmental model

| Parameter                  | proLPH_h+c | Lactase |
|----------------------------|------------|---------|
| Mass (kDa)                 | 200        | 140     |
| Mass (% of total)          | 12.0 ± 1.6 | 54.0 ± 8.0 |
| Production rate (µg·g tissue^-1·h^-1) | 10.5 ± 1.6 | 7.02    |
| FCR (h^-1)                 | 0.88 ± 0.13 | 0.13    |
| Mean residence (h)         | 1.14 ± 0.17 | 7.83    |
| Conversion (%)             | 65.7       | 100.0   |

* Total mass: 66 ± 10 µg/g tissue.
* Fractional catabolic rate.

This technique because the amino acid pools from which the proteins were synthesized were not measured. Further, it is not known whether the values obtained in vitro accurately reflect synthesis rates in vivo. The few in vivo studies have estimated K_s values for brush border hydrolases in combined precursor and mature isoforms and/or under conditions in which the precursor amino acid pools were not at steady state (4, 22, 29). The former leads to variation in calculated synthesis rates with time (2), and the latter greatly reduces the precision and accuracy of K_s estimates.

The measurement of intestinal enzyme synthesis in vivo is complicated by the constant fluctuation of the free amino acid pools from which the enzymes are formed. In the mucosa both the enzyme precursors and the amino acid pools can be derived from arterial circulation, from intracellular proteolysis, and from luminal-serosal transport. Thus, a systemically administered amino acid entering the free amino acid pools from which the enzymes are formed. In the mucosa both the enzyme precursors and the amino acid pools can be derived from arterial circulation, from intracellular proteolysis, and from luminal-serosal transport. Thus, a systemically administered amino acid entering the free amino acid pools from which the enzymes are formed.
shown that the free amino acid pool from which SI is synthesized is kinetically heterogeneous and that the relationship between the labeling of SI and that of the bulked mucosal amino acid pool varies with the feeding status of the animal (2).

In attempts to measure LPH turnover, investigators have encountered an additional problem: uncertainty as to the identity of the three protein bands separated by SDS-polyacrylamide gel electrophoresis. Pulse-chase studies in a variety of mammals have suggested a precursor-product relationship between the 200- and the 140-kDa polypeptides (17, 21). The identity of the 220 kDa band has, however, never been clear, and N-terminal amino acid sequencing has not been reported. These studies generally support the idea of a precursor-product relationship between the 200- and the 140-kDa polypeptides. Isotope was first detected in the 200-kDa polypeptide and accumulated with time in the 140 kDa band. Because the isotope was continuously infused in the present study, however, the possibility that the 140 kDa band is an unrelated, slowly synthesized protein could not be eliminated. N-terminal amino acid sequencing of the three polypeptides further clarified their identity. The 200-kDa polypeptide was homogeneous, but shared no homology with the N-terminal amino acid of the 140- or the 220-kDa protein. The 200-kDa protein revealed an approximate 90% homology with the N-terminal amino acids of proLPH reported for the rabbit and 95% homology with those reported for the human (17, 22, 30, Table II). The 140-kDa protein showed approximately 80% homology with rabbit brush border lactase and 70% homology with human brush border lactase. Because the SR of the 220 kDa band increased with time at approximately the same rate as the 140-kDa protein, and because the 140 kDa band has been shown repeatedly to be the brush border form of LPH (1, 21), we conclude that the 220 kDa band is, as previously described, a dimer of the brush border polypeptide rather than an intermediate product on the pathway of synthesis (24).

The labeling strategy and design of the present study were planned to overcome some of the problems described above and to enable in vivo estimates of the synthesis rate of purified SI and LPH precursor and mature isoforms under conditions such that their precursor pool was at isotopic equilibrium. By infusing the animals with large quantities of Phe, large amounts of amino acid were introduced into the free amino acid pools of the blood and mucosa. In this manner we minimized, but did not eliminate, the isotope dilution effect of both amino acid absorption from the luminal contents and intracellular degradation. We were able to attain isotopic steady state in the free amino acid pools of the blood and mucosa within 10–20 min. Equilibrium was maintained by the continued infusion of small amounts of amino acid solution. Although the SR of both the plasma and mucosal free amino acids fell gradually, they fell in parallel. The SR of the free amino acid pool in the mucosa was close to that in the blood and from 30 to 140 min was a constant percentage of the blood value (on average 54%). Because this ratio is twice the value we were able to attain in fed animals using a primed continuous tracer infusion, the problem of label dilution has been partially overcome (2). In addition, we avoided the problems of compartmentalization of the free amino acid pool in the mucosa (2) by using SR of the free amino acid pool in the blood as a forcing function to drive the compartmental model.

The compartmental model used for these analyses was the simplest model that could be adapted to the sequence of processing events suggested by pulse-chase experiments (14, 21), corroborated by these continuous infusion studies, and which afforded a reasonable fit to the data. The model is not unique. The fate of the tracer in each compartment was either conversion to the active protein in the cascade or irreversible catabolism. More complex patterns of tracer flux between compartments were adequately fit but discarded because they failed to adequately fit the experimental data. There is the possibility of more complexity within the system, particularly with regard to SI (for example, the de novo/parallel synthesis of proSI, or proSL). Because radiolabel was infused continuously during these studies, however, and reached steady state quickly in most compartments, we were unable to distinguish more complexity than was embodied in our model. A single bolus injection of tracer would have resulted in more information about the rate of processing events and permitted additional complexity within the compartmental model. A single bolus injection, however, requires long experiments, more experimental animals to in order have more time points, and a prohibitively large dose of radiolabel to permit measurement of the specific radioactivity in individual isoforms.

The in vivo K, for brush border sucrose calculated from the compartmental model (414%/day) is remarkably similar to the in vivo value we have previously reported for brush border sucrose processing (400%/day) using primed continuous tracer infusion and the SR of proSI (combined proSI, proSI, and proSI isoforms) as the basis for the calculation (2). The K, of lactase (307%/day) is slightly slower. These findings contrast sharply with those of Olsen et al. (29), who have reported a K, of 140%/day for SI. Olsen and co-workers, however, estimated turnover in pooled samples of proSI isoforms and sucrase during the first 10 min of infusion using the flooding dose infusion technique described by Garlick et al. (11, 29). Our results show that initial incorporation of label into the SI isoforms shows a significant time lag, presumably because the isotopic must first be incorporated into tRNA and the pre-proenzyme. Furthermore, during the first 10 min of infusion, label incorporation is confined largely to proSI, a polypeptide that represents less than 6% of the total mass of SI (mature sucrase and isomaltase constitute 80%-90% of the total mass). Thus, turnover estimates for combined isoforms made during the first few minutes of infusion would necessarily appear to be low because of the disproportionate mass of unlabeled mature polypeptide.

Despite differences in their modes of attachment to the brush border membrane and a 5-fold difference in the quantity of enzyme/gram of mucosa, SI and LPH appear to be synthesized at approximately the same rate. The mean intracellular residence times of 59 min for SI and 68 min for lactase are not different and closely parallel values reported for in vitro systems (10). Furthermore, under the conditions of this study, the two disaccharidases appear to be synthesized from the same intracellular amino acid pool, which, as in our previous study using tracer infusion techniques (2), appears to be a subcompartment of the free amino acid pool. ProSI, proSI, proSI, and proLPH are all reach steady state plateaus that are not significantly different from one another but are significantly lower than the free amino acid pool in the mucosa.

Under the conditions of this study, the best fit of the SR data to a compartmental model was one in which only small amounts of the two disaccharidases are degraded as the enzymes are glycosylated in the endoplasmic reticulum/Golgi and translocated to the brush border. Approximately 65% of the tracer incorporated into proSI was converted to mature sucrase. Of the label in proLPH, 72% was converted to the brush border form of the enzyme. These values are not statistically different from the values that would be obtained if there were 100% conversion of the primary translation prod-
uct to the brush border isofoms (approximately 46–47% for sucrase and 65% for lactase, as calculated from previous reports of molecular mass) (1, 21, 25). Thus, under normal physiologic conditions, precursor missorting, possibly followed by intracellular degradation as reported in some cases of congenital SI deficiency, does not appear to be a significant factor (25).

In contrast to the turnover rate for brush border enzymes, the turnover rate for mucosal protein was 139%/day, a value consistent with previously reported measurements using the flooding dose technique (31, 32). The rapidly synthesized brush border proteins comprise a significant proportion of the total mucosal protein; for example, sucrase alone is thought consistent with previously reported measurements using the flooding dose technique (31, 32). The rapidly synthesized brush border proteins comprise a significant proportion of the total mucosal protein; for example, sucrase alone is thought to represent more than 10% of brush border protein. It seems likely, therefore, that the cytoplasmic, mitochondrial, and structural proteins within the enterocyte are turning over at a considerably slower rate than the proteins of the brush border.

Acknowledgments—We are extremely indebted to Cynthia Fedrick for manuscript preparation, E. Rosland Klein and Jerry D. Eastman for editorial assistance, and Adam C. Gillum for photographic support.

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