Biochimica et Biophysica Acta, 323 (1973) 113–124

BBA 76446

PROTEINS AND GLYCOPROTEINS OF THE HUMAN INTESTINAL BRUSH BORDER MEMBRANE*

D. MAESTRACCI,** J. SCHMITZ,*** H. PREISER and R. K. CRANE §

College of Medicine and Dentistry of New Jersey, Rutgers Medical School, Department of Physiology, Piscataway, N. J. 08854 (U.S.A.)

(Received May 1st, 1973)

SUMMARY

Proteins associated with human jejunal and ileal epithelial brush borders and their subfractions were solubilized with dodecyl sulfate and separated by electrophoresis on acrylamide gels. Twenty-three bands, at least, have been found in the brush border membrane protein patterns, corresponding to a heterogeneous group of polypeptides of molecular weights ranging from 25000 to over 400000. The protein patterns obtained from jejunum and ileum are strikingly different. Thirty bands have been found in the protein subunit patterns with the most prominent band centered at 46000–50000 daltons. Two classes of proteins were present in the brush border, the largest polypeptides belonging to the membrane, the smallest to the core fraction. About 13 glycoproteins with apparent molecular weight varying from 50000 to over 400000 have been detected in the brush border membrane. Most of them have been identified as membrane proteins.

INTRODUCTION

The development of a variety of methods for the isolation and purification of plasma membranes for erythrocyte, liver, kidney, intestine and other tissues (for review see ref. 1) and the introduction of detergents into the field protein chemistry has permitted fractionation and analysis of membranes. The most commonly used method involves solubilization of the proteins by sodium dodecyl sulfate and their subsequent electrophoresis. The proteins are converted into disorganized, random coiled polypeptides, to which sodium dodecyl sulfate binds very tightly and in a constant mass ratio[2]. The molecular weights of the polypeptides thus obtained can be estimated by sodium dodecyl sulfate–gel electrophoresis[3,4].

* Portions of this paper were reported at the fifteenth Annual Meeting of the Canadian Federation of Biological Societies, and the 14th Czechoslavak Congress of Gastroenterology in Prague, June, 1972.

** Present address: Département de Médecine, Centre Hospitalier Universitaire, Sherbrooke (P.Q.) Canada.

*** Present address: Unité de Recherches de Génétique Médicale Hôpital des Enfants-Malades, Paris, France.

§ To whom reprint requests should be addressed.

With this technique, it was possible to establish the general heterogeneity of plasma membrane proteins (for review see ref. 5). Although previous data were few, the same heterogeneity seemed to occur in the brush border membrane of the intestinal epithelial cells[6–8]. In these earlier studies, however, electrophoresis was carried out in a continuous system. Since discontinuous techniques are far superior[9,10] and provided high resolution patterns of sodium dodecyl sulfate-solubilized membranes, a multiphasic buffer system[10] was used in the present investigation. The protein composition of human intestinal brush border membranes prepared from jejunal and ileal surgical specimens was studied by sodium dodecyl sulfate electrophoresis with and without disulfide-bond cleavage. Information has been obtained on the number and size distribution of the proteins, protein subunits and glycoproteins of the brush border membrane. This is the subject of this paper.

MATERIALS AND METHODS

Chemicals

Acrylamide, *N,N'*-methylene-bisacrylamide, ammonium persulfate, *N,N,N',N'*-tetramethylethylenediamine and 8-anilino-1-naphthalene sulfonic acid (magnesium salt) were purchased from Eastman Kodak Co. Mercaptoethanol, dithiothreitol, HIO_4, Tris, were obtained from Sigma Chemical Co. Sodium dodecyl sulfate was purchased from Mallinckrodt and Coomassie brilliant blue from Schwartz–Mann. The other chemicals were obtained from Fisher Scientific Co.

Membrane preparation

Membranes isolated as described in the preceding paper, were in a high state of purity as judged by electron microscopy and by enzymatic analyses[11]. They showed unchanging gel patterns during two weeks when stored at −20 °C.

Suspensions of membranes were prepared in 0.05 M Na_2CO_3 or distilled water and were clarified by the addition of 2% sodium dodecyl sulfate. When the subunits were desired, 10% by volume of β-mercaptoethanol was added.

Acrylamide-gel electrophoresis

Polyacrylamide-gel electrophoresis was performed using the multiphasic buffer system calculated from theory by Jovin *et al.*[12] and modified by Neville[10], for sodium dodecyl sulfate (running pH 9.5). According to Hjerten[13] upper and lower gels are 3.2×6.25 and 11.1×0.9, respectively. They are polymerized with 0.15% *N,N,N',N'*-tetramethyethylenediamine and 0.05% ammonium persulfate at room temperature using a 40% sucrose solution to insure flat reproducible surfaces. After complete polymerization (30 min) the sucrose is removed prior to casting the upper gel. Distilled water is layered over the upper gel solution[14] and removed after polymerization (30 min). The gels 0.5 cm in diameter, 10 cm long are run at 1.5 mA per tube at room temperature during 30 min before layering the samples[15]. For electrophoresis, 2 parts of solubilized brush borders or their subfractions were mixed with 2 parts of upper gel buffer. 20–60 μg of protein (as determined by the procedure of Lowry *et al.*[16]) were generally applied except for the study of glycoprotein when loads from 100 to 200 μg of membrane protein were used. After electrophoresis carried out at 1.5 mA per gel for 90 min at room temperature the gels were removed and fixed in 12.5% trichloroacetic acid for 1 h[17].

Protein staining and destaining

This procedure was done according to Weber and Osborn[4] as modified by Neville[10] except that the destaining temperature was increased to 65 °C.

Schiff staining

In order to avoid artifactual staining of some non-carbohydrate-containing proteins, we have used the method of Glossmann and Neville[18]. 8-Anilinonaphthalene sulfonic acid is used to visualize the protein band after Schiff staining[18,19].

Determinations of apparent molecular weights

Relative mobilities were calculated by comparison with the migration of the marker dye bromophenol blue.

Proteins used as markers are listed in Table I along with their sources and molecular weights[10,20]. Individual protein concentrations generally ranged between 0.8 and 3 μg per sample. When available, chromatographically purified or electrophoretically homogeneous proteins were purchased. Solutions of marker proteins were prepared according to Neville[10]. When the dimer form of thyroglobuin and the tetramer forms of phosphorylase A and catalase were desired, the proteins were dissolved in 0.1% sodium dodecyl sulfate without heating or the addition of β-mercaptoethanol. Molecular weights were calculated from the migration of markers of known molecular weights (Table I). The position of each marker relative to the dye front was measured after staining and a plot of the logarithm of molecular weight *versus* relative mobility was drawn. The molecular weights of the brush border membrane proteins were then determined from this curve. Molecular weights higher than that of phosphorylase A (370000) were estimated by extrapolation of the plot.

The gels were photographed using a plexiglass gel support[21] and a medium yellow filter. The cataloguing of the membrane protein bands was done according to Neville and Glossmann[22], wherein, for example, the number 1.420 indicates a relative intensity of 1, as the highest and a molecular weight of 420000 Daltons.

TABLE I
PROTEINS USED AS MARKERS

Code	*Protein*	*Molecular weight* $\times 10^{-3}$*	*Source*
1	thyroglobulin (dimer)	669	Schwarz/Mann
2	phosphorylase A (tetramer)	370	Sigma
3	catalase (tetramer)	232	Worthington
4	β-galactosidase	130	Sigma
5	phosphorylase a	94	Sigma
6	bovine serum albumin	68	Sigma
7	ovalbumin	45	Worthington
8	chymotrypsinogen A	25	Worthington
9	myoglobin	17.2	Schwarz/Mann
10	cytochrome *c*	12.38	Sigma
11	glucagon	3.5	Sigma
12	bacitracin	1.45	Schwarz/Mann
13	trypsin inhibitor (soy bean)	0.196	Calbiochem

* References for molecular weights[10,20].

RESULTS

Log of molecular weight versus relative mobility

The sulfate–borate system stacks protein–dodecyl sulfate complexes over a molecular weight range of 200 to more than 370000. Fig. 1 shows the curve obtained when values of relative mobility (calculated from 11.1×0.9 gels) are plotted against the molecular weight on a semilog scale. When data from 5×0.9 gels are plotted

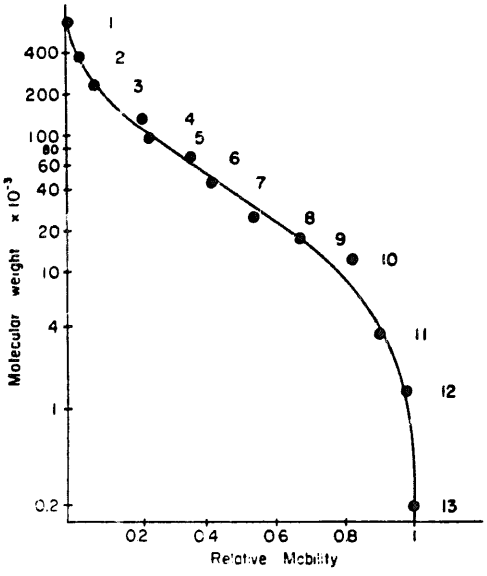

Fig. 1. Plot of the logarithm of the protein molecular weights of a series of protein–sodium dodecyl sulfate complexes *versus* their relative mobilities on gel (11.1 × 0.9). Numbers refer to protein code in Table I. Relative mobilities were calculated as described under Materials and Methods. Each point in the figure represents the mean of eleven determinations. The standard errors of the means of the relative mobilities were less than 0.01. This value is too small to be represented as it falls within the filled circles used.

in the same manner, the results are similar and the impression is given that the true nature of the function is sigmoidal. The curve connecting the high-mol.-wt points is hyperbolic. In the range of 15000–120000 a straight line can be drawn and four markers lie on the line (deviations in mol.wt less than 5%). The points for cytochrome *c*, chymotrypsinogen and phosphorylase A shows respective deviations of 25, 20 and 15% from this line. In the range of 370000 to 669000, the true nature of the curve remains to be confirmed. Thyroglobulin does not enter the gel, phosphorylase A does, and the curve between their corresponding points was drawn by extrapolation.

Electrophoretic fractionation of brush border membrane proteins

The jejunal and ileal protein patterns observed in four different preparations were remarkably reproducible and revealed polypeptide heterogeneity (Figs 2 and 3). In all preparations studied, the core fraction (IV) was not pure but always contaminated with variable amounts of brush border membranes[11].

Jejunal protein pattern

Comparison of gels P_2 (brush border) and I, II, III, IV (fractions obtained

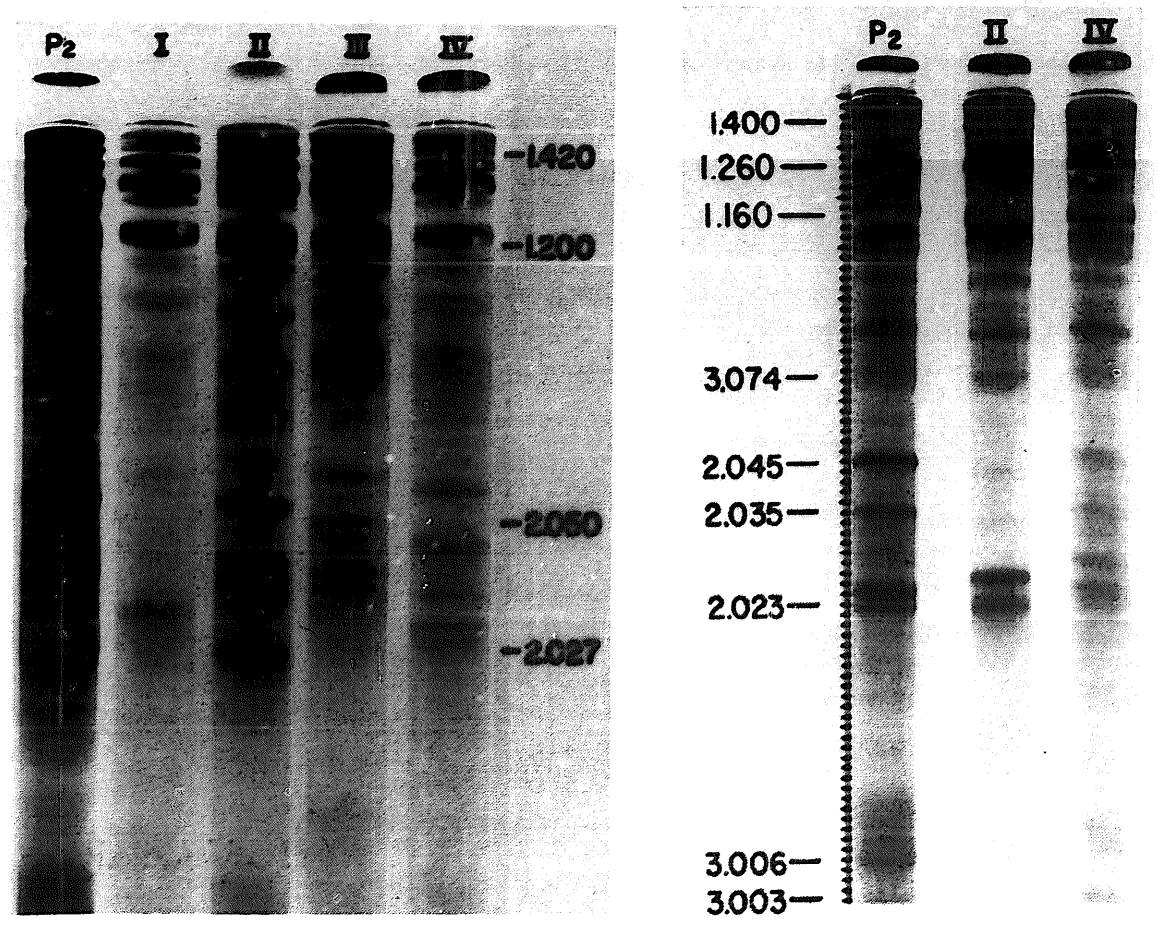

Fig. 2. Electrophoretic protein patterns obtained from jejunum. Gels P_2 (brush border) II (membrane) and IV (core fraction) were loaded with 53, 56 and 48 μg of protein, respectively. Some proteins cannot enter the upper gel. Electrophoresis (anode at the bottom) was carried out at 1.5 mA per gel for 90 min. Staining and photography were described under Materials and Methods.

Fig. 3. Electrophoretic protein patterns obtained from ileum. The gels were loaded with 45 μg of protein and stained with Coomassie brilliant blue. Fractions P_2, II and IV are respectively, entire brush borders, brush borders membranes and core fraction. The bands 2.045, 2.035, 3.006, 3.003 present in Fraction P_2 are missing in II.

after Tris disruption of the brush borders[11]) shows (Fig. 2) that each fraction has between 3 and 5 prominent bands and at least 16 less intense bands. Gels P_2 (brush border) and II (membrane) have prominent bands centered at positions corresponding to about 50000, 200000 and 320000 daltons. Two other bands with a molecular weight higher than 400000 are prominent too. 23 bands are found in the membrane fraction (Gel II); 21 with molecular weight varying from 27000 to 420000, two others with a molecular weight higher than 420000.

Ileal protein patterns

The patterns obtained from entire brush borders (Fraction P_2), brush border membranes (Fraction II) and core fraction (Fraction IV) are shown in Fig. 3. Although poorly seen in the photograph, the bands 2-045, 2-035, 3-006 and 3-003 present in Fraction P_2 and Fraction IV are missing in Fraction II. 26 proteins were found in the brush border membrane with molecular weights varying from 23000 to more

than 400000, two major proteins only, having a molecular weight less than 74000. Comparison of gels of Fractions P_2 and II shows that the membrane fraction includes many, but not all of the proteins found in the brush border.

Comparison between the protein patterns obtained from jejunum and ileum

The comparison of the gels of Fraction II (brush border membranes) obtained from jejunum (Fig. 2) and ileum (Fig. 3) shows marked differences in their electrophoretic patterns. However, these electrophoreses were performed at different times and so, for a proper comparison, brush border membrane prepared from jejunum

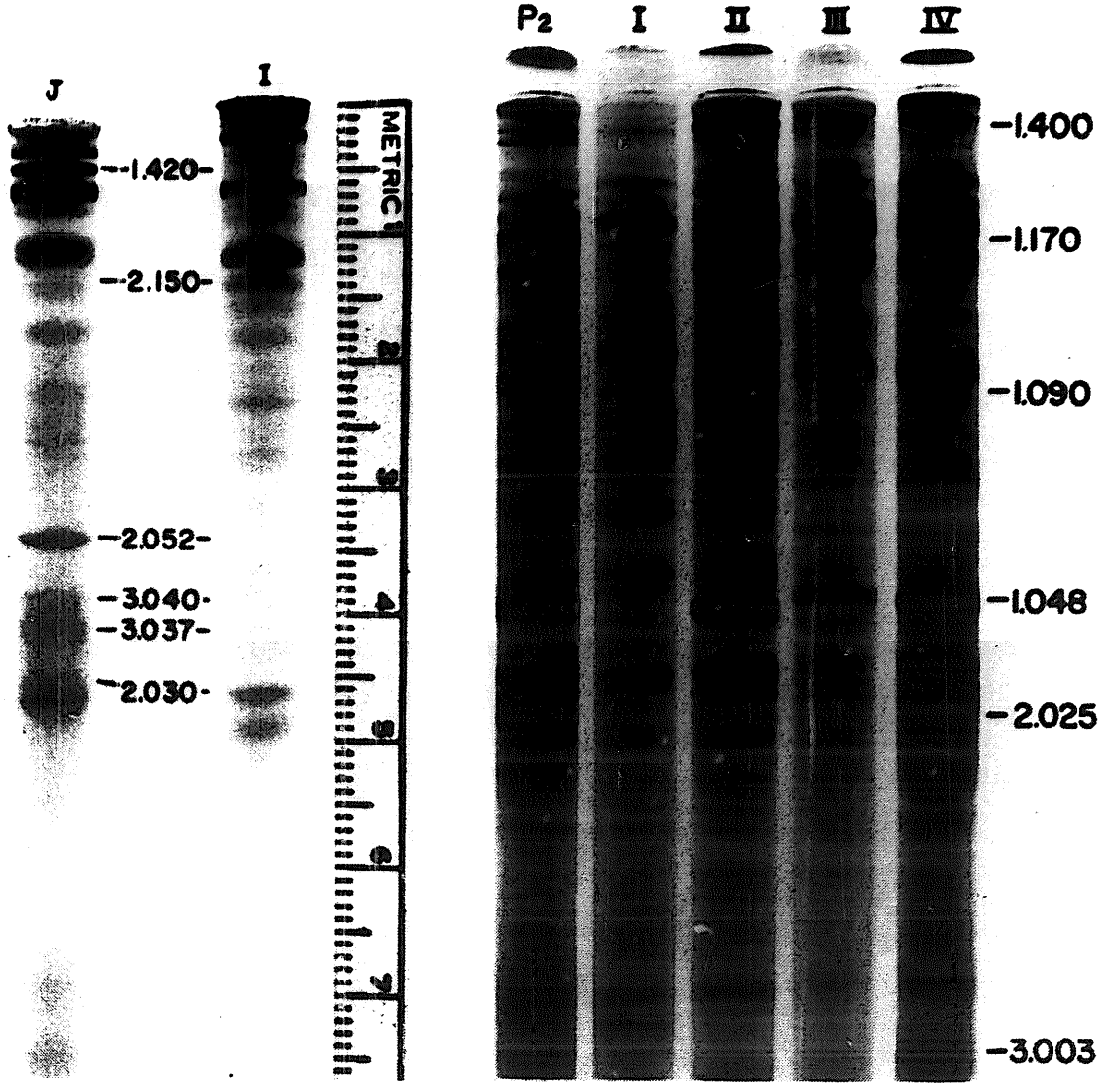

Fig. 4. Jejunal and ileal protein patterns. 36 μg of brush border membranes prepared from jejunum (J) and ileum (I) were electrophoresed and stained with Coomassie brilliant blue. The bands 2.052, 3.040, 3.037 are missing in the ileum. Anode is at the bottom.

Fig. 5. Electrophoretic protein subunit patterns. Brush borders (Fraction P_2) and their subfractions (I, II, III, IV) obtained from ileum were solubilized in sodium dodecyl sulfate and β-mercaptoethanol and electrophoresed in (11.1 × 0.9) gels. The gels were loaded with 43 μg of protein (except gel Fraction I, 20 μg). Anode is at the bottom.

and ileum were electrophoresed at the same time, under exactly the same conditions, including the amount of protein loaded on the gel (see Fig 4).

The differences are striking! The intensity of the band 1-420 in jejunum is diminished in ileum and ileum lacks the 2-052, 3-040, 3-037 bands. Among the bands common to jejunum and ileum, most are of weaker intensity in the ileum. The intensity of the bands 2.150 is higher in the ileum.

Protein subunit composition

The electrophoretic patterns of entire brush borders (Fraction P_2) and their subfractions (Fractions I, II, III, IV) are shown in Fig. 5. About 30 bands could be resolved in Fraction P_2 (brush border), II (brush border membrane) and IV (core fraction).

Gels of Fraction P_2, II and IV have a prominent band centered at the position corresponding to 48000 daltons. The bands 1-400, 1-230, 1-200, 1-170, 1-150, 1-110, 1-090, 2-030, 2-027, 2-250 are prominent too. The other bands appear from their staining intensities to be minor components. Although poorly seen in the photograph, comparison between gels of Fractions P_2, II and IV shows that 5 bands of molecular weight lower than 25000 found in Fractions P_2 and IV are missing in Fraction II. It is apparent that the membrane fractions included many, if not all, of the large proteins in the brush border but contained almost no detectable protein with a molecular weight less than 25000.

Comparison of protein and protein subunits patterns

Electrophoresis of brush border membranes treated with sodium dodecyl sulfate only (protein pattern, Fraction II) or dodecyl sulfate and β-mercaptoethanol (protein subunit pattern, Fraction II) were carried out at the same time.

22 and 29 bands were found respectively, in the protein and the protein subunit patterns obtained from jejunum (Fig. 6). The differences between these patterns are striking. Two bands, one located at the top of the gel (arrow), the other centered at the position corresponding to 420000 daltons in Fraction II are missing in Fraction II_M. New bands of lower molecular weight appear in this gel: 180000, 110000; six bands between 100000 and 50000 and 5 bands with a molecular weight lower than 25000. Fraction II_M has a prominent band centered at 46000–48000 daltons.

20 and 31 bands were found respectively in the protein and the protein subunit patterns obtained from ileum (Fig. 7). In Fraction II the prominent bands were at the top of the gel and centered at positions corresponding to 420000, 260000, 180000 and 150000 daltons. The band located at the top of the gel and the band 1-260 found in Fraction II are missing in Fraction II_M while in this gel, new bands appear: 2-110, 1-090, 2-060, 1-046, 2-036, 2-027, 2-025. Gel Fraction II_M has a prominent band centered at 46000–48000 daltons.

Glycoprotein patterns

12–13 bands with apparent molecular weight varying from 50000 to over 400000 were stained with Schiff's reagent in the glycoprotein and glycoprotein subunit patterns obtained from brush borders and brush border membranes. Three bands only were stained heavily. The major carbohydrate containing band in the glycoprotein and glycoprotein subunit patterns was centered at 320000–330000

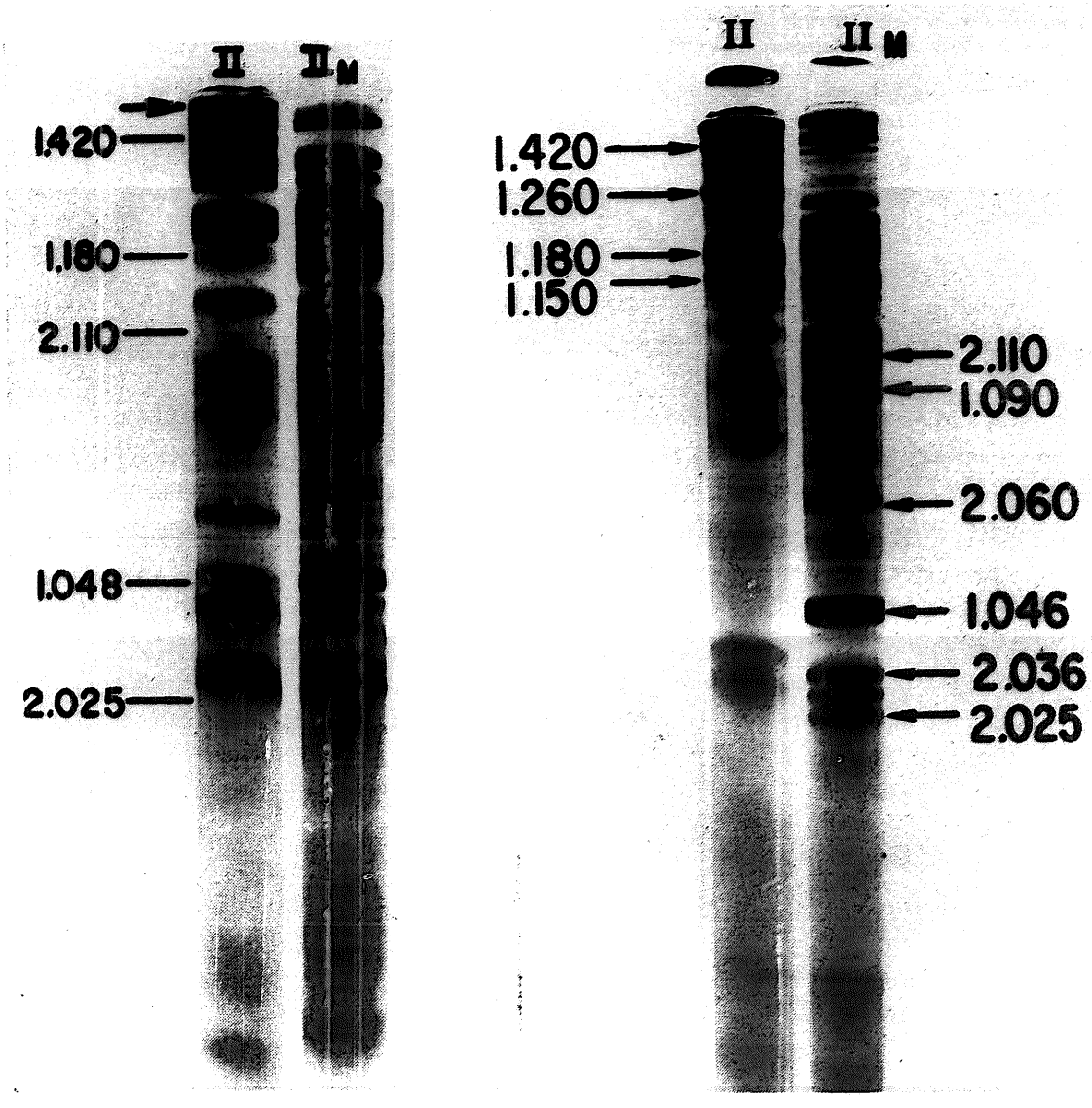

Fig. 6. Protein and protein subunit patterns obtained from jejunal brush border membranes. Fractions II and II_M are the protein and protein subunit patterns, respectively. Electrophoresis was performed at 1.5 mA per gel for 90 min. The gels were loaded with 56 μg of protein.

Fig. 7. Protein and protein subunit patterns obtained from ileal brush border membranes. 45 μg of protein was applied to each gel. Electrophoretic conditions and legends are the same as in Fig. 6. Coomassie brilliant blue stain. Anode is at the bottom.

and 420000–440000 daltons, respectively. Comparison of the membrane profile of gels stained for proteins and carbohydrates suggest that all of the glycoproteins could be identified as membrane proteins. The prominent Schiff positive components centered at 4000 daltons are probably glycolipids[18,23–26].

DISCUSSION

Solubilization with 2% sodium dodecyl sulfate and subsequent electrophoretic fractionation show the heterogeneity of the intestinal brush border membrane proteins. This dodecyl sulfate concentration completely solubilizes these proteins

and inhibits their proteolysis[26]. Although, according to Maddy[27], the interpretation of the results thus obtained would not be straightforward, our experiments did provide highly resolved and reproducible patterns for 8 jejunal and 7 ileal specimens. Only minor variations were observed from one specimen to another and were possibly due to differences in specimen origin and in the precise degree of membrane purification.

The curves of log mol. wt *versus* relative mobility obtained with 5×0.9; 7.5×0.9; and 1.1×0.9 gels are sigmoidal in nature, confirming Neville's results[10]. Since theoretical justification for calculating molecular weight from relative mobilities obtained at a single gel concentration has been provided[10] the molecular weights were determined only from the curve obtained with 11.1×0.9 gels (Fig. 1) which produced sharper and more highly resolved bands. In order to determine more precisely the extrapolated region of this curve, apoferritin (mol.wt 480000) was tried but the sample available was electrophoretically heterogeneous. Extrapolation probably does not accurately describe the behavior of polypeptides larger than phosphorylase. However, determinations of enzyme activities in the gel have shown (Maestracci, D., unpublished) that the "heavy maltase" was centered at 420000–440000 daltons in agreement with the mol.wt of 400000 reported earlier[28]. These results are evidence that proteins of mol.wt higher than 400000 enter the gel.

Although no technique can be used alone for the absolute determination of the number of polypeptides because of possible overlaps[29], 20 and 22 bands, at least, were present in ileal and jejunal protein patterns, respectively. Those numbers are increased to 29 and 31 in the protein-subunit patterns. 10–15 protein or subunits were found in hamster[6], chicken[7], and rat[8] intestinal brush border electrophoretic patterns. In these studies, the gels were polymerized in presence of dodecyl sulfate and the electrophoresis carried out in a continuous system. In our experiments dodecyl sulfate was not present in the gel, and because a discontinuous system was used, more highly resolved patterns were obtained[29].

The molecular weights of the 20–22 polypeptide chains separated on the gel range from 23000 to 400000. Very high-mol.-wt polypeptide chains have also been found in erythrocyte[22,24,30] platelet[31] and liver[22] plasma membranes and in kidney brush borders[22]. However, the presence of predominant bands with mol.wt about 400000 in protein and protein subunit patterns of intestinal brush border membrane seems to differentiate this "structure" from the other plasma membranes. While the function of these very large polypeptides is not yet defined in most of the plasma membranes, the physiological role of some enzymes of very high molecular weight associated with the intestinal brush border membrane[32–42] is well known.

Most proteins of the core have a mol.wt lower than 46000–48000, while membrane proteins are higher than 23000. However, the core and the membrane fractions are not pure so that it is unclear to which brush border fraction the proteins of mol.wt from 23000 to 46000 belong. Although less evident, the same conclusions can be deduced from the protein subunit patterns. Similar findings were also obtained using hamster intestine (Maestracci, D., unpublished). Our results and Alpers'[8] seem to suggest that two classes of proteins are present in the brush border. However, in Alpers' study the smaller proteins observed might be subunits resulting from larger membrane proteins since mercaptoethanol was used. Our results, obtained with sodium dodecyl sulfate only, and sodium dodecyl sulfate and β-mercaptoethanol,

seem clearly to suggest that proteins or protein subunits smaller than 23000–25000 are associated only with the core fraction.

Disaccharidases activities are lower in ileum than in jejunum[36,43] while it is the contrary for leucylnaphthylamide hydrolase activity[43]. This finding and the regionalization of some transport functions to the ileum[41] are consistent with our data showing marked differences between the patterns obtained from jejunum and ileum.

About 13 glycoprotein bands with apparent molecular weight varying from 50000 to 400000 were detected in the brush border membrane. Three only were stained heavily. Most of them were identified as membrane proteins. It is interesting to note that maltase[28] alkaline phosphatase[28,44] sucrase–isomaltase[45] and enterokinase[46] all enzymes of mol.wt higher than 140000 and associated with the intestinal brush border membrane are glycoproteins. In view of previously reported anomalous behavior of glycoproteins in dodecyl sulfate gels[47,48] and since it was shown that the calculated mol.wt depended upon the percentage of acrylamide in the gels used[29], the molecular weights should be regarded as approximate. However, the molecular weights found for maltase, sucrase, isomaltase and alkaline phosphatase were consistent with earlier determinations by other physical means[28,44,45].

The most striking feature of the border membrane subunit pattern is the prominent band centered at 48000. A major protein subunit of similar size was obtained in liver plasma membranes, kidney brush border, erythrocyte ghost[22]. According to Tilney and Mooseker[7] the core within each microvillus consist of actin subunit which provides the most prominent band (mol.wt 46000) in electrophoretograms of isolated intestinal brush border. In our study, the protein subunit band at 46000–50000 daltons was found in brush border, brush border membrane and in the core fraction so that is it unclear to which brush border fraction this subunit belongs. Since, electron micrographs of brush border membrane have shown that core material was included into 30–40% of the brush membrane vesicles, this contaminating material could account for the prominent band centered at 46000–50000 daltons. However, there may be multiple proteins for each protein band, so that it is impossible to be certain how much, if any, of the protein of 46000–50000 daltons may be actin. Recently, the association of actin filaments with preparation of purified plasma membranes was demonstrated[49,50].

The procedure described in this paper used in conjunction with gel-slicing techniques and methods for removing the sodium dodecyl sulfate from protein–sodium dodecyl sulfate complexes[51,52] should allow the association of various physiological and biochemical properties of the enterocyte brush border membrane with specific polypeptide chains.

ACKNOWLEDGEMENTS

This work was supported by a grant from the National Institutes of Health and an award to D.M. from the Medical Research Council of Canada.

REFERENCES

1 DePierre, J. W. and Karnovsky, M. L. (1973) *J. Cell Biol.* 56, 275–303
2 Pitt-Rivers, R. and Impiombato, F. S. A. (1968) *Biochem. J.* 109, 825–830

3 Shapiro, A. L., Vinuela, E. and Maizel, Jr, J. V. (1967) *Biochem. Biophys. Res. Commun.* 28, 815–820
4 Weber, K. and Osborn, K. (1969) *J. Biol. Chem.* 244, 4406–4412
5 Guidotti, G. (1972) in *Annual Review of Biochemistry* (Snell, E. E., ed.), Vol. 41, pp. 731–752, Annual Reviews, Palo Alto
6 Critchley, D. R., Howell, K. E. and Eichholz, A. (1970) *Fed. Proc.* 29, 596
7 Tilney, L. G. and Mooseker, M. (1971) *Proc. Natl. Acad. Sci. U.S.* 68, 2611–2615
8 Alpers, D. H. (1972) *J. Clin. Invest.* 51, 2621–2630
9 Petropakis, H. J., Montgomery, M. W., Davidson, W. D. and Anglemier, A. F. (1969) *Can. Inst. Food Technol. J.* 2, 108–111
10 Neville, Jr, D. M. (1971) *J. Biol. Chem.* 246, 6328–6334
11 Schmitz, J., Preiser, H., Maestracci, D., Ghosh, B. K., Cerda, J. J. and Crane, R. K. (1973) *Biochim. Biophys. Acta* 323, 98–112
12 Jovin, T. K., Dante, M. L. and Chrambach, A. (1971) *Multiphasic Buffer Systems Output*, Federal Scientific and Technical Information, United States Departmant of Commerce, Springfield, Virginia
13 Hjerten, S. (1962) *Arch. Biochem. Biophys.* Suppl. 1, 147–151
14 Davis, B. J. (1964) *Ann. N. Y. Acad. Sci.* 121, 404–427
15 Mitchell, W. M. (1967) *Biochim. Biophys. Acta* 147, 171–174
16 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* 193, 265–275
17 Chrambach, A., Reisfeld, R. A., Wyckoff, M. and Zaccari, J. (1967) *Anal. Biochem.* 20, 150–154
18 Glossmann, H. and Neville, Jr, D. M. (1971) *J. Biol. Chem.* 246, 6339–6346
19 Nerenberg, S. T., Ganger, C. and DeMarco, L. (1971) *Anal. Biochem.* 43, 564–576
20 Darnall, S. W. and Klotz, I. M. (1972) *Arch. Biochem. Biophys.* 149, 1–14
21 Patterson, R. and Phillips, M. (1971) *Anal. Biochem.* 43, 628–630
22 Neville, Jr, D. M. and Glossmann, H. (1971) *J. Biol. Chem.* 246, 6335–6338
23 Lenard, J. (1970) *Biochemistry* 9, 1129–1132
24 Carraway, K. L. and Kobyla, D. (1970) *Biochim. Biophys. Acta* 219, 238–241
25 Carraway, K. L., Lam, A., Kobyla, D. and Huggins, J. (1972) *Anal. Biochem.* 45, 325–331
26 Fairbanks, G., Steck, T. L. and Wallach, D. F. H. (1971) *Biochemistry* 10, 2606–2617
27 Maddy, A. H. (1972) *Sub-Cell. Biochem.* 1, 293–301
28 Forstner, G. G. (1971) *Biochem. J.* 121, 781–789
29 Kobyla, D., Kettry, A., Shin, B. C. and Carraway, K. L. (1972) *Arch. Biochem. Biophys.* 148, 475–487
30 Gwynne, J. T. and Tanford, C. (1970) *J. Biol. Chem* 245, 3269–3271
31 Nachman, R. L. and Ferris, B. (1972) *J. Biol. Chem* 247, 4468–4475
32 Gardner, J. D., Brown, M. S. and Laster, L. (1970) *N. Engl. J. Med* 283, 1196–1202
33 Gardner, J. D., Brown, M. S. and Laster, L. (1970) *N. Engl. J. Med.* 283, 1246–1271
34 Gardner, J. D., Brown, M. S. and Laster, L. (1970) *N. Engl. J. Med.* 283, 1317–1324
35 Crane, R. K. (1972) *Present Concepts in Internal Medicine*, Vol. V, 57–73, Hellerman General Hospital, San Francisco
36 Semenza, G., Auricchio, S. and Rubino, A. (1965) *Biochim. Biophys. Acta* 96, 487–497
37 Eichholz, A. and Crane, R. K (1965) *J. Cell. Biol.* 26, 687–691
38 Eichholz, A. (1967) *Biochim. Biophys. Acta* 135, 475–482
39 Rhodes, J. B., Eichholz, A. and Crane, R. K. (1967) *Biochim. Biophys. Acta* 135, 959–965
40 Nishi, Y. Yoshida, T. O. and Takesue, Y. (1968) *J. Mol. Biol.* 37, 441–444
41 Greenberger, N. J. (1969) *Am. J. Med. Sci.* 258, 144–149
42 Rubin, W. (1971) *Am. J. Clin. Nutr.* 24, 45–64
43 Welsh, J. D., Preiser, H., Woodley, J. F. and Crane, R. K. (1972) *Gastroenterology* 62, 572–582
44 Kaplan, M. M. (1972) *Gastroenterology* 62, 452–468
45 Cogoli, A., Mosimann, H., Vock, C., VonBalthazar, A. K. and Semenza, G. (1972) *Eur. J. Biochem.* 30, 7–14
46 Maroux, S., Baratti, J. and Desnuelle, P. (1971) *J. Biol. Chem.* 246, 5031–5039
47 Segrest, J. R., Jackson, R. L., Andrews, E. P. and Marchesi, V. T. (1971) *Biochem. Biophys. Res. Commun.* 44, 390–395

48 Bretscher, M. S. (1971) *Nat. New Biol.* 231, 229–232
49 Korn, E. D. and Wright, P. L. (1973) *J. Biol. Chem.* 248, 439–447
50 Pollard, T. D. and Korn, E. W. (1973) *J. Biol. Chem.* 248, 448–450
51 Weber, K. and Kuter, D. J. (1971) *J. Biol. Chem.* 246, 4504–4509
52 Lenard, J. (1971) *Biochem. Biophys. Res. Commun.* 45, 662–668