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DEVELOPMENT OF INTESTINAL BRUSH BORDER MEMBRANE PROTEINS IN THE RAT

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Summary

1. The proteins of the intestinal microvillus membrane have been studied during post-natal development in the rat (days 12—37).

2. In suckling animals (up to age 20 days), the majority of alkaline phosphatase, glucoamylase and lactase activities in the distal half of the intestine were located in the supernatant fraction (100000 × *g*, 60 min). These enzymes were attached to the membrane from the proximal intestine at all ages.

3. Alkaline phosphatase, maltase and lactase activities in the supernatant fractions chromatographed in Sephadex G-200 in positions similar to the corresponding membrane enzyme. Corresponding activities for lysosomal counterparts of maltase and lactase present in the supernatant fraction chromatographed differently. Moreover, pH optimum of the soluble enzymes was 9.2 for phosphatase and 5.5—6.0 for glycoamylase and lactase. The soluble lactase and alkaline phosphatase were inhibited minimally by *p*-chloromercuribenzoate, and sodium fluoride respectively. L-Phenylalanine (20 mM) did inhibit the soluble phosphatase by 90%. Thus, the soluble enzymes are not mainly of lysosomal origin, but have characteristics of membrane-bound enzymes.

4. Polyacrylamide gel electrophoresis in sodium dodecyl sulfate revealed 18 protein bands which were present in adult membranes. Two other proteins were unique for membranes of distal intestine in suckling rats. The proteins corresponding to known enzyme activity changed as expected with age (e.g. sucrase, maltase increased, lactase decreased). Most of the other proteins were also altered in amount during development. Thus, the changes in the microvillus membrane during development in the rat are not limited to specific enzymes.

Introduction

The small intestinal mucosa undergoes important changes during post-natal development. The activity of many brush border enzymes can be influenced by

hormones like cortisone [1,2,3] and thyroxine [4]. In addition, one of the brush border enzymes, maltase, had been found to be present in the soluble fraction in the suckling rat [5].

There have been reports of the protein composition of the microvillus membranes obtained from human jejunum [6], suckling rat jejunum [7] and from Swiss mouse duodenum [8]. However, systematic studies on all the brush border proteins during development of the rat from suckling to adult are lacking. In the present paper, we report the changes as a function of age involving brush border protein components and with soluble enzymes whose substrate specificity resembles certain brush border enzymes, e.g. lactase, maltase and glucoamylase.

Materials and Methods

Animals and tissue preparation

The rats used are the NLR Wistar strain obtained from the National Laboratory Animal Company, Creve Coeur, Missouri. The young rats remained with their mothers until the age of 12—28 days. The animals were fasted overnight and killed the next morning. The intestinal lumen was flushed with 50 ml of normal saline and excess fluid gently expressed by manual pressure. Intestinal homogenates, 10% (w/v), were prepared in 0.25 M mannitol, and the cytosol prepared by centrifugation at $100000 \times g$ for 1 h in a Beckman model L ultracentrifuge.

For the isolation of the membranes, the intestines were washed in 0.9% cold NaCl and cut into two halves to produce proximal and distal segments. The method of Schmitz et al. [9] used for isolation of membranes from human tissue was employed. When this method was used to isolate membrane from rat intestine, the microvillus membrane was slightly lighter with a density of 1.05 for suckling rats and about 1.105 for adult rats. These density values agree with the values reported by Galand and Forstner [7] for rats, and are slightly less than calculated value for 1.115 for human membranes [9].

Chemicals

Acrylamide, *N,N'*-methylene-bisacrylamide, ammonium persulfate, and *N,N,N',N'*-tetramethylethylene diamine were purchased from Eastman Kodak Company. β-Mercaptoethanol, Tris and Sephadex G-200 were obtained from Sigma Chemical Company. Sodium dodecyl sulfate was purchased from Mallinckrodt Chemical Company and Coomassie Brilliant Blue from Schwarz-Mann. All other chemicals were reagent grade and were obtained from Fisher Scientific Company. The molecular weight marker proteins were purchased from Sigma Chemical Company (St. Louis, Missouri) except for trypsin (Worthington Biochemicals, Freehold, N.J.).

Enzyme assays

Sucrase and lactase were used as brush border marker enzymes for the adult and suckling rats. The disaccharidases were estimated by the method of Dahlqvist [10], and leucyl napthylamidase as described by Nicholsen et al. [11]. Alkaline phosphatase was assayed according to the method of Forstner et al. [12]. Protein was estimated by the method of Lowry et al. [13].

Sodium dodecyl sulfate-gel electrophoresis

Slab gel electrophoresis with sodium dodecyl sulface (SDS) was done using 50 μg of the brush border membrane protein heated at 90°C for 5 min in 0.11% SDS by the method of Neville [14]. After staining and destaining, gels were scanned at 555 μm using a Gilford gel scanner. To locate specific enzymes on the gel, SDS was added to the membranes without heating and the electrophoresis was performed immediately afterwards at 5°C. The gel was sliced by a set of razor blades held between two solid supports and the width of each slice was 1.75 mm. Each gel slice was homogenized in 1.0 ml of 10 mM Tris · HCl, pH 7.4, for phosphatase and 10 mM sodium phosphate buffer, pH 6.0, for disaccharidase measurements. The homogenates were centrifuged at 20 000 × *g* for 30 min. The clear supernatants were used for enzyme assays as described by Maestracei et al. [15] except that lactase was assayed using 5-methyl umbelliferyl-β-D galactoside as substrate as described earlier [16]. For the determination of apparent molecular weights of the different protein bands on the gel the following marker proteins were used: β-galactosidase dimer (*Escherichia coli*, 260000), β-galactosidase (*E. coli*, 130000), phosphorylase *a* (muscle, 94000), catalase (liver, 60000), α-amylase (hog pancreas, 45000), trypsin (hog pancreas, 23300) and hemoglobin (15000). Solutions of these marker proteins were prepared according to Neville [14]. After staining the gels the position of each marker protein relative to dye front was calculated and a plot of the relative mobility versus logarithm of molecular weights was obtained. Molecular weight of brush border membrane proteins was then calculated from this curve. In all the gel runs an acrylamide concentration of 11% was employed. Gels analyzed quantitatively were stained for 16 h, destained for 72 h in the dish, and analyzed at 555 nm using a Gilford linear scanner. The area under each protein was calculated by planimetry. The total area under all peaks and the percent present as each protein were derived from these data.

Electron microscopy

The microvillus membrane fraction was fixed from 2 h to overnight at 4°C in 3% glutaraldehyde buffered with 0.2 M Na-cacodylate buffer, pH 7.4, containing 0.01 M $CaCl_2$. The fractions were washed several times in buffer, pelleted and suspended in 1% agar. The agar was cut into 1 mm cubes, post fixed in 1% OsO_4 in cacodylate buffer for 1 h at room temperature, washed in several changes of buffer, dehydrated in a graded ethyl alcohol series and embedded in Spurr's [17] embedding resin. Thin sections were cut out with a diamond knife on a Porter Blum MT 2 Ultra-microtome, stained with uranyl acetate and lead citrate and examined with a Hitachi HU-11C electron microscope at 75 KV.

Gel filtration on Sephadex G-200

90 ml of the 105000 × *g* supernatant from distal intestinal homogenates of 16 day rats were concentrated in an Amicon concentrator using a UM-10 membrane and were placed on a Sephadex G-200 column (2.5 × 90 cm). The proteins were eluted with 10 mM Tris · HCl buffer, pH 7.4, and 5-ml fractions were collected at a flow rate of 10 ml/h.

Solubilization of membrane-bound enzymes

The proximal intestinal homogenates (100.0 ml) were spun at 105000 × *g*

for 1 h. The resulting pellet was suspended either in 10 mM Tris · HCl buffer, pH 7.4, or 10 mM potassium phosphate buffer, pH 6.0. Maltase and lactase were solubilized, dialyzed and concentrated on an Amicon concentrator using a UM-10 membrane. Solubilization was effected by papain according to Galand and Forstner [5]. Suspension in 10 mM Tris · HCl buffer was used to solubilize alkaline phosphatase. The solubilization, dialysis and concentration were done essentially according to Doellgast and Fishman [18] except that the alkaline phosphatase activity was precipitated between 40 and 65% saturation of ammonium sulphate. The concentrated solubilized fractions were processed on a Sephadex G-200 column as described above. The enzyme activities recovered for the three enzymes after solubilization were in excess of 75%, resulting in purification of 7- to 12-fold over the starting material.

Results

Preparation of brush border membrane from suckling and adult rats

Lactase for suckling rats and sucrase for adult rats were used as brush border membrane markers since these two enzymes are present in the brush border membrane [7] and high levels of each are found in suckling and adult rats respectively [19]. There was an approximately 14- to 36-fold increase of specific activity of these enzymes during membrane purigication (Table I). Recovery of enzyme activity was greater (15—24%) from proximal intestine than from distal intestine (6—10%). Electron microscopy of the membrane preparation revealed a fairly homogenous preparation of vesicles from proximal bowel of suckling and adult animals (Fig. 1). A few vesicles that contain electron dense material were found in membrane preparations, particularly from the adult rats (Fig. 1, B and D). The dense vesicles probably comprise both microvillus core and membrane. Organelles other than electron dense vesicles could not be identified. Fig. 1C demonstrates a close up of membranes in vesicle and tubule form with attached fuzzy coat. Fig. 1D shows a high power view of dense material surrounded by a similar membrane. This material seems to represent a microvillus core surrounded by a microvillus membrane. Organelles other than microvilli could not be identified.

TABLE I

PURIFICATION OF MICROVILLUS MEMBRANE FROM SUCKLING AND ADULT RATS

The values represent mean ± S.E. on 4 separate membrane purifications. Suckling rats were 12—14 days old and adult 37 days old. For each purification 6 suckling rats and 2 adult rats were used.

Fraction	Lactase from suckling rats		Sucrase from adult rats	
	Total activity (units)	Specific activity (units/mg protein)	Total activity (units)	Specific activity (units/mg protein)
Homogenate				
Proximal	12.67 ± 1.2	0.044 ± 0.003	50.00 ± 7.00	0.042 ± 0.003
Distal	10.30 ± 1.3	0.044 ± 0.002	12.56 ± 1.40	0.017 ± 0.002
Membrane				
Proximal	1.83 ± 0.1	1.59 ± 0.16	11.90 ± 1.10	1.07 ± 0.10
Distal	0.60 ± 0.04	0.65 ± 0.06	1.20 ± 0.11	0.24 ± 0.03

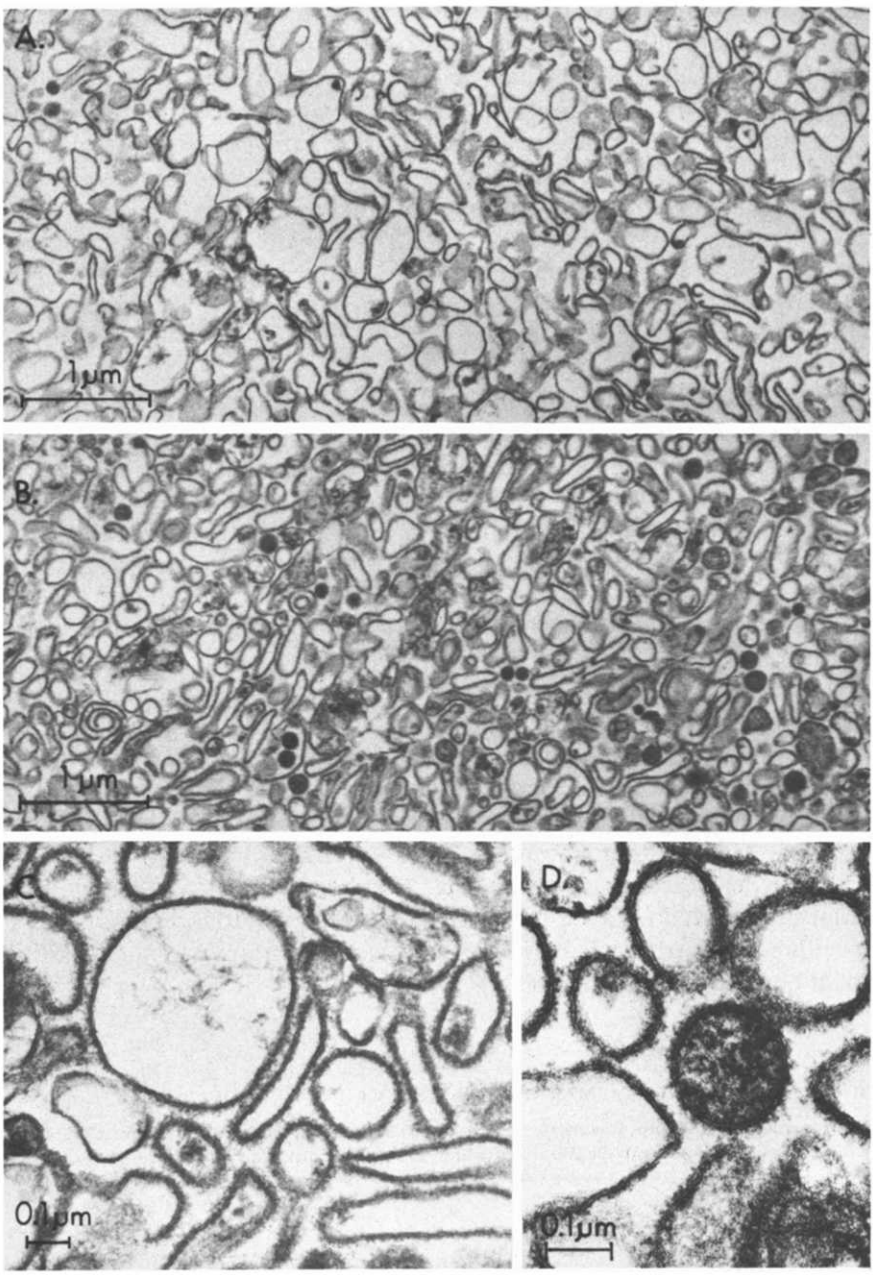

Fig. 1. Electron microscopic representation of microvillus membranes from proximal intestine. A. 12-day old rats. This photograph is a typical picture chosen after examination of 42 photographs from six grids obtained from six blocks in three different membrane preparations. B. 37-day old rats. This represents a typical appearance chosen from 24 photographs taken from four grids from four blocks in two different membrane preparations. C. Membrane fragments from vesicles or tubules of various sizes of 37-day old rats. Both sides of the membrane show some fuzzy material. D. Membrane surrounding electron dense material from 37-day old rats.

Solubility of brush border enzymes as a function of age

In the proximal part of the intestine, membrane-bound enzymes, alkaline phosphatase, lactase and maltase-glucoamylase were mostly particulate, with only 10—25% of the activity being soluble in suckling rats (Fig. 2A). This percentage slightly decreased at 37 days of age. However, in the distal half of the intestine, these enzyme activities were distributed differently so that about 45—70% were recovered in the soluble fraction after centrifugation at 105000 $\times g$ for 1 h (Fig. 2B). The proportion of activity which was soluble decreased to about 2—25% in the adult rats. This phenomenon was not observed with any other brush border enzyme (e.g. sucrase, aminopeptidase, trehalase). We next examined the various enzyme activities in the soluble fraction in suckling animals to see whether they had properties similar to those of their membrane-bound counterparts, or whether they were lysosomal, since lysosomal enzyme activity is higher in suckling than adult intestine.

Gel filtration on Sephadex G-200

The 105000 $\times g$ supernatant fraction from the distal intestinal homogenates of 16-day old suckling rats was fractionated in order to separate the different enzyme activities by size. Alkaline phosphatase emerged as a major peak near the void volume (Fig. 3), identical to the position of brush border phosphatase. Alkaline phosphatase activity also eluted at this point (Fig. 3, arrow). The neutral maltase-sucrase which is present at low levels in suckling rats, emerged near the void volume, whereas the neutral maltase-glucoamylase emerged later on, since it is retarded by Sephadex. Again, it position was similar to that for the brush border enzyme. Acid maltase, however, eluted earlier than neutral maltase activity. The neutral lactase activity (pH 6.0) was found in two peaks distinctly separated. The first lactase peak (pH 6.0) also showed activity against the substrate lactose at pH 3.0, albeit at a slower rate. Acid lactase activity eluted somewhat later than the neutral lactase peak. These results are given in Fig. 3. Alkaline phosphatase, maltase and lactase solubilized by butanol and papain from the proximal intestinal particulate fraction of 16-day old rats were

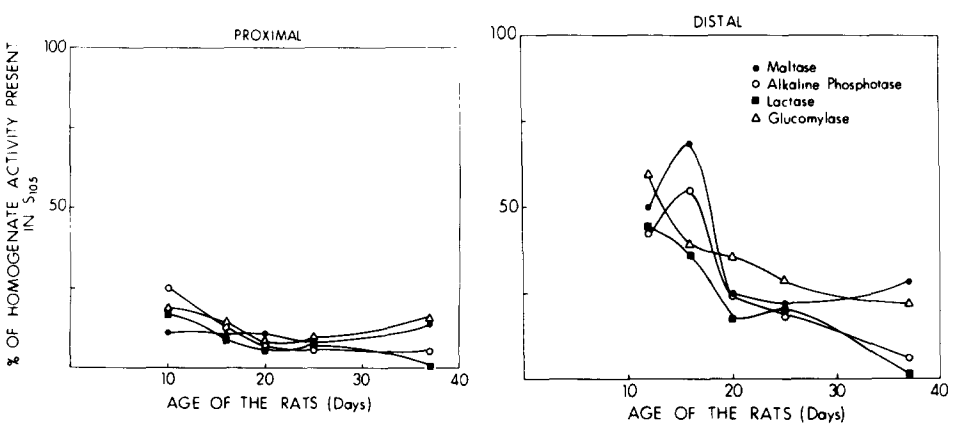

Fig. 2. Enzyme activity in the supernatant fraction of intestinal homogenates. Tissue was fractionated and enzyme activity assayed as described in Materials and Methods. These calculations are average of six determinations.

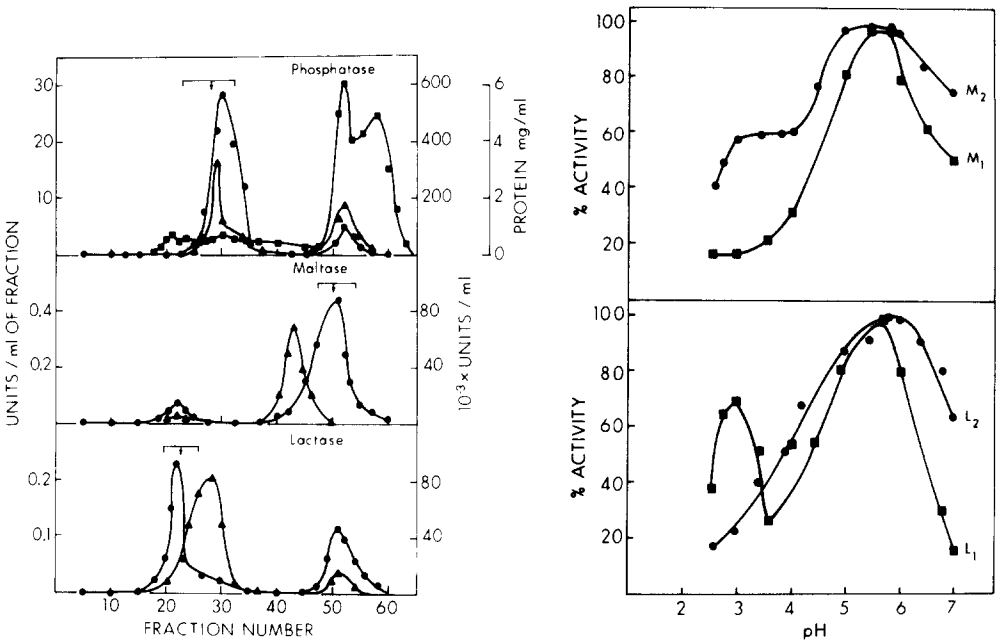

Fig. 3. Chromatography of a concentrated 105000 × *g* supernatant fraction from distal intenstinal homogenates of 16-day old rats on a Sephadex G-200 column. Tissue fractionation and assay of enzyme activity was carried out as described in Materials and Methods. ■, protein; ●, enzyme activity measured at pH 9.2 (phosphatase) and pH 6.0 (maltase and lactase); ▲, enzyme activity measured at pH 5.2 (phosphatase) and pH 3.0 (maltase and lactase). The total volume of the column was 441 ml: void volume was 130 ml. The arrows represent the location of the three enzymes from the solubilized extracts of a 105000 × *g* pellet fraction obtained using proximal intestinal homogenates of 16-day old rats.

Fig. 4. pH profiles of enzyme activity eluted from Sephadex G-200 chromatography. 0.1 M sodium citrate-phosphate buffer was used. L_1 and M_1 correspond to enzyme activity eluting at or near the void volume, L_2 and M_2 correspond to activity eluting after the void volume. Enzyme activity was analyzed as described in Materials and Methods.

eluted in identical positions to the enzyme activity from the supernatant fractions, as indicated by the arrows in Fig. 3.

pH optima of the soluble enzymes

The influence of pH on the activity of the different soluble enzymes separated on Sephadex G-200 was examined to determine if this property was similar to that of the brush border enzyme. As can be seen in Fig. 4, the higher molecular weight maltase (M_1) had a pH optima of 5.5 and is the maltase-sucrase which is present in very low amounts in suckling rats. The maltase-glucoamylase which was eluted later on (M_2) had a shoulder at pH 3.0, but a pH of optimal hydrolysis at 5.5. This peak could represent a mixture of brush border and lysosomal maltase-glucoamylase, but is clearly not all lysosomal enzyme, which has a pH optima of 3.0 [5]. The major soluble alkaline phosphatase from distal intestine had a pH optima of 9.2 (not shown) similar to that for the phosphatase of brush border origin. Each of the two lactase peaks separated on Sephadex G-200 had a pH optimum at pH 6.0, but the higher molecular weight lactase (L_1) also had a second peak of activity at pH 3.0.

Inhibition of soluble enzymes

Neither peak of lactase activity measured at pH 6.0 was inhibited significantly by the addition of 0.1 mM *p*-chloromercuribenzoate (>90% activity retained). This concentration uniformly inhibits lysosomal lactase activity [20].

The major alkaline phosphatase activity from Sephadex G-200 chromatography was inhibited by L-phenylalanine as expected for brush border phosphatase. L-Phenylalanine (4 and 20 mM) caused a decrease in activity of 50% and 90%, respectively. On the other hand, sodium fluoride, which inhibits acid phosphatase but not brush border phosphatase, had no effect on the soluble enzyme activity at concentrations up to 20 mM.

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis in SDS was carried out to examine the protein components of the membrane brush border during development. Electrophoresis was performed after treating the brush border membrane preparation (usually 50 μg protein) with 1% SDS at 90°C for 5 min. The densitometric tracing of the protein stain with Coomassie Blue is shown in Fig. 5 for the proximal and the distal parts for ages ranging from 12 to 37 days. As can be seen, 18 proteins and/or subunits with a molecular weight range of 34—380 · 10^3 can be identified. Those proteins with associated enzymatic activities have been identified. Table II gives a complete list of the membrane protein and subunits from proximal intestine, with their molecular weights and the changes they undergo with development.

Certain patterns can be seen which were found in common along the entire intestine. First, amounts of protein corresponding to known enzymes follow approximately as expected from the previously determined patterns of enzyme activity. That is, maltase (protein 2), sucrase (protein 6), aminopeptidase (protein 7) and trehalase (protein 11) increase with age, whereas lactase (protein 4) decreases. Second, more extensive changes occur than can be appreciated by measuring enzyme activity alone. Proteins 9,10, 15, and 17 increase with age and proteins 12 and 13 decline. Protein 15 has a molecular weight similar to that of actin [21] and could possibly represent that protein. Third, one protein located between proteins 6 and 7 in both proximal and distal intestine disappears with increasing age. Finally, some proteins in the distal intestine behave uniquely. Protein 13a is only present during suckling and disappears with development. Protein 12 is much more prominent in distal suckling gut and diminishes markedly with age.

The changes observed qualitatively in Fig. 5 were defined quantitatively by determining the area under the curve for each protein. The total of all these areas was obtained and the area for each protein measured as a percent of this total. In this way, the data recorded in Table II is comparable to a specific activity/mg protein for the membrane.

The patterns seen in Fig. 5 referred to above were confirmed by this analysis. Protein 15 rises to about 30% of brush border protein. This increase could represent either the degree of attachment of this protein (possibly actin) to the true membrane or a change in its synthetic or degradation rate. At first glance, the increase in protein 15 might partially explain the anomalous fall in the percent of membrane protein as alkaline phosphatase (protein 8) with increasing

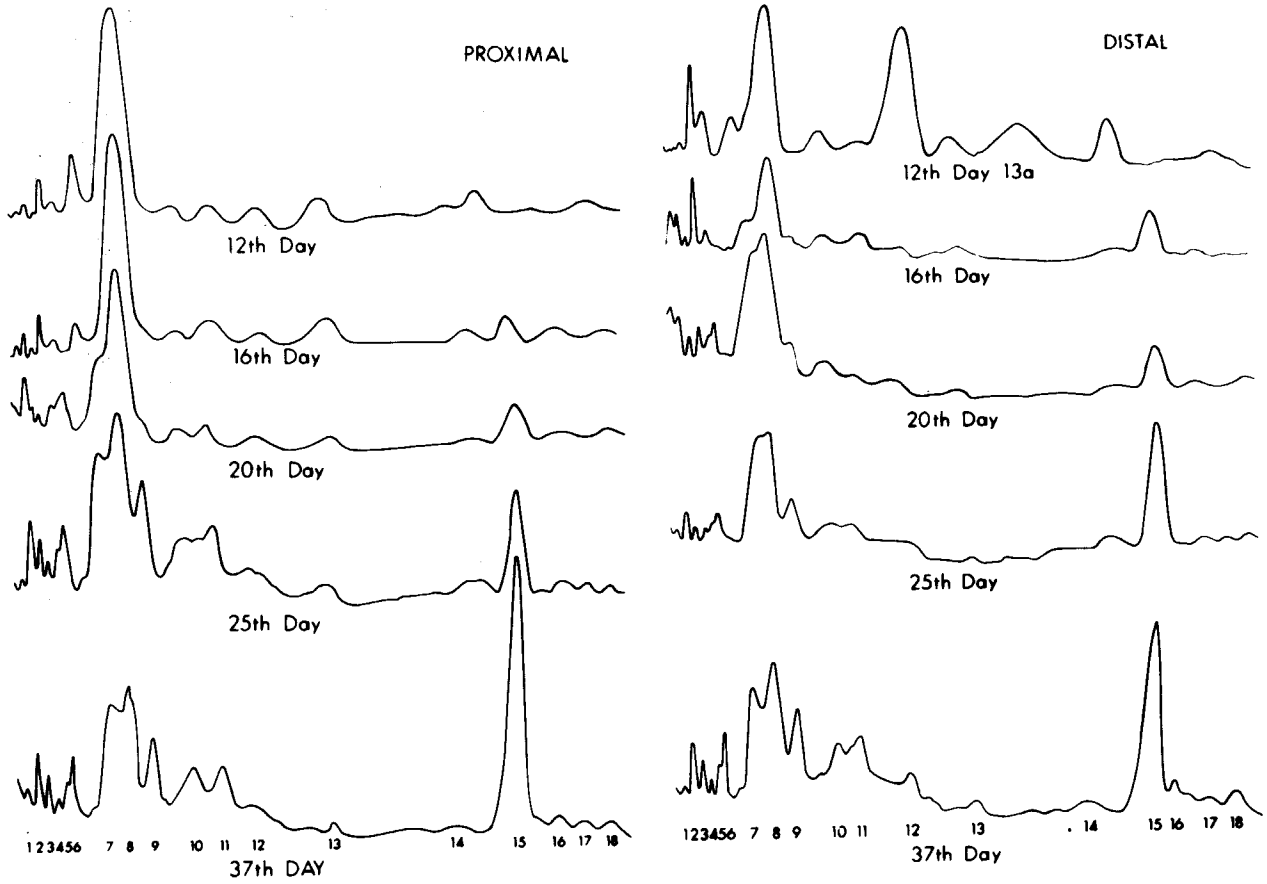

Fig. 5. Scans of polyacrylamide SDS gel of microvillus membranes from proximal and distal rat intestine. Membranes were purified and treated as described in Materials and Methods. The densitometric tracing was done after staining membrane proteins with Coomassie Brilliant Blue. The origin of the gel is at the left (anode) and the migration of the proteins is toward the right (cathode).

TABLE II

COMPOSITION OF PROXIMAL MICROVILLUS MEMBRANE PROTEINS DURING DEVELOPMENT

The time of development (12, 20 and 37 days) refers to time after birth. Enzyme activities were located in SDS gels described in Materials and Methods. Gels were stained, analyzed at 555 nm, and the area under the curve calculated as described in Materials and Methods. The data are the mean of two separate electrophoretic patterns from each of two separate experiments.

Protein (No.)	Molecular weight ($\times 10^{-3}$)	Enzyme activity	Membrane proteins		
			12 d (%)	20 d (%)	37 d (%)
1	380		0.4	0.7	1.2
2	340	Glucoamylase	0.6	4.5	2.4
3	300		0.1	1.3	1.0
4	270	Lactase	2.4	0.9	0.5
5	240		2.1	1.0	0.8
6	215	Sucrase	0	7.3	2.2
6a	200		6.5	0.8	0.4
7	175	Aminopeptidase	0	12.8	12.5
8	145	Alkaline phosphatase	59.5	40.0	11.7
9	130		0	3.5	6.1
10	107		3.9	3.1	9.7
11	95	Trehalase	4.3	3.1	9.7
12	77		4.3	2.1	3.8
13	65		6.1	2.4	0.5
13a	56		0.70	—	—
14	48		5.5	1.4	1.2
15	43.5		0.2	9.6	31.4
16	39.0		0.4	3.1	1.8
17	36.5		0	0	1.2
18	34.0		0	2.6	1.0

age. Alkaline phosphatase, whose specific activity rises during development, shows a marked decline when expressed as per cent of total membrane protein (Table II). However, if the increase in protein 15 (and other proteins) were the major reason for the decline in phosphatase content, then other proteins should also show a marked decrease in per cent. When the data in Table II is recalculated after elimination of protein 15, the percentages for other proteins vary very little, and the direction of change remains the same in all cases. For example, alkaline phosphatase represents 59.5%, 40% and 11.7% in the different age groups (Table II). If protein 15 is treated as a membrane "contaminant" and not included in the calculations, the percentages would be 59.5%, 44% and 17% respectively. On an absolute basis, moreover, phosphatase also shows a decline with age, since the height of protein 8 decreases with age (Fig. 5).

Discussion

Interpretation of changes in brush border proteins depends upon the degree of purification of the organelle. Morphologically the membrane preparations from both the suckling and the adult rats were satisfactorily pure for this study. A few dense particles could be seen, probably representing contamination by brush border cores. The core has been shown to contain mainly low molecular weight proteins [22]. The preparation from distal intestine did not

appear as purified as that from proximal intestine, either morphologically or by enzyme activity (Table I).

Not all brush border enzyme activity is associated with the brush border during development. Galand and Forstner [5] reported that in suckling rats nearly 44% of the brush border maltase activity is recovered in the supernatant fraction. We confirm this finding, and our results show that this high solubility of a brush border-like enzyme is not unique to maltase alone (Fig. 2). Other enzymes of the brush border (e.g., alkaline phosphatase and lactase) are also present in high amounts in the supernatant fraction.

These soluble enzymes are most likely related to their brush border counterparts. In the case of maltase, the major peak on Sephadex G-200 corresponds with the brush border maltase-glucoamylase. The pH curve, however, suggests some contamination from the lysosomal acid maltase. This fraction has been separated by Galand and Forstner into two fractions of equal activity representing in equal amount the brush border maltase-glucoamylase and the lysosomal glucoamylase [5]. Our major peak of maltase activity also probably has an equal mixture of the two enzymes, since neutral maltase has about 18% activity at pH 3.0 and acid maltase had nearly 58% activity at pH 6.0. In the case of alkaline phosphatase, nearly all of the soluble phosphatase activity at pH 9.2 is due to the brush border-like enzyme since the activity of acid phosphatase at pH 9.2 and the activity of alkaline phosphatase at acid pH is virtually zero. L-Phenylalanine, a potent inhibitor of brush border alkaline phosphatase [23], also inhibits soluble enzyme. Sodium fluoride, on the other hand, inhibits acid phosphatase [24] and not alkaline phosphatase, again showing that the bulk of the soluble phosphatase is of brush border origin. Once again, as with maltase, lactase of brush border origin seems to be the major lactase present in the cytosol of suckling rats. Both peaks from Sephadex G-200 chromatography have a pH optimum of 5.5 and very slight (10%) inhibition by *p*-chloromercuribenzoate was found. However, contamination of the L_1 peak by lysosomal acid β-galactosidases is possible.

The presence in the cytosol of enzyme activities similar to those usually associated with membranes (alkaline phosphatase, maltase and lactase) in the distal half of suckling rat intestine cannot yet be explained. These enzymes could appear in the soluble fraction as the result of mechanical fragility of the membrane during preparation. However, the soluble enzymes appear to have the size of individual enzymes and not membrane fragments (Fig. 3). Solubilization has been suggested as a prerequisite of degradation by the lysosomes [25]. Solubilization by luminal enzymes seems to be unlikely because (a) the level of pancreatic proteases in suckling rats is very low [26] and (b) alkaline phosphatase is neither inactivated nor solubilized by trypsin [27]. Finally, membrane bound enzymes may simply be held less rigidly to membranes during suckling or may not yet have become attached to the membrane. Since these larger proteins would become glycosylated in the Golgi apparatus, it would seem unlikely that they were free in the cytosol.

In the present study, 18 distinct protein bands were identified in microvillus membranes isolated from both proximal and distal parts of the intestine. The molecular weight ranged from 380000 to 34000. In their studies on the membrane proteins of mouse duodenum, Billington and Nayadu [8] have obtained

17 protein bands with molecular weight ranges from 250000 to 16000. Maestracci et al. [6], using human intestinal membranes isolated from jejunum and ileum, have identified 23 protein bands with molecular weights ranging from 400000 to 25000. The difference between these studies could be due to the species to the different degrees of purity of the membrane preparations or to difficulties encountered in estimating large molecular weight proteins.

In the present study, there is a clear indication of molecular reorganization of most of the membrane proteins during development. Proteins that are added onto the developing proximal membrane are (a) maltase (protein 2), molecular weight 340000; (b) sucrase (protein 6), molecular weight 215000; (c) aminopeptidase (protein 7), molecular weight 175000; (d) protein 9, molecular weight 130000; (e) protein 15 molecular weight 43500; and (f) proteins 17 and 18, molecular weights 36500 and 34000 respectively. More or less similar patterns exist for the distal intestinal membranes. Among the proteins which decrease with development are (a) lactase (protein 4), molecular weight 270000; (b) protein 6, molecular weight 145000; and (d) protein 13, molecular weight 65000. Uniquely in the proximal intestine, protein 13a levels are very low, whereas in the distal intestine it represents about 13.6% of the membrane protein in suckling rats and disappears during development. Similar changes can also be noticed with protein 12 in the distal intestine.

One of the striking freatures in the present study is the prominence of protein band 15 of molecular weight 43500 and the steady increase in the levels from suckling to adulthood. It is possible that this apparent increase during development may be due to an increased attachment of this protein to the membrane rather than altered synthesis. Our electron micrographs show more dense vesicles in the adult than in the suckling animals (Fig. 1, A and B), suggesting that the protein may be a component of these electron dense structures.

Our results confirm prior results concerning changes in some intestinal enzyme levels during development. Rubino et al. [19] have shown that maltase activity is maximal around 20 days of age and decreases to about one-half the maximal levels in adults. Doell and Kretchmer [28] have shown that lactase activity is highest at birth and falls to less than 10% of the levels in adults. Our results show a similar decrease of lactase protein.

Galand and Forstner [7] have shown that cortisone induces the appearance of proteins of molecular weight 400000, 170000 and 140000. Our studies show that proteins of molecular weight 380000, 175000 and 130000 appear during development when cortisone levels are rising. Although the normal developing membrane and the cortisone-inducing maturation may not be the same, at least in this respect they appear to be similar.

One of the important aspects of the developing intestinal membranes is the high proportion of alkaline phosphatase protein in suckling-rat membranes. During development the per cent falls from about 58 to 10 for proximal membranes and from about 37 to 17 for distal membranes. This does not correspond with the rise in enzymatic activity of alkaline phosphatase seen during development. Cortisone is known to activate pre-existing enzymatic proteins like alkaline phosphatase in cells cultures [29] and it may have a similar effect in the maturation of the mouse intestine [30], which at 11 days contains an enzymically inactive material that cross reacts with antibodies of the alkaline

phosphatase of the mature state [31]. This activation of membrane-bound enzymes by cortisone without induction of more enzymic protein may occur only with alkaline phosphatase and not with maltase and sucrase, since Galand and Forstner [7] have shown that cortisone actually increases maltase and sucrase protein. It is possible, of course, that protein 8 is not entirely composed of alkaline phosphatase during the suckling period, but that another (or other) protein, which later decline in amount, comigrate with it.

Acknowledgements

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