

Lectin Binding by *Giardia lamblia*

DAVID R. HILL, ERIK L. HEWLETT, AND RICHARD D. PEARSON*

Divisions of Geographic Medicine and Infectious Diseases, Department of Medicine, University of Virginia School of Medicine, Charlottesville, Virginia 22908

Received 4 June 1981/Accepted 25 August 1981

Surface carbohydrates of *Giardia lamblia* were examined using six plant lectins chosen because of their specificity for major carbohydrate moieties. The binding to axenically grown *G. lamblia* trophozoites was assessed in both a quantitative microagglutination assay and a fluorescence assay. Of the six lectins tested, wheat germ agglutinin (WGA) agglutinated the highest percentage ($22.9 \pm 3.7\%$) of live trophozoites, and fluorescein-labeled WGA ($100 \mu\text{g/ml}$) bound to $98 \pm 5\%$ of them. Since the carbohydrate specificity of WGA includes both *N*-acetyl-D-glucosamine (GlcNAc) and sialic acid, inhibition experiments were performed. GlcNAc inhibited the binding of WGA to *G. lamblia* in both assays to a greater extent than did sialic acid. Binding of WGA was not altered by prior treatment of trophozoites with neuraminidase, suggesting that WGA was binding to GlcNAc moieties on *G. lamblia* and not to sialic acid. The remaining five lectins either bound nonspecifically or exhibited low percentages of binding. The apparent presence of GlcNAc but not sialic acid or other exposed surface carbohydrates may be important in the interaction of *G. lamblia* with its human host.

Giardia lamblia is an intestinal protozoan which is a frequent cause of both endemic and epidemic diarrheal illness worldwide. Several recent water- and foodborne epidemics have occurred in the United States (6, 10, 15, 21), causing significant morbidity. Despite this prevalence of giardiasis, little is known about the pathogenesis of disease, the interaction of the organism with host defense mechanisms, and the basic parasitology of the protozoan, including its cell surface characteristics.

Microbial surfaces and membrane-associated glycoproteins are important in many stages of disease (27). These may include pathogen adherence and colonization (2, 14), host humoral and cell-mediated immune response, and microbial susceptibility to complement, phagocytosis, and intracellular killing (11, 27). In a number of instances, the basic properties of microbial cell surfaces have been shown to impair host immune responses (4). In the case of *Giardia*, it is likely that its surface plays a role in adherence to intestinal mucosa and the subsequent interaction with humoral and cellular host defense mechanisms. Therefore, a study of the surface characteristics of *G. lamblia* was undertaken to enhance our understanding of both the parasite and the pathogenesis of giardiasis.

The recent development of an axenic culture technique (28) for *G. lamblia* made possible this *in vitro* study of a human isolate (18). Using oligosaccharide-specific plant lectins as probes,

we examined representative surface carbohydrates of *Giardia* in microagglutination and fluorescence assays. *Giardia* organisms bind the lectin wheat germ agglutinin (WGA), but in contrast to other pathogenic protozoa (8, 17, 22), they appear to expose relatively little surface carbohydrate.

MATERIALS AND METHODS

Parasites. *G. lamblia*, Portland 1 strain, was a human isolate originally obtained by E. A. Meyer (18). The *Giardia* trophozoites were axenically grown in Trypticase Panmede Serum (TPS-1) medium using the modification by Meyer (18) and Visvesvara (28) of Diamond's *Entamoeba histolytica* medium (7). Major medium components included Trypticase (BBL Microbiology Systems, Cockeysville, Md.), Panmede (Paines and Byrne Ltd., Greenford, England), 10% (vol/vol) heat-inactivated fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.), and 3% (vol/vol) NCTC vitamin mixture no. 107 (GIBCO). Reduced oxygen tension was maintained by adding 0.1% (wt/vol) L-cysteine hydrochloride and placing 16 ml of culture medium in tightly capped 16-by-125-mm borosilicate tubes. *Giardia* were cultured at 37°C.

After 2 to 6 days of growth, *Giardia* trophozoites were harvested in the following manner. Culture tubes were immersed in ice for 10 min and then vigorously shaken to dislodge trophozoites attached to the glass. Trophozoites were pelleted at $250 \times g$ for 5 min, washed four times in phosphate-buffered saline (PBS) (pH 7.2), and resuspended in PBS for all studies.

Agglutination assay. A microagglutination assay was developed to assess lectin binding to *Giardia*

since, in preliminary experiments, macroagglutination by the standard slide agglutination test did not occur with parasite concentrations up to 2.5×10^6 /ml.

The lectins used—WGA, phytohemagglutinin (PHA), concanavalin A (ConA), peanut agglutinin (PNA), soybean agglutinin (SBA), and fucose binding protein—were obtained from Sigma Chemical Co., St. Louis, Mo., and prepared in PBS (pH 7.2). The monosaccharides used to specifically inhibit these lectins were: *N*-acetyl-D-glucosamine (GlcNAc) (1, 12) and *N*-acetyl-D-neuraminic acid (NANA) (3, 23) for WGA; *N*-acetyl-D-galactosamine (GalNAc) (5, 13) for PHA and SBA; D-mannose and D-glucose (13) for ConA; D-galactose (13) for PNA, and L-fucose (13) for fucose binding protein. Monosaccharides were obtained from Sigma.

Giardia trophozoites (5×10^5) were incubated with 0, 1, 10, 25, 50, 100, 250, and 500 μ g of lectin in 1 ml of PBS (pH 7.2) at 22°C for 15 min. The lectin-trophozoite suspension was then centrifuged at $250 \times g$ for 5 min, 0.7 ml of supernatant was discarded, and the pellet was gently dispersed in the remaining volume. This was placed on a hemacytometer and examined microscopically. Agglutination was assessed by counting the number of trophozoites that were agglutinated in groups of ≥ 3 and determining the percent of agglutinated trophozoites. At least 200 total parasites were counted.

Specific inhibition of lectin-induced agglutination was studied by incubating the lectin and trophozoites with 5 mM monosaccharides for 30 min before assessing agglutination.

Fluorescein-labeled lectin assay. Fluorescein isothiocyanate (FITC)-labeled lectins were also used to determine lectin binding to *G. lamblia*. Live, washed trophozoites (10^6) were incubated with 100 μ g of FITC-labeled WGA (Miles-Yeda Ltd., Rehovot, Israel), PHA, ConA, PNA, or SBA (Cappel Laboratories, Inc., Cochranville, Pa.) in 1 ml of PBS (pH 7.2) at 4°C for 30 min. The parasites were then washed twice and suspended in 0.2 ml of PBS. At least 100 live trophozoites were examined in a fresh wet mount using a Zeiss Research Microscope equipped with phase-contrast and epifluorescence optics. Photomicrographs were made with a Zeiss Axiomat Microscope. Fluorescence was graded as 0 = no apparent fluorescence, 1+ = weak fluorescence, and 2+ = strong fluorescence.

Inhibition of FITC-lectin binding to *Giardia* was studied by incubating both lectin and *Giardia* trophozoites with 100 mM monosaccharides for 30 min before performing the fluorescence assay.

Neuraminidase treatment of *Giardia*. WGA binds to both GlcNAc and sialic acid moieties (1, 23). Therefore, the saccharide specificity of WGA binding to *Giardia* was assessed by pretreating live trophozoites with neuraminidase (*Clostridium perfringens*, type IX, Sigma; EC 3.2.1.18), which specifically cleaves sialic acid residues from membrane glycoproteins and glycolipids (25). *Giardia* trophozoites (3×10^6) were incubated with 0.1 U of neuraminidase per ml in 1 ml of PBS (pH 6.0) at 37°C for 1 h. After treatment, trophozoites were cooled in an ice bath and then washed three times in PBS (pH 7.2) at 4°C. Agglutination or fluorescence by WGA (100 μ g/ml) was then

assayed. An equal number of non-enzyme-exposed, but otherwise identically treated, *Giardia* served as a control to the enzyme-exposed group.

The efficacy of neuraminidase treatment was verified by concomitantly treating human erythrocytes and determining the ability of PNA (5 μ g/ml) to agglutinate a 1.5% suspension of cells. PNA agglutinates human erythrocytes only after they have been desialated, thus exposing D-galactose residues (16).

RESULTS

Agglutination assay. The agglutination of *Giardia* trophozoites by lectins of various saccharide specificities is shown in Fig. 1. WGA significantly agglutinated *Giardia* at concentrations of 25 μ g/ml ($P < 0.004$, paired *t* test) and greater. Maximum agglutination by WGA was observed at 250 μ g/ml, at which concentration $22.9 \pm 3.7\%$ (standard error of the mean) of *Giardia* agglutinated. A mean of 5.2 ± 0.4 trophozoites was observed in each agglutination group, with flagellar-body, body-body, and flagellar-flagellar attachment seen.

GlcNAc (5 mM) reduced WGA (100 μ g/ml) agglutination by $56.7 \pm 5.0\%$ ($P < 0.0005$, paired *t* test), and NANA (5 mM) reduced it by $38.8 \pm 7.7\%$ ($P < 0.03$). The difference between GlcNAc and NANA inhibition of agglutination was significant ($P < 0.05$, unpaired *t* test). No inhibition was observed with GalNAc, D-galactose, D-mannose, or D-fucose, confirming the specificity of the WGA-*Giardia* binding.

Neuraminidase treatment of *Giardia* trophozoites did not significantly alter agglutination by WGA (100 μ g/ml), nor did it result in *Giardia* agglutination in the absence of WGA. Ninety-five percent of trophozoites were viable after enzyme treatment, as assessed by trypan blue dye exclusion (24).

PHA and SBA agglutinated *Giardia* significantly ($P < 0.04$, paired *t* test) only at concentrations ≥ 100 μ g/ml, ConA agglutinated only at 250 μ g/ml ($P < 0.02$), and PNA agglutinated at 500 μ g/ml ($P < 0.03$). Even at these lectin concentrations, however, the number of agglutinated parasites was small. The maximum percentage of *Giardia* that agglutinated with PHA was $8.3 \pm 1.6\%$, with SBA was $3.4 \pm 0.3\%$, with ConA was $6.7 \pm 0.9\%$, and with PNA was $5.4 \pm 0.2\%$. Fucose binding protein agglutinated $10.3 \pm 2.6\%$ of *Giardia* at 500 μ g/ml, and although this lectin produced significant ($P < 0.04$) agglutination at concentrations ≥ 25 μ g/ml, agglutination was not inhibited by L-fucose (5 mM) or GlcNAc (5 mM). This suggested a nonspecific lectin-parasite interaction.

For all lectins, percent agglutination generally increased with increasing lectin concentrations

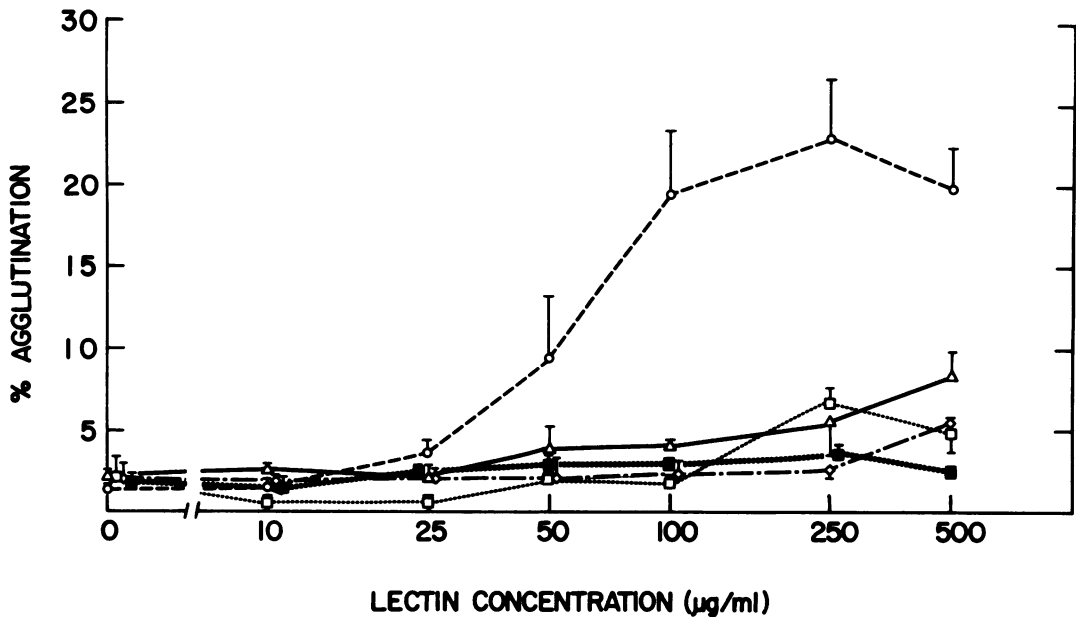


FIG. 1. Percent agglutination of *G. lamblia* trophozoites by WGA (○), PHA (△), ConA (□), PNA (◇), and SBA (■). Each point represents the mean percent agglutination determined from at least three experiments done in duplicate. Brackets indicate the standard error of the mean.

(Fig. 1). In the absence of lectin, $1.7 \pm 0.2\%$ of *Giardia* spontaneously agglutinated.

Fluorescein-labeled lectin assay. The relative fluorescence of *Giardia* trophozoites after treatment with FITC-labeled lectins with and without inhibiting monosaccharides present is shown in Table 1. With FITC-WGA (100 µg/ml), $98 \pm 5\%$ of *Giardia* trophozoites exhibited fluorescence; $93 \pm 3\%$ of them were at the 2+ level. FITC-WGA bound to both agglutinated and unagglutinated trophozoites (Fig. 2). As in the microagglutination assay, GlcNAc and NANA inhibited FITC-WGA binding. Only $13 \pm 8\%$ of *Giardia* were fluorescent in the presence of GlcNAc (100 mM), and $66 \pm 18\%$ were fluorescent in the presence of NANA (100 mM). At this inhibitory concentration of NANA, the trophozoites no longer appeared viable by morphological and motility criteria. Pretreatment with neuraminidase did not decrease fluorescence with FITC-WGA.

In contrast to the findings using FITC-WGA, we found that using FITC-PHA, FITC-ConA, FITC-PNA, and FITC-SBA (100 µg/ml) resulted in much lower percentages of fluorescent cells (Table 1), and when present, fluorescence was generally 1+ rather than 2+. Although there was a suggestion of inhibition of FITC-PHA fluorescence by GalNAc (100 mM) and of FITC-ConA fluorescence by D-mannose (100 mM), the differences were too small to establish specific-

TABLE 1. Fluorescein-labeled lectin assay^a

Lectin (n) ^b	% Fluorescence ^c		
	0	1+	2+
WGA (7)	2 ± 1	5 ± 2	93 ± 3
+ GlcNAc ^d (2)	87 ± 8	13 ± 8	0 ± 0
+ NANA (2)	34 ± 11	60 ± 15	6 ± 3
PHA (5)	81 ± 4	11 ± 2	8 ± 5
+ GalNAc (2)	93 ± 1	7 ± 1	0 ± 0
ConA (6)	80 ± 4	16 ± 3	4 ± 1
+ D-Mannose (2)	94 ± 0	6 ± 0	0 ± 0
PNA (2)	92 ± 2	6 ± 2	2 ± 0
SBA (2)	98 ± 1	2 ± 1	0 ± 0

^a For details of assays see the text.

^b n, Number of experiments.

^c Mean percent fluorescence ± standard error of the mean.

^d Inhibition by 100 mM monosaccharides.

ity. Inhibition experiments were not performed with FITC-PNA and FITC-SBA because of the low percentage of *Giardia* that bound these lectins.

A rim pattern of fluorescence was noted with all lectins. There was no evidence for patching or capping of lectin when the trophozoites were warmed to 22 or 37°C for 30 min.

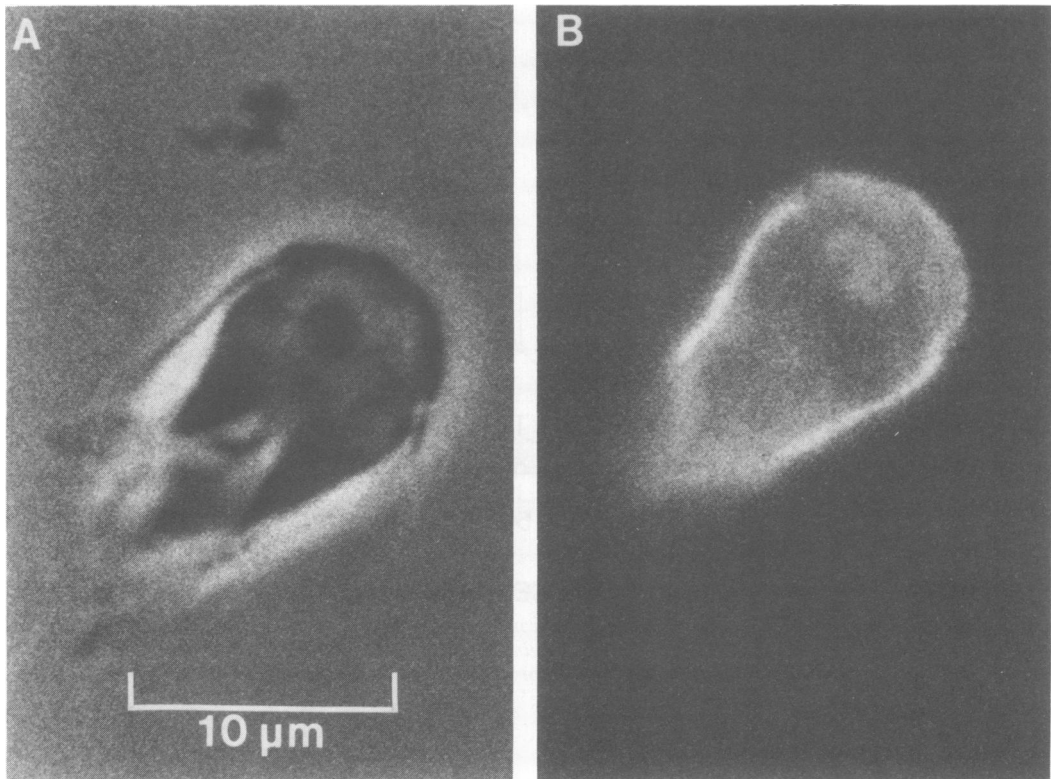


FIG. 2. (A) Phase-contrast photomicrograph of a *G. lamblia* trophozoite exposed to FITC-WGA (100 μ g/ml). (B) The same trophozoite viewed under the fluorescence microscope. Bright fluorescence is observed in a rim pattern.

DISCUSSION

G. lamblia trophozoites exhibited carbohydrate-specific lectin binding in both microagglutination and fluorescence assay systems. This binding was most pronounced with the lectin WGA. Of the six lectins tested, WGA specifically agglutinated the highest percentage of *Giardia* trophozoites, agglutinated *Giardia* at the lowest lectin concentration, and bound to greater than 95% of the trophozoites in the fluorescence assay.

The carbohydrate binding specificity of WGA is twofold. It specifically binds both terminal and internally situated, but exposed, GlcNAc and its β -(1 \rightarrow 4) linked oligomers (1, 12), and it also binds to NANA (3, 23). Both of these saccharides may compete for some of the same combining sites (29). Our studies suggest that WGA is binding to *G. lamblia* via GlcNAc or its oligomers. First, GlcNAc (5 mM) reduced WGA agglutination of *Giardia* by $56.7 \pm 5.0\%$. This inhibition of agglutination was significantly greater than that with NANA. The concentration of GlcNAc used compares closely with the 30 mM concentration required to inhibit by 50%

both WGA agglutination of rabbit erythrocytes (1) and hapten-induced WGA precipitation (12), and the 12.5 mM concentration used to inhibit agglutination of human type O erythrocytes (3). Second, there was greater reduction of fluorescence by GlcNAc (100 mM) than by NANA (100 mM); 87% of *Giardia* showed no fluorescence with GlcNAc, as compared to 34% with NANA (Table 1). Lastly, treatment of *Giardia* with a glycosidase, neuraminidase, did not significantly decrease WGA binding in either the agglutination or fluorescence assays. These results suggest the relative paucity of sialic acid residues on the surface of *G. lamblia* and the presence of exposed GlcNAc or its oligomers.

Although statistically significant agglutination was demonstrated for PHA, ConA, PNA, and SBA, the number of agglutinated parasites was small. In contrast to WGA, which bound to 98% of *Giardia* in the fluorescence assay, the low levels of agglutination produced by these lectins correlated well with low levels of binding observed in the fluorescence assay. The results suggest that, apart from GlcNAc, *G. lamblia* exposes little of the representative carbohydrate moieties on its surface.

Giardia differs from other protozoan parasites in the following two lectin binding characteristics. There was neither macroagglutination of *Giardia* in the standard slide agglutination test, nor significant specific binding of lectins, except WGA, in concentrations less than 100 $\mu\text{g}/\text{ml}$. Virulent strains of *E. histolytica* were strongly agglutinated in a macroagglutination system by low concentrations (10 $\mu\text{g}/\text{ml}$) of ConA (17). In a fluorescence assay Sethi et al. (26) were able to demonstrate specific binding of FITC-conjugated ConA, WGA, and SBA in 10- $\mu\text{g}/\text{ml}$ concentrations to isolated brain cysts of *Toxoplasma gondii*. Animal-passaged trophozoites, however, did not specifically bind these lectins. *Leishmania donovani* has been agglutinated by ConA (30 to 500 $\mu\text{g}/\text{ml}$) and PHA (120 to 500 $\mu\text{g}/\text{ml}$) (8). Dwyer demonstrated macroagglutination of bloodstream and cultured forms of *Trypanosoma lewisi* trypomastigotes by ConA, SBA, WGA, and fucose binding protein (9). Of the lectins tested, ConA produced specific agglutination at the lowest concentration, 10 $\mu\text{g}/\text{ml}$. Recently, Pereira et al. (22) examined various forms of *Trypanosoma cruzi* and found differential agglutination of epimastigotes, bloodstream and cultured trypomastigotes, and amastigotes by lectins which bind GlcNAc, GalNAc, D-galactose, D-mannose, and sialic acid. In contrast to the findings for *G. lamblia*, they determined that the WGA receptor on epimastigotes was not GlcNAc but sialic acid.

The failure of lectins to produce macroagglutination of *G. lamblia* necessitated the development of a microagglutination system. Even when this technique was used with WGA, fewer than 30% of the trophozoites were agglutinated in groups of three or more. The relatively low percentage of agglutination seen with WGA could not be ascribed to the selection of a small, unique subpopulation of *Giardia* trophozoites with exposed GlcNAc residues, since FITC-WGA was shown to bind to 98% of the trophozoites. Therefore, the failure of all lectins to produce macroagglutination of *Giardia*, and the small proportion of trophozoites which did agglutinate in the microsystem, appear to be related to inherent cell-surface characteristics.

Agglutination of cells by lectins is dependent upon several variables which include the valency of the lectin (a minimum number of carbohydrate combining sites sufficient for cross-linking of cells), the number of cells in the assay system, the ability of the cell membrane to cap, shed, or ingest bound lectin, the number of surface carbohydrate residues, and general membrane fluidity (5, 19, 20). WGA is a tetravalent lectin which easily agglutinates cells in other systems (1, 3). In our microagglutination assay system,

the *Giardia*-lectin suspension was gently pelleted to assure adequate cell-to-cell contact. Furthermore, there was no evidence for capping or shedding of bound FITC-WGA at 37°C. Therefore, the lack of macroagglutination of *Giardia* by WGA appeared to be due either to a relatively low number of exposed GlcNAc residues, or to a lack of parasite membrane fluidity which allowed only minimal receptor clustering. Further study is needed to determine the role that these surface characteristics play in the pathogenesis of giardiasis.

ACKNOWLEDGMENTS

This investigation was supported in part by the Rockefeller Foundation and by Public Health Service grant T32-AI-07046 from the National Institute of Allergy and Infectious Diseases.

We thank Bonita Shannon and Uma Ritchie for their secretarial assistance, and James Sullivan for his technical assistance. We are particularly indebted to Jay Brown for helpful advice and discussions throughout the course of this work.

LITERATURE CITED

- Allen, A. K., A. Neuberger, and N. Sharon. 1973. The purification, composition and specificity of wheat-germ agglutinin. *Biochem. J.* 131:155-162.
- Beachey, E. H. 1981. Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surfaces. *J. Infect. Dis.* 143:325-345.
- Bhavanandan, V. P., and A. W. Katlic. 1979. The interaction of wheat germ agglutinin with sialoglycoproteins. The role of sialic acid. *J. Biol. Chem.* 254:4000-4008.
- Bloom, B. R. 1979. Games parasites play: how parasites evade immune surveillance. *Nature (London)* 279:21-26.
- Brown, J. C., and R. C. Hunt. 1978. Lectins. *Int. Rev. Cytol.* 52:277-349.
- Center for Disease Control. 1980. Waterborne giardiasis—California, Colorado, Oregon, Pennsylvania. *Morbidity Mortal. Weekly Rep.* 29:121-123.
- Diamond, L. S. 1968. Techniques of axenic cultivation of *Entamoeba histolytica* Schaudinn, 1903 and *E. histolytica*-like amoeba. *J. Parasitol.* 54:1047-1056.
- Dwyer, D. M. 1974. Lectin binding saccharides on a parasitic protozoan. *Science* 184:471-473.
- Dwyer, D. M. 1976. Cell surface saccharides of *Trypanosoma lewisi*. II. Lectin-mediated agglutination and fine-structure cytochemical detection of lectin-binding sites. *J. Cell. Sci.* 22:1-19.
- Dykes, A. C., D. D. Juranek, R. A. Lorenz, S. P. Sinclair, W. Jakubowski, and R. Davis. 1980. Municipal waterborne giardiasis: an epidemiologic investigation. *Ann. Intern. Med.* 92:165-170.
- Edwards, M. S., A. Nicholson-Weller, C. J. Baker, and D. L. Kasper. 1980. The role of specific antibody in alternative complement pathway-mediated opsonophagocytosis of Type III, Group B Streptococcus. *J. Exp. Med.* 151:1275-1287.
- Goldstein, I. J., S. Hammarström, and G. Sunblad. 1975. Precipitation and carbohydrate-binding specificity studies on wheat germ agglutinin. *Biochim. Biophys. Acta* 405:53-61.
- Goldstein, I. J., and C. E. Hayes. 1978. The lectins: carbohydrate-binding proteins of plants and animals. *Adv. Carbohydr. Chem. Biochem.* 35:127-340.
- Jones, G. W. 1977. The attachment of bacteria to the surfaces of animal cells, p. 139-176. *In* J. L. Reissig

- (ed.), *Microbial interactions: receptors and recognition*, series B, vol. 3. Chapman and Hall, London.
15. Lopez, C. E., A. C. Dykes, D. D. Juranek, S. P. Sinclair, J. M. Conn, R. W. Christie, E. C. Lippy, M. G. Schultz, and M. H. Mires. 1980. Waterborne giardiasis: a communitywide outbreak of disease and a high rate of asymptomatic infection. *Am. J. Epidemiol.* **112**: 495-507.
 16. Lotan, R., E. Skutelsky, D. Danon, and N. Sharon. 1975. The purification, composition, and specificity of the anti-T lectin from peanut (*Arachis hypogaea*). *J. Biol. Chem.* **250**:8518-8523.
 17. Martinez-Palomo, A., A. Gonzalez-Robles, and M. de la Torre. 1973. Selective agglutination of pathogenic strains of *Entamoeba histolytica* induced Con A. *Nature (London) New Biol.* **245**:186-187.
 18. Meyer, E. A. 1976. *Giardia lamblia*: isolation and axenic cultivation. *Exp. Parasitol.* **39**:101-105.
 19. Nicolson, G. L. 1974. The interactions of lectins with animal cell surfaces. *Int. Rev. Cytol.* **39**:89-190.
 20. Nicolson, G. L. 1976. Transmembrane control of the receptors on normal and tumor cells. I. Cytoplasmic influence over cell surface components. *Biochim. Biophys. Acta* **457**:57-108.
 21. Osterholm, M. T., J. C. Forfang, T. L. Ristinen, A. G. Dean, J. W. Washburn, J. R. Godes, R. A. Rude, and J. G. McCullough. 1981. An outbreak of food-borne giardiasis. *N. Engl. J. Med.* **304**:24-28.
 22. Pereira, M. E. A., M. A. Loures, F. Villalta, and A. F. B. Andrade. 1980. Lectin receptors as markers for *Trypanosoma cruzi*. *J. Exp. Med.* **152**:1375-1392.
 23. Peters, B. P., S. Ebisu, I. J. Goldstein, and M. Flashner. 1979. Interaction of wheat germ agglutinin with sialic acid. *Biochemistry* **18**:5505-5511.
 24. Phillips, H. J. 1973. Dye exclusion tests for cell viability, p. 406-408. In P. F. Kruse and M. K. Patterson (ed.), *Tissue culture*. Academic Press, New York.
 25. Rosenberg, A., and C. L. Schengrund. 1976. Sialidases, p. 295-359. In A. Rosenberg and C. L. Schengrund (ed.), *Biological roles of sialic acid*. Plenum Publishing Corp., New York.
 26. Sethi, K. K., A. Rahman, B. Pelster, and H. Brandis. 1977. Search for the presence of lectin-binding sites on *Toxoplasma gondii*. *J. Parasitol.* **63**:1076-1080.
 27. Smith, H. 1977. Microbial surfaces in relation to pathogenicity. *Bacteriol. Rev.* **41**:475-500.
 28. Visvesvara, G. S. 1980. Axenic growth of *Giardia lamblia* in Diamond's TPS-1 medium. *Trans. R. Soc. Trop. Med. Hyg.* **74**:213-215.
 29. Wright, C. S. 1980. Crystallographic elucidation of the saccharide binding mode in wheat-germ agglutinin and its biological significance. *J. Mol. Biol.* **141**:267-291.