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LIPIDS ASSOCIATED WITH RAT SMALL-INTESTINAL MUCUS GLYCOPROTEIN*,†

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ABSTRACT

The lipid content and composition of rat small-intestinal mucus, and the purified mucus glycoprotein before and after Pronase digestion were investigated. The mucus, obtained by the instillation of intestine with 2M NaCl, was fractionated on Bio-Gel A-50 in the presence of 6M urea and the mucus glycoprotein free of noncovalently bound protein was isolated. A portion of the purified glycoprotein was subjected to Pronase digestion to yield glycopeptides. The native mucus, and the purified glycoprotein and glycopeptides were extracted with chloroform–methanol, and the lipids contained in the extracts were analyzed. The lipids accounted for 17.6 of the dry weight of mucus, 26.4 of the mucus glycoprotein, and 25.3% of the glycopeptides. In comparison to mucus, the lipids associated with mucus glycoprotein contained 1.9 times more phospholipids and 2.1 times more glycolipids, showed a 26% increase in neutral lipids, and were virtually free of glycosphingolipids. Treatment of the purified glycoprotein with Pronase led to a moderate (22.3%) loss in neutral lipids, 4.3-fold decrease in phospholipids, and 52.3% increase in glyceroglucolipids. The results indicate that while the interaction of mucus glycoprotein with phospholipids involves its Pronase-susceptible region, the interaction with glyceroglucolipids occurs in the glycosylated region of the glycoprotein that is resistant to proteolysis.

INTRODUCTION

The surface epitelium of gastrointestinal tract is covered by an extracellular and renewable layer of a viscous mucus, the function of which is to protect the underlying mucosal surfaces from physical injury and proteolytic enzymes[1,2]. This mucus layer consists of proteins, glycoproteins, and lipids in the form of a gel in-

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bibed with water and electrolytes[1–4]. The molecules responsible for the viscoelastic properties of mucus are the high-molecular-weight ($\simeq 2.0 \times 10^6$) glycoproteins called mucins[2,5].

Concerted efforts of numerous laboratories over the past 20 years have provided considerable amount of information on the composition of gastrointestinal mucins, their immunological properties, and the structure of their carbohydrate chains[2,6–13]. More recently, the macromolecular organization of gastric and intestinal mucins has been also described[5,14]. Until now, however, only scant attention has been paid to identifying other components of gastrointestinal mucus which can affect the physicochemical and physiological properties of mucins, namely lipids[3,4]. In this report, we present the evidence that, in intestinal mucus, lipids are associated with mucus glycoprotein.

EXPERIMENTAL

Materials. — Male Sprague–Dawley rats of the same age and mean weight (250 g) fasted overnight prior to sacrifice, were used for intestinal mucus preparation. Bio-Gel A-50 (50–100 mesh), Bio-Gel P-100 (100–200 mesh), and silicic acid Bio-Sil A (100–200 mesh) were obtained from Bio-Rad (Richmond, CA). DEAE-Sephadex A-25 was supplied by Pharmacia (Piscataway, NJ) and HETLC Uniplates (10 × 10 cm) by Analtech (Newark, DE). Fatty acid methyl esters, neutral lipids, and phospholipids standards were purchased from Supelco (Bellefonte, PA), and Pronase from Calbiochem (San Diego, CA).

Analytical methods. — The protein content of samples was measured by the method of Lowry *et al.*[15] with bovine serum albumin as a standard. Hemoglobin was quantitatively determined according to the procedure of Hanks *et al.*[16]. The phenol–sulfuric acid method was used for monitoring the carbohydrate content in column fractions[17] and the carbohydrate content of samples was determined by g.l.c. following methanolysis, *N*-reacetylation, and treatment with a silylating reagent[18]. Gel electrophoresis in 1% $NaDodSO_4$ was performed with 7.5% polyacrylamide gels[19]. Samples of glycoprotein (200–400 μg) were incubated in 0.2M phosphate buffer–$NaDodSO_4$, pH 7.2, for 3 min at 100°, and then applied to the gels. After electrophoresis, the gels were stained for protein with Coomassie Brilliant Blue, and for carbohydrate by the periodate–Schiff method[20].

T.l.c. of lipids was performed on Bio-Sil A plates (10 × 10 cm) activated for 1 h at 120°. The neutral lipids, separated into individual compounds, were made visible with iodine vapor, identified by comparison with chromatograms of authentic standards, and quantitatively determined[3,21]. The neutral and acidic glycolipids, separated by t.l.c. into individual bands, were differentiated into glycospingolipids and glyceroglucolipids by detection with benzidine and orcinol reagents[21,22], and quantitatively determined by g.l.c.[21]. The phospholipids were identified on thin-layer plates by cochromatography with appropriate standards and quantitatively determined by the procedure of Lowry and Tinsley[23]. G.l.c. was performed with a

Beckman GC-65 instrument equipped with glass columns (180 × 0.2 cm) packed with 3% SE-30 on Gas Chrom Q. For the analysis of trimethylsilyl derivatives of glycerol, long-chain bases, and methyl glycosides, the temperature was programmed at 2°/min from 100 to 210°. The analysis of fatty acid methyl esters was carried out at 2°/min from 150 to 290°.

Preparation of mucus. — Rat small-intestine (proximal, 30–50 cm), excised immediately after sacrifice, was rinsed with cold water to remove its content, and the ligature was placed at its distal end. The lumen of the intestine was instilled to capacity with 2M sodium chloride kept for 30 min, and the content recovered. The instillates recovered from each of the 28 intestinal segments were individually assayed for hemoglobin[17], and those (25) free of blood were pooled and filtered through Millipore HA (0.45 μm) filters. The filtrate was dialyzed against distilled water and lyophilized. A part (10 mg) of the lyophilizate was set aside for lipid analysis and the remaining material was used for mucus glycoprotein isolation.

Isolation of mucus glycoprotein. — The mucus was dissolved in 6M urea (to give a 10 mg/mL solution) and applied, in 5-mL portions, to a Bio-Gel A-50 column (2.0 × 150 cm) equilibrated with 6M urea. Elution was achieved with 6M urea at a flow rate of 4–6 mL/h. The eluted fractions (4.8 mL) were monitored for protein and carbohydrate. Fractions containing mucus glycoprotein were pooled, dialyzed against distilled water, and lyophilized. The lyophilizate was dissolved in 6M urea and the residual protein removed by rechromatography on a Bio-Gel A-50 column. The excluded peak, which contained purified mucus glycoprotein, was collected, dialyzed against distilled water, and lyophilized.

Pronase digestion. — A portion (20 mg) of the purified glycoprotein was dissolved (0.5 mg/mL) in 50mM phosphate buffer–mM calcium chloride, pH 7.8, and digested with Pronase in an enzyme-to-glycoprotein ratio of 1:40 (w/w) at 37° under a layer of thymol crystals. Equal amounts of Pronase were added at 24 and 48 h. After incubation for 72 h, the digest was lyophilized, dissolved in 6M urea, and applied to a column (2.0 × 160 cm) of Bio-Gel P-100, equilibrated in and eluted with 0.5M sodium chloride. Fractions (5 mL) were collected and monitored for protein and neutral sugars[17]. The glycopeptide-containing fractions (excluded peak) were pooled, dialyzed against distilled water, and lyophilized.

Extraction and purification of lipids. — Dried samples (10 mg) of mucus, mucus glycoprotein, and glycopeptide were extracted twice, each time for 24 h, with 2:1 (v/v) chloroform–methanol. Each extract was filtered through a Millipore FH (0.5 μm) filter to retain the insoluble protein residue and dried[21]. The lipids were dissolved in a small volume of chloroform and separated on a silicic acid column (0.5 × 20 cm) into three major lipid fractions: neutral lipids eluted with chloroform, glycolipids eluted with 4:1(v/v) acetone–methanol, and phospholipids eluted with methanol[21]. Separation of the neutral lipid fractions into individual lipid components was accomplished by a two-stage t.l.c.[24]. The glycolipids were fractionated on a DEAE-Sephadex column (0.5 × 10 cm) into neutral and acidic fractions, and then chromatographed into individual compounds on thin-layer

plates[25]. The thin-layer plates used for separation of phospholipids were developed in two dimensions with 13:5:1 (v/v) chloroform–methanol–conc. ammonium hydroxide, followed by 3:4:1:1 (v/v) chloroform–acetone–methanol–acetic acid–water.

RESULTS

Gel filtration of the solubilized, rat small-intestinal mucus on Bio-Gel A-50 column gave the elution pattern shown in Fig. 1 (upper). The excluded peak (Mr $\approx 2 \times 10^6$) contained mucus glycoprotein, and the included fractions represented 25–29% of the carbohydrate-containing substance originally present in the solubilized mucus and consisted mainly of protein. $NaDodSO_4$–polyacrylamide electrophoresis of the excluded fraction indicated the presence of lower-molecular-weight protein contaminants. These were removed by rechromatography of the excluded fraction on Bio-Gel A-50 in the presence of 6M urea. Of the total hexose

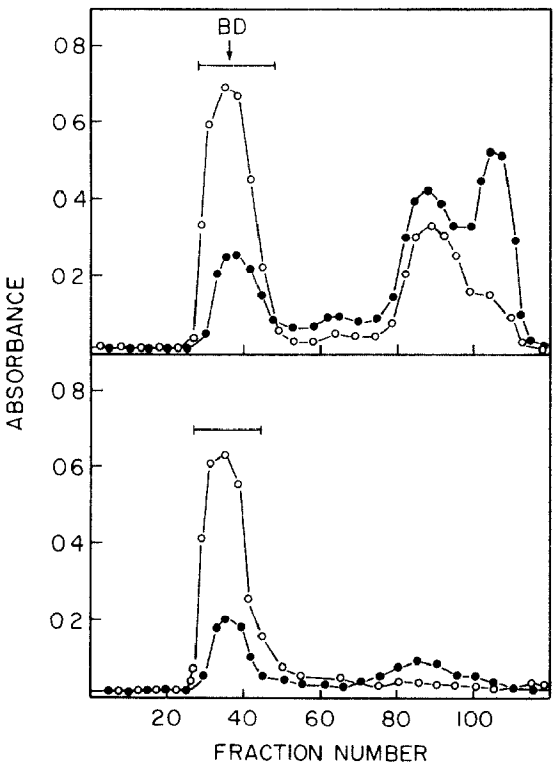

Fig. 1. Gel filtration on a Bio-Gel A-50 column of rat small-intestinal mucus. Upper part. The sample (50 mg) dissolved in 6M urea (5 mL) was applied to a column (2.0 × 150 cm) equilibrated with 6M urea. Fractions (4.8 mL) were collected and monitored for protein (absorbance at 280 nm, ●-●-●) and neutral sugar (phenol–sulfuric acid, method, ○-○-○). The void volume material was pooled as indicated, dialyzed, and concentrated to 5 mL. Lower part: This material was rechromatographed on Bio-Gel A-50 under the conditions just described. BD, Blue Dextran.

TABLE I

CONTENT OF PROTEIN AND LIPIDS IN THE RAT SMALL-INTESTINAL MUCUS AND IN THE PURIFIED MUCUS GLYCOPROTEIN BEFORE AND AFTER PRONASE DIGESTION[a]

Constituent (mg/100 mg)	*Mucus*	*Glycoprotein*	*Glycopeptide*
Protein	54.6 ±7.1	10.2 ±0.8	7.9 ±0.5
Total lipids[b]	17.6 ±2.3	26.4 ±2.9	25.3 ±3.1
Neutral lipids[c]	9.6 ±1.1	12.1 ±1.7	9.4 ±1.0
Glycolipids	4.1 ±0.5	8.6 ±1.0	12.7 ±1.4
Phospholipids	2.7 ±0.3	5.2 ±0.4	1.2 ±0.2

[a]Each value represents means ±SD of four separate determinations. [b]Determined gravimetrically. [c]By summation of individual neutral lipid components.

applied to the column, 95% was recovered in the excluded fraction, and the rest was eluted as a long trailing, protein-rich fraction (Fig. 1, lower). $NaDodSO_4$–polyacrylamide gel electrophoresis of the rechromatographed glycoprotein revealed a strong band staining with the periodate–Schiff reagent at the origin where the glycoprotein did not enter the gel, and no extra protein bands staining with Coomassie Blue were detected.

Results of the analyses of the purified glycoprotein showed that carbohydrates constitute 62.1% of the dry weight of the glycoprotein and consisted mainly of fucose, galactose, 2-acetamido-2-deoxyglucose, 2-acetamido-2-deoxygalactose, and sialic acid. Lesser proportions (1.5–1.8% of total glycoprotein) of glucose were also detected. The ratios of individual sugars were in the range of those found for other preparations of rat intestinal-mucus glycoproteins[2,10]. Following Pronase digestion of the glycoprotein, the carbohydrate content of the isolated glycopeptide fraction increased to 65.4%, but the ratios of the individual sugars remained unchanged.

Table I shows the percentage composition of protein and lipids in rat small-intestinal mucus, and in the purified mucus glycoprotein before and after Pronase digestion. The results indicated that the purified glycoprotein contains 50% more lipids/g of dry sample than the native mucus, and that the treatment of the glycoprotein with Pronase had only marginal effect on the total lipid content of the resulting glycopeptides. However, considerable differences in the proportions of the major lipid classes were noted between the analyzed samples. Of the total lipids from mucus, 58.5% were represented by neutral lipids, 25% by glycolipids, and 16.5% by phospholipids. In mucus glycoprotein, the neutral lipids constituted 46.7% of the total lipids, glycolipids 33.2%, and phospholipids 20.1%. The lipids derived from the glycopeptide fraction contained 40.3% of neutral lipids, 54.5% of glycolipids, and 5.2% of phospholipids.

The neutral lipids derived from each type of sample exhibited similar lipid composition, and consisted of free fatty acids, cholesterol, cholesteryl esters, and

TABLE II

NEUTRAL LIPID COMPOSITION OF RAT SMALL-INTESTINAL MUCUS AND ITS MUCUS GLYCOPROTEIN BEFORE AND AFTER PRONASE DIGESTION[a]

Constituent	*Neutral lipids (mg/100 mg) in*		
	Mucus	*Glycoprotein*	*Glycopeptide*
Free fatty acids	57.5 ±8.1	44.6 ±5.3	36.9 ±4.5
Mono- and di-glycerides	1.1 ±0.2	0.3 ±0.0	1.0 ±0.2
Triglycerides	11.5 ±1.9	6.6 ±0.8	4.8 ±0.6
Cholesterol	24.1 ±2.6	43.8 ±4.1	44.1 ±4.9
Cholesteryl esters	5.8 ±0.8	4.7 ±0.6	13.2 ±2.8

[a]Values represent the means ±SD of four to six separate determinations.

TABLE III

PHOSPHOLIPID COMPOSITION OF RAT SMALL-INTESTINAL MUCUS AND ITS MUCUS GLYCOPROTEIN BEFORE AND AFTER PRONASE DIGESTION[a]

Phospholipid	*Total lipid phosphorus (%) in*		
	Mucus	*Glycoprotein*	*Glycopeptide*
Phosphatidylcholine	42.2	37.1	33.4
Phosphatidylethanolamine	13.4	12.2	16.8
Sphingomyelin	8.4	14.1	8.6
Phosphatidylglycerol	12.5	19.6	19.4
Diphosphatidylglycerol	3.8	5.2	7.4
Phosphatidylserine	3.1		
Phosphatidylinositol	2.3	3.3	5.2
Lysophosphatidylcholine	4.6	1.4	1.6
Lysophosphatidylethanolamine	1.1	2.3	2.0
Phosphatidic acid	3.2	0.5	0.9
Unidentified	5.4	4.3	4.7

[a]Values represent the means of triplicate analyses performed on each sample

mono-, di- and tri-glycerides. However, significant quantitative differences were noted (Table II). The neutral lipids from the glycoprotein contained 22.4% less free fatty acids, 42.6% less triglycerides, and 81.7% more cholesterol than that of the mucus. Neutral lipids of the glycopeptide, in comparison to glycoprotein, contained 1.2 times less free fatty acids, 1.4 times less triglycerides, and 2.8 times more cholesteryl esters.

The phospholipids constituted 2.7% of the dry weight of mucus, and their content increased to 5.2% in the purified mucus glycoprotein, and dropped to 1.2% following digestion of the glycoprotein with Pronase. The proportions of individual phospholipid classes present in all three types of samples are shown in Table III. The major phospholipids identified in these samples were phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, and phosphatidylglycerol.

These four compounds accounted for 76.5% of the total phospholipids in mucus, 83% in the mucus glycoprotein, and 78.2% in the glycoprotein digested with Pronase. The glycopeptide fraction, in comparison to mucus glycoprotein, exhibited higher contents of diphosphatidylglycerol (42.3%) and phosphatidylethanolamine (37.7%), whereas the level of sphingomyelin was 1.6 times higher in the mucus glycoprotein.

T.l.c. of the glycolipids, eluted from silicic acid columns with acetone–methanol, revealed in each type of sample the presence of several orcinol-positive bands. In the glycopeptide sample, none of the glycolipids reacted with benzidine reagent, thus indicating the presence of glyceroglucolipids only. The glycolipids of mucus and mucus glycoprotein were composed mainly of glyceroglucolipids, but each sample also contained simple glycosphingolipids. The latter compounds constituted 7.3% of the total glycolipids of mucus and 0.2–0.3% of the glycolipids of mucus glycoprotein, and were comprised primarily of glucosyl- and lactosylceramide. Following chromatography on DEAE–Sephadex, the acidic glycolipids from all three types of samples were fractionated on thin-layer plates into two components. These glycolipids corresponded, in composition and chromatographic migration, to the sulfated monoacylmonoalkyltri- and -tetra-glucosylglycerol standards[26], and accounted for 45% of the glyceroglucolipid fraction in each type of the analyzed sample (Table IV). The neutral glyceroglucolipids from mucus, mucus glycoprotein, and glycopeptide samples consisted mainly of tri-, hexa-, and octa-glucosylglyceroglucolipids. The proportions of individual glyceroglucolipids present in each type of sample are given in Table IV.

The fatty acid composition of lipids from mucus, mucus glycoprotein, and glycopeptide is given in Table V. The lipids derived from all three samples exhib-

TABLE IV

COMPOSITION OF GLYCEROGLUCOLIPIDS IN THE RAT SMALL-INTESTINAL MUCUS AND ITS MUCUS GLYCOPROTEIN BEFORE AND AFTER PRONASE DIGESTION[a]

Type of glyceroglucolipid and number of Glc residues	*Glyceroglucolipids (mol/100 mol) in*		
	Mucus	*Glycoprotein*	*Glycopeptide*
Neutral			
1	5.5	3.8	4.4
2	3.7	4.9	4.9
3	10.0	9.7	10.3
4	7.2	7.2	6.9
6	15.7	15.8	15.6
8	12.1	13 9	12.8
Sulfated			
3	24.6	23.1	24.3
4	21.2	21.6	20.8

[a]Values represent the means of triplicate analyses performed on each sample.

TABLE V

FATTY ACID COMPOSITION OF LIPIDS FROM RAT SMALL-INTESTINAL MUCUS AND MUCUS GLYCOPROTEIN BEFORE AND AFTER PRONASE DIGESTION

Fatty acid[a]	*Total fatty acids (%) in*		
	Mucus	*Glycoprotein*	*Glycopeptide*
16:0	24.0	20.8	20.3
16:1	1.3	1.5	1.6
18:0	31.6	22.0	25.9
18:1	28.2	37.3	37.6
18:0, α-OH	1.5	2.1	3.2
22:1	1.6	3.5	2.1
24:1	8.4	8.2	4.8
Unidentified	3.4	4.6	4.5

[a]The number before the colon denotes the number of carbon atoms, the number following the colon is the number of double bonds, and α-OH refers to 2-hydroxy fatty acids

ited high content of hexadocanoic, octadocanoic, octadecenoic and tetracosenoic acids. The analyses established that these four compounds account for 92.2% of the total fatty acids in mucus, 88.3% in the mucus glycoprotein, and 88.6% in the glycoprotein digested with Pronase.

DISCUSSION

Our previous studies on the composition of gastrointestinal mucus established that this viscous, slimy covering of the epithelial surfaces of the mucosa, in addition to proteins and glycoproteins, contains substantial quantities of lipids[3,4]. In gastric mucus, these lipids are represented mainly by neutral lipids and glycolipids of glyceroglucolipid type[3,25,26]. In the study presented herein, we have investigated the lipids of rat small-intestinal mucus and shown that they are associated with mucus glycoprotein.

Analyses of the lipid component of mucus revealed that lipids constitute 17.6% of the dry weight of mucus and are comprised of neutral lipids (58.5%), glyceroglucolipids (23.2%), phospholipids (16.5%), and glycosphingolipids (1.8%). The same lipids, although in different proportions, were also present in mucus glycoprotein purified from mucus by a nondegradative method. The mucus glycoprotein, free of noncovalently bound protein, was found to contain 26.4% of lipids. In comparison to mucus, the lipids associated with mucus glycoprotein contained 1.9 times more phospholipids and 2.1 times more glycolipids, showed a 26% increase in neutral lipids, and were essentially devoid of glycosphingolipids. These data are quite similar to those reported earlier for the lipids of gastric mucus and submandibular saliva, where the mucus glycoproteins were found to be enriched in glyceroglucolipids and phospholipids[27,28].

The presence of lipids in mucus and in the purified mucus glycoproteins is not restricted only to the alimentary tract, as they are also found in the mucus and mucus glycoproteins of normal and pathological secretion of the respiratory tract[29–33]. The interaction of lipids with mucus glycoproteins, although of noncovalent type, is quite strong and is not disrupted by such dissociating agents as 6M urea or cesium bromide, which have been used to free the glycoproteins of noncovalently bound proteins[27,33]. Moreover, variation in lipid binding gives rise to the different buoyant densities exhibited by mucus glycoproteins[33]. These data, however, provide only limited information as to the topography of lipids within the mucus glycoprotein molecule and the requirement of the glycoprotein for lipid binding.

Since the intestinal mucus glycoprotein consists of two domaines, a glycosylated domain that is resistant to proteolytic digestion and a domain free of carbohydrates that is susceptible to proteolysis[5,34], we subjected the purified glycoprotein to Pronase digestion, and analyzed the lipid content and composition of the isolated glycopeptides. The results indicate that removal of the nonglycosylated, hydrophobic region from the glycoprotein was accompanied by a moderate (22.3%) loss in neutral lipids, 4.3-fold decrease in phospholipids, and 52.3% increase in glyceroglucolipids. These data suggest that the interaction of phospholipids with rat small-intestinal mucus glycoprotein involves the nonglycosylated domain of the glycoprotein that is susceptible to Pronase digestion. On the other hand, the interaction of glyceroglucolipids with mucus glycoprotein appears to involve the glycosylated domain of the glycoprotein that is resistant to proteolytic attack.

Little is known about the function of lipids in mucous secretions. Available data, however, indicate that lipids contribute to the rheological properties of mucins, affect the penetration of the mucosa by lipophilic substances, and are capable of alteration of the interaction of mucins with calcium ions[2,35–37]. The elevated levels of lipids in mucous secretions of patients with cystic fibrosis point toward their involvement in the alteration of physicochemical and physiological properties of mucus associated with this pathological state[32,38]. The higher content of lipids, in mucus derived from antral area of the stomach as compared to other stomach regions, has been linked to the ability of antral mucus to protect the underlying mucosa against high acidity and duodenal refluxes to which the antral area of the stomach is most frequently exposed[25,26]. This protective function of lipids in gastric mucus may be the result of their ability to modulate the cytolytic activity of the duodenal content. The involvement of glyceroglucolipids in the regulation of peptic activity in the stomach has been recently demonstrated[39,40]. Lipids associated with mucins may also augment such functions of gastrointestinal mucus as lubrication, interaction with bacteria, and waterproofing.

REFERENCES

1 G. B. J. GLASS AND B. L. SLOMIANY, in M. EISTEIN AND D. V PARKE (Eds), *Mucus in Health and Disease*, Plenum, New York, 1977, pp 311–347.
2 J. F. FORSTNER, *Digestion*, 17 (1978) 234–263.
3 A SLOMIANY, S YANO, B L SLOMIANY AND G B. J. GLASS, *J Biol Chem* , 253 (1978) 3785–3791.
4 B. L SLOMIANY AND A. SLOMIANY, *ACS Symp Ser* , 128 (1980) 149–176
5 A. ALLEN, in L. R JOHNSON (Ed.), *Physiology of the Gastrointestinal Tract*, Vol 1, Raven, New York, 1981, pp 617–639.
6 K. O. LLOYD, S BEYCHOK, AND E. A. KABAT, *Biochemistry*, 6 (1967) 1448–1454.
7 K O. LLOYD AND E. A. KABAT, *Proc Natl Acad. Sci. U S A* , 61 (1968) 1470–1477
8 W. NEWMAN AND E. A KABAT, *Arch. Biochem Biophys* , 172 (1976) 535–550
9 G. FORSTNER, J. STURGESS AND J FORSTNER, in M. ELSTEIN AND D V PARKE (Eds), *Mucus in Health and Disease*, Plenum, New York, 1977, pp 349–369
10 A BELLA AND Y. S. KIM, *Arch. Biochem Biophys* , 150 (1972) 679–689
11 B. L SLOMIANY AND K. MEYER, *J. Biol. Chem.*, 247 (1972) 5062–5070
12 B. L. SLOMIANY, V. L. N. MURTY, AND A. SLOMIANY, *J. Biol. Chem* , 255 (1980) 9719–9723
13 B L SLOMIANY, Z. BANAS-GRUSZKA, E. ZDEBSKA AND A SLOMIANY, *J Biol Chem* , 257 (1982) 9561–9566.
14 A ALLEN, in J. W HARMON (Ed), *Basic Mechanisms of Gastrointestinal Mucosal Cell Injury and Protection*, Williams and Wilkins, Baltimore, 1981, pp. 351–367.
15 O H. LOWRY, N J ROSEBROUGH, A L. FARR AND R J. RANDALL, *J Biol Chem* , 193 (1951) 265–275.
16 G E HANKS, M. CASSELL, R N. RAY, AND H. CHAPLIN, *J Lab Clin Med* , 56 (1960) 486–489.
17 M. DUBOIS, K A GILLES, J K HAMILTON, P. A REBERS, AND F SMITH, *Anal Chem* , 28 (1956) 350–356.
18 K W HUGHES AND J R CLAMP, *Biochim. Biophys Acta*, 264 (1972) 418–425
19 K WEBER AND M OSBORNE, *J Biol Chem.*, 244 (1969) 4406–4412.
20 G FAIRBANKS, T I STECK, AND D. F. H. WALLACH, *Biochemistry*, 10 (1971) 2606–2617
21 B L. SLOMIANY, V L N. MURTY, M AONO, A SLOMIANY AND I D MANDEL, *Arch Oral Biol.*, 27 (1982) 803–808.
22 M. D. BISCHEL AND J. H. AUSTIN, *Biochim Biophys Acta*, 70 (1963) 598–600
23 R. R. LOWRY AND I. J TINSLEY, *Lipids*, 9 (1974) 491–492.
24 J BITMAN, D. L WOOD, AND I. M RUTH, *J. Liquid Chromatogr* , 4 (1981) 1007–1021
25 A. SLOMIANY, N. I GALICKI, K KOJIMA, Z. BANAS-GRUSZKA, AND B L. SLOMIANY, *Biochim Biophys Acta*, 665 (1981) 88–91
26 B L SLOMIANY, N. I. GALICKI, K KOJIMA AND A. SLOMIANY, *Eur J Biochem* , 111 (1980) 259–263.
27 B L SLOMIANY, Z BANAS-GRUSZKA, AND A SLOMIANY, *IRCS Med. Sci* , 9 (1981) 757.
28 B. L SLOMIANY, H WITAS, V L N. MURTY, A. SLOMIANY, AND I D MANDEL, *J Dent Res* , 62 (1983) 24–27.
29 S. SAHU AND W. S. LYNN, *Am Rev. Respir Dis.*, 115 (1977) 233–239.
30 M LHERMITTE, G LAMBLIN, P DEGAND, P ROUSSEL AND M MAZZUCA, *Biochimie*, 59 (1977) 611–620
31 C GALBERT, M. FILLIAT, AND G LAMBLIN, *Bull Eur. Physiopathol Respir* , 17 (1981) 197–208.
32 A. SLOMIANY, V L N. MURTY, M. AONO, C E SNYDER, A HERP, AND B L. SLOMIANY, *Biochim Biophys Acta*, 710 (1982) 106–111
33 H. WOODWARD, B HORSEY, V P BHAVANANDAN, AND E A DAVIDSON, *Biochemistry*, 21 (1982) 694–701,
34 M. MANTLE, D MANTLE, AND A. ALLEN, *Biochem J* , 195 (1981) 277–285
35 R. W LEWIS, *J Theor. Biol.* 61 (1976) 21–25.
36 I A SIEGEL AND K. T IZUTSU, *J. Dent. Res* , 59 (1980) 1604–1605.
37 J. C VOEGEL AND A. BELCOURT, *Arch Oral Biol.*, 25 (1980) 137–139
38 B. L. SLOMIANY, M AONO, V L. N. MURTY, A SLOMIANY, M J LEVINE AND I A TABAK, *J Dent. Res* , 61 (1982) 1163–1166
39 B L. SLOMIANY, K KOJIMA, AND A SLOMIANY, *J. Appl Biochem* , 3 (1981) 531–534
40 B L. SLOMIANY, K KOJIMA, H WITAS, AND A. SLOMIANY, *J Appl Biochem* , 4 (1982) 86–89.