JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1986, p. 1079–1083
0095-1137/86/121079-05$02.00/0

Vol. 24, No. 6

Physical and Chemical Characterization of a *Giardia lamblia*-Specific Antigen Useful in the Coprodiagnosis of Giardiasis

JOHN D. ROSOFF†* AND HENRY H. STIBBS

Department of Pathobiology, School of Public Health and Community Medicine, University of Washington, Seattle, Washington 98195

Received 26 February 1986/Accepted 26 August 1986

We recently reported the isolation and identification of a *Giardia lamblia*-specific antigen (GSA 65) that is shed in the stool of giardiasis patients. In the present study, this antigen was affinity purified from sonic extracts of axenically cultured *G. lamblia* trophozoites and characterized to better understand its biological function and its potential usefulness in the design of coprodiagnostic assays for giardiasis. GSA 65 was resistant to proteolytic digestion with trypsin, chymotrypsin, and protease but was sensitive to treatment with $NaIO_4$ as assessed by Western blotting. This antigen was also stable during prolonged storage at 4 and −20°C in 10% Formalin or distilled H_2O as assessed by counterimmunoelectrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and isoelectric focusing gel banding patterns, in conjunction with protein and carbohydrate assays and lectin binding studies, confirmed that this antigen is a highly glycosylated glycoprotein. The resistance of GSA 65 to proteolytic degradation, together with previous immunofluorescence data that indicate the antigen is an integral part of the *G. lamblia* cyst wall, suggests that this molecule may play a role in maintaining the integrity of the cyst in vivo. The ability of GSA 65 to maintain its antigenic structure under a wide variety of conditions makes it an ideal antigen around which to design sensitive immunodiagnostic assays for giardiasis.

We recently reported the isolation and identification of a *Giardia lamblia*-specific antigen (GSA 65) that is shed in the stool of giardiasis patients (18). This antigen was shown to have an approximate molecular weight of 65,000 and to be present in *G. lamblia* cysts and trophozoites.

An understanding of the chemical nature of this *G. lamblia* antigen would be helpful in efforts to design tests for detecting and quantitating levels of the antigen in stool specimens and would also enable us to understand how it is produced, stored, and released by the parasite in vivo. Furthermore, chemical characterization of GSA 65 may lend insight into the biological function of this molecule. In this report, we describe the results of a series of experiments aimed at the physical and chemical characterization of GSA 65.

MATERIALS AND METHODS

Antiserum preparation. A monospecific rabbit antiserum raised against GSA 65 and a polyvalent rabbit antiserum raised against whole *G. lamblia* WB trophozoites were prepared as previously described by Rosoff and Stibbs (18) and Einfeld and Stibbs (5), respectively. Immunoglobulin G fractionation of the monospecific antiserum was accomplished with a protein A-Sepharose column (Pharmacia Fine Chemicals, Piscataway, N.J.) as described by Goding (8). Goat anti-rabbit immunoglobulin G (heavy and light chain specific) was purchased from Cooper Biomedical, Inc., Malvern, Pa.

Parasite culture and antigen preparation. *G. lamblia* WB ATCC 30957 trophozoites were axenically cultured in antibiotic-free medium at 37°C as described by Einfeld and Stibbs (5). After harvest in the late log phase, trophozoites were chilled on ice for 20 min, pelleted by centrifugation at 800 × *g*, and washed four times in cold phosphate-buffered saline (PBS) or cold 0.14 M saline–1% glucose. After being washed, cultures were suspended in PBS containing 0.05% Tween 20 (PBS-T) and sonicated with six 10-s bursts on ice. Particulate matter from sonic extracts was removed by centrifugation at 12,000 × *g* for 6 min, and the supernatant was assayed for protein by the method of Bradford (3).

Affinity purification of GSA 65 was accomplished as described by Grundy (9). Briefly, 60 mg of monospecific rabbit anti-GSA 65 immunoglobulin G was covalently coupled to 2 g of Sepharose CL-4B, transferred to a 10-ml column, and equilibrated with PBS-T. Sonicated trophozoites (100 mg) were applied to the immunoadsorbent column and reacted for 2 h at 4°C. Unadsorbed antigens were removed by washing the column with 100 ml of PBS-T. Nonspecifically adsorbed antigens were eluted with 50 ml of PBS-T containing 1 M NaI. GSA 65 was eluted with 25 ml of PBS-T containing 3 M NaI, exhaustively dialyzed against distilled water at 4°C, and lyophilized. Lyophilized GSA 65 was reconstituted in distilled water and assayed for protein by the method of Bradford (3) and for hexose content by the orcinol-H_2SO_4 method (2). Affinity-purified GSA 65 was stored at −20°C until use.

Characterization of GSA 65. Affinity-purified GSA 65 was characterized to elucidate the chemical nature of the molecule. The antigen was boiled for 10 min to assess its heat stability. Stability under oxidizing conditions was assessed by periodate treatment: a solution of 100 mM $NaIO_4$ was prepared in 20 mM acetate buffer (pH 4.5), and 30 μl was added to 30 μl of antigen in a polypropylene centrifuge tube. Controls received 30 μl of buffer alone. Oxidation was carried out at 4°C for 24 h in the dark. The reaction was immediately quenched with 30 μl of 0.5 M $NaBH_4$, followed by drying under N_2 for 2 h. The proteolytic sensitivity of GSA 65 was determined by incubation with trypsin, chymotrypsin, and protease (all from Sigma Chemical Co., St. Louis, Mo.). Trypsin and chymotrypsin were used at an

* Corresponding author.
† Present address: Seattle Biomedical Research Institute, Seattle, WA 98109-1651.

enzyme concentration of 200 μg/ml in 0.1 M Tris hydrochloride buffer (pH 8.0). GSA 65 was digested for 24 h at 37°C. Protease was used at a concentration of 10 mg/ml in 0.1 M Tris hydrochloride (pH 8.0) containing 1 mM $CaCl_2$. GSA 65 was incubated for 15 min at 37°C, followed by digestion at 60°C for 24 h. Controls received 0.1 M Tris hydrochloride alone. All digestions were terminated by boiling for 3 min. The effect of these treatments was evaluated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by Western blotting with monospecific rabbit anti-GSA 65 antiserum (methods described below).

The carbohydrate composition of GSA 65 was assessed in lectin binding studies. A sample (5 μg) of GSA 65 was applied to pieces of (1.5 by 1.5 cm) of nitrocellulose. The nitrocellulose pieces were incubated for 12 h at 23°C in TBS-CS (10 mM Tris hydrochloride, 150 mM NaCl [pH 8.0], 10% fetal calf serum) to block the remaining binding sites. The TBS-CS solution was replaced with freshly prepared concanavalin A-peroxidase, castor bean agglutinin-peroxidase (RCA-I), or wheat germ agglutinin-peroxidase, (all from Sigma) solutions at 50 μg/ml in TBS-CS containing 5 mM $CaCl_2$ and 5 mM $MgCl_2$. Each strip was incubated for 30 min at 23°C, followed by three 5-min washes in TBS-CS to remove unbound lectin-peroxidase conjugates. Bound peroxidase was detected by the addition of a freshly prepared solution of 0.06% (wt/vol) 4-chloro-1-naphthol (Sigma)–0.01% hydrogen peroxide in 0.1 M Tris buffer. The specificity of binding was assessed by the addition of the appropriate competing carbohydrate (1) to the reaction mixture at a concentration of 100 mM.

Electrophoretic techniques. (i) SDS-PAGE. SDS-PAGE was used to assess the stability of affinity-purified GSA 65 after proteolytic enzyme digestion, oxidation, and boiling. SDS-PAGE was done basically as described by Laemmli (11). A 5% stacking gel and an 8% separating gel were used. Samples were mixed with an equal volume of sample buffer consisting of 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.004% bromophenol blue, and 0.2 M Tris hydrochloride (pH 6.8) and boiled for 2 min before addition to the sample slots. After SDS-PAGE, some slabs were used for Western blotting, whereas others were silver stained for protein detection by the method of Merril et al. (13) or stained for carbohydrate detection by the periodate oxidation-silver stain method described by Tsai and Frasch (23).

(ii) Western blotting. Antigens separated by SDS-PAGE were transferred to nitrocellulose paper and analyzed by the method of Towbin et al. (22) as modified by Burnette (3). After the transfer, nitrocellulose sheets were incubated for 1 h at room temperature in blocking buffer {160 mM NaCl, 5 mM tetrasodium EDTA, 0.25% gelatin, 0.1% Tween 20 in 10 mM TES [*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid] [pH 7.3]}. The sheets were transferred to fresh blocking buffer containing 0.32% monospecific rabbit anti-GSA 65 antiserum or rabbit anti-whole trophozoite antiserum and incubated with slow shaking for 1 h, followed by six 5-min washes in PBS-T. The sheets were placed in fresh blocking buffer containing 0.1% goat anti-rabbit horseradish peroxidase-conjugated antibody. (Antibodies Inc., Davis, Calif.), followed by six 5-min washes in PBS. Nitrocellulose sheets were developed by the addition of a freshly prepared solution of 0.06% (wt/vol) 4-chloro-1-naphthol (Sigma) and 0.01% hydrogen peroxide in TBS.

(iii) IEF. Affinity-purified GSA 65 was analyzed by isoelectric focusing (IEF) with agarose as described by Marine Colloids (12) with the following modifications. The agarose gels contained 1% IsoGel agarose and 3% ampholyte (IsoGel ampholyte [pH 3.0 to 10.0]; Marine Colloids, Rockland, Maine). No other additives, such as sorbitol, were used. Gels were cast to a thickness of 1 mm at 65°C and stored overnight at 4°C until use.

IEF was accomplished with a thermally controlled Isobox and PS-2500 programmable power supply (both from Hoefer Scientific Instruments, San Francisco, Calif.). An application mask allowed samples of 4 μl to remain in contact with the gel surface for 10 to 20 min to allow sample absorption before the run. The anolyte was 0.5 M acetic acid and the catholyte was 1.0 M NaOH. Samples were initially electrophoresed for 5 min at 500 V. Then, the sample application mask was removed and IEF was continued at a constant power of 7 W for 25 min. The voltage was then increased to 2,000 V for 5 min before termination of the run. The gel was immediately removed from the Isobox and subjected to precipitation in trichloroacetic acid (12).

(iv) CIE. Counterimmunoelectrophoresis (CIE) with monospecific rabbit anti-GSA 65 antiserum was used to assess the stability of GSA 65 after Formalin fixation and prolonged storage at −20 and 4°C in stool eluates previously verified to contain GSA 65 (10). With glass slides (1 by 3 in. [2.54 by 7.62 cm]) covered with 2.5 ml of 0.9% agarose in electrophoresis buffer (20 mM ethanesulfonic acid, [CHES], 16 mM diethyloglycine [bicine] containing 0.05% Triton X-100 [pH 8.86]), two 3-mm holes separated by 5 mm were punched in the agarose so that they were parallel with the electric field. The holes closest to the anode were filled with 10 μl of rabbit anti-GSA 65 monospecific antiserum. The cathodal holes were filled with stool eluate from giardiasis patients (18). Electrophoresis with a field strength of 5 V/cm was performed for 90 min. Before drying and staining plates with Coomassie blue R-250, we washed them for 24 h in TBS [pH 8.0] and rinsed them for 24 h in distilled water.

RESULTS

Assessment of GSA 65 purity. Before characterization experiments, the purity of affinity-purified GSA 65 was assessed by SDS-PAGE, followed by Western blotting and silver staining. Nitrocellulose sheets were developed with antitrophozoite antiserum and monospecific anti-GSA 65 antiserum to assess whether trophozoite antigens in addition to GSA 65 had nonspecifically bound and been eluted from the affinity column. A single diffuse band characteristic of GSA 65 developed at a molecular weight of 65,000 (Fig. 1). Periodic acid-Schiff silver stains of polyacrylamide gels also revealed a single dark staining region in the 65,000-molecular-weight range (not shown). No contaminants were noted in either technique.

Characterization of GSA 65. GSA 65 was characterized by proteolytic digestion, boiling, periodate oxidation, and lectin binding experiments. Affinity-purified GSA 65 was not labile after proteolytic digestion with trypsin, chymotrypsin, or protease. After proteolytic digestion, GSA 65 retained its reactivity with monospecific antiserum. All digests retained the same Western blotting patterns as the undigested controls (Fig. 2). GSA 65 was labile after periodate oxidation but stable after boiling. After oxidation with $NaIO_4$ and reduction with $NaBH_4$, GSA 65 lost immunoreactivity and could not be detected in immunoblots. After boiling, GSA 65 retained its immunoreactivity and electrophoretic mobility in Western blots (Fig. 3). Controls showed no change in immunoreactivity, mobility, or molecular weight.

In lectin-binding studies, GSA 65 bound RCA but not concanavalin A-peroxidase or wheat germ agglutinin-

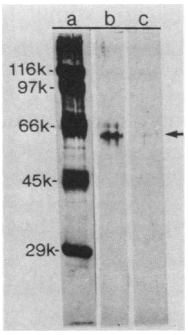

FIG. 1. SDS-PAGE Western blots of affinity-purified GSA 65. Lanes: a, protein molecular weight standards of the indicated molecular weights (top to bottom showing β-galactosidase, phosphorylase *b*, bovine serum albumin, egg albumin, carbonic anhydrase); b, GSA 65 as detected by 0.32% monospecific rabbit anti-GSA 65 antiserum; c, GSA 65 as detected by 0.32% rabbit anti-whole *G. lamblia* trophozoite antiserum. This antiserum detected only GSA 65 (arrow), indicating that affinity-purified preparations did not contain other giardial contaminants.

peroxidase. RCA binding was specific and could be blocked by the addition of 100 mM D-(+)-galactose to the reaction mixture.

In agarose IEF gels, GSA 65 migrated with the low pH standards but failed to resolve into a single well-focused band. Instead, GSA 65 smeared broadly from pH 5.4 to a region far below that of the protein standard with the lowest pI (amyloglycosidase [pI 3.6]). The most intense banding was seen below pH 4, indicating that GSA 65 is very acidic. This smearing was reproducible from run to run. All pH standards were well resolved and stained intensely with Coomassie blue R-250 (Fig. 4).

Stability of GSA 65 under storage conditions. *G. lamblia* cyst-positive stool eluates that were previously verified GSA 65 positive by CIE were resistant to Formalin fixation and prolonged storage. Formalin-fixed and untreated GSA 65-positive stools gave characteristic precipitin arcs in CIE after storage for 6 months at 4 or −20°C (Fig. 5). No precipitin arcs were observed in negative controls.

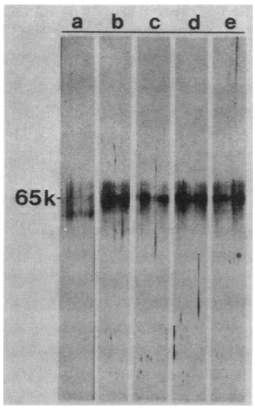

FIG. 2. SDS-PAGE Western blots of proteolytically treated GSA 65 as detected by 0.32% monospecific rabbit anti-GSA 65 antiserum. Lanes: a, undigested (control) GSA 65 as detected by india ink staining of nitrocellulose paper; b, undigested (control) GSA 65; c, trypsin-digested GSA 65; (d) chymotrypsin-digested GSA 65; e, protease-digested GSA 65. GSA 65 remained unaffected by proteolytic enzyme treatment.

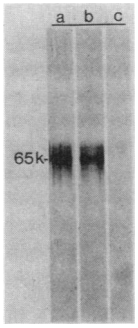

FIG. 3. SDS-PAGE Western blots of heat-treated and oxidized GSA 65 as detected by 0.32% monospecific rabbit anti-GSA 65 antiserum. Lanes: a, untreated (control) GSA 65; b, GSA 65 boiled for 10 min; c, periodate-treated GSA 65, followed by $NaBH_4$ reduction. After oxidation, GSA 65 could not be detected in Western blots. Heat treatment had no effect on GSA 65.

DISCUSSION

An earlier report from this laboratory describes the isolation and identification of GSA 65 from stool eluates of giardiasis patients and *G. lamblia* cysts and trophozoites (18). In the present study, we characterize this antigen.

GSA 65 resistance to proteolytic degradation and boiling and its sensitivity to periodic acid oxidation suggest that this antigen contains carbohydrate. GSA 65 behaves as a glycoprotein: it is highly water soluble, precipitates in ammonium sulfate and trichloroacetic acid, and stains intensely with periodic acid-Schiff reagent in polyacrylamide gels. SDS-PAGE and IEF banding patterns confirmed that GSA 65 is a glycoprotein. Because of differential glycosidation, glycoproteins have a variable charge-to-mass ratio and hence often fail to resolve into single bands characteristic of a pure protein by most electrophoretic techniques (21). GSA 65 gave this characteristic glycoprotein fingerprint in Western blots and in agarose IEF gels.

Carbohydrate and protein assays suggested that GSA 65 is highly glycosidated. GSA 65 has approximately a 1:4 carbohydrate/protein mass ratio, which translates to roughly 1 hexose monosaccharide per 9 to 10 amino acids. This is comparable to the glycosidation ratio found in many mucins (20). Mucins, also known as mucoproteins, contain long

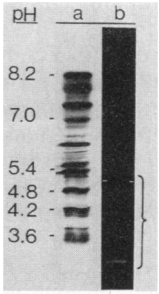

FIG. 4. IEF gel of affinity-purified GSA 65. Lanes: a, Coomassie blue-stained protein standards with the indicated IEF points (top to bottom showing whale myoglobin (major), horse myoglobin (minor), β-lactoglobulin, ovalbumin, glucose oxidase, amyloglucosidase); b, trichloroacetic acid precipitate of GSA 65 as photographed in a light diffraction box. Bracket at the right indicates the acidic pH region in which GSA 65 smeared. The most intense banding occurred in the lowest pH ranges. White lines denote the sample application origin.

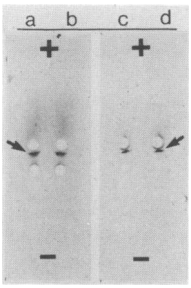

FIG. 5. CIE analysis of GSA 65-positive stool eluates after Formalin fixation, storage for 6 months, or both. Monospecific anti-GSA 65 antiserum was used for antigen detection. Lanes show storage in distilled H_2O at 4°C (a) and at −20°C (b) and in 10% formalin at 4°C (c) and at −20°C (d). GSA 65 retained its antigenic structure under the above conditions. Characteristic immunoprecipitin lines were seen (arrows) in each case.

polysaccharide chains complexed with protein and are widely distributed in eucaryotic systems (17, 20, 24). Lectin studies support the idea that GSA 65 may be a mucoprotein. Like many mucoproteins, GSA 65 contains terminal galactose residues and selectively binds RCA (1, 17, 20). Mucoproteins also usually contain uronic acids and sulfate groups, resulting in molecules with unusually high electronegativities (20). We previously observed GSA 65 to have an uncommonly strong negative charge in crossed immunoelectrophoresis; low pH banding in IEF gels also indicates that this antigen is strongly electronegative.

Several research groups suggest that glycosidation serves a protective function against proteolytic enzyme degradation (6, 7, 19, 25). Fibronectin is highly resistant to proteases in its glycosidated form but susceptible to degradation when deglycosylated (16). Enzymatic removal of the terminal neuraminic acid from ovine submaxillary gland mucoprotein enhances its susceptibility to trypsin digestion (15). Our previous immunofluorescence studies showed that GSA 65 is a constituent of the *G. lamblia* cyst wall (18). In light of this information, it is possible that the extensive glycosidation of GSA 65 is responsible for the stability of this antigen in the presence of proteases and may serve a function in preserving the integrity of the cyst as it passes down the intestinal tract of the host.

Currently, we have no idea what function GSA 65 serves in the *G. lamblia* trophozoite. Trophozoites have been previously shown to contain significantly less of this molecule than do *G. lamblia* cysts. Unlike cysts, the trophozoite outer membrane yielded no detectable GSA 65 in immunofluorescence studies. We have not detected GSA 65 in *G. lamblia* trophozoite culture supernatants; therefore, it is unlikely that this molecule is shed directly into the surrounding environment as an in vitro excretory-secretory product, as has been previously shown for other giardial antigens (14). Whether this antigen is shed by the trophozoite in vivo as it encysts is not known. Regardless of the functional role of GSA 65 in cysts and trophozoites, it is freely shed into the stool of giardiasis patients in high amounts. The possibility exists that GSA 65 is liberated into the stool as a result of cyst degradation by a yet unknown mechanism. This theory is supported by our previous findings of high concentrations of GSA 65 in the stool of symptomatic giardiasis patients who were low cyst excretors (unpublished data).

The presence of GSA 65 in stool eluates of giardiasis patients and its apparent lack of cross-reactivity with other intestinal protozoa originally stimulated our interest in the potential usefulness of this antigen in the design of coproimmunodiagnostic assays for giardiasis. Soon it became apparent that if GSA 65 was to be a useful diagnostic antigen, it would have to retain its antigen structure under the wide variety of conditions found in the host intestinal tract, especially proteases and broad changes in pH. It would also be necessary for GSA 65 to be resistant to the transport and storage conditions commonly used with specimens submitted to the ova and parasite diagnostic laboratory; in particular, maintenance of its antigenic structure in 10% Formalin and during periods of cold storage would be a high priority.

With the above considerations in mind and in light of what we now know about this antigen, GSA 65 appears to be an ideal antigen around which to design immunodiagnostic tests for giardiasis. Its resistance to proteolytic enzymes provides assurance that when passed in the stool, this antigen remains intact and can be detected immunologically. Stability during Formalin treatment and prolonged storage at 4 or −20°C implies that the traditional methods of stool collection and storage need not be altered. Stools may be Formalin treated, shipped, and stored at ambient temperature before assay. At this time, assay by CIE seems to be the most feasible means of detecting GSA 65. However, work is also underway to develop enzyme-linked immunosorbent and particle agglutination assays for this antigen.

ACKNOWLEDGMENTS

We thank Kwane Nyame of the Department of Zoology at the University of Georgia and Stephen J. Murray of the Department of Pathobiology at the University of Washington for their technical advice.

This reseach was funded by grant C6/181/142(A) from the World Health Organization, Diarrhoeal Diseases Control Programme, Geneva, Switzerland.

LITERATURE CITED

1. **Alroy, J., A. A. Ucci, and M. E. A. Pereira.** 1984. Lectins: histochemical probes for specific carbohydrate residues, p. 68–88. *In* R. A. DeLellis (ed.), Advances in immunohistochemistry. Masson Publishing U.S.A., Inc., New York.
2. **Balazs, R., B. W. L. Brooksbank, A. J. Patel, A. L. Johnson, and D. A. Wilson.** 1971. Incorporation of [^{35}]sulphate into brain constituents during development and the effects of thyroid hormone on myelination. Brain Res. **30**:273–293.
3. **Bradford, M. N.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
4. **Burnette, W. N.** 1981. "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal. Biochem. **112**:195–203.
5. **Einfeld, D. A., and H. H. Stibbs.** 1984. Identification and characterization of a major surface antigen of *Giardia lamblia*. Infect. Immun. **46**:377–383.
6. **Garoff, H., and R. T. Schwarz.** 1978. Glycosidation is not necessary for membrane protein insertion and cleavage of Semliki virus membrane proteins. Nature (London) **274**:487.
7. **Geisow, N.** 1981. A carbohydrate signal for intracellular transit. Nature (London) **290**:15.
8. **Goding, J. W.** 1976. Conjugation of antibodies with fluorochromes: modifications to the standard methods. J. Immunol. Methods **13**:215–226.
9. **Grundy, M. S.** 1982. Preliminary observations using a multilayer ELISA method for the detection of *Entamoeba histolytica* trophozoite antigens in stool samples. Trans. R. Soc. Trop.

Med. Hyg. **76**:396–400.
10. **Kenny, G. E.** 1983. Counterimmunoelectrophoresis for the diagnosis of pneumococcal respiratory and other infections, p. 105–111. *In* J. D. Coonrod, L. J. Kunz, and M. J. Ferraro (ed.), Direct detection of microorganisms in clinical samples. Academic Press, Inc., Orlando, Fla.
11. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
12. **Marine Colloids.** 1982. Methodology for agarose isoelectric focusing, immunofixation, and related techniques. Marine Colloids Technical Bulletin. Marine Colloids, Rockland, Maine.
13. **Merril, C. R., D. Goldman, S. A. Sedman, and M. H. Ebert.** 1981. Ultrasensitive stain for proteins in polyacrylamide gels shows regional variation in cerebrospinal fluid proteins. Science **211**:1437.
14. **Nash, T. E., F. D. Gillin, and P. D. Smith.** 1983. Excretory-secretory products of *Giarida lamblia*. J. Immunol. **131**:2004–2010.
15. **Olden, K., R. M. Pratt, and K. M. Yamada.** 1979. Role of carbohydrate in biological function of the adhesive glycoprotein fibronectin. Proc. Natl. Acad. Sci. USA **76**:3343–3347.
16. **Olden, K., J. B. Parent, and S. L. White.** 1982. Carbohydrate moieties of glycoproteins. Biochim. Biophys. Acta **650**:209–232.
17. **Roehrig, K. L.** 1984. Carbohydrate biochemistry and metabolism. Avi Publishing Co., Inc., Westport, Conn.
18. **Rosoff, J. D., and H. H. Stibbs.** 1986. Isolation and identification of a *Giardia lamblia*-specific stool antigen (GSA 65) useful in coprodiagnosis of Giardiasis. J. Clin. Micro. **23**:905–910.
19. **Schwarz, R. T., M. F. Schmidt, and R. Datema.** 1979. Inhibition of glycosidation of viral glycoproteins. Biochem. Soc. Trans. **7**:322.
20. **Sharon, N.** 1974. Glycoproteins. Sci. Am. **230**:78–86.
21. **Sharon, N.** 1975. Complex Carbohydrates. Addison-Wesley Publishing Co., Inc., Reading, Mass.
22. **Towbin, H., T. Staehlin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:607–610.
23. **Tsai, C. M., and C. E. Frasch.** 1982. A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. Anal. Biochem. **119**:115–119.
24. **White, A., P. Handler, and E. L. Smith.** 1973. Principles of biochemistry, 5th ed. McGraw-Hill Book Co., New York.
25. **White, D. A., and B. K. Speak.** 1979. Protein glycosidation in animal secretory tissues. Biochem. Soc. Trans. **7**:326.