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GLYCOPROTEINS AND GLYCOLIPIDS OF OXYNTIC CELL MICROSOMES

I. GLYCOPROTEINS: CARBOHYDRATE COMPOSITION, ANALYTICAL AND PREPARATIVE FRACTIONATION

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SUMMARY

Isotopically-labeled sugars were incorporated into glycoproteins of isolated bullfrog gastric mucosa. The majority of the label was found in gastric microsomal fractions which were shown to contain membranes derived from the oxyntic cell tubular membrane system and were not significantly contaminated with mucus. The tubular membranes contained exceptionally large quantities of carbohydrate (approx. 260 μg/mg protein). Most of the sugar (73%) was associated with protein in the following molar ratios: hexose, 1.0; fucose, 0.42; hexosamine, 0.62; sialic acid, <0.02. The remaining sugar, predominantly hexose, could be extracted into lipid solvents and was presumably glycolipid.

Gastric microsomes were dissolved in sodium dodecyl sulfate and subjected to acrylamide gel electrophoresis and Sephadex G-200 fractionation. The latter preparative procedure yielded several molecular weight classes, each of which contained different sets of proteins and/or glycoproteins; however, the molar ratios of the sugars found in the two carbohydrate containing classes were quite similar.

Significant quantities of carbohydrate were also found in gastric microsomal fractions from other species, *e.g.* pig and rabbit. Furthermore, characteristic proteins and glycoproteins were not present in tadpole gastric microsomes until the later stages of metamorphosis when HCl secretory capability had been established. The above findings suggest that glycoproteins may play an important role in oxyntic cell functions; the possibility of a membrane protective role is discussed.

INTRODUCTION

Many attempts have been made to elucidate the molecular structure and chemical composition of cell membranes in the belief that this knowledge will lead to a better understanding of membrane function. Earlier studies were mainly concerned with the protein and lipid moieties while membrane associated carbohydrates were largely ignored. More recent studies, however, have included an assessment of membrane glycoproteins and glycolipids since these ubiquitous carbohydrate-containing compounds appear to be involved in a number of important membrane-mediated processes (see reviews of Winzler[1] and Kraemer[2]).

The oxyntic, or acid-secreting, cell of the gastric mucosa is characterized by an abundant smooth surfaced membrane system which is believed to be intimately involved in the acid secretory process[3–6]. Although the enzymatic activities associated with these oxyntic cell membranes have been the subject of intense investigation, relatively little is known about the nature of individual membrane components. Through the application of histochemical techniques it has recently been shown that this membrane system has abundant quantities of carbohydrate associated with it[7–9].

The purpose of the present study was to further investigate the nature of oxyntic cell membrane carbohydrates and to partially characterize the lipid, protein and glycoprotein components of this highly functional membrane system. Microsomal fractions isolated from bullfrog gastric mucosa appeared to have originated from the oxyntic cell smooth surfaced tubular membrane system. The gastric microsomes contained an exceptionally large quantity of carbohydrate which was separated into several classes based on (1) its association with protein or lipid and (2) the molecular weights of the glycosylated compounds. Preliminary studies were extended to assess the carbohydrate content of gastric microsomes from other species and to correlate the appearance of typical gastric microsomal proteins and glycoproteins with morphological and functional changes occurring in the metamorphosing tadpole.

METHODS

Isolation of subcellular fractions

Animals were sacrificed by pithing and the stomach removed immediately. The mucosa was separated from underlying muscle coats by blunt dissection and desquamated as previously described* (ref. 10). Oxyntic cells were harvested using a glass slide, suspended in cold (0–4 °C) homogenization medium containing 220 mM sucrose, 10 mM Tris–HCl, 0.25 mM EDTA (pH 7.3) and homogenized with a teflon and glass homogenizer.

Two fractionation procedures were employed, both being carried out at 0–4 °C. In the most frequently used procedure the homogenate was centrifuged at $1935 \times g$ for 20 min. This low speed pellet was rehomogenized and centrifuged as above to pellet the sediment fraction. Mitochondrial and microsomal fractions were obtained from the combined supernatants by sedimentation at $12000 \times g$ for 10 min and $40000 \times g$ for 90 min, respectively. The above fractionation procedure was used for isolation of subcellular fractions from pig, rabbit (New Zealand white) and tadpole (*Rana catesbiana*) mucosa and from frog (*Rana catesbiana*) mucosa after incorporation of labeled sugars. For speed and convenience, when only microsomal fractions of adult bullfrog gastric mucosa were required they were isolated according to the following procedure. The homogenate was centrifuged at $15000 \times g$ for 15 min to remove debris, nuclei and mitochondria and subsequently at $40000 \times g$ for 90 min to obtain the microsomal fraction.

In order to remove sucrose, all fractions were resuspended and washed at least three times in cold (0–4 °C) isotonic medium containing 110 mM KCl, 10 mM Tris–HCl, 0.25 mM EDTA (pH 7.3) and finally resuspended in KCl medium or double-distilled water. The effective removal of sucrose was verified by Sephadex chromato-

* Because of the fragility of the tissue, tadpole gastric mucosa was not desquamated prior t o harvesting the cells.

graphy of microsomal materials and by comparison with fractions prepared in the absence of sucrose.

Sodium dodecyl sulfate solubilization of microsomes

Native or lipid extracted microsomes were incubated overnight at room temperature in 10% sodium dodecyl sulfate (up to 10 mg of protein in 0.2 ml of 10% sodium dodecyl sulfate). This procedure solubilized more than 95% of the microsomal proteins as judged by assays of the supernatant after centrifugation at $40000 \times g$ for 90 min. These solutions could subsequently be diluted to as little as 0.1% sodium dodecyl sulfate without significant loss of protein solubility. When desired, excess sodium dodecyl sulfate was precipitated by cooling overnight at 0–4 °C and removed by centrifugation at $27000 \times g$ for 15 min. More than 95% of the protein remained in the supernatant.

Incorporation of isotopically-labeled sugars

Isolated bullfrog gastric mucosa were mounted in a chamber similar to those previously described[11]. Both sides of the mucosa were bathed in nutrient solution[12] continuously aerated with O_2-CO_2 (95:5, v/v). The appropriate isotopically-labeled sugar was added to the serosal side and incorporation allowed to proceed at room temperature for 4 h. At the end of the incubation period the bathing solutions were removed and the mucosa rinsed in four changes of cold (0–4 °C) nutrient solution to remove most of the unincorporated label. Cell fractions were prepared according to the first fractionation scheme given above.

Samples were solubilized in 10 ml of Aquasol (New England Nuclear) and counted in a Packard Tri-Carb liquid scintillation counter. Control experiments showed Aquasol to be as efficient as organic bases (*e.g.* NCS, Amersham/Searle) for solubilization of gastric mucosal fractions for scintillation counting.

In order to determine the identity of the labeled sugar after incorporation, samples were sealed in ampules under vacuum and subsequently hydrolyzed in 4 M HCl for 4 h at 100 °C. HCl was removed by several evaporations *in vacuo* over KOH and conc. H_2SO_4. The hydrolysate was added to a mixture of known sugars (glucose, galactose, mannose, glucosamine, galactosamine, *N*-acetylneuraminic acid and fucose), spotted in triplicate on Whatman No. 1 paper and chromatographed in a solvent system described by Fischer and Nebel[13]. Sugar spots were identified by the alkaline $AgNO_3$ staining procedure[14] and corresponding unstained sections were cut out, eluted with water overnight and the eluant counted.

Acrylamide gel electrophoresis

Sodium dodecyl sulfate acrylamide gel electrophoresis was performed as described by Fairbanks *et al.*[15]. Electrophorograms were stained for protein with Coomassie blue[15] or for sugar with periodic acid–Schiff reagent[15]. For molecular weight determination, gels were calibrated with human γ-globulin, bovine serum albumin, chymotrypsinogen, myoglobin and cytochrome *c* (Sigma Chemical Co.).

After appropriate staining procedures, electrophorograms containing isotopically-labeled materials were sliced according to the banding pattern, dissolved overnight at room temperature in approximately 0.3 ml of 30% H_2O_2 and counted in Aquasol.

Miscellaneous methods

Lipids were extracted as described by Rosenberg and Guidotti[16]. Total phospholipid was calculated from inorganic phosphorus analysis according to Bartlett[17] after digestion in H_2SO_4, assuming an average phospholipid molecular weight of 800. Cholesterol was assayed as described by Zlatkis *et al.*[18].

Protein was measured by the method of Lowry *et al.*[19] with bovine serum albumin as standard. Fucose was determined by the cysteine–sulfuric acid reaction[20] and amino sugars by the method of Gatt and Berman[21] after hydrolysis under N_2 in 4 M HCl at 100 °C for 4.5 h with glucosamine as standard. Sialic acids were determined by the thiobarbituric acid method[22] after 1 h hydrolysis in 0.05 M H_2SO_4 at 80 °C and hexoses were determined by the orcinol assay[23]. For the latter, mannose was used as a standard and corrections were made for the contribution by fucose. In all sugar assays, corrections were made for nonspecific color development by including tubes containing the unknowns but without the specific chromogenic compound.

RESULTS

Distribution of labeled sugars

The distribution of radioactivity among gastric cell fractions after incubation with D-[6-^{3}H]glucosamine is shown in Table I. Although less than 30% of the particulate protein was harvested as the microsomes, this fraction contained more than 70% of the incorporated radioactivity. Thus, the specific activity of the microsomal fraction was 23.3 and 2.1 times that of the sediment and mitochondrial fractions, respectively. 75–81% of the counts could be recovered as amino sugar after hydrolysis and paper chromatography. Similar profiles of specific activity were obtained after incorporation of D-[1-^{14}C]glucosamine or L-[1-^{3}H]fucose. Only a small percentage (< 5%) of the incorporated sugars could be extracted into lipid solvents.

TABLE I

INCORPORATION OF [^{3}H]GLUCOSAMINE INTO VARIOUS PARTICULATE FRACTIONS DERIVED FROM BULLFROG GASTRIC MUCOSA

5 cm^2 of gastric mucosa was incubated for 4 h in 9 ml of nutrient solution containing 50 μCi of D-[6-^{3}H]glucosamine (1.3 Ci/mmole).

Fraction	*Total protein (mg)*	*Total dpm*	*dpm/mg protein*
Sediment	0.78	1 196	1 533
Mitochondria	0.24	3 960	16 667
Microsomes	0.40	13 508	35 777

Chemical composition

The chemical composition of frog gastric microsomes is shown in Table II. The relatively high ratio of phospholipid and cholesterol to protein (0.80) reflects the membranous nature of the microsomal fraction. Moreover, an abundance of carbohydrate was associated with these membranes. The hexoses were the most

TABLE II

CHEMICAL COMPOSITION OF BULLFROG GASTRIC MICROSOMES

	Native microsomes		*Lipid-extracted microsomes*	
	*μg/mg**	*moles**/mole hexose*	*μg/mg**	*moles**/mole hexose*
Total carbohydrate	257	—	187	—
Hexose	176	1.0	93	1.0
Fucose	31	0.20	36	0.42
Hexosamine	50	0.28	58	0.62
Sialic acid	< 3	< 0.01	< 4	< 0.02
Phospholipid	543			
Cholesterol	260			
Glycolipid***	69			

* Values expressed as μg per mg of microsomal protein.

** Calculated assuming that hexosamines are not acetylated. Sialic acid as *N*-acetylneuraminic acid.

*** Determined from the difference in carbohydrate content of native and lipid-extracted microsomes.

plentiful species, representing over 65% of the total sugar. Significant quantities of hexosamine and fucose were also present while the acidic sugar, sialic acid, was found in very small amounts.

Lipid extraction of these membranes resulted in a 27% decrease in their carbohydrate content. This loss was completely accounted for by a 47% reduction of hexose suggesting that hexose-containing glycolipids are associated with these membranes. The small increase in the other sugars, on a per mg protein basis, after lipid extraction was probably due to removal of some "Lowry reactive" substances, possibly protein[16], into the lipid solvents.

Although our results are in general agreement with those of Forte and Forte[8], we report a somewhat larger total sugar content and a somewhat reduced proportion of hexosamine in the microsomes. These differences are most likely due to differences in the methods employed for isolation and assay of carbohydrate containing materials.

Acrylamide gel electrophoresis

A frequently used method of demonstrating the complexity and diversity of membrane constituents has been acrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The electrophoretic fractionation of frog gastric microsomal proteins and glycoproteins is seen in Fig. 1. More than 20 proteins varying in apparent molecular weight from greater than 150000 to less than 20000 are revealed. A particularly intensely staining band (CB1) with a molecular weight of approximately 95000 is seen in the upper third of this gel.

Similar electrophorograms stained for carbohydrate reveal at least 12 periodic acid–Schiff-positive bands, some of which are not discernible in the photographic reproduction. Although the majority of these bands are thought to be glycoproteins, the intensely staining material seen near the tracking dye front was, as suggested by

others[15,24], probably lipid and/or glycolipid in nature since (a) this material did not stain with Coomassie blue and (b) was not found when lipid-extracted microsomes were electrophoresed (see Fig. 5). Upon close inspection, the dark region near the middle of the gel could be resolved into two glycoproteins (bands PAS2 and PAS3) with mobilities corresponding to molecular weights of approximately 70000 and 60000 while the band just below the origin (band PAS1) appeared to be a single glycoprotein of considerably larger size.

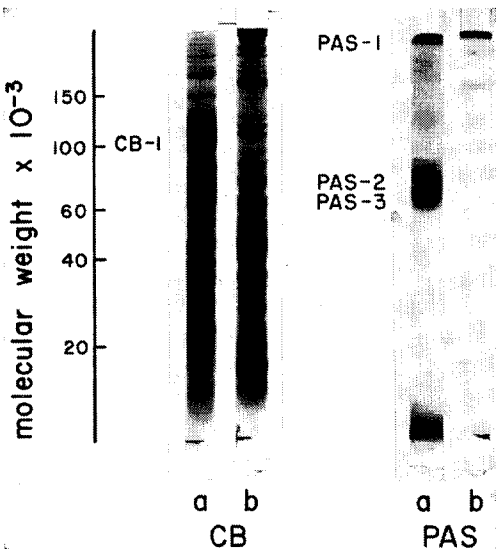

Fig. 1. Electrophorograms of bullfrog gastric microsomes (a) and gastric mucus (b). 80 μg of microsomal protein was applied to gels stained for protein (CB) while 140 μg was applied to those stained for carbohydrate (PAS). The major microsomal protein and glycoprotein bands are designated by CB-1 and PAS-1, 2 and 3, respectively. The scale at the left indicates the estimated molecular weights as determined by calibration of identical gels with standard proteins. The dark line at the lower end of the gel marks the position of the tracking dye. Details of sample preparations, electrophoresis and staining are given in the Methods section.

[^{14}C]Glucosamine-labeled microsomes were also subjected to acrylamide gel electrophoresis. The distribution of radioactivity in these gels is shown in Fig. 2. Although all the glycoproteins appear to have incorporated some [^{14}C]glucosamine, the major glycoproteins (PAS bands 1, 2 and 3), were the most heavily labeled. It should be noted that, consistent with the analytical data suggesting that the glycolipid carbohydrates are predominantly hexoses, no [^{14}C]glucosamine was found in the lipid–glycolipid band.

For purposes of comparison, electrophorograms of frog gastric mucus are also shown (Fig. 1). This material was fractionated into many protein and several glycoprotein bands. Even though several bands had similar mobilities, the overall pattern clearly distinguished the microsomes from mucus materials. Note in particular that the major microsomal protein (CB1) and glycoproteins (PAS 1, 2 and 3) were absent, or present in only negligible amount in mucus.

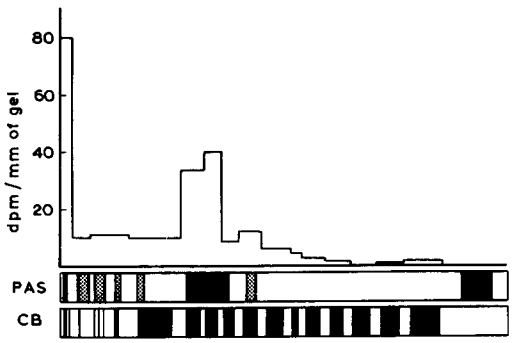

Fig. 2. Distribution of radioactivity on sodium dodecyl sulfate acrylamide gels. 70 μg of [^{14}C] glucosamine labeled microsomes (19000 dpm/mg protein) was applied to each gel. The carbo hydrate (PAS) and protein (CB) staining patterns of these gels are depicted below the histogran (see Methods for details of the procedures employed).

Preparative fractionation of glycoproteins

Lipid-extracted microsomes were solubilized in sodium dodecyl sulfate anc fractionated on a Sephadex G-200 column. A typical elution profile is shown in Fig. 3 From inspection of the absorption curve at 280 nm, the microsomal proteins appea to have been eluted as three major peaks (I, II and IV). However, further analysi of Peak IV fractions showed that they contained negligible amounts of protei (Lowry) and had $A_{260\ nm}/A_{280\ nm}$ ratios approaching 2.0, suggesting that this pea contained low molecular weight nucleotides[25]. Furthermore, the hexose content o these fractions was quite low. 80–90% of the anthrone-positive materials were elutec as two peaks roughly corresponding to protein Peaks I and II.

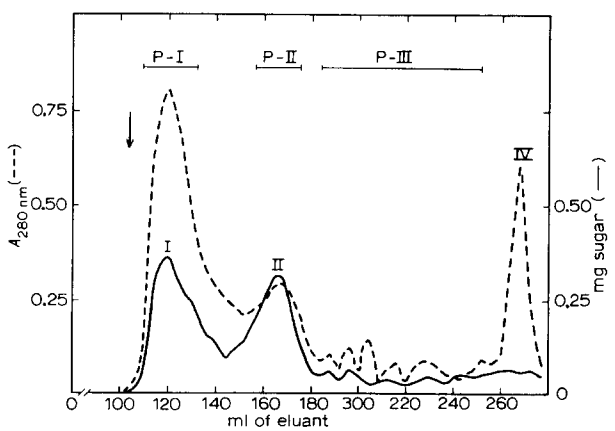

Fig. 3. Sephadex G-200 chromatography of microsomal proteins. Approx. 36 mg of lipid-extracte microsomal protein was dissolved in 4.5 ml of 3.6% sodium dodecyl sulfate and applied to 2.5 cm × 53 cm Sephadex G-200 column equilibrated and eluted with 0.1% sodium dodecyl sulfate The flow rate was approx. 10 ml/h; 4-ml fractions were collected. Elution was monitored b 260 and 280 nm absorption and fractions were assayed for carbohydrate with the anthron procedure[20]. Ultraviolet absorbing materials were eluted in several major peaks (I, II and IV) For further analytical work fractions were pooled as indicated by the horizontal bars (P-I, P-I and P-III). The arrow designates the void volumn.

Fractions from Sephadex chromatography were pooled as shown in Fig. 3 (Pools I, II and III) and subsequently subjected to acrylamide gel electrophoresis. From the electrophoretic patterns shown in Fig. 4, it may be seen that the proteins were roughly segregated into high (Pool I), medium (Pool II) and low (Pool III) molecular weight groups. The carbohydrate staining pattern shows that the major glycoproteins (PAS 2 and 3) were found almost exclusively in Pool II, while the higher molecular weight glycoproteins, including PAS1, were restricted to Pool I.

Corresponding pools from two Sephadex G-200 columns were combined, con-

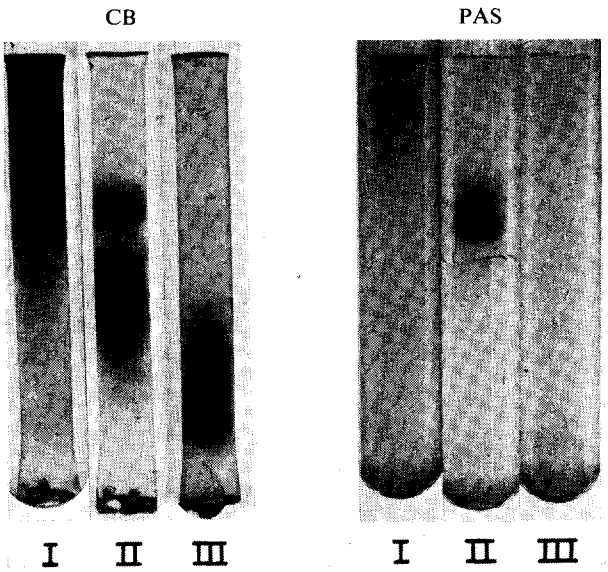

Fig. 4. Electrophorograms of pools obtained from Sephadex G-200 chromatography of sodium dodecyl sulfate-solubilized lipid-extracted microsomes. I, II and III refer to pools shown in Fig. 3. Gels were stained for protein (CB) and carbohydrate (PAS).

TABLE III

COMPOSITION OF POOLS OBTAINED FROM SEPHADEX CHROMATOGRAPHY OF MICROSOMAL PROTEINS

Pools obtained from Sephadex G-200 chromatography of 70 mg of microsomal protein. Approx. 65% of the applied protein and sugar was recovered in Pools I and II.

Pool	*Total protein*	*μg sugar/mg protein*				*moles/mole hexose**			
		Hexose	*Fucose*	*Hexos-amine*	*Sialic acid*	*Hexose*	*Fucose*	*Hexos-amine*	*Sialic acid*
I	32.4	70	26.5	45.4	<2.4	1	0.42	0.65	<0.02
II	15.1	135	50.5	72.2	<5.7	1	0.42	0.54	<0.02

* Calculated assuming that hexosamines are not acetylated. Sialic acid as *N*-acetyl-neuraminic acid.

centrated by lyophilization and partially freed of sodium dodecyl sulfate as describe under Methods. Aliquots were then assayed for protein and carbohydrate (Table III) Although comparison of electrophorograms showed that a separation of differen groups of glycoproteins had been achieved, the molar ratios of the various sugars i these two pools were quite similar, the only exception being the slightly increase proportion of hexosamine in Pool I.

Developmental studies

Changes in electrophysiological parameters and the development of cell struc tures and enzymes associated with acid secretion have made the gastric mucosa o the metamorphosing bullfrog tadpole a useful system for studying the developmen of HCl secretory capability and its association with particular subcellular organ elles[6,26,27]. Electrophorograms of gastric microsomes from several tadpole stage (staged according to Taylor and Kollros[28]) and from an adult are shown in Fig. 5 The protein patterns of all three stages were similar to each other and to the adul with the exception of the major protein band (CB 1) which did not appear unti Stage XXIV. The glycoprotein patterns, on the other hand, were quite different Stages XX and XXII microsomes showed little periodic acid–Schiff-positive material whereas glycoprotein bands analogous to those of the adult were seen in Stage XXIV Thus, the major protein (CB 1) and glycoproteins are not present in significan quantities in microsomal fractions until metamorphosis has progressed to the late stages (Stage XXIV).

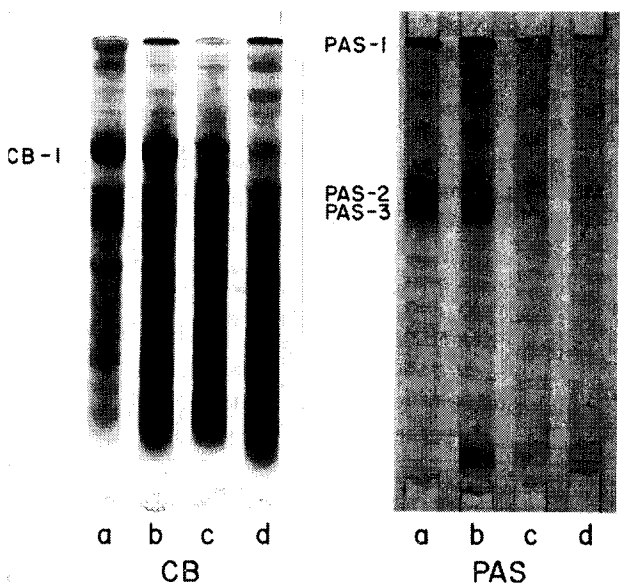

Fig. 5. Electrophorograms of gastric microsomes from an adult (a) and Stage XXIV, XXII an XX tadpoles (b, c and d, respectively). Approx. 80 μg of protein was applied to each gel. Gel were stained for protein (CB) and carbohydrate (PAS). The adult microsomes were lipid extracte before solubilization and electrophoresis; the microsomal preparations from tadpoles were no extracted of lipids (see Methods). CB-1 and PAS-1, 2 and 3 designate major protein and glyco protein bands corresponding to those seen in Fig. 1.

Carbohydrates of mammalian gastric microsomes

The presence of relatively large amounts of sugar in gastric microsomal fractions is not peculiar to the frog. As shown in Table IV, microsomes isolated from pig and rabbit gastric mucosa also contained considerable quantities of carbohydrate, although both the amount of sugar per mg protein and the amounts of fucose and hexosamine relative to hexose are somewhat below the analogous values for frog (*cf.* Table II). The predominate sugars were the hexoses while sialic acid was present in negligible quantities. As noted for the frog, the presence of hexose-containing glycolipids in the mammalian microsomes was suggested by the reduced amount of hexose associated with the residual protein after lipid extraction.

TABLE IV

CHEMICAL COMPOSITION OF MICROSOMES DERIVED FROM PIG AND RABBIT GASTRIC MUCOSA

	Pig		*Rabbit*	
	*μg/mg**	*moles**/mole hexose*	*μg/mg**	*moles**/mole hexose*
Native microsomes				
Total carbohydrate	161		147	
Hexose	122	1.00	116	1.00
Fucose	9	0.08	4	0.04
Hexosamine	30	0.25	27	0.23
Sialic acid	< 2	< 0.01	< 4	< 0.02
Lipid-extracted microsomes				
Total carbohydrate	112		127	
Hexose	67	1.00	92	1.00
Fucose	11	0.18	4	0.05
Hexosamine	34	0.51	31	0.34
Sialic acid	< 2	< 0.02	< 4	< 0.03
Lipid extract				
Phospholipid	506		564	
Cholesterol	125		155	
Carbohydrate***	49		20	

* Values expressed as μg per mg of microsomal protein.

** Calculated assuming that hexosamines are not acetylated. Sialic acid as *N*-acetylneuraminic acid.

*** Determined from the difference in carbohydrate content of native and lipid-extracted microsomes.

DISCUSSION

Source of microsomal constituents

A serious problem encountered when isolating and studying components of a complex tissue is the determination of the source of a particular constituent both with regard to cell type and subcellular organelle of origin. Bullfrog gastric mucosa

is such a tissue being composed of several cell types besides the oxyntic cell[10]. Hype tonic desquamation of this tissue allows the removal of virtually all of the surfac epithelial and mucous neck cells, thus permitting the harvesting of a relatively hom geneous population of oxyntic cells[10]. One of the subcellular fractions of the oxynt cells, the microsomes, was of particular interest to us since (a) this fraction containe the largest amount of newly synthesized glycoproteins and (b) similar fractions hav been shown to contain significant quantities of carbohydrate[8] as well as several en zymes which are thought to be involved in the acid secretory process[29,30].

Morphological, chemical and histochemical evidence has been used to sugge that gastric microsomes are composed primarily of the tubular membrane syste found in the apical portion of oxyntic cells[8,30]. Additional support for this notic can be derived from our ontogenetic experiments with tadpoles. Gastric microsom isolated from young tadpoles (*e.g.* Stages XX and XXII), which do not secrete HC and whose gastric glands contain relatively undifferentiated cells, do not have th characteristic microsomal proteins and glycoproteins found in the adult. The materials are not present in gastric microsomal fractions until metamorphosis ha progressed to later stages (*e.g.* Stage XXIV) when typical oxyntic cells with apic tubular membrane systems are present in the gastric glands and HCl secretory cap bility has been established[6,25].

Contamination of the gastric microsomes with non-tubular membrane materia must also be considered. Electrophorograms demonstrated that mucus, which ha been virtually eliminated during the desquamation procedure, could not have co tributed significantly to the carbohydrate content of the microsomes. Minor contam nation with organelles derived from other cellular sources has not been rigorous excluded. Application of a density gradient technique, such as suggested by Gans and Forte[31], might serve to further purify the tubular membrane system. Howeve since this same study showed that the carbohydrates remained with the purified mem brane fraction, it is likely that the percentage of glycoproteins associated with the membranes would be enhanced. Therefore, the weight of the evidence presente above strongly indicates that the carbohydrate materials found in gastric microsom fractions are primarily derived from the tubular membrane system of the oxyntic cell

Solubilization and fractionation of oxyntic cell tubular membranes

One approach to the study of membrane constituents involves their separatic on the basis of several chemical and physical parameters. A necessary first step this approach is the solubilization of these components. Numerous systems have bee devised with this end in mind including the use of chelating agents[32], urea[33], hig salt concentration[32], butanol[33], pyridine[34], phenol[35], ionic[32,33] and non-ionic dete gents[32]. Preliminary experiments in our laboratory involving a number of the systems showed the ionic detergent sodium dodecyl sulfate to be the most effecti solubilizing agent for native and lipid extracted gastric microsomes, thus permitti the application of analytical and preparative fractionation techniques to the materials.

Analytical acrylamide gel electrophoresis in the presence of sodium dodec sulfate provides exceptional resolution of complex protein mixtures as well as a estimate of the size of these components[35]. Electrophoretic fractionation demo strated that gastric microsomes, like membranes from a number of other source

contain a complex mixture of proteins and glycoproteins. The major protein, designated CB1, has a molecular weight similar to that of the major protein found in red blood cell membranes of numerous species[37] (Component III[15]). However, it is inappropriate to generalize that similar molecular weight proteins (approx. 100000) are primary membrane structural components since they do not appear to be major constituents of membranes from several other cell types[38,39]. Furthermore, mini proteins, low molecular weight (5000–6000) polypeptides, which Laico *et al.*[40] suggest are fundamental subunits of biological membranes appear to be present in only small amounts, if at all, in gastric microsomal fractions.

Unfortunately, the sodium dodecyl sulfate gel electrophoresis technique cannot be used for accurate determinations of the molecular weights of glycoproteins containing large amounts of carbohydrate. These molecules may exhibit decreased mobilities, increased apparent molecular weights, due to lower levels of sodium dodecyl sulfate binding and the resultant decrease in charge density[41]. Nevertheless, this electrophoretic technique has provided a rapid method for (1) fractionating membrane proteins, (2) comparing the constituents of various membranes when samples are run under identical conditions, (3) estimating protein size and (4) perhaps providing an upper limit to the molecular weight of glycoproteins.

The fact that the microsomal membranes contain many proteins with a fairly uniform distribution of molecular weights makes the preparative isolation of the individual components difficult. The use of sodium dodecyl sulfate further compounds the problem since the very properties which make it an effective solubilizing agent, its tenacious binding to the hydrophobic portion of proteins[43] and its negative charge, preclude the use of certain fractionation techniques such as ion-exchange chromatography. Further, the presence of sodium dodecyl sulfate in developing solutions decreases the apparent pore size of gel filtration resins and therefore limits their usefulness to the larger, less discriminating sizes (unpublished observation).

Nevertheless, we were able to fractionate gastric microsomal constituents into several molecular weight classes. It is of interest that although electrophorograms clearly demonstrated that each class contained a different set of proteins and glycoproteins, the molar ratios of the sugars in each of the two carbohydrate containing classes were similar. This finding can be interpreted in a number of ways; for instance the covalently-linked oligosaccharide units may be identical for the different classes of glycoproteins or they may differ in size and degree of branching but may be similar in overall sugar composition and contain similar sugar sequences. Such possibilities as these would not be particularly unusual since diverse glycoproteins often possess oligosaccharides containing the same or very similar monosaccharide sequences and branching patterns[2].

Carbohydrates of oxyntic cell tubular membranes

A very distinctive feature of bullfrog oxyntic cell tubular membranes concerns the carbohydrate content which, in comparison to other membranes, is exceptionally large and unusual in composition. For instance, on a per mg protein basis, gastric microsomes contain from 1.5 to more than 3 times as much carbohydrate as red blood cell ghosts[1,16,33,34,39], liver plasma membranes[39,43,44], and kidney brush border[39,45]. For the latter membrane sources sialic acids account for 13–32% of the protein-bound carbohydrate, whereas this acidic sugar represents less than 2% of

the sugar associated with gastric microsomal glycoproteins. Further, in the latte system the abundance of fucose suggests that this species substitutes for sialic acid as terminal residues of the oligosaccharides.

Glycoproteins and glycolipids appear to have an asymmetric distribution i the oxyntic cells of a number of species. On the basis of histochemical staining several investigators[8,9] demonstrated that the bulk of the sugars were localized o the apical plasma membranes, the tubular membrane system and the Golgi apparatus Other subcellular organelles as well as the lateral and basal plasma membrane showed little or no staining. Further, their evidence suggested that the sugars as sociated with the apical plasma membrane were located at the external or lumena surface. Similarly, the carbohydrate present on the tubular membranes appeared t be predominantly disposed at the membrane surface which, upon stimulation of aci secretion, would become exposed to the lumen of the gastric glands[46,47]. Therefore in view of the abundance of the carbohydrate, its asymmetric distribution, and evi dence suggesting that large segments of membrane proteins are sequestered in th hydrophobic interior of the membrane (see ref. 48), it would appear that carbo hydrates must comprise a major proportion of the surface membrane component exposed to the lumen of oxyntic cell gastric glands.

The function of this lumenal carbohydrate coat is as yet unknown althoug several possible modes of involvement in the acid secretory process have been previ ously suggested[7,8]. Among these is the possibility that the membrane glycoprotein and glycolipids play a role in preventing digestion of the stomach by the harsh acidi and proteolytic environment. Survival of the cells of the gastric epithelium appear to be dependent upon the integrity of their surface membranes[49]. Although it ha been proposed that gastric mucus protects the surface epithelial cells[50], only mino amounts of this material are present deep in the gastric glands where the highes concentration of H^+ and pepsin are likely to be found. One might speculate, therefore that the rich carbohydrate coat covering the lumenal surface of oxyntic cells protect them by providing a mechanical barrier to the approach of proteolytic enzymes. I addition the hydrophilic nature of oligosaccharides and their capacity to forn hydrogen bonds may endow the membrane surface components with a degree of con formational stability rendering them resistant to denaturation by the high concen tration of H^+ found in the stomach.

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