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THE POSSIBLE ROLE OF PANCREATIC PROTEASES IN THE TURNOVER OF INTESTINAL BRUSH BORDER PROTEINS

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SUMMARY

1. Intestinal brush border enzymes have heterogeneous rates of turnover, the largest proteins having the fastest turnover. Since the membrane faces the intestinal lumen, the effects of pancreatic factors were examined in mediating this turnover. Surgical subtotal pancreatectomy was used as an experimental model to study the turnover of brush border proteins in the absence of most pancreatic secretions.

2. Subtotal (95 %) pancreatectomy of rats was found to cause elevations by about 50 % of total activity and specific activities of certain brush border enzymes (maltase, sucrase, lactase), but not of others (alkaline phosphatase, trehalase). Rats were judged to be functionally deficient in pancreatic proteolytic enzymes (a) by demonstration of vitamin B-12 malabsorption, which was corrected by trypsin, and (b) by the finding of only about 20 % of proteolytic activity appearing in the lumen after a test meal when compared to control.

3. To measure protein turnover in vivo the method of double labelling was used, where [^{3}H]- and [^{14}C]valine were administered intraduodenally in sequence 10 h apart. With this technique, a high $^3H/^{14}C$ ratio is correlated with rapid turnover. Proteins with apparent molecular weights of about 200 000–270 000 were found to turn over more rapidly than smaller proteins. $^3H/^{14}C$ ranged from 4.7 to 6.2 in animals without pancreatic insufficiency. In the face of decreased pancreatic proteolysis, the $^3H/^{14}C$ ratio was 2.3–3.1, similar to that of proteins with a slow half life.

4. Estimates of relative synthetic rates of large brush border proteins were lower than normal in pancreatectomized animals, but were constant over the period of the labelling experiment. The high enzyme levels in the face of lower synthetic rates confirms that, at the new steady rate, degradation rates must be slower for large brush border proteins in pancreatic insufficiency.

5. In vitro, using purified brush borders, unfractionated pancreatic enzymes were found to remove sucrase, maltase and lactase, but not alkaline phosphatase and trehalase. The enzyme most potent in this respect was the pancreatic protease, elastase. Non-proteolytic enzymes (amylase, lipase, phospholipase A) were inactive in removing enzyme from the brush border. The addition of elastase to pancreatecto-

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mized animals in vivo restored the rapid turnover rate of large brush border proteins.

6. A model is thus proposed for the normal catabolism of some large intestinal brush border proteins. It is suggested that the surface of intestinal absorptive cells is being constantly remodelled, and that certain surface enzymes are in part removed from the membrane by the action of pancreatic proteases. A possible special role for elastase is suggested.

INTRODUCTION

Intestinal brush border disaccharidases have been shown to have a turnover rate which is more rapid than the rest of the brush border proteins [1]. Moreover, there may be other large molecular weight brush border proteins distinct from the disaccharidases with equally rapid turnover rates [2]. It seems unlikely that the mechanism for rapid turnover of the disaccharidases would depend on intracellular proteases as proposed by Schimke and Doyle [3]. The disaccharidases are functionally extracellular enzymes [4] and are released earlier than other brush border enzymes by papain treatment [5]. They are, thus, probably exposed to the external surface of the cell membrane, and would have to return to the cell to be removed by intracellular processes. Membrane fragments could be internalized by pinocytosis and degraded by intracellular proteases. However, disaccharidases are very resistent to known proteases. Therefore, it seems reasonable to look for other mechanisms to account for their turnover. It seems possible that increased disaccharidase turnover could take place by extracellular mechanisms releasing enzyme into the intestinal lumen.

Many workers have obtained data implicating bile or pancreatic enzymes in the release of enterokinase [6, 7] and other brush border enzymes [8] from the membrane itself. Since the disaccharidases are located on the external cell membrane, we hypothesized that the release of brush border enzyme from their membrane might be mediated, in part at least, by intraluminal pancreatic proteases. Although most studies on protein turnover have emphasized a protein-degrading system as the mechanism of turnover, it seemed equally reasonable to examine the importance of a system that released proteins and removed them from the cell surface. We therefore designed experiments to study the degradation, synthesis and mechanisms of release of large brush border proteins from the membrane, in normal conditions and in pancreatic insufficiency, using this pathological state as a model system for investigating the role of pancreatic proteases. Our data demonstrate that pancreatic proteases may be important in removing the large proteins, including disaccharidases, from the brush border membrane.

METHODS

95 % pancreatectomy was performed in male Wistar rats, 150–180 g (National Laboratory Animal Co., Creve Coeur, Mo.) by the method of Scow [9]. Only the pancreas between the common bile duct and the duodenum was left intact (parabiliary portion) [10]. Animals were fed thereafter with Ralston-Purina rat chow for 3–6 months. Weight at the time of sacrifice ranged from 340 to 530 g. Functional assessment of pancreatic protease deficiency was determined by vitamin B-12 absorp-

tion and by response to a test meal. Vitamin B-12 absorption was tested according to Toskes and Deren [11]. ^{57}Co-labeled vitamin B-12 (15 Ci/g) obtained from Amersham-Searle (Chicago, Ill.) was given by stomach tube (0.1 μCi), and stools collected for 5 days thereafter. Stools and urine were collected separately in a metabolic cage. Stools were dried and counted in a Packard model 3320 liquid spectrometer. Results are reported as percent of administered dose absorbed (i.e. not excreted). Trypsin when used was added separately via stomach tube at a dose of 2 mg per animal.

1 h before sacrifice each rat was given two Purina rat chow pellets, which were totally consumed in all cases. That was to provide stimulus to pancreatic secretion, and it was found that in their fasted state, the rats rapidly consumed two pellets. At the time of sacrifice, the upper third of the intestine was removed from the pylorus and washed out carefully with 2.0 ml of normal saline, repeated three times. The combined washings were assayed for proteolytic activity using casein as a substrate [12]. Results are reported as mg of trypsin equivalent in the upper jejunum at the time of sacrifice. Since total protein output was lower in pancreatectomized animals, results are reported as total, not specific activity.

The upper third of the small intestine was homogenized in 3 vol of 50 mM potassium phosphate buffer, pH 6.0, and disaccharidase activity measured according to Dahlqvist [13]. Alkaline phosphatase was measured using *p*-nitrophenyl phosphate as substrate [14]. Protein was assayed according to Lowry et al. [15]. Results are reported as total jejunal activity (activity/ml per ml) or specific activity (activity/mg protein).

Estimation of intestinal protein turnover

Two types of radioisotope labelling experiments were performed. [^{3}H]Valine (6.2 mCi/μmol) and [^{14}C]valine (230 Ci/mol) were purchased from New England Nuclear Corp., Boston, Mass.). First, control and pancreatectomized animals were given only a single-labelled amino acid in order to determine relative synthetic rates at different times of the day. Animals were anesthetized with ether and their abdomens opened. [^{3}H]Valine (100 μCi in 2 ml of normal saline) was administered by direct intraduodenal injection via a 27 guage needle, and the hole sealed with Eastman adhesive 910. The distal extent of the fluid was marked by a loose ligature, but usually corresponded to a point of one fourth to one third of the length of the small intestine. Injection was made at 2 p.m. or 12 p.m. to animals made fasting the previous evening. Animals were sacrificed 6 h later, at which time incorporation of label into brush border proteins is maximal [1], although the soluble pool of amino acids and incorporation into total protein is maximally labelled by 1 h. Intestine was removed from the pylorus to the loosely tied ligature. Brush borders were isolated according to Forstner et al. [16], boiled 5 min in 3 % sodium dodecyl sulfate and 1 % 2-mercaptoethanol, and separated on 5 % acrylamide gels, in the presence of 1 % 2-mercaptoethanol and 3 % sodium dodecyl sulfate [2]. After electrophoresis, gels were removed and stained according to Fairbanks et al. [17] in Coomassie Blue. Densitometry was performed on the stained gels by scanning with a Gilford model 2410 linear transport system at 555 nm as previously reported [2]. Gels were sliced at the appropriate protein bands, and the protein solubilized as described by Ferroluzzi-Ames [18]. Efficiency of counting was 44 % and recovery of counts from the gels was 70–75 %. Corresponding samples from two gels (200 μg protein/gel) were combined for count-

ing. All samples were counted for at least 2000 counts above background, equivalent to a probable counting error of 1.5 %. Results are described as cpm/inch^2 where the inch^2 refers to area under the curve produced for each protein peak by the spectrophotometric scan [2].

Double-labelled isotope experiments were performed to study relative rates of degradation of protein and were carried out as described earlier [2]. This technique was designed [3] to provide information about turnover rates of protein without having to isolate specific proteins and measure specific activity. Separation of solubilized proteins on polyacrylamide gels in the presence of detergent allowed measurement of $^3H/^{14}C$ ratios in groups of proteins with the same molecular weight. Proteins turning over rapidly would have lost a large portion of initial label (^{14}C) and would incorporate a large amount of the second label (^3H), thus giving a high $^3H/^{14}C$ ratio. Under ether anesthesia after overnight fasting, 100 μCi of [^{14}C]valine was injected intraduodenally into control and pancreatectomized rats as described above at 2 p.m. At midnight, the procedure was repeated, using 200 μCi of [^3H]valine. Animals were sacrificed 10 h after the second injection. 1 h before sacrifice, each animal was given two pellets of rat chow, as described above. After sacrifice, intestine was removed between the pylorus and the loose ligature and homogenized in 100 vols of 5 mM EDTA. Aliquots were removed for enzyme studies, and brush borders were then isolated. 250 μg of brush border protein were applied to acrylamide gels containing sodium dodecyl sulfate [2], after solubilization in 1 % 2-mercaptoethanol and 3 % sodium dodecyl sulfate, and boiling for 5 min. About 3000–5000 ^3H cpm and 2000–2500 ^{14}C cpm were applied to the acrylamide gels. After electrophoresis at 8 mA per gel for 10 h, the unstained gels were sliced into equal 3-mm fractions with a multiple razor blade device, and prepared for counting as described above. A simultaneously run sample stained with Coomassie Blue provided location of protein bands. A sample of gel, cut from the area ahead of the tracking dye, was used for background. All samples were counted for at least 2000 counts above background, with an efficiency for ^{14}C of 48 % and for ^3H of 28 %. A high $^3H/^{14}C$ ratio was associated with rapid turnover.

Uptake of the amino acid valine by rings of proximal jejunum removed at the time of sacrifice of the above animal was studied by methods previously used [19]. Incubation with [^{14}C]valine (0.2 μCi, 0.065 mM) for 30 min was carried out in Krebs-Ringer bicarbonate buffer, and the resulting distribution ratio calculated, correcting for inulin space. Cell migration rate was measured in control and pancreatectomized animals by migration of intraperitoneally administered [^3H]thymidine along the crypt, as described earlier [20].

In vitro studies

Duodenal fluid was collected from male Wistar rats, 150–180 g, by the following procedure. Animals were fasted overnight and anesthetized with ether. A polyethylene catheter (PE 120) with a flanged end was inserted into the duodenal loop through the muscular wall, and a purse string suture used to anchor the tube in place. The duodenum was then ligated distal to the catheter. The abdomen was closed, allowing the catheter to come out at the incision line. Animals were placed in restraining cages and given 5 % dextrose in normal saline to drink ad libitum. Duodenal juice was collected for about 6 h, collecting from 4 to 8 ml. Undiluted juice contained

about 12 mg protein/ml.

For in vitro studies of enzyme release, purified intestinal brush borders were prepared [16]. For the various incubations brush border was added to a concentration of about 0.15 mg/ml protein in 50 mM sodium phosphate buffer, pH 7.0. Other additions were made according to the experimental design in Table IV. At the various time points, aliquots were removed for total enzymatic assay, and a duplicate sample centrifuged at $45\,000 \times g$ for 30 min to separate membrane-bound from membrane-released material. Centrifugation at $100\,000 \times g$ for 60 min gave identical results and was not used because it was more cumbersome. In addition, the sucrase activity present in the $45\,000 \times g$ supernatant fraction was eluted from a Sephadex G-200 column identical to rat sucrase purified in our laboratory after papain solubilization, and entered a sucrose gradient, sedimenting with an S value of 12. Furthermore, after dialysis to remove bile salts, enzyme activity remained in the supernatant fraction after centrifugation at $100\,000 \times g$ for 60 min. Thus, it seems unlikely that enzyme release reflected enzyme attached to small pieces of membrane, but rather that the enzyme was truly soluble. Aliquots were removed from each sample prior to incubation and assayed to give the initial value for each parameter. Data are recorded as percent of total activity which was released from the membrane.

Biochemical analyses

Peptide bonds liberated were determined by a micro modification of the ninhydrin assay [21]. Fasting blood sugar was determined by the glucose oxidase method on venous tail vein samples obtained the day prior to sacrifice.

RESULTS

Evidence of pancreatectomy

All animals undergoing 95 % pancreatectomy were tested for vitamin B-12 absorption and proteolytic enzyme activity in the intestinal lumen. The results depicted in Table I correspond to representative animals used for enzyme and double-labelled isotope experiments. Animals 5–9 had vitamin B-12 absorption of less than 50 % on repeated occasions, and four of these five responded to the addition of trypsin intragastrically, as reported by Toskes et al. [22]. Animals 5–9 also had proteolytic activity, measured 1 h after ingestion of two pellets, which was only about 20 % of the control animals. Amylase activity (not shown) was similarly depressed. Thus, these animals were considered as functionally having pancreatic insufficiency and in particular, protease deficiency. In addition, their stool was grossly loose and light colored, consistent with steatorrhea and lipase deficiency. However, they were not diabetic, since fasting blood sugar was within the normal range of 140–155 mg/100 ml, and they gained weight at rates comparable to control animals possibly because they ingested more food. Animals 1–4 had also undergone 95 % pancreatectomy and had at one time shown vitamin B-12 malabsorption. However, with time, this malabsorption was lost, probably due to pancreatic regeneration [23]. At the time of sacrifice, all of these animals had vitamin B-12 absorption greater than 50 %, which did not increase with the addition of trypsin (Table I). Proteolytic activity of their intestinal juice was high, and comparable to that obtained from rats never operated upon (2.48 ± 0.42). Thus, animals 1–4 were considered to have recovered pancreatic function, and were used as controls.

TABLE I

EVIDENCE FOR PANCREATECTOMY IN EXPERIMENTAL ANIMALS

All animals were subjected to 95 % pancreatectomy and allowed to grow for 3–6 months. Rats 1–4 recovered the ability to absorb vitamin B-12 and were felt to have regenerated their pancreas. Rats 5–9 continued to show malabsorption of vitamin B-12 and were considered as having functional partial pancreatectomy.

Animals	Vitamin B-12 absorption (%)		Proteolytic activity of intestinal juice (mg trypsin equivalent)	Fasting blood sugar (mg/100 ml)
	Vitamin B-12	Vitamin B-12 and trypsin		
1	58	57	2.24	152
2	52	55	3.13	160
3	55	49	1.32	138
4	61	60	1.84	143
5	26	62	0.246	165
6	22	20	0.348	153
7	8	88	0.382	132
8	42	56	0.159	147
9	13	77	0.225	158

Brush border enzyme activity after 95 % pancreatectomy

Activity of certain brush border enzymes was tested in the nine animals described in Table I. Maltase, sucrase and lactase were used as examples of proteins with rapid turnover rates [1], which are removed from the membrane by proteolytic enzymes, whereas trehalase and alkaline phosphatase are not solubilized by proteolytic enzymes. Lactase, sucrase and maltase all had increased levels of specific activity in pancreatectomized animals as seen in Table II. The increase of about 50 % was statistically significant at the $p = 0.05$ level when examined by student's t-test. Total

TABLE II

BRUSH BORDER ENZYME ACTIVITY AFTER 95 % PANCREATECTOMY

Rats described in Table I were stunned, and the proximal third of small intestine removed, homogenized, and assayed as described in Methods. Results are reported as the mean ±standard error of the mean. Alkaline phosphatase activity is reported as μmol of *p*-nitrophenyl phosphate released per h. Results were compared statistically using a student's *t*-test. n.s., not significant.

	Total jejunal activity			Specific activity		
	Control (units)	Pancreatectomized (units)	*P*	Control (units/mg protein)	Pancreatectomized (units/mg protein)	*P*
Maltase	456±46	681±31	<0.1	5.1 ±0.3	6.4 ±0.13	<0.05
Sucrase	39±1.5	76±7	<0.05	0.45±0.05	0.79±0.06	<0.05
Lactase	30±0.65	54±1	<0.05	0.10±0.007	0.17±0.005	<0.05
Trehalase	179±18	192±18	n.s.	1.45±0.13	1.47±0.13	n.s.
Alkaline phosphatase	15±1.6	21±2.1	n.s.	0.04±0.002	0.04±0.001	n.s.

jejunal activity was also significantly elevated, slightly less so for maltase, because of the wide range of values. On the other hand, trehalase and alkaline phosphatase values remained unchanged by pancreatectomy.

Relative degradation rates of brush border proteins

The mechanism of elevated enzyme levels of pancreatectomized animals was next investigated. For this purpose, relative degradation rates of brush border proteins were measured using the double-labelled method of Dehlinger and Schimke [24], as modified for intestine [2]. Such a method is based on the fact that rapidly turning over proteins will lose a greater proportion of the initially administered isotope and, because of their increased synthetic rate, incorporate more of the second isotope. Thus, when [^{14}C]valine was given first the $^{3}H/^{14}C$ ratio would reflect rapid turnover relative to proteins with a lower ratio. Fig. 1 gives the results of such an experiment. Groups of brush border proteins were separated according to molecular weight on polyacrylamide gels in the presence of 2-mercaptoethanol and sodium dodecyl sulfate. In the control animals, heterogeneous turnover rates were observed, with a higher $^{3}H/^{14}C$ ratio for those proteins with high molecular weights. Included among these proteins are the disaccharidases, with molecular weights of about 210 000. In fact, the band of protein at 205 000 stains histochemically for maltase [2]. Pancreatectomy, on the other hand, altered the turnover rate of these large proteins, as evidenced by the fact that the $^{3}H/^{14}C$ ratio for these proteins was now identical to other smaller brush border proteins. This result was duplicated in two separate experiments.

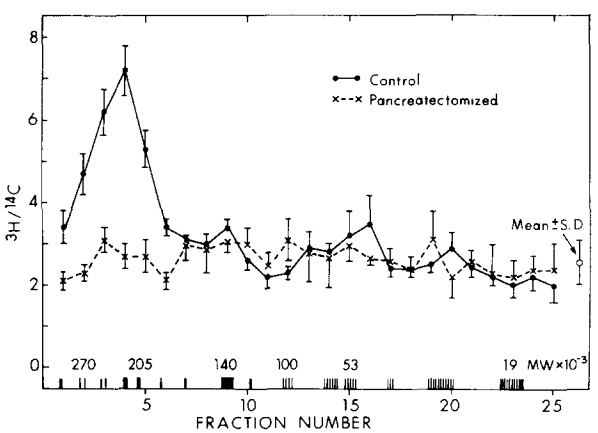

Fig. 1. Effect of 95 % pancreatectomy on turnover of brush border proteins. Animals were prepared and experiments performed as described in Methods. Two animals each were used for control and pancreatectomized groups. [^{14}C]Valine was injected at 2 p.m., [^{3}H]valine at 12 midnight. Animals were sacrificed at 10 a.m. Brush borders were isolated from the upper jejunum. Three separate sodium dodecyl sulfate-acrylamide gels were used for each curve, and the fractions pooled. To each gel was applied 250 μg of brush border protein containing approx. 5000 ^{3}H counts and 2500 ^{14}C counts. When both labels were given simultaneously, the $^{3}H/^{14}C$ ratios of this control experiment for the whole gel gave a mean value of 2.7 ± 0.55. This is depicted graphically on the right of the figure. The protein bands corresponding to the gel fractions are depicted at the bottom of the figure, with their apparent molecular weights.

For a double-labelled experiment as depicted in Fig. 1 to be meaningful, certain assumptions must be valid. These include (a) that proteins are undergoing degradation at the time of sacrifice, (b) that labelled precursor is not metabolized, (c) that decay of labelled proteins follows first-order kinetics, (d) that rates of synthesis are the same at the time of addition of both labelled precursors and (e) that cell migration time is not altered in control and experimental conditions. These conditions have been shown to be met for non-pancreatectomized rats [2]. They also seem to be true for rats pancreatectomized months earlier and growing normally. Firstly, specific activity of brush border proteins begins to fall after 6 h, so that by 10 h after it is administered, the proteins are being degraded. Secondly, label in brush border protein occurred in valine (over 90 %) and not other amino acids, although the label is reutilized [25]. Thirdly, relative rates of synthesis of total brush border proteins do not vary according to the time of day in fasting rats [25]. However, it was still possible that individual groups of proteins might show diurnal variations and thus affect the results of the double-labelled experiment.

Accordingly, [^{3}H]valine (100 μCi) was given intraluminally to control and pancreatectomized rats at 2 p.m. and midnight, the times used for the double-labelled experiment. Incorporation into protein was allowed to proceed for 6 h, that is until maximal incorporation had occurred [1]. Proteins were separated on sodium dodecyl sulfate-containing acrylamide gels, and amino acid incorporation measured in groups of proteins of like molecular weight. The specific activity of each group of proteins did not vary, regardless of time of injection or size of proteins, except for the large ($>$ 205 000 mol. wt) proteins (Table III). Large proteins (gel fractions 2, 4 and 5) in control animals had higher specific activity than in pancreatectomized animals, at both times tested. No significant differences were seen in relative synthetic rates of

TABLE III

RELATIVE SYNTHETIC RATES OF INTESTINAL BRUSH BORDER PROTEINS

Four control and four pancreatectomized rats similar to those described in Table I were fasted overnight. Half of the rats were given 100 μCi of [^{3}H]valine in the jejunum at 2 p.m., and at midnight in the other half. Rats were killed 6 h after exposure to isotope. Brush borders were isolated and 200 μg of protein were applied to gels, electrophoresed, scanned, sliced and counted as described in Methods and as depicted in Fig. 1. The results are the mean of the two animals in each group. Statistical significance was determined by analysis of variance for 2 $\times$ 2 factorial design. In no case was the interaction of time by treatment statistically significant. n.s., not significant.

Gel fraction	Approximate molecular weight	Protein synthesis (cpm/inch^2)				Significance levels	
		2 p.m.–8 p.m.		Midnight–6 a.m.		Control vs Pancreatectomized	Time
		Control	Pancreatectomized	Control	Pancreatectomized		
2	270	210	160	190	137	<0.05	n.s.
4	210	240	134	218	148	<0.001	n.s.
5	205	180	148	202	152	<0.05	n.s.
9	140	135	122	164	158	n.s.	n.s.
12	100	158	172	133	148	n.s.	n.s.
15	53	138	155	149	137	n.s.	n.s.
23	19	120	130	140	116	n.s.	n.s.

smaller proteins (mol. wt 140 000 or less). In no protein fraction did the time of injection make a significant difference. Differing degrees of amino acid transport did not seem to explain the differences between control and pancreatectomized animals, since the distribution ratio (as a measure of uptake) in intestinal rings was 11.3±2.1 for control and 12.4±3.6 for pancreatectomized animals.

Finally, differences in the rate of cell migration could conceivably affect the $^{3}H/^{14}C$ ratio, although large brush border proteins turn over at a rate faster than the cells migrate [2]. Thus, in control and pancreatectomized animals, the fractional distance from the top of the villus of the leading edge of labelled cells after [^{3}H]-thymidine was measured. The value for jejunum at 24 h was 38.3±4.2 % for controls and 36.3±6.2 % for pancreatectomized animals. This migration rate is similar to that observed (46.4±8.3 %) for rats not previously operated upon [20].

In vitro effect of pancreatic enzymes on brush border proteins

The in vivo data suggested that pancreatic secretions might be important in the turnover of large brush border proteins. We next looked at the effect of components of pancreatic secretions on isolated brush borders.

Isolated brush borders were used as a source of large proteins on which to study effect of pancreatic enzymes on release of proteins. It seems likely that those factors which were responsible for release of enzyme activity might in some way correspond to the in vivo degradation of the same proteins. When brush borders were

TABLE IV

FACTORS RESPONSIBLE FOR ENZYME RELEASE FROM BRUSH BORDERS

Brush borders (150 μg protein/ml) were incubated for 30 min at 37 °C in 50 mM potassium phosphate buffer, pH 7.0, in a final volume of 0.1 ml. Duodenal juice was added to a final protein concentration of 0.84 mg/ml, elastase, typsin and chymotrypsin to 0.2 mg/ml, pancreatic enzymes to 0.4 mg/ml and amylase and lipase to 1.0 mg/ml. When boiled duodenal juice was used, a volume equal to that used to give a protein concentration of 0.84 mg/ml was added. Pancreatic enzymes refers to the protein fractions obtained from Sephadex G-25 chromatography of duodenal juice.

Additions	Enzyme released		
	Sucrase (%)	Maltase (%)	Alkaline phosphatase (%)
Buffer	3	5	2
Duodenal juice	84	88	3
Boiled duodenal juice	3	5	5
Boiled duodenal juice and pancreatic enzymes	95	92	2
Pancreatic enzymes	3	4	3
Elastase	4	6	3
Boiled duodenal juice and trypsin and chymotrypsin	28	32	6
Boiled duodenal juice and elastase	82	93	4
Boiled duodenal juice and amylase	3	4	2
Boiled duodenal juice and lipase	3	5	3

incubated in buffer alone, very little sucrase or maltase (used as markers for large brush border proteins) were released from the membrane in soluble form (Table IV). However, the addition of duodenal juice caused 84 % of total sucrase activity and 88 % of maltase activity to be non-membrane bound. When duodenal juice was boiled, all releasing activity was destroyed. After separating macromolecular components of duodenal juice from bile salts, lipids and salts on Sephadez G-25, all the pancreatic enzyme activity was in this macromolecular fraction. Adding enzyme activity to boiled pancreatic juice restored releasing activity, although the enzymes alone had no effect. Trypsin and chymotrypsin could not substitute for the total pancreatic proteolytic activity, but elastase could do so under the conditions used. However, elastase alone had little effect.

The data in table IV suggested that total proteolytic activity and enzyme release were not necessarily correlated since elastase was much more capable of promoting release than trypsin or chymotrypsin. The pH necessary for enzyme release also suggested that factors other than proteolysis itself play an important role. The optimum pH for release of sucrase by duodenal juice was 7.0, although considerable release occurred between pH 6 and 8. On the other hand, proteolytic activity was much higher at pH 8 than pH 7, consistent with the pH optimum of some pancreatic proteases. Thus, it would appear that some relatively specific peptide bonds are broken at pH 7, leading to release of some large brush border proteins.

Relative specificity of enzyme release from brush borders

Both pancreatic enzymes and boiled duodenal juice were needed to promote enzyme release from brush borders (Table IV). The enzymes needed were most likely proteases since neither commercially purified amylase, lipase (Table IV), phospholipase C, ribonuclease, nor deoxyribonuclease could replace the enzyme fraction. Elastase was more active than trypsin or chymotrypsin. Furthermore, trypsin inhibitor (0.5 mg/ml) abolished the effect of elastase (0.2 mg/ml). Thus, no non-proteolytic contaminants of elastase were responsible for the observed activity. Using brush borders in vitro, the maximal sucrase release could be achieved by concentrations of purified elastase in the order of 0.1–0.2 mg/ml. When a large concentration of proteases was used in vitro ($>$ 1 mg/ml), the difference between elastase and trypsin diminished. These large concentrations were comparable to those found in vivo, elastase concentration in duodenal juice (using elastin as substrate) being 2.5 mg/ml, trypsin concentration 2.5 mg/ml, and cymotrypsin 1.3 mg/ml. Thus, elastase may not be uniquely active in enzyme release from brush borders.

The release of enzyme activity not only demonstrated specificity for the components of the duodenal juice which mediate this effect, but also for the brush border proteins solubilized. Under conditions where over 80 % of sucrase and maltase were released (Table IV), no alkaline phosphatase or trehalase were solubilized, and total activity of these latter enzymes remained constant. This is consistent with the location of the alkaline phosphatase within the brush border membrane. Furthermore, only 32 % of the total membrane protein was released, further documenting the specificity of the membrane protein solubilization.

Role of elastase in vivo

Because elastase was the most active protease releasing sucrase in vitro, we

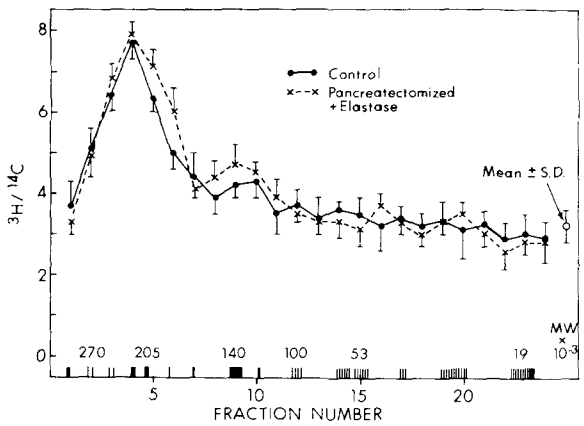

Fig. 2. Effect of elastase on turnover of brush border proteins in pancreatectomized animals. Experimental design was identical to Fig. 1, except that at 10 h before the [^{14}C]valine was injected and at each injection of isotope, 1.0 μg of elastase was injected intrajejunally. The $^3H/^{14}C$ ratio for a control experiment run at the same time was 3.2 ± 0.3.

tested its efficacy in vivo. This seemed especially important, since the in vitro data showed release of enzyme from brush borders using duodenal juice diluted 1 : 10. Thus, there are surely factors in vivo which protect the brush border against the effects of duodenal juice. Thus, the role of elastase in affecting brush border protein turnover was examined in vivo.

A double-labelled experiment was performed identical to that shown in Fig. 1, except that 10 h before labelled amino acid was given, and at each injection of labelled valine, 1.0 mg of elastase (Worthington Biochemicals) was added directly to the jejunal segment. As can be seen in Fig. 2, the addition of elastase restored the turnover of large brush border proteins to its normal high rate. The animals used for these experiments were judged to have pancreatic insufficiency because of vitamin B-12 absorption of 19 and 27 % and stools consistent with steatorrhea. Therefore, it seemed unlikely that turnover of large membrane proteins would have been normal without the addition of elastase, at least based on the data in Fig. 1.

DISCUSSION

The present results and our earlier data are consistent with the following scheme. New epithelial cells migrate along the intestinal villus, making protein at all levels [25]. Since enzyme content does not increase along the length of the villus, presumably enzyme turnover must occur to make room for the newly made brush border enzymes. Rapid disaccharidases turnover does in fact, occur [1, 2]. The brush border membrane is covered by a carbohydrate-rich "fuzzy coat" and protected by mucus, but is also exposed to high concentrations of pancreatic and biliary secretions. It would appear that pancreatic enzymes could play a role in stripping at least some of the proteins from the brush border. Since the disaccharidases function extracellularly [4], it is reasonable that they might be located externally on the membrane and be removed by intraluminal factors. Which of the pancreatic components is most

important in vivo is still unclear. Arvenitakis and Olsen [26] have reported an increased specific activity of disaccharidases in patients with chronic pancreatitis, lending support in man to the concept outlined above, based on data in small animals.

The data presented in Fig. 1 and Table II present evidence that in vivo pancreatic secretions play some role in the turnover of large brush border proteins. In partially pancreatectomized animals it was found that, compared with normal animals, disaccharidase levels were higher, relative turnover of large brush border proteins (including disaccharidases) was decreased, and relative synthetic rates were lower. Absolute synthetic rates cannot be determined because of the extensive reutilization of label in the intestinal mucosa [27]. This data can best be explained by a decrease in the degradation rate of large brush border proteins. Moreover, the pancreatic enzyme, elastase, leads to an increased turnover of these large proteins. The double-labelled experiments provide key, but not the only information, concerning degradation rates. Control experiments show that the conditions required for double-labelled turnover experiments have been met. Decreased turnover of protein should lead to increased amount of protein, and synthetic rates then fall to restore steady-state conditions. This was, in fact, observed, as enzyme activity increased (Table II) while relative synthetic rates fell (Table III). The change in enzyme activity and the observed in vitro release of disaccharidases suggested that those proteins involved in the turnover may be included in the pancreatic secretions.

Our data and that of Nordstrom [8] clearly differ from other experiments studying specificity of release of brush border enzymes in a different experimental setting. Nordstrom [6] found that cholecystokinin and secretin stimulated release of enterokinase and alkaline phosphatase (approx. 15 %) from the intestinal mucosa in vivo, whereas only 7–10 % of sucrase was released. The effect seemed related to the action of proteases and bile salts on the mucosa itself. However, there was no specificity of enzyme released, so that the phenomenon observed seemed to be different from the present data. Gotze et al. [7] found that cholecystokinin enhanced release of enterokinase, sucrase and alkaline phosphatase into the lumen of the small intestine of the rat and this release was augmented by bile salts. The effect was seen even with the pancreatic duct ligated. The explanation for the phenomena reported by these two groups is not clear. Perhaps these workers are reporting phenomena related to extrusion of whole cells into the lumen so that all brush border enzymes are involved. The lack of effect on alkaline phosphatase (Tables II and IV) seems to suggest that the present data may reflect different mechanisms.

Much of protein turnover in cell membranes is thought to be intracellular, but recent studies show that surface proteins are shed into the extracellular fluid. Agglutinin receptors and histocompatibility antigens are shed rapidly into the medium from cells in culture [28]. However, trypsin treatment of the cell did not change the rate of turnover, suggesting that replacement was independent of membrane damage. Our data show that pancreatic proteases may be necessary for the shedding of macromolecules from the brush border surface without necessarily producing membrane damage.

It is of interest that elastase might be active in the release of enzyme from brush borders, since its specificity is very broad and almost identical with that of papain [29], the plant enzyme most commonly used to solubilize brush border enzymes. It is intriguing to wonder whether one of the functions of elastase in the body is to help to regulate brush border protein turnover.

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