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MICROVILLUS MEMBRANE VESICLES FROM PIG SMALL INTESTINE

PURITY AND LIPID COMPOSITION

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Microvillus membrane vesicles from pig small intestine were isolated by a method based on hypotonic lysis, Mg^{2+}-aggregation of contaminants and differential centrifugation. The purity of the membrane vesicles were established by measuring the activity of marker enzymes and the RNA and DNA content. The membranes were found free of contamination by other subcellular membrane fragments, except for a minor contamination with basolateral plasma membranes. The lipid composition was established and, based on weight percentage, the membrane contained neutral lipids, phospholipids, neutral glycolipids and gangliosides in the weight ratio of 18 : 50 : 29 : 2%. The amount of individual phospholipids and glycolipids were quantitated. Phosphatidylethanolamine, -choline, -serine, -inositol and sphingomyelin made up 17, 17, 6, 5 and 5%, respectively of the total lipid. The major glycolipids were two monohexosylceramides containing glucose and galactose as the carbohydrate component, a dihexosylceramide containing galactose as the only carbohydrate component and two pentahexosylceramides containing fucose, galactose, glucose and hexosamine (either *N*-acetylglucosamine or *N*-acetylgalactosamine) in the molar ratio of 1 : 2 : 1 : 1.

Introduction

Much interest has been focused on the isolation and structure-function relationship of the proteins from the microvillus membrane of the small intestine [1,2]. However, so far little work has been carried out for characterization of the lipids of the microvillus membrane, and with respect to the lipid composition of the pig microvillus membrane, nothing is known. This information is essential for understanding the fluidity of the lipid bilayer, which may affect the activity of various plasma membrane enzymes and transport systems, as indicated by the experimental data accumulated during the last 10 years [3–6]. The present study describes the purity and lipid composition of the microvillus membrane vesicles isolated by a modification of the procedure described by Kessler et al. [7].

Materials and Methods

Chemicals. Pig small intestines were kindly delivered by the Department of Experimental Pathology (Rigshospitalet, Copenhagen, Denmark). The organic solvents were of analytical reagent grade and were distilled prior to use. Standard phospholipids were obtained from Serdary Research Laboratories, London, Canada, and standard glycolipids were obtained from Supelco, Inc., Pennsylvania, U.S.A.

Isolation of microvillus membrane vesicles. Microvillus membrane vesicles were prepared by a modification of the method described by Kessler et al. [7]. In order to prevent hydrolysis of phospholipids by mem-

Abbreviation: EGTA, ethyleneglycol bis(β-aminoethyl ether)-*N*,*N*'-tetracetic acid.

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brane-bound phospholipases, $CaCl_2$ was substituted by $MgCl_2$ and in order to improve the purity of the preparation, two more centrifugation steps were introduced. In short, 150 g of pieces of frozen inverted pig intestine [8] from the upper part of ileum were thawed in 500 ml of a cold solution of 0.3 M mannitol containing 12 mM Tris-HCl, pH 7.1. The intestine was vibrated for 90 s on a Vibro-mixer, and the suspension poured onto a Büchner funnel (1 mm holes) without filter paper and without suction, in order to remove connective tissues and muscle fibers. The resulting mucosal cell suspension was diluted to 2800 ml with cold distilled water, and homogenized at full speed for 30 s in a blender (Kenwood). A concentrated solution of $MgCl_2$ was added in order to make the final concentration 10 mM, and after standing at 4°C for 20 min, the homogenate was centrifuged at $2900 \times g$ for 15 min. The supernatant was filtered through cheese cloth and the centrifugation step repeated. The pellet from the centrifugation step was discarded and the supernatant was centrifuged, $28000 \times g$ for 30 min. The pellet containing the microvillus membrane vesicles was further purified after homogenization in 80 ml 50 mM Tris-HCl, pH 7.0 in a Potter-Elvehjem homogenizator by centrifugation at $800 \times g$ for 15 min. The supernatant which contained the purified microvillus membrane vesicles was pelleted at $28000 \times g$ for 30 min. The pellet was suspended in 50 mM Tris-HCl, pH 7.0 and aliquotes were taken for protein, RNA and DNA determinations and for measuring marker enzymes, which were assayed immediately or on vesicles which had been stored at −80°C. The rest of the membrane vesicles were used for lipid extraction.

Enzymes and other assays. Aminopeptidase activity (EC 3.4.11.2) was determined with L-alanyl-*p*-nitroanilide as substrate [8] at 37°C by use of the Reaction Rate Analyzer LKB 8600 (LKB Products AB, Stockholm, Sweden). 5′-Nucleotidase activity (EC 3.1.3.5) was measured at 37°C at pH 7.4 with 0.1 mM AMP as substrate and with the addition of 30 mM phosphate in order to distinguish it from alkaline phosphatase as described by Gratecos et al. [9]. $Na^+ + K^+$-dependent ouabain-sensitive ATPase (EC 3.6.1.3) was measured at 37°C in a coupled assay [9]: after recording the total ATPase activity for 3 min, ouabain was added to a concentration of 0.5 mM and the new activity recorded. The difference in the slopes between the two lines is a measure of the ouabain-inhibited activity. Cytochrome oxidase (EC 1.9.3.1) [10], rotenone insensitive NADPH-cytochrome *c* reductase (EC 1.6.2.3) [11], RNA [12], DNA [12] and protein [13] were assayed by established procedures.

Electron microscopy. A suspension of microvillus membrane vesicles was fixed in 3% glutaraldehyde in 0.1 M cacodylate, pH 7.2 by standing at 4°C for 18 h. The fixed membrane vesicles were pelleted and post-fixed in 1% osmiumtetraoxide in 0.1 M cacodylate buffer, pH 7.2 at 4°C for 1.5 h. After rinsing in buffer, the specimen was dehydrated though a series of ethanol solutions (70–90%) and finally in propylenoxide, before embedding in araldite. Thin sections were cut and stained with uranylacetate and examined in a Philips 300 Electron Microscope.

Lipid extraction. Lipid extraction of the purified membrane vesicles was performed as described by Suzuki [14], with 20 vol. of chloroform/methanol (2 : 1, v/v). After filtration, the residue was extracted with 10 vol. of chloroform/methanol (1 : 2, v/v) containing 5% of water. The filtrates were combined and an aliquote was taken for making a total lipid extract, by removing the solvent in a rotatory evaporator, and dissolving the residue in a known amount of chloroform/methanol (19 : 1) saturated with water. The amount of total lipid was determined by weighing an aliquot on a Cahn electrobalance. The quantitative analysis of all lipids was performed on this total lipid extract (see later).

For structural studies, the rest of the chloroform/methanol extract was partitioned into Folch's upper and lower phase [15]. After addition of chloroform in order to obtain a chloroform/methanol ratio of 2 : 1 (v/v), 0.2 vol. of 0.88% KCl was added. The lower phase was washed once with 0.2 vol. of the theoretical upper phase containing KCl (chloroform/methanol/0.88% KCl; 3 : 48 : 47, v/v) and once with the theoretical upper phase containing water. The combined upper phases were concentrated in a rotatory evaporator and dialyzed against two changes of distilled water at 4°C for 24 h in a dialysis bag which had been extensively washed in boiling water. The dialyzate was lyophilized, and the residue was dissolved in a known amount of chloroform/methanol/water (10 : 10 : 3, v/v).

From the lower phase, neutral lipids, glycolipids

and phospholipids were separated by chromatography on silicic acid (Unisil, Clarkson Chemical Co., Williamsport, U.S.A.) by successive elution with chloroform, acetone and methanol [16]. The eluates containing each of the three lipid classes were concentrated and the residues were dissolved in a known amount of chloroform (neutral lipids) or chloroform/methanol (2 : 1, v/v) (glycolipids and phospholipids). The column separation, judged from thin-layer chromatography on silica gel plates (Merck) in the solvent system chloroform/methanol/water (65 : 25 : 4, v/v), was found complete except for the fucose-containing glycolipid (see later) which appeared in the methanol eluate.

Quantitative measurements of individual lipids. Quantitative measurements of individual lipids were performed on the total lipid extract. Cholesterol [17] and free fatty acids [18] were determined on silica gel spots after thin-layer chromatographic separation of these components from each other and from the glycolipids and the phospholipids in the solvent system petroleum ether (b.p. 40–60°C)/diethyl ether/acetic acid (70 : 30 : 1, v/v). The amount of individual glycolipids was determined as the amount of sphingosine [19] and phospholipids as the amount of lipid phosphorous [20] on silica gel spots after either two-dimensional thin-layer chromatography in chloroform/methanol/water (65 : 25 : 4, v/v) in the first dimension and *n*-butanol/acetic acid/water (60 : 20 :20, v/v) in the second dimension or one-dimensional thin-layer chromatography in chloroform/methanol/2-propanol/0.25% KCl/ethyl acetate (30 : 9 : 25 : 6 : 18, v/v) [21] developed three times. The amount of gangliosides was determined by measuring the sialic acid content [22]. A conversion factor of 25 was used for calculating the amount of phospholipids from determined amount of lipid phosphorus. Molecular weights of 723, 866, 1 396 and 1 503 were used for calculating the amount of, respectively, mono-, di- and pentahexosylceramides and ganglioside (G_{M1}) from the sphingosine and the sialic acid content.

Identification of individual glycolipids and phospholipids. From the phospholipid fractions isolated by silicic acid column chromatography, individual phospholipids were separated by thin-layer chromatography on silica gel plates (Merck). The phospholipids were identified by comparing the R_F values with those of known phospholipids in different solvent systems, and by detection with specific spray reagents [23,24].

The mono- and dihexosylceramides were isolated by preparative thin-layer chromatography of the glycolipid fraction obtained after silicic acid column chromatography. The pentahexosylceramides which partitioned both into Folch's upper and lower phase were isolated either from Folch's upper phase or from the phospholipid fraction obtained after silicic acid column chromatography, as they eluted with methanol rather than with acetone. After brief exposure of guidestrips to iodine vapour, the appropriate areas of the chromatograms were marked and the glycolipids eluted with 2 × 5 ml chloroform/methanol (1 : 1, v/v) or in the case of the pentahexosylceramides 2 × 5 ml chloroform/methanol/water (10 : 10 : 1, v/v). The purity of the glycolipids were analyzed by thin-layer chromatography in the solvent systems chloroform/methanol/water (65 : 25 : 4, v/v or 60 : 35 : 8, v/v) or chloroform/methanol/2-propanol/0.25% KCl/ethyl acetate (30 : 9 : 25 : 6 : 18, v/v) developed three times, and their R_F values were compared to those of standards. The glycolipids were visualized with α-naphtol reagent [25]. In case of the pentahexosylceramides, care was taken to separate this from ganglioside (G_{M1}) as these compounds moved on thin-layer close to each other. The separation was confirmed by the nonreactivity of the pentahexosylceramides with resorcinol [26].

Gas chromatography. Methylation of individual glycolipids (0.5–1.0 mg) were performed with 3 ml 1 M HCl in anhydrous methanol for 16 h at 80°C [27]. Fatty acids were removed by extraction with hexane, and the methanol phase was concentrated to dryness under a stream of nitrogen. During evaporation, methanol (2 × 1.0 ml) was added in order to prevent hydrolysis of the methylglycosides as a result of a temporary increase in the acid concentration. The residue was re-acetylated in order to convert methyl glycosides of amino sugars to *N*-acetyl derivatives. The re-acetylation was carried out by addition of 500 µl of methanol/acetic acid anhydride (3 : 1, v/v) plus 30 mg of silver acetate and the mixture left in the dark at room temperature overnight [28]. The samples were filtered and the solvent evaporated first under N_2, later in vacuum. Trimethylsilylation was performed by addition of 50 µl of Sil-A (Sigma,

U.S.A.) for 15 min at room temperature. The solvent was removed under N_2, and the trimethylsilylated methylglucosides were extracted into petroleum ether, b.p. 40–60°C. An aliquote was injected into a gas chromatograph (F-11, Perkin-Elmer), equipped with a column (200 × 0.2 cm) containing 3% SE 30 on chromosorb W. (acid washed) 100–120 mesh. The temperature was kept at 160°C until elution of the trimethylsilyl ether of mannitol (added as internal standard), then the temperature was programmed at 3°C/min until 220°C. To determine relative detector response of the individual sugars, weighed samples of standard monosaccharides were analyzed by gas-liquid chromatography under similar conditions.

Hydrolysis of glycolipids. The carbohydrate composition of the individual glycolipids were also determined by thin-layer chromatography after aqueous hydrolysis. To 100–500 μg of glycolipid was added 100 μl 3 M HCl and the mixture was heated for 1 h at 100°C. The monosaccharides, after removal of the sphingosin and fatty acids by extraction with 100 μl of chloroform/methanol (2 : 1, v/v), were separated by thin-layer chromatography on silica gel plates (Merck) in the solvent system ethyl acetate/pyridine/water (8 : 2 : 1, v/v) or in the solvent system methanol/water (1 : 1, v/v) developed three times. This last system allowed glucosamine and galactosamine to move from the application line. The spots were visualized in ultraviolet light by spraying the plates with a 10% solution of ethylendiaminesulphate followed by heating for 10 min at 120°C [29], and the R_F values compared to those of standards.

Results

Preparation and purity of microvillus membrane vesicles

From measurements of marker enzyme activities, RNA and DNA content (Table I), the microvillus membrane vesicle preparation was found satisfactorily free of contamination with nuclei (DNA content), mitochondria (cytochrome oxidase activity) and endoplasmic reticulum (RNA content and rotenone-insensitive NADPH-cytochrome *c* reductase activity) as revealed by enrichment factors less than 1 for these components and enzyme activities. The microvillus membrane enzyme, aminopeptidase was enriched by a factor of 13 compared to the homogenate. Other digestive enzymes, sucrase-isomaltase and alkaline phosphatase were also enriched in the microvillus membrane fraction to a similar extent as the aminopeptidase (data not shown). The ouabain-sensitive $(Na^+ + K^+)$-ATPase which is a marker enzyme for the basolateral plasma membrane gave rather variable activity, judged from the standard error (Table I), probably because of a rather high basal Mg^{2+}-ATPase activity in the microvillus membrane fraction, and therefore only a small difference

TABLE I

ENZYME ACTIVITIES, RNA AND DNA CONTENT OF HOMOGENATE AND MICROVILLUS MEMBRANE FRACTION OF PIG INTESTINE

The enzyme activities are expressed as nmol substrate converted/min per mg protein. The RNA and DNA contents are expressed as μg/mg protein. The figures are the mean ± S.E.; number of preparations is given in parenthesis. The enrichment factor is the ratio between the specific activity in the microvillus membrane fraction and the homogenate.

Fraction	Homogenate	Microvillus membrane	Enrichment factor
Protein (mg)	5 935 ± 92 (10)	161 ± 5 (11)	
Aminopeptidase	184 ± 6 (5)	2 350 ± 65 (8)	13
5′-Nucleotidase	8 ± 0.4 (7)	50 ± 2 (8)	6.3
$(Na^+ + K^+)$-ATPase	58 ± 3 (6)	89 ± 9 (7)	1.5
Cytochrome *c* oxidase	307 ± 27 (3)	39 ± 2 (3)	0.13
NADPH-cytochrome *c* reductase	15 ± 2 (3)	5 ± 1 (3)	0.30
RNA	43 ± 4 (3)	27 ± 2 (3)	0.63
DNA	71 ± 8 (3)	9 ± 0.5 (3)	0.13

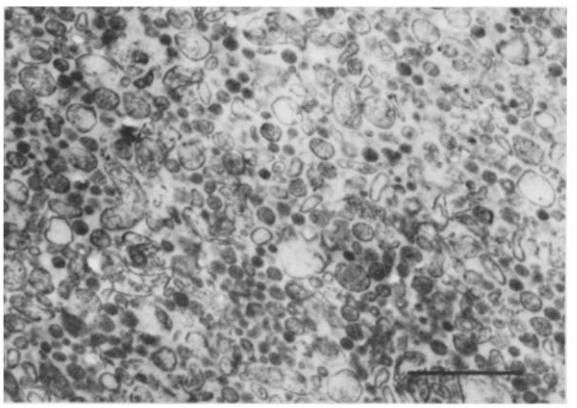

Fig. 1. Thin section of microvillus membrane vesicles of pig small intestine. Scale mark 1.0 μm.

in ATPase activity with and without ouabain was seen. In most preparations, the ouabain-sensitive $(Na^+ + K^+)$-ATPase activity was in the same order of magnitude as the homogenate, indicating a minor contamination with basolateral plasma membrane fragments. The 5′-nucleotidase activity was enriched in the microvillus membrane vesicle preparation by a factor 6. However, as recently stated by Colas and Maroux [30] for membranes of rabbit enterocytes, the 5′-nucleotidase is an enzyme which probably is located both in the microvillus membrane and in the basolateral membrane.

Electron micrographs of the microvillus fraction (Fig. 1) revealed that the preparation is not entirely of spherical vesicles, but elongated structures are also seen corresponding to sections cut through different planes of the intact microvilli, and thus resembles the preparation recently described for rabbit brush border membranes [30].

Lipid composition

The composition of the microvillus membrane is presented in Table II. The average lipid to protein weight ratio is 0.5, as also found for the rabbit and rat microvillus membrane [31,32]. The lipid is made up of cholesterol, phospholipids, neutral glycolipids and gangliosides in the weight ratio of 12 : 50 : 29 : 2%. Small amounts of free fatty acids were detected, however, no lyso compounds could be detected by thin-layer chromatography, which indicates that the amount of free fatty acids observed probably are not due to hydrolysis, but could be fatty acid associated with the intestine during absorption. Hydrolysis of lipids in our preparation is prevented by using $MgCl_2$ instead of $CaCl_2$ in the preparation medium and by rapid extraction of the membranes into chloroform/methanol. The molar ratio of cholesterol to phospholipid is approx. 1 : 2. The major phospholipids in pig small intestine microvillus membranes are phosphatidylcholine and phosphatidylethanolamine, which each makes up about 34% of the total phospholipid fraction. The rest is phosphatidylserine, phosphatidylinositol and sphingomyelin.

The glycolipids of the microvillus membrane are mainly neutral glycolipids which make up approx. 30% (by weight) of the total lipid fraction. Only 2% of the lipids of the microvillus membrane are the more complex, sialic acid-containing gangliosides. Three major types of cerebrosides are measured,

TABLE II

CHEMICAL COMPOSITION OF MICROVILLUS MEMBRANE VESICLES FROM PIG SMALL INTESTINE

The values are the average and S.E. of double determinations from different preparations. The number of preparations are indicated in parenthesis. FFA, free fatty acid; C, cholesterol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; SpH, sphingomyelin; CMH, CDH and CPH; mono-, di- and pentahexosylceramide.

	Mean ± S.E. (mg)	% of total
Protein	104 ± 6 (4)	65
Lipid	56 ± 4 (4)	35
Lipid	56 ± 4 (4)	100
		% of lipid
FFA	3.2 ± 0.8 (4)	6
C	6.9 ± 0.8 (4)	12
PE	9.5 ± 1.0 (4)	17
PC	9.4 ± 0.6 (4)	17
PS	3.6 ± 0.2 (4)	6
PI	2.8 ± 0.6 (4)	5
SpH	2.8 ± 0.3 (4)	5
CMH	1.6 ± 0.2 (4)	3
CDH	8.4 ± 0.8 (4)	15
CPH	6.2 ± 0.2 (4)	11
Ganglioside	1.0 ± 0.14 (7)	2

where a dihexosylceramide and two fucose-containing pentahexosylceramides make up about 90% of the total glycolipid fraction.

Identification of the carbohydrates of the glycolipids

The purity of the glycolipids were judged from thin-layer chromatography in different solvent systems. The number of carbohydrate residues was tentatively established by comparing the R_F value of the individual glycolipids with that of standards. As seen in Fig. 2, the major glycolipids of the microvillus membrane of pig small intestine had R_F values which indicate that they were mono-, di- and pentahexosylceramides. The number and types of carbohydrate residues were finally established by gas-chromatography of the trimethylsilyl ether derivatives of the methylglycosides, and confirmed by thin-layer chromatography of the free monosaccharides librated by aqueous hydrolysis of the glycolipids. As seen in Table III, the carbohydrate residues of the two monohexosylceramides (CMH_1 and CMH_2) are glucose and galactose, respectively. The major dihexosylceramide contains two galactose residues (CDH_2). Small amounts (less than 5% of the total glycolipids) of a dihexosylceramide (CDH_1) which contained both glucose and galactose was also present. The pentahexosylceramides (CPH_1 and CPH_2) contained fucose, galactose, glucose and hexosamine (either *N*-acetylglucosamine or *N*-acetylgalactosamine) in the molar ratio of 1 : 2 : 1 : 1. No attempt was made to separate the two pentahexosylceramides which in all tested thin-layer chromatographic systems (see Materials and Methods) moved very close to each other. The relative amounts of the two fucoglycolipids varied from preparation to preparation, but in most cases equal amounts of *N*-acetylglucosamine and *N*-acetylgalactosamine were present.

TABLE III

GAS-CHROMATOGRAPHIC ANALYSIS OF THE CARBOHYDRATE COMPOSITION OF GLYCOLIPIDS FROM MICROVILLUS MEMBRANE VESICLES FROM PIG SMALL INTESTINE

The values represent the relative molar ratio of monosaccharides in each glycolipid. The abbreviations are the same as in Table II. The glycolipid designated 1 had a greater R_f value than the glycolipid designated 2.

Glycolipids	Glucose	Galactose	Fucose	Hexosamine
CMH_1	1.0	0.2	–	–
CMH_2	0.4	1.0	–	–
CDH_1	0.7	1.0	–	–
CDH_2	0.0	1.0	–	–
$CPH_1 + CPH_2$	1.0	2.0	0.8	0.9

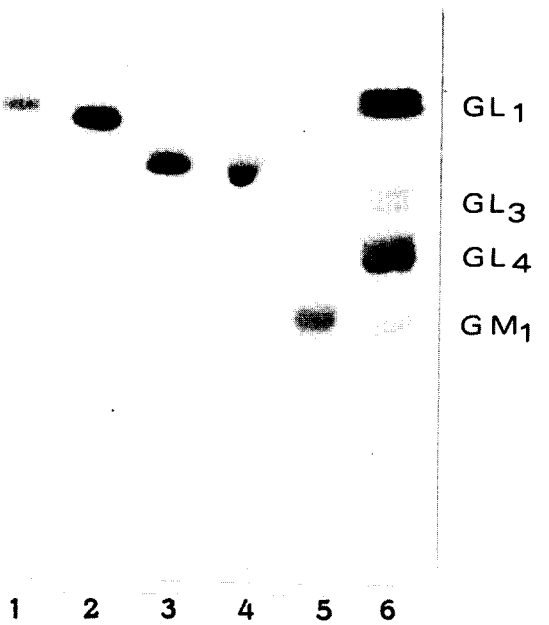

Fig. 2. Thin-layer chromatogram of glycolipids of microvillus membrane vesicles of pig small intestine. Lane 1–5: the purified glycolipids, CMH_1, CMH_2, CDH_1, CDH_2 and CPH_{1+2} (fucoglycolipids) in the amount of approx. 10 μg. Lane 6: a mixture of standard glycolipids (glucocerebroside and galactocerebroside (GL_1), trihexosylceramide (GL_3), globoside (GL_4) and ganglioside (GM_1), 10 μg of each. The thin-layer plates (Silica gel (Merck)) was developed in the solvent system chloroform/methanol/water (60 : 35 : 8, v/v) and the glycolipids were visualized with α-naphtol reagent as purple-blue spots (the fucoglycolipids had a characteristic red-orange colour).

Discussion

Compared to the data of Kessler et al. [7] for DNA content and marker enzymes of rat microvillus membrane vesicles, our modification of the method of preparing pig microvillus membrane vesicles which

introduces a few more centrifugation steps and uses $MgCl_2$ instead of $CaCl_2$, gave a microvillus membrane fraction with at least the same degree of purity when contamination with nuclei, mitochondria and endoplasmic reticulum is considered. From their data on ouabain-sensitive K^+-dependent phosphatase activity, an activity exhibited by the ouabain-sensitive ($Na^+ + K^+$)-ATPase [32] (enrichment factor of 2.2) and our data on ouabain-sensitive ($Na^+ + K^+$)-ATPase activity (enrichment factor of 1.5), contamination with basolateral plasma membrane fragments in our preparation was obviously smaller than in their preparation.

The minor contamination with basolateral plasma membrane fragments was estimated to approx. 10% from the preparation of 100% pure microvillus membranes by the immunosorbent technique (Carlsen, J., Christiansen, K. and Bro, B., unpublished data), and will not influence significantly the lipid composition. The high cholesterol content is typical for plasma membranes. The molar ratio of cholesterol to phospholipid is 1 : 2 and is similar to that found in rabbit microvillus membranes [31]. The phospholipid composition is also similar to the one reported for rabbit intestinal microvillus membranes, thus the serine- and ethanolamine-containing phospholipids accounted for 42% of the total lipid phosphorus, and choline-containing phospholipids accounted for 43% of the total lipid phosphorus. The rather high content (10%) of phosphatidylinositol is also similar to that found for mouse and rabbit intestinal microvillus membranes [34,31]. In this connection, it is worth mentioning that intestinal mucosa contains a very active Ca^{2+}-requiring phosphodiesterase which is specific for phosphatidylinositol [35]. The rapid turnover of the phosphate moiety of phosphatidylinositol in many tissues, suggest an important physiological function of inositol-containing phospholipids, probably in relation to transport processes [36]. The phosphatidylinositol content of microvillus membrane vesicles may thus be of importance and thus $CaCl_2$ should always be replaced with $MgCl_2$ in preparing microvillus membrane vesicles, as also used by Hauser et al. [31] for preparing rabbit small intestinal brush border membranes. However, in contrast to their observation we do not find it necessary to add EGTA to all buffers in order to prevent formation of lysophospholipids, as these compounds were not detected at all in our preparation.

Glycolipids make up a significant proportion of the total lipids of small intestine, but the amount and type of glycolipids vary from species to species [37]. Glycolipids are present at the cell surface and are connected with a number of surface membrane phenomena such as differentiation and regulation [38]. However, except for the early work of Forstner and Wherrett [32] on rat intestinal microvillus membrane vesicles, no systematic study on quantitation and characterization of glycolipids of intestinal microvillus membrane vesicles have been carried out. In pig microvillus membrane vesicles, we found three major types of glycolipids, two monohexosylceramides, one containing glucose and one containing galactose as the carbohydrate moiety, a dihexosylceramide, which is a digalactosylceramide and two pentahexosylceramides containing fucose, galactose, glucose and *N*-acetylglucosamine or *N*-acetylgalactosamine in the molar ratio of 1 : 2 : 1 : 1. The digalactosylceramide and the two fucoglycolipids are quantitatively the dominating glycolipids, making up approx. 50 and 40% each of the total glycolipid fraction. This is in contrast to the work on rat microvillus membrane vesicles where a monohexosylceramide was found to be the dominating glycolipid [32]. Furthermore, the fucoglycolipid of rat small intestine is a trihexosylceramide, the structure of which was established recently [39].

Neutral fucoglycolipids which contain fucose, galactose, glucose and *N*-acetylglucosamine or *N*-acetylgalactosamine in the molar ratio of 1 : 2 : 1 : 1 have been isolated from human erythrocyte membranes [40], dog small intestine [41] and porcine intestine [42]. The structure of the oliosaccharide portion of one of the dog intestinal fucolipids is identical with a fucoglycolipid isolated from human erythrocyte membranes. The structure of the porcine whole intestine fucoglycolipids has not been established. In the present paper, no attempt was made to establish the complete structure of any of the glycolipids, but rather to give an overall quantitative picture of the complex mixture of lipids involved in the structure of the pig microvillus membrane. As stated by Brasitus et al. [43] and Brasitus and Schachter [44] for the rat microvillus membrane, the lipid composition may influence the fluidity of the membrane and thereby the activity of microvillus enzymes with 'intrinsic' activities. Thus, the present

study supplies basic compositional data which are prerequisites before further studies on lipid-protein interaction in pig microvillus membrane can be carried out.

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