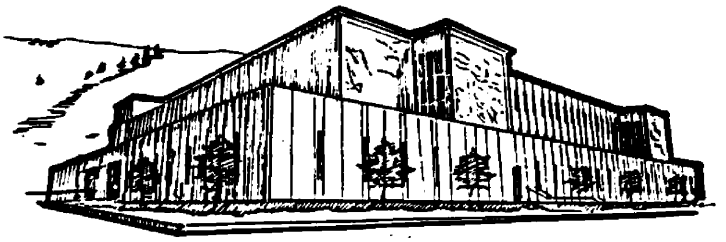
1990

# Identification and partial characterization of glycoproteins in the serum of Biomphalaria glabrata and on the surface of Schistosoma mansoni

Nicholas John Vietri
*The University of Montana*



# IDENTIFICATION AND PARTIAL CHARACTERIZATION OF GLYCOPROTEINS IN THE SERUM OF *BIOMPHALARIA GLABRATA* AND ON THE SURFACE OF *SCHISTOSOMA MANSONI*

by

Nicholas John Vietri
B.S. University of Idaho, 1987

presented in partial fulfillment of the requirements
for the degree of
Master of Science
University of Montana
1990

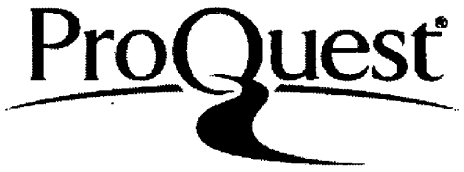
Approved by:

Willard A. Granath Jr.
Chairman, Board of Examiners

Dean, Graduate School

Oct. 17, 1990
Date




ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

Vietri, Nicholas J., M.S. September, 1990 Microbiology

Identification and Partial Characterization of Glycoproteins in the Serum of Biomphalaria glabrata and on the Surface of Schistosoma mansoni (81 pp.)

Director: Willard O. Granath, Jr. WOG.

Serum agglutinin molecules have been postulated as factors which influence schistosome-snail interactions by functioning as bridging molecules between parasite and host. Such molecules must recognize lectin binding sites on the surface of both the tegument of Schistosoma mansoni and on hemocytes of Biomphalaria glabrata. In this thesis the role that glycoproteins in snail serum (cell free hemolymph) may play in antigenic modification of the sporocyst tegument was examined. Specifically, this study characterized and compared glycoproteins in the serum of schistosome-resistant (10-R2) and susceptible (M-line) strains of B. glabrata as well as those associated with S. mansoni sporocysts. Comparison of serum by SDS-PAGE, immunoblotting, and a series of peroxidase labeled lectins with different sugar specificities revealed that 10-R2 snails possessed at least three glycoproteins that were not present in M-line snails. These three serum glycoproteins, found only in schistosome-resistant snails, consisted of a 205 kDa glycoprotein with either N-acetyl-D-glucosamine or N-acetyl-neuraminic acid residues, a 116 kDa glycoprotein with a N-acetyl-D-galactosamine sugar residue, and a 70 kDa glycoprotein with an α-D-mannose or α-D-glucose sugar. These glycoproteins from 10-R2 snail serum only became visible when serum was depleted of hemoglobin by ultracentrifugation. They were not observed in whole serum samples. Results from this study also revealed that sporocysts incubated in 10-R2 or M-line serum absorbed serum glycoproteins on their surface, and these glycoproteins altered the binding of lectin probes. Altered binding of the lectin probes resulted in the visualization of low molecular weight components of snail serum. Moreover, differences existed between the glycoproteins absorbed from 10-R2 and M-line snail serum and serum from 10-R2 and M-line snails induced changes in native S. mansoni glycoproteins during in vitro incubation. Finally, serum from B. glabrata was probed with anti-lectin antibodies. Anti-wheat germ lectin recognized two polypeptides in the serum of 10-R2 snails that were not present it the serum of M-line B. glabrata. These data suggest that snail serum glycoproteins are immunologically important and may be intimately associated with the mechanisms of snail susceptibility or resistance to schistosome infection.

## Acknowledgements

I would like to thank my thesis director, Dr. Bill Granath for the guidance and support he has given me during my graduate studies. I would also like to thank the members of my committee, Dr. Ralph Judd and Dr. Kerry Foresman for their advice and for the time they spent reviewing this thesis. I would also like to acknowledge my grandparents, Mary and Wallace Chunyk, and my parents, Marge and Nick Vietri, for their support and encouragement. This thesis is dedicated to my wife Andrea, whose love and steadfast faith in my goals, have been the motivating force behind my graduate career.

Table of Contents

## List of Figures

# List of Tables

Introduction:

Schistosomiasis, a chronic and debilitating disease caused by a metazoan parasite of humans and other mammals, is one of the most widespread diseases of mankind. Presently endemic in 75 countries world wide, infection with this parasite results in anemia, diarrhea, abdominal pain and sometimes death (Rollinson and Simpson, 1987). Although identification of one of the helminth worms responsible for this disease was first described by the parasitologist Theodore Bilharz over a century ago, it only has been since 1915 that the life cycle of these digenetic trematodes has been known (Leiper, 1915). Moreover, there is evidence indicating that man most likely has been exposed to this devastating disease for thousands of years. For example, ancient papyri of Egypt contain at least 50 references to schistosomiasis (Cheng, 1986), and some medical historians believe that Joshua's curse, which led to the abandonment of Jericho, was due to the introduction of schistosome bearing snails into the communal well by invaders (Hulse, 1971). Thus, schistosomiasis is a disease of antiquity. Although vast amounts of research into many aspects of schistosomiasis have been undertaken, and effective chemotherapeutic agents to cure infected individuals are available, approximately 271 million people still remain infected with this parasite (Schmidt and Roberts, 1989). Further, schistosome-associated disability and death is

estimated at 800 thousand persons annually (Capron et al., 1987).

There are five schistosome species which infect humans. The species with the greatest prevalence and most widespread distribution are Schistosoma mansoni, S. haematobium, and S. japonicum. Two other species S. mekongi and S. intercalatum are considered to be of lesser importance due to their restricted host range. Of these human infecting schistosomes, the most important pathogen causing human schistosomiasis in both the New and Old Worlds is S. mansoni (Rollinson and Southgate, 1987).

S. mansoni, a dioecious trematode which lives in the blood vessels of man, has a complex life cycle involving both a vertebrate definitive host and an invertebrate intermediate host (Cheng, 1986). Briefly, the life cycle of the parasite consists of male and female adult worms residing in the smaller branches of the inferior mesenteric veins of the vertebrate host. Throughout their 3.5 to 12 year life span, adult female S. mansoni can produce about 300 eggs daily. These oval, non-operculated eggs have a characteristic lateral pointed extension of the shell called a spine. Eggs deposited in the venules, pass through the vessel wall and intestinal parenchyma. The exact mechanisms of how this is accomplished is unknown, but blood pressure, the lateral spine, peristalsis, and proteolytic enzymes(s) secreted by the miracidium within the egg, have all been

implicated (Jourdane and Theron, 1987). The passage of eggs to the intestinal lumen, and subsequent deposition of eggs into fresh water via the feces, is necessary for the continuance of the life cycle.

Once excreted into fresh water, osmotic pressure seems to be the most important parameter that influences the hatching of S. mansoni eggs resulting in the release of miracidia (Jourdane and Theron, 1987). Freshly hatched, free-swimming miracidia actively penetrate an appropriate snail intermediate host guided by host chemical stimuli (Chernin, 1970). Within the snail, (certain species of the genus Biomphalaria for S. mansoni) miracidia undergo a morphological transformation and develop into mother sporocysts. Mother sporocysts reproduce asexually giving rise to daughter sporocysts. Eventually large numbers of furcocercous cercariae are produced asexually within the daughter sporocysts. Free swimming, phototrophic cercariae emerge from the snail, and with the aid of an anteriorly located penetration gland, actively seek and burrow into the skin of a definitive mammalian host. After penetration of a definitive host, another transformation occurs, with cercariae giving rise to schistosomules. After approximately 2 days, the schistosomules migrate via the blood stream to the lungs. Following several days in the lungs, the schistosomules are transported via the pulmonary artery circulation to the heart, and eventually become

deposited in the liver via the hepatic portal system. It is here that the schistosomules mature into adult worms. Finally, approximately 23 days post-penetration by cercariae, S. mansoni adult worms pair and take residence in the veins surrounding the large intestine (Cheng, 1986).

Adult schistosome worm pairs mate throughout most of their life span, and as a result, female S. mansoni worms lay eggs for many years. Egg deposition by the female worms occurs in the smaller vessels associated with the host's large intestine. It has been estimated that 50 percent of the eggs deposited in the host never reach the lumen of the small intestine. Instead they remain where they were deposited or become dislodged and swept up in the portal circulation (Katz et al., 1989). Thus, it is the eggs entrapped within the host that give rise to the pathology associated with schistosomiasis. These embolic eggs are deposited in various tissues throughout the body, with the liver being the most affected target organ, where eggs obstruct the pre-sinusoidal capillaries. Within the liver, T-cell dependent granuloma formation occurs stimulated by soluble egg antigens released through submicroscopic egg-shell pores. This host hypersensitivity leads to the formation of advanced fibrovascular lesions which obstruct critical vessels in the liver. Pipestem fibrosis, cirrhosis, hepatic decompensation, encephalopathy due to liver dysfunction, and coma can result (von Lichenberg,

1987). These sequelae are indicative of a poor prognosis for the infected individual.

Failure of modern science to control schistosomiasis, in spite of our understanding of how the disease is transmitted and with the existence of effective chemotherapy, demonstrates that schistosomiasis can not be addressed merely as a biological problem. That is, schistosomiasis is endemic in tropical and subtropical countries, precisely those countries that have less economic resources to invest in schistosome control projects. In some cases, well intended projects such as dam and irrigation construction, have increased the incidence of schistosomiasis due to inadvertently creating new snail habitat (Jordan and Webbe, 1982). Furthermore, since many individuals lack an understanding of the schistosome life cycle and basic hygiene, indiscriminate urination and defecation puts their communities at risk of disease (Mott, 1987). Finally, political instability in endemic countries impedes the procurement of supplies and equipment, prevents adequate data collection, and hinders personnel administering treatment.

It would be naive to suggest, however, that the control of schistosomiasis is simply a problem that can be solved with an increase of monetary resources and education. With regards to infectious diseases, parasitic trematodes, such as S. mansoni, present a formidable obstacle to those

attempting to understand the complex relationship that occurs between parasite and host. As in any host parasite relationship, a parasitic organism must gain access and establish infection in a suitable host while at the same time be able to avoid host-associated immune responses. S. mansoni not only manages to establish infections in a wide range of mammalian species, but also propagates infections in a genus of pulmonate snails (eg. B. glabrata) to continue its life cycle. Establishment of infection in two very different types of hosts, molluscs and mammals, each possessing unique host defense mechanisms, demonstrates the superb ability that S. mansoni has developed to exploit host defense mechanisms. Thus, this parasite, which is so adept at avoiding host immune responses, will present a considerable challenge to researchers attempting to develop vaccines to prevent human schistosomiasis.

As in any host parasite system, the genetics of the host plays a pivotal role in determining whether or not a parasite will be able to establish and continue an infection. Since S. mansoni naturally infects a wide range of mammals (38 species spanning 7 different orders) (Rollinson and Southgate, 1987), the genetic range of the definitive hosts of S. mansoni is quite diverse. In contrast, of the 17 or so well defined species of Biomphalaria in the Americas, only 3 species, B. glabrata, B. straminea and B. tenagophila have been found to be

naturally infected with S. mansoni. Of these, B. glabrata is the major intermediate host of S. mansoni in the Americas (Rollinson and Southgate, 1987). Thus the intermediate hosts of S. mansoni represent a very restricted gene pool as compared with the parasite's vertebrate hosts. This gene pool can be further manipulated since Biomphalaria species are hermaphroditic and can self-fertilize (Richards, 1972). This feature allows for the development and maintenance of genetically-defined strains of B. glabrata. For example, different strains of B. glabrata that vary in their susceptibility to infection with S. mansoni, have been well characterized. One such strain of B. glabrata, designated 10-R2, is 100% resistant to schistosome infection whereas the PR albino M-line strain is 95 to 100% susceptible to parasite infection (Richards, 1975a, 1975b). Since defined strains of B. glabrata exist which vary in their susceptibility to infection, these invertebrate hosts present an opportunity to study parasite interactions with hosts of a defined genetic makeup. Since the genetic variability of the host is restricted, differences between susceptible and resistant hosts should be more easily observed and interpreted. Such differences between susceptible and resistant snails should give researchers insight into those factors which play a role in mediating schistosome-snail compatibility.

Literature review:

In order to understand how S. mansoni is able to initiate and sustain infection in certain strains of B. glabrata, while other strains of the snail are actively resistant to this parasite, one must first understand the components of the snails' immune system. Since knowledge of the specific mechanisms involved in molluscan defenses is far from complete, a review of the current understanding of general invertebrate immunity will be presented. Immunity in invertebrates simply can be described as the recognition of non-self. This is in contrast to the immune responses of higher vertebrates, which contains well understood humoral and cellular effector components, along with the ability to induce specific memory. Although invertebrates lack immunoglobulins and a complement system (Cheng, 1979; Renwrantz, 1983), they still manage to keep their internal tissues sterile. Considering their rather sessile environment this is no small accomplishment; however, our understanding of the mechanisms involved in invertebrate immunity is rudimentary.

In reviewing the literature, one sees much evidence that phagocytosis and encapsulation responses are a universal phenomenon used by invertebrates in response to non-self material. Numerous studies have demonstrated that non-self materials are quickly recognized and digested intra-cellularly by hemocytes, cells believed to be the

ancestors to the vertebrate phagocyte (Olafson, 1986). If a foreign body is too large to be endocytosed by hemocytes, the non-self material may be quickly enveloped by a multilayered cellular capsule (Cheng and Rifkin, 1970). Besides motile phagocytes, some invertebrates also possess a group of non-circulating fixed phagocytes which participate in the clearance of foreign substances from the blood during circulation (Renwrantz and Mohr, 1978; Sminia et al., 1979).

Although invertebrates lack the complexities associated with the vertebrate immune system, such as specific memory and enhanced secondary responses (Lackie, 1980), invertebrate recognition of foreign material is by no means primitive. For example, the crayfish Procambarus clarkii is able to discern self from non-self by clearing various foreign proteins from its circulation and concentrating these proteins in the gills. In their study, Sloan et al. (1975) reported that the immune system of the crayfish was able to differentiate between egg white lysozyme, keyhole limpet hemocyanin, human gamma globulin and bovine serum albumin. The investigators thus hypothesized that the crayfish had naturally occurring receptors for at least three groups of foreign proteins. Similarly, the immune system of the chiton Liolophura gaimardi can distinguish between its own hemocyanin and the very similar hemocyanins of four other closely related species of chitons. Moreover, the rate of clearance of the foreign molecules by

L. gaimardi was related to the degree of structural difference between the homologous and the test hemocyanin. The less the serological cross reactivity, the more rapidly these molecules were eliminated from the circulation (Crichton and Lafferty, 1975). These two studies demonstrate that the immune system of invertebrates used to recognize and phagocytize foreign material has a highly specific discriminatory ability, and that multiple receptor systems apparently have evolved.

The lack of humoral immunoglobulin in invertebrates does not mean that humoral factors are unimportant in invertebrate defense mechanisms. In contrast, hydrolytic enzymes such as lysozyme are known to be released by hemocytes during phagocytosis or encapsulation, and the role of elevated serum lysozyme in molluscan defense molecules has been extensively reviewed by Cheng (1978). In addition, other humoral blood factors such as bacteriocidins (Anderson et al., 1973) and phenoloxidase (Nappi, 1973), have also been implicated. However, humoral factors which have generated the most attention as molecules which may be intimately associated with invertebrate immune responses are the haemagglutinins.

Haemagglutinins, so named because of their ability to agglutinate human or animal erythrocytes, are commonly called lectins. Although initially thought to be exclusively confined to plants, lectins now appear to be

ubiquitous as they are found in almost all living organisms (Sharon and Lis, 1989). These divalent or multivalent carbohydrate binding proteins agglutinate cells through their interactions with membrane glycoproteins or glycolipids. These binding interactions occur primarily via the lectin's carbohydrate binding site. However, secondary forces such as charge interactions and hydrophobicity are also involved in bond stabilization (Rudiger, 1980; Olafsen, 1986). It was suggested by Vasta et al. (1982) that humoral lectins could theoretically function as non-self recognition molecules by binding to glycosylated molecules on the surface of hemocytes and sugar residues on the surface of foreign particles. The humoral lectin would thus act as an opsonin and promote hemocyte attachment, resulting in phagocytosis and/or encapsulation.

There is substantial evidence to suggest that serum lectins function as opsonins in invertebrates. For example, Prowse and Tait (1969) showed that Helix aspersa hemolymph promoted the in vitro phagocytosis of sheep erythrocytes and yeast cells by H. aspersa hemocytes. This humoral mediated increase in phagocytosis (opsonization) has also been demonstrated in vitro with other molluscan sera (Arimoto and Trip, 1977). In vitro studies with the snail Helix pomatia revealed that hemolymph factors were involved in the removal of injected human A or B erythrocytes from the circulation of H. pomatia (Renwrantz and Mohr, 1978).

The nature of these naturally occurring molluscan opsonins has been investigated. For example, an albumin gland extract of the gastropod mollusc Otala lactea, that was shown to stimulate in vitro phagocytosis of formalin-fixed sheep erythrocytes by Olata hemocytes, was identified as a hemagglutinin (Anderson and Good, 1976). Further work on the humoral opsonin in H. pomatia responsible for the in vivo clearance of erythrocytes was conducted by Renwrantz et al. (1981). The investigators demonstrated that the opsonin responsible for erythrocyte clearance possessed carbohydrate-specific binding sites. The nature of this opsonin was further identified to be a hemolymph agglutinin, further implicating lectins as humoral factors involved in foreign cell recognition.

The role of lectins in invertebrate immunity should not be confined to the concept of humoral lectins functioning solely as bridging molecules. As suggested by Vasta et al. (1982), cell membrane associated lectins could also function as a true cell surface receptor, initiating attachment for phagocytosis and encapsulation by recognizing foreign carbohydrate determinants. Evidence supporting the function of lectins as cell surface receptors in invertebrates has been provided by various researchers. For example, rabbit polyclonal anti-sera generated against a naturally occurring snail serum agglutinin of Lymnaea stagnalis cross-reacted with L. stagnalis hemocytes (van der Knaap et al., 1981).

This suggested that L. stagnalis hemocytes synthesize a surface exposed lectin which presumably functions as a receptor for non-self materials. Likewise, polyclonal rabbit anti-sera raised against an Ascidia malaca purified serum lectin bound to a portion of the A. malaca hemocytes as visualized by fluorescence microscopy (Parrinello and Arizza, 1988). However, since immunological cross reactivity between serum and hemocyte associated lectins could have been due to the presence of serum agglutinin molecules passively absorbed onto the hemocyte surface, the existence of a true cellular lectin receptor present on the hemocyte surface remained unconfirmed. However, a true cell membrane associated lectin was finally identified by Vasta et al. (1982). In their experiments Vasta et al. characterized a hemocyte surface associated lectin from the oyster Crassostrea virginica which co-purified with the plasma membrane of disrupted hemocytes during sucrose density gradient centrifugation. Because of this co-purification the investigators contended that the oyster lectin was truly a hemocyte membrane associated lectin rather than an absorbed serum agglutinin. They further speculated that this hemocyte membrane associated lectin was a true integral membrane protein, and therefore may function as a membrane receptor during non-self recognition by molluscan hemocytes.

The fact that both humoral and membrane associated

lectins were found to be important in invertebrate defense mechanisms indicates that invertebrates make use of both cellular and humoral effector mechanisms, analogous to what is known to take place in vertebrate animals. Although invertebrate defense mechanisms are understood less than the defense mechanisms of higher animals, there is some evidence that both cellular and humoral factors may be operating simultaneously in invertebrates. In studies with the mussel Mytilus edulis, Renwrantz and Stahmer (1983) demonstrated that yeast cells, with prior incubation in M. edulis hemolymph, were phagocytized at a greater rate in vitro by M. edulis hemocytes compared to yeast cells incubated in Tris-buffered saline. This study thus demonstrated the presence of a serum opsonin in M. edulis. However, the addition of calcium ions to the yeast in Tris-buffered saline increased the rate of phagocytosis by M. edulis hemocytes. This experiment suggested that the calcium dependent increase in hemocyte activity was due to activation of divalent cation dependent recognition molecules (lectins) on the hemocyte surface. Furthermore, the attachment of the yeast cells to the hemocyte surface was greatly inhibited by the glycoprotein mucin. Since this same glycoprotein also inhibited the agglutinating activity of the isolated humoral lectin, these experiments suggested that a cell bound and a humoral lectin, with very similar recognition sites, were both functioning to remove foreign

cells from the circulation of M. edulis.

With regards to the S. mansoni-B. glabrata system, a body of knowledge has been accumulating recently. The central theme of much of this research has been the elucidation of why certain strains of B. glabrata are susceptible to infection by S. mansoni, while other strains are actively resistant to this parasite. In attempting to understand this phenomenon, numerous studies have been undertaken in an attempt to determine the mechanism(s) whereby the 10-R2 strain of B. glabrata resists infection by the NIH-Sm-PR-1 (PR-1) strain of S. mansoni, and whether or not these mechanisms are absent or inactive in the susceptible M-line snail strain. The 10-R2 strain of B. glabrata actively resist infection since their hemocytes quickly recognize and encapsulate larval S. mansoni. However, the 10-R2 strain of B. glabrata can be induced to become a compatible host for S. mansoni. That is, when 10-R2 B. glabrata defense mechanisms were weakened with a co-infection by irradiated larval Echinostoma paraensei, 10-R2 B. glabrata became susceptible to S. mansoni infection (Lie et al., 1979).

With the presumption that both humoral as well as cellular effector components are most likely operating in invertebrate defense mechanisms, researchers studying the B. glabrata-S. mansoni system have focused their attention on both snail hemocytes as well as serum factors. Bayne

et al. (1980a) showed that the killing of schistosome larvae by the 10-R2 strain of B. glabrata was via a hemocyte-mediated cytotoxic mechanism. Thus, hemocytes were shown to be the effector cells which destroyed S. mansoni primary sporocysts. The comparison of both resistant and susceptible snail hemocytes to identify strain specific differences has been undertaken by various researchers. Using fluorescence-labeled lectin probes, Yoshino (1983) demonstrated that no qualitative differences in surface structure exist between hemocytes of M-line and 10-R2 B. glabrata. However, the broad reactivity of the lectins used in this study would make it difficult to observe minor differences in hemocyte surface membrane structure. Therefore, in an attempt to identify more subtle differences between hemocytes from resistant and susceptible snails, Yoshino and Granath (1983) generated a series of monoclonal antibodies to the hemocyte surface. Antibodies produced from one clone (designated IID7.1-Bg) did identify antigenic heterogeneity between the hemocytes of resistant and susceptible strains of B. glabrata. However, the role these antigen differences may play in mediating resistance or susceptibility to schistosome infection was not elucidated. Interestingly, Bayne et al. (1984) showed hemocytes from both resistant and susceptible B. glabrata could encapsulate S. mansoni sporocysts in vitro, but only hemocytes from the resistant strain were able to damage the sporocyst tegument

during encapsulation. Thus it seems that recognition of S. mansoni by B. glabrata hemocytes is not the only step that leads to parasite destruction. This suggests that other events are required for the activation of hemocytes to a cytotoxic state after recognition has occurred.

In attempts to identify specific hemocyte activation factors, researchers have directed their attention to serum components of B. glabrata. This avenue of research has been fruitful since a series of experiments have shown that resistant, 10-R2 B. glabrata possess a serum factor that can activate hemocytes from susceptible M-line snails to become cytotoxic, and destroy larval S. mansoni. Bayne et al. (1980), utilizing an in vitro assay system, demonstrated that hemocytes from 10-R2 snails destroyed S. mansoni sporocysts while hemocytes from M-line snails did not damage the parasite. However, when cell free hemolymph from 10-R2 snails was added to hemocytes from M-line B. glabrata, these cells became cytotoxic and destroyed S. mansoni sporocysts within 24 hours. Hemocytes from 10-R2 snails remained cytotoxic even when incubated in serum from M-line B. glabrata or when incubated in culture media absent of all snail components. Furthermore, serum from 10-R2 snails had no cytotoxic effects when incubated with S. mansoni sporocysts demonstrating that, although not responsible for parasite killing, serum from resistant snails had a specific activating factor which induced B. glabrata hemocytes to

become cytotoxic.

Similar results to the above also have been obtained from experiments conducted *in vivo*. Granath and Yoshino (1984) were able to passively transfer *S. mansoni* resistance to susceptible M-line snails by injecting cell-free 10-R2 serum into M-line snails one hour before parasite exposure. These susceptible M-line snails did not become infected with *S. mansoni* while a control group of susceptible M-line snails that received M-line sera by passive transfer developed a high level of infection. In addition, another control group of M-line snails, which had received passive transfer of fetal calf serum, also developed a high level of infection after exposure to *S. mansoni*, indicating that the protective effect of 10-R2 serum was not a non-specific response to foreign protein. Furthermore, it was shown that the 10-R2 serum resistance factor was heat labile; heating of resistant serum to 70° C for 30 minutes abolished its ability to confer protection. These two experiments clearly showed that humoral factors were pivotal in the ability of *B. glabrata* to resist infection by *S. mansoni*.

The identity of the serum factor that confers resistance to susceptible snails remains elusive. Comparison of the serum proteins of both resistant and susceptible strains of *B. glabrata* has been carried out by various researchers. For example Bayne *et al.* (1985) failed to find consistent, strain specific differences between the

serum proteins of resistant and susceptible snails when comparing serum polypeptide profiles by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Spray and Granath (1988) showed that sera from both resistant and susceptible strains of B. glabrata had similar polypeptide profiles as assessed by SDS-PAGE and immunoblotting techniques, although they reported that some specific differences (both qualitative and quantitative) did exist.

The failure to identify polypeptide differences between susceptible and resistant strains of B. glabrata by SDS-PAGE does not mean that these two snail sera have the same biological activities. On the contrary, Bayne et al. (1985) reported that serum from resistant B. glabrata agglutinated glutaraldehyde-fixed S. mansoni sporocysts while serum from susceptible B. glabrata did not agglutinate the parasites. Since all invertebrate agglutinins characterized to date have been shown to be lectins (Lackie, 1980), it follows that the agglutinating factor observed in resistant serum of B. glabrata was also a lectin. However, since a comparison of resistant and susceptible serum of B. glabrata using SDS-PAGE did not reveal a specific resistant strain agglutinin, the investigators have postulated that agglutinins with different binding specificities could all have similar electrophoretic mobilities. This is analogous to the similar migration of vertebrate immunoglobulins with

different binding specificities within a single class (Bayne et al., 1985).

Additional evidence that M-line snails lack a serum factor for hemocyte activation in response to S. mansoni was presented by Boswell and Bayne (1985). This in vitro study reported that M-line hemocytes became cytotoxic for S. mansoni sporocysts if the parasites were first incubated with the tetravalent lectin Con A. The cytotoxicity of M-line hemocytes towards Con A treated sporocysts was equal to that of hemocytes from resistant snails with untreated sporocysts. Since pre-treatment of susceptible strain B. glabrata hemocytes with Con A did not appreciably kill untreated S. mansoni sporocysts, this study suggested that to activate hemocytes to a cytotoxic state, the lectin must be on the surface of the parasite. The investigators further postulated that resistant snails have a naturally occurring plasma agglutinin which bridges the host hemocyte and parasite and that susceptible snails lack this serum agglutinin. Thus, this research further implicated lectin bridging molecules functioning as hemocyte activating factors.

If serum agglutinins activate B. glabrata hemocytes to a cytotoxic state by functioning as bridging molecules, both the hemocyte and the surface of S. mansoni sporocysts must have lectin binding sites. As previously stated, lectin binding sites have been documented on the surface of

B. glabrata hemocytes (Yoshino, 1983). S. mansoni primary sporocysts have lectin binding sites as well (Yoshino et al., 1977). Moreover, these carbohydrate epitopes are potent immuno-stimulating molecules, since Boswell et al., (1987) demonstrated that mouse monoclonal antibodies raised against in vitro transformed primary sporocysts, all recognized carbohydrate epitopes on the sporocyst surface. This further implicates a dynamic relationship between sporocyst tegumental carbohydrates and serum agglutinins.

Identification of serum lectin differences between susceptible and resistant strains of B. glabrata is not a trivial undertaking. Agglutination of cells, the most visible method to identify lectins, is a complex process affected by numerous factors. These include molecular properties of the lectin such as the number of binding sites and molecular size. Properties of the cell surface, such as number and accessibility of lectin binding sites, and membrane fluidity and metabolic state, are also important. In addition, temperature, cell concentration and mixing also affect agglutination studies. Furthermore isolation of lectins from biological materials by affinity chromatography requires a knowledge of the sugar specificity of the lectin to be isolated (Sharon and Lis, 1989).

Although actual serum lectin isolation presents a formidable challenge to those studying invertebrate defense mechanisms, the role of lectins in schistosome-snail

interactions can still be evaluated. Namely, an analysis of lectin binding sites on the surface of S. mansoni primary sporocysts could be accomplished by using a series of commercially available peroxidase-labeled lectins with different sugar specificities. Such characterization of the sporocyst surface is important since S. mansoni sporocysts rapidly acquire components of B. glabrata serum on their surface within minutes of being placed in snail serum (Bayne et al., 1986). Obviously, then, snail serum can alter the parasite surface in terms of antigenic composition. Further, the binding of snail serum proteins to the surface of S. mansoni sporocysts is likely to alter lectin binding sites on the parasite surface, since B. glabrata possesses serum proteins that are glycosylated. These glycoproteins, by nature of their carbohydrate chains, could alter or mask lectin binding sites by the presentation of new carbohydrate epitopes on the parasite surface. If hemocyte cytotoxicity is activated by a resistant snail strain serum agglutinin acting as a bridge between parasite and hemocyte, any alterations in the sporocyst's lectin binding sites due to snail serum glycoproteins could be important in determining whether or not the serum hemocyte activating factor could bind to the parasite surface.

If differences in serum glycoprotein composition between susceptible and resistant B. glabrata do exist, these differences could be important parameters of snail

susceptibility or resistance to schistosome infection. Differential binding of serum glycoproteins from resistant and susceptible snails to S. mansoni sporocysts could either enhance or impede the recognition of S. mansoni sporocysts by snail serum agglutinins, the very molecules postulated to activate B. glabrata hemocytes to become cytotoxic.

Because of the above, the goal of this research was to conduct a detailed characterization of glycoproteins associated with the serum of schistosome-resistant and susceptible B. glabrata. Furthermore, an investigation of whether glycoproteins from susceptible or resistant B. glabrata bound differently to the surface of S. mansoni sporocysts (and thus altered lectin binding sites on the parasite surface) was conducted. For these studies SDS-PAGE, electroblotting, $^{125}$I-surface radiolabeling and peroxidase-conjugated lectins were used as probes to identify glycoproteins in the serum of B. glabrata and carbohydrate epitopes on the surface of S. mansoni sporocysts. Incubation studies were also conducted to determine if sporocysts incubated in B. glabrata serum showed evidence of differential acquisition of serum glycoproteins. Finally, B. glabrata serum was probed with commercially available lectin anti-sera to determine the extent of cross-reactivity with well defined plant agglutinins.

Materials and Methods:

Snails and Serum Preparation. Strains of Biomphalaria glabrata that are susceptible (PR albino, M-line) and resistant (10-R2) to NIH-Sm-PR-1 Schistosoma mansoni were maintained in 38 liter aquaria which received constant filtration and aeration. Snails were fed leaf lettuce ad libitum and were subjected to a constant 26°C water temperature with a 12 light:12 dark hour photoperiod.

Hemolymph was collected from snails by head foot puncture (Sminia and Barendsen, 1980) and pooled in 1.5 ml microfuge tubes. The pooled hemolymph was then centrifuged for 10 minutes at 1000 xg to pellet the hemocytes. The supernatant was withdrawn using a pulled pasteur pipette, and this serum was used in various experiments. In order to analyze non-hemoglobin (Hb) associated proteins, Hb-depleted serum fractions were prepared. Such samples were obtained by serum ultracentrifugation as described by Granath et al. (1987).

All chemicals and reagents used were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise noted.

Sporocysts. Eggs of S. mansoni were obtained from the livers of Swiss Webster mice. The mice had been individually exposed to 200 cercariae 7 weeks earlier. The liver of each infected mouse was removed aseptically. Three

mouse livers were placed in a sterile Waring blender and 150 ml of sterile 0.15M NaCl was added. The livers were blended for 30 seconds at the highest speed. The blended liver was poured into a 250 ml sterile graduated cylinder and was allowed to settle. The liver suspension was then decanted until only approximately 30 ml remained. This suspension was then transferred into a sterile sidearm flask, painted black. The flask was then filled with sterile artificial spring water (Ulmer, 1970) and sealed with a rubber stopper. The tip of the sidearm was illuminated with a Nicholas illuminator (Cambridge Instruments, Buffalo, NY) to attract the miracidia. Aliquots containing miracidia were withdrawn from the flask with a sterile Pasteur pipette and placed into 15 ml sterile conical centrifuge tubes that were on ice, to immobilize the miracidia. Immobilized miracidia were then transformed to sporocysts by first evacuating the artificial spring water and then adding 10 ml of RPMI-1640 tissue culture medium (GIBCO, Grand Island, NY) buffered with 10 mM Hepes to pH 7.2. The suspension was gently mixed, lightly capped and allowed to sit at room temperature for 18 hours. Following this, the tubes were centrifuged at 5 xg for 1 minute. All except 1 ml of the supernatant was removed with a pasteur pipette. Sporocysts were then transferred to a sterile 9 well culture dish and washed 5 times with Chernin's balanced salt solution (CBSS) (Chernin, 1963). The ciliary plates were removed with a finely pulled

Pasteur pipette. The sporocysts were then washed 2 times with phosphate buffered saline isotonic with B. glabrata tissue (sPBS; 8.35mM dibasic sodium phosphate; 1.65mM monobasic sodium phosphate; and 45mM sodium chloride). Parasites were then solubilized for SDS-PAGE or used in incubation studies.

SDS-PAGE. To visualize the polypeptide profiles of both snail and parasite samples, serum proteins of both snail strains, as well as sporocysts, were separated by SDS-PAGE using the Tris-glycine system of Laemmli (1970) as modified by Judd (1982). Polypeptide separation was done on 10 percent acrylamide (acrylamide: N,N, methylene bis-acrylamide ratio, 30:0.8) slab gels with a 2.5 percent acrylamide stacking gel. Whole serum and Hb-depleted fractions from both snail strains, as well as sporocysts that were suspended in 150 $\mu$l of sPBS, were first solubilized for 10 minutes at 100° C in a 1:1 ratio with a solution of 4% (wt/vol) SDS (British Drug House. Poole, England), 10% (vol/vol) 2-mercaptoethanol, 10% (vol/vol) glycerol-0.1M Tris (pH 6.8) and bromphenol blue (tracking dye). The resulting samples were then applied to slab gels and subjected to electrophoresis at 10W constant power until the tracking dye reached the bottom of the gel.

Electroblotting. After separation by SDS-PAGE, serum

and sporocyst proteins were electroblotted onto nitrocellulose paper (NCP; Millipore Corp., South San Francisco, CA) using a transblot chamber. Electroblotting was carried out in a 20mM phosphate buffer, pH 7.4 at 0.5 A for 18 hours at 20 V. The NCP was then blocked for 2 hours at room temperature with a PBS-Tween solution (8.35mM dibasic sodium phosphate; 1.65mM monobasic sodium phosphate; 145mM NaCl- 0.5% (vol/vol) Tween 20). Following blockage, peroxidase-labeled lectins, diluted 1:500 in PBS-Tween, were added to the NCP and incubated overnight at room temperature. The peroxidase labeled lectins used in this study and their sugar specificities, are listed in Table I.

Table I. Taxonomic name and sugar specificity of the peroxidase-labeled lectin probes.

| Lectin | Sugar Specificity |
|---|---|
| Canavalin ensiformis | α-D-man, α-D-glc |
| Tetregonolobus purpureas | α-D-fuc |
| Dolichos biflorus | α-D-galNAc |
| Erythrina crystagalli | ß-D-gal(1-4)-D-glcNAc |
| Arachis hypogaea | ß-D-gal(1-3)-D-galNAc |
| Ricinus communis (agglutinin) | ß-D-gal |
| Triticum vulgaris | $(D\text{-}glcNAc)_2$, NeuNAc |
| Glycine max | D-galNAc |

Following incubation, the NCP was washed 4 times during a 1 hour period with fresh PBS-Tween. Lectin binding was detected by the addition of a developer (60 mg of 4-chloro-1-naphthol, 20 ml of methanol, 100 ml of 20mM Tris-500mM NaCl, pH 7.2, and 200 $\mu$l $H_2O_2$). Controls consisted of

incubating replicated samples in specific inhibiting sugars. These sugars were 0.5M methyl-α-D mannopyranoside for Canavalin enisoformis (Con A), 0.5M N-acetyl-D-glucosamine for Triticum vulgaris (wheat germ), and 0.5M N-acetyl-D-glactosamine for Glycine max (soybean). The inhibiting sugars were added before the addition of the peroxidase conjugated lectin. In addition, blots were counter-stained with 1% Indian Ink to monitor the transfer of proteins.

Radioiodination. Whole serum from both resistant and susceptible strains of B. glabrata were radiolabeled with $^{125}I$. This was accomplished by placing 100 μl of whole serum into Iodogen tubes. To prepare these tubes, 20 μl of a 1 mg/ml solution of Iodogen (1,3,4,6, tetrachloro-3-α-6α-diphenylglycoluril (Pierce Chemical Co., Rockford, IL)) in chloroform was placed in siliconized microfuge tubes and allowed to air dry. After the addition of serum, four microliters of $^{125}I$ (as NaI; 50 μCi/μl) (ICN Pharmaceuticals, Irvine, CA) was added to the iodogen tube, which was then placed on ice for 1 hour. Next, the radiolabeled serum was transferred to a new microfuge tube and aliquots were removed and solubilized for SDS-PAGE.

In order to determine if B. glabrata serum proteins bind to S. mansoni sporocysts, the following study was done. Live in vitro-transformed sporocysts (approximately 500 suspended in 150 μl in sPBS) were added to 100 μl of freshly

radiolabeled snail serum in siliconized microfuge tubes. The tubes were gently rocked for 2 hours at room temperature. The sporocysts were then washed to remove unbound iodine by using a pasteur pipette to add ice-cold sPBS to the tubes. The sporocysts were allowed to settle by gravity. Using a finely pulled pasteur pipette the supernatant was withdrawn until only 150 $\mu$l of liquid remained. This supernatant was counted for radioactivity with a Beckman gamma counter (Redmond, WA). These washings continued until the supernatant counts stabilized. The sporocysts were then solubilized for SDS-PAGE.

In vitro-transformed S. mansoni sporocysts also were surface radiolabeled to identify proteins associated with the outer tegument. Approximately 500 sporocysts suspended in 150 $\mu$l of sPBS were transferred to a siliconized iodogen tube and 8 $\mu$l of $^{125}$I was added. The tube was then placed on ice for 30 minutes. After this time the sporocysts were washed with ice-cold sPBS to remove unbound iodine, and solubilized for SDS-PAGE as previously described.

Following SDS-PAGE of radiolabeled samples, the gels were placed in a fixing solution (25% (vol/vol) isopropyl alcohol; 7% (vol/vol) acetic acid) for at least four hours. The gels were then placed in a plastic bag and used to expose Kodak X-OMAT AR film (Kodak, Rochester, NY) for 16 hours. A lightening plus intensifying screen (Dupont, Wilmington, DE), was used to enhance the autoradiographic

image.

To summarize, the samples analyzed in this set of experiments included:

1. $^{125}$I-labeled serum from 10-R2 and M-line *B. glabrata*.
2. Sporocysts incubated in either radiolabeled 10-R2 or M-line serum.
3. $^{125}$I-surface labeled sporocysts.

*Incubation Experiments*. In order to determine if *B. glabrata* serum had the ability to alter lectin binding sites on *S. mansoni* sporocysts, the following experiments were performed. Approximately 500 *in vitro*-transformed sporocysts suspended in 150 µl of sPBS were transferred to siliconized microfuge tubes. To these tubes, 300 µl of M-line or 10-R2 serum, or CBSS (control) was added. The microfuge tubes were gently rocked for 2 hours at room temperature. Next, the sporocysts were washed 7 times with ice-cold sPBS, suspended to a final volume of 150 µl in sPBS, and then solubilized. Protein concentrations of the solubilized samples were determined via ultraviolet absorption at 280 nm as described by Stoscheck (1990). Samples were adjusted with sPBS so they had the same total protein concentration. SDS-PAGE separated samples were then transferred to NCP and probed with various peroxidase conjugated lectins as previously described.

Immunoblotting of Sporocysts and Serum. In order to characterize surface reactive epitopes of S. mansoni sporocysts, SDS-PAGE separated proteins were probed with polyvalent rabbit anti-sporocyst sera. Rabbit immune sera raised against whole sporocysts was diluted 1:20 in PBS-Tween. After SDS-PAGE, separated proteins were transferred to NCP as previously described. The NCP was then blocked for 2 hours in PBS-Tween, and then incubated overnight in the rabbit anti-sera. Following washing of the NCP with PBS-Tween for 2 hours, a 0.1% (vol/vol) solution of protein G-peroxidase (Gamma Bind G-HRP; Genex Corp. Gaithersburg, MD) was added. Following overnight incubation, the NCP was washed for 1 hour at room temperature to remove any unbound protein G. Antibody binding was detected by the addition of the peroxidase developer previously described. Non-specific binding of rabbit serum to sporocysts was controlled by using a 1:20 dilution of normal rabbit serum.

To determine the extent of cross reactivity of B. glabrata serum proteins with well characterized plant lectins, the following was performed using polyvalent rabbit anti-lectin sera. Whole snail serum and Hb-depleted serum samples were first separated by SDS-PAGE, transferred to NCP and blocked with PBS-Tween as described above. The NCP was incubated overnight in a 1:160 dilution of either anti-Con A, anti-wheat germ or anti-soybean sera. Next, the NCP was washed with PBS-Tween, and incubated with protein G-

peroxidase overnight. After washing the NCP with PBS Tween to remove any unbound protein G, antibody binding was detected using the peroxidase developer previously described. A 1:160 dilution of normal rabbit serum was used as a control.

Results:

Specific differences in serum glycoprotein composition between schistosome-resistant and susceptible B. glabrata were revealed using various lectin probes. Three lectin probes that recognized strain specific serum differences were Canavalin ensiformis (Con A), Glycine max (soybean), and Tritiucm vulgaris (wheat germ).

Figure 1 shows the results of serum proteins separated by SDS-PAGE, and then probed with Con A (recognizes α-D-mannose and α-D-glucose). This figure indicates that the Hb-depleted fraction of schistosome-resistant (10-R2) serum had a 70 kDa (*) glycoprotein that was absent from Hb-depleted serum of schistosome-susceptible (M-line) snails. In addition, Con A recognized a wide variety of sporocyst associated glycoproteins ranging in molecular weight from greater than 205 kDa to less than 46 kDa. Likewise, specific snail strain glycoprotein differences were noted when snail serum was probed with soybean lectin (recognizes N-Acetyl-D-galactosamine) (Figure 2). Soybean lectin recognized a 116 kDa (*) glycoprotein present in the Hb-depleted serum of 10-R2 snails, which was absent from Hb-depleted serum of M-line snails. Furthermore, sporocyst associated glycoproteins, varying widely in molecular weight, were also visualized with this lectin, suggesting that S. mansoni sporocysts possess a large number of glycosylated proteins.

Figure 1. Electroblot of whole serum (SER) and Hb-depleted supernatants (SUP) of the 10-R2 and M-line strains of B. glabrata. The electroblot was probed with the lectin Con A. Differences between the electroblot of resistant and susceptible snail sera are indicated (*). Molecular weight markers (MW) are in thousands of daltons (K).

Figure 2. Electroblot of whole serum (SER) and Hb-depleted supernatants (SUP) of the 10-R2 and M-line strains of B. glabrata. The electroblot was probed with the lectin soybean. Differences between the electroblot of resistant and susceptible snail sera are indicated (*). Molecular weight markers (MW) are in thousands of daltons (K).

Fig.1

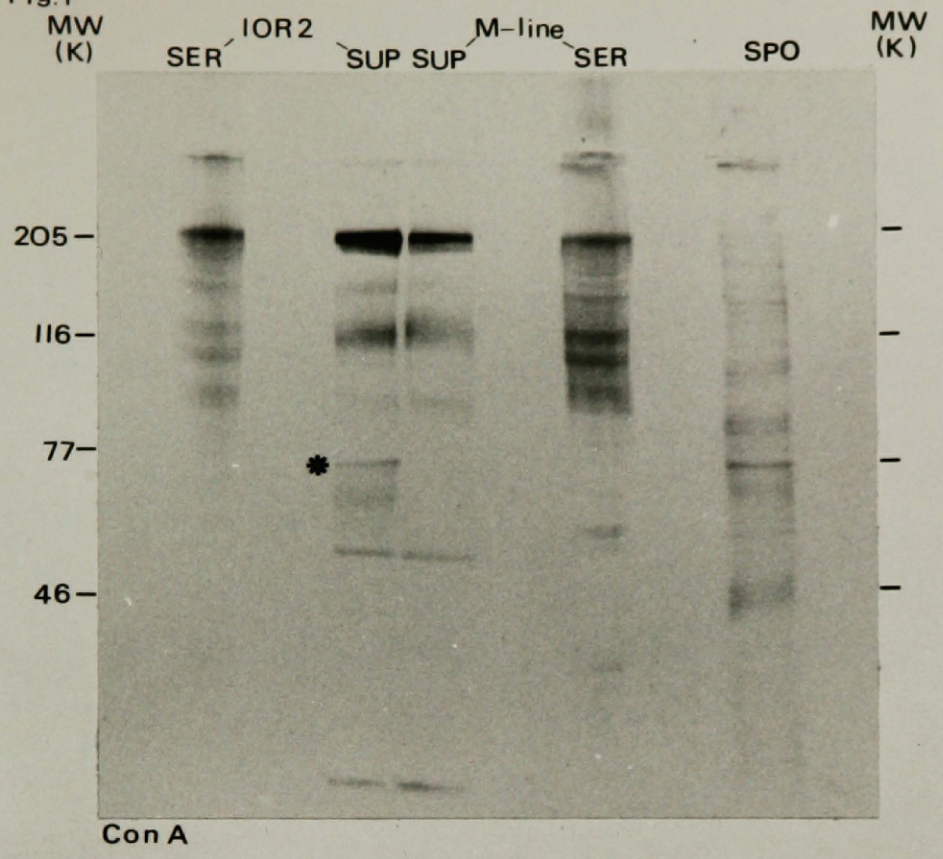
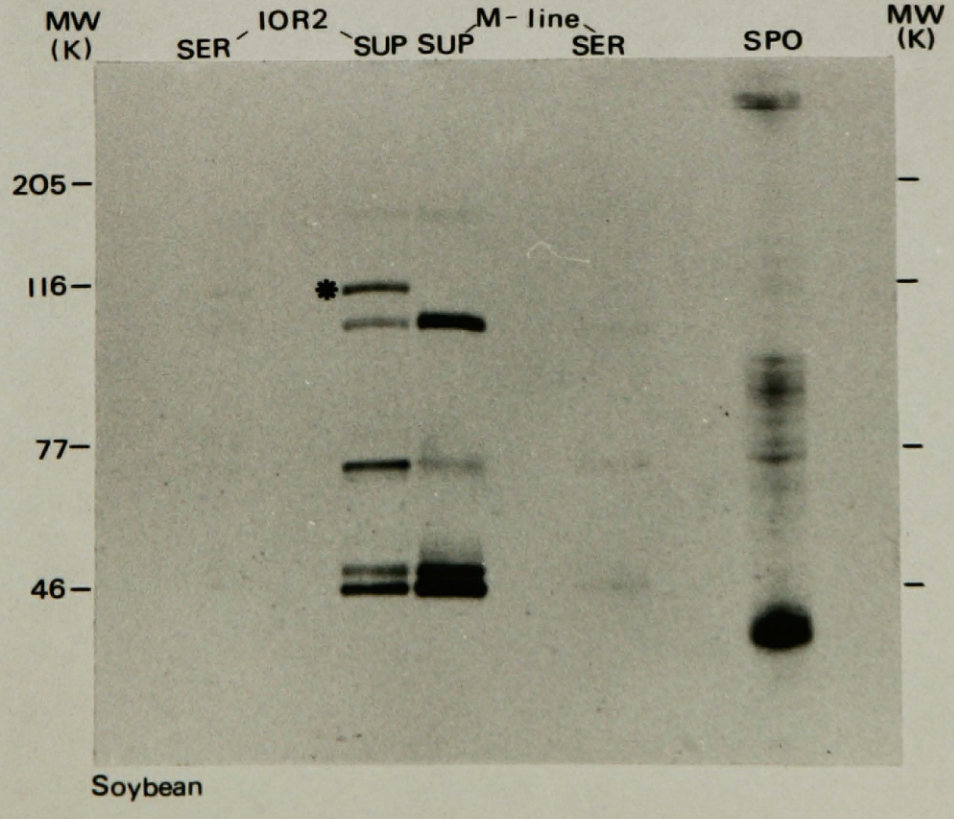

MW (K)
SER
IOR2
SUP
SUP
M-line
SER
SPO
MW (K)
205
116
77
46
Con A

Fig.2


MW (K)
SER
IOR2
SUP
SUP
M-line
SER
SPO
MW (K)
205
116
77
46
Soybean

A third lectin, wheat germ, also revealed differences between the serum of resistant and susceptible snails. This lectin, which is specific for N-acetyl-D-glucosamine and N-acetyl neuraminic acid, revealed two glycoproteins at 116 kDa (*) and 205 kDa (*) in Hb-depleted serum from 10-R2 snails that were absent from M-line snails (Figure 3). As with Con A and soybean lectins, wheat germ also recognized many sporocyst associated glycoproteins which varied greatly in molecular weight.

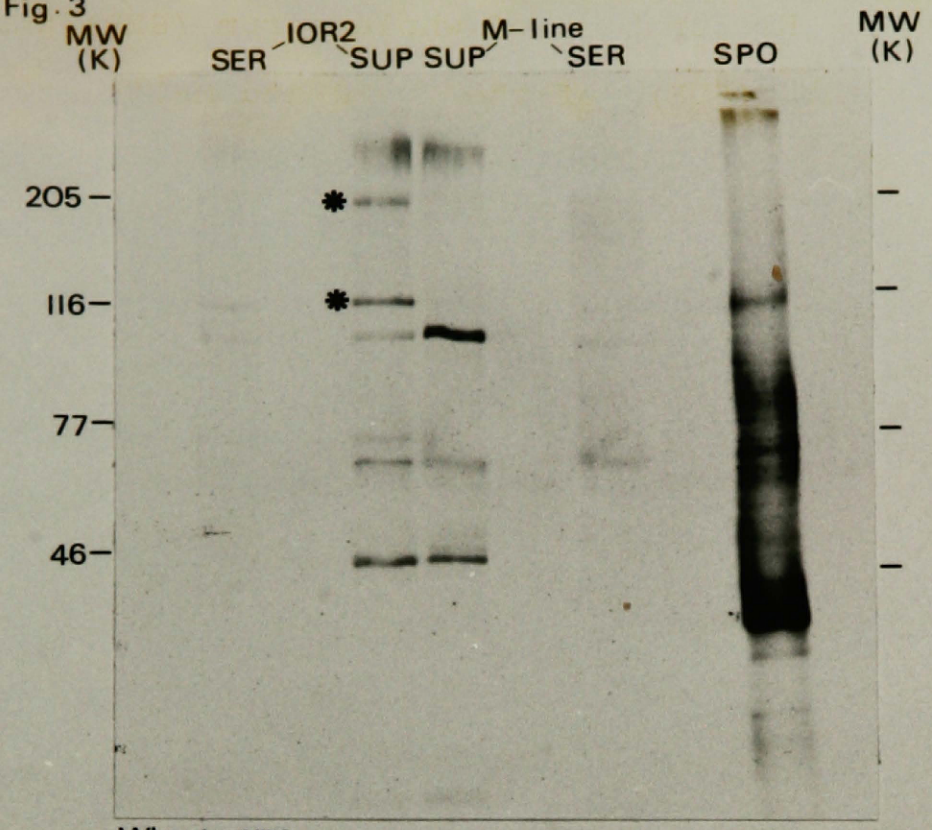
To rule out non-specific lectin binding to snail serum and sporocyst glycoproteins, specific inhibitory sugars were used as controls. In all cases, 0.5M solutions of the appropriate inhibitory sugar blocked lectin binding, indicating that the lectins were indeed recognizing glycosylated proteins via their sugar residues.

In addition to Con A, soybean and wheat germ, five other lectin probes were used to identify snail strain serum differences. Dolichos bifloris lectin (horse gram), which recognizes N-acetyl-D-galactosamine, revealed a glycoprotein profile pattern similar to soybean lectin, with a dominant 116 kDa glycoprotein in 10-R2, but not M-line serum. Arachis hypogaea lectin (peanut), which is specific for ß-D-galactose (1-3) N-acetyl-D-galactosamine residues, revealed no differences between the serum of susceptible or resistant snails. Further, Tetragonolobus purpureas (winged pea), specific for α-L-fructose, and Ricinus communis agglutinin

Figure 3. Electroblot of whole serum (SER) and Hb-depleted supernatants (SUP) of the 10-R2 and M-line strains of B. glabrata. The electroblot was probed with the lectin wheat germ. Differences between the electroblot of resistant and susceptible snail sera are indicated (*). Molecular weight markers (MW) are in thousands of daltons (K).

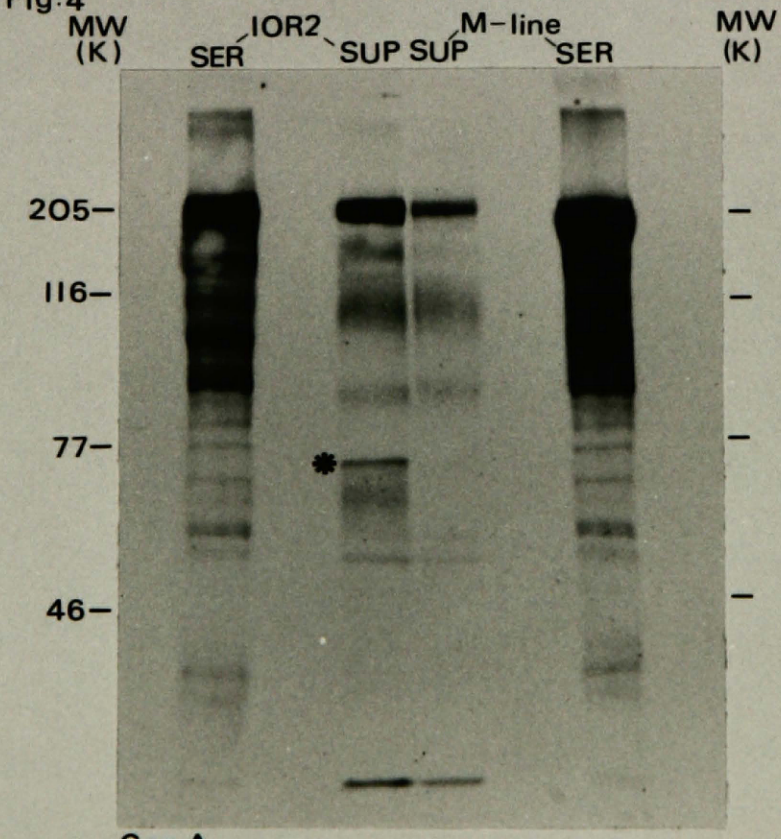
Figure 4. Electroblot of whole serum (SER) and Hb-depleted supernatants (SUP) of the 10-R2 and M-line strains of B. glabrata. The protein concentration of the 10-R2 and M-line serum samples are equal. The electroblot was probed with the lectin Con A. Differences between the electroblot of resistant and susceptible snail sera are indicated (*). Molecular weight markers (MW) are in thousands of daltons (K).

Fig. 3




Wheat germ

Fig. 4




Con A

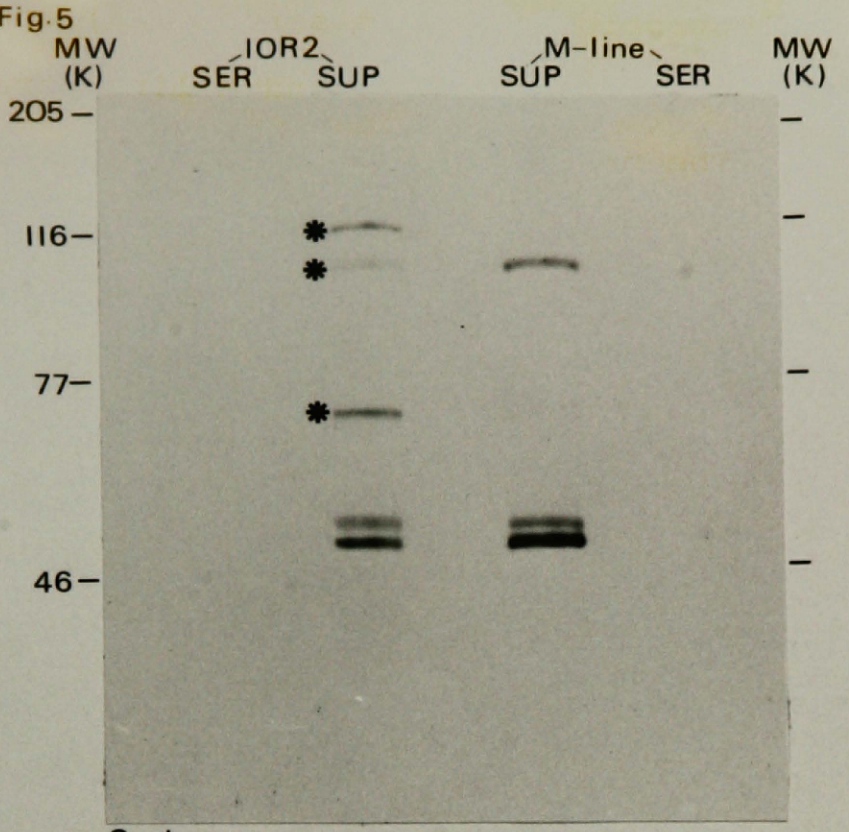
(caster bean), specific for ß-D-galactose, did not bind to any snail serum proteins. Finally, Erythrina crystagalli (coral tree lectin), which is specific for ß-D-galactose (1-4) N-acetyl-D-glucosamine residues, failed to recognize any snail serum proteins as well. Lectins which did not recognize serum differences between schistosome-resistant and susceptible snails, were not used to probe S. mansoni sporocysts.

Since it has been documented that M-line serum contains more total protein than 10-R2 B. glabrata (Spray and Granath, 1988), the above experiments were repeated with samples containing equivalent protein concentrations. Figure 4 shows 10-R2 and M-line serum samples with equal amounts of protein, probed with the lectin Con A. Results similar to those in Figure 1 were observed, with a 10-R2 specific glycoprotein present at about 70 kDa (*). In Figure 5, serum samples with equal amounts of protein were probed with soybean lectin. The 116 kDa (*) glycoprotein previously observed in 10-R2 snails was visualized, as were intensity differences between serum of the two snail strains in bands at about 110 and 70 kDa (*). Lastly, Figure 6 illustrates serum samples with equal amounts of protein probed with wheat germ lectin. Figure 6 reveals results that are essentially identical to those shown in Figure 3, with the prominent 116 kDa and 205 kDa (*) 10-R2 specific glycoproteins visible. Thus, these last three experiments,

Figure 5. Electroblot of whole serum (SER) and Hb-depleted supernatants (SUP) of the 10-R2 and M-line strains of B. glabrata. The protein concentration of 10-R2 and M-line serum samples are equal. The electroblot was probed with the lectin soybean. Differences between the electroblot of resistant and susceptible snail sera are indicated (*). Molecular weight markers (MW) are in thousands of daltons (K).

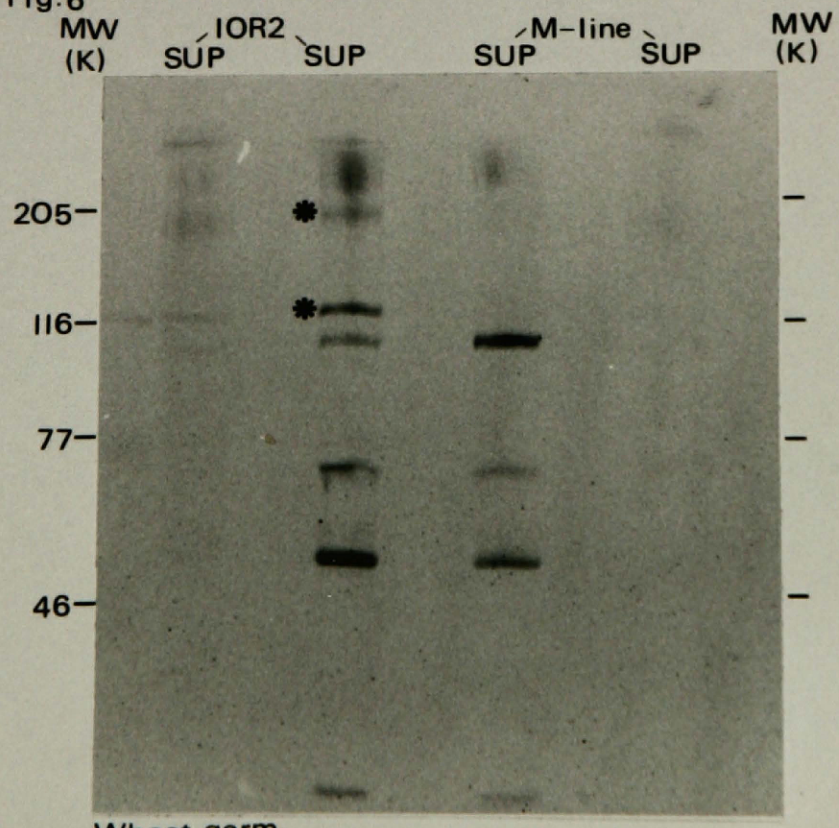
Figure 6. Electroblot of whole serum (SER) and Hb-depleted supernatants (SUP) of the 10-R2 and M-line strains of B. glabrata. The protein concentration of the 10-R2 and M-line samples are equal. The electroblot was probed with the lectin wheat germ. Differences between the electroblot of resistant and susceptible snail sera are indicated (*). Molecular weight markers (MW) are in thousands of daltons (K).

Fig.5




Soybean

Fig.6




Wheat germ

which accounted for protein concentration differences between the serum of two B. glabrata strains, gave the same results as those using equal volumes of snail serum.

Radiolabeling Experiments. The results of radiolabeling whole serum from both schistosome-resistant and susceptible strains of B. glabrata are shown in Figure 7. This figure demonstrates that both M-line and 10-R2 strains of B. glabrata had similar polypeptide profiles when labeled with $^{125}I$ and examined by autoradiography. Serum polypeptides from greater than 200 kDa to less than 45 kDa can be visualized. The results of surface radiolabeling of S. mansoni sporocysts with $^{125}I$ are presented in Figure 8. In this figure, 10 surface-associated polypeptides ranging in molecular weight from greater than 200 kDa to less than 45 kDa are evident. Finally, Figure 9 shows the results of sporocysts incubated in radiolabeled serum from resistant or susceptible B. glabrata. This autoradiograph demonstrates that $^{125}I$-labeled serum proteins bound to the sporocyst surface even after extensive washing. Furthermore, most serum polypeptides that were labeled with $^{125}I$, ranging in molecular weight from 200 kDa to less than 45 kDa, also bound to S. mansoni sporocysts during incubation.

Figures 7, 8, and 9. Autoradiographs of $^{125}$I-labeled samples separated by SDS-PAGE (10%). Samples include $^{125}$I-labeled serum from M-line and 10-R2 strains of *B. glabrata*, (Figure 7), $^{125}$I-surface labeled *S. mansoni* sporocysts, (Figure 8), and *S. mansoni* sporocysts incubated with $^{125}$I-labeled M-line or 10-R2 *B. glabrata* serum.

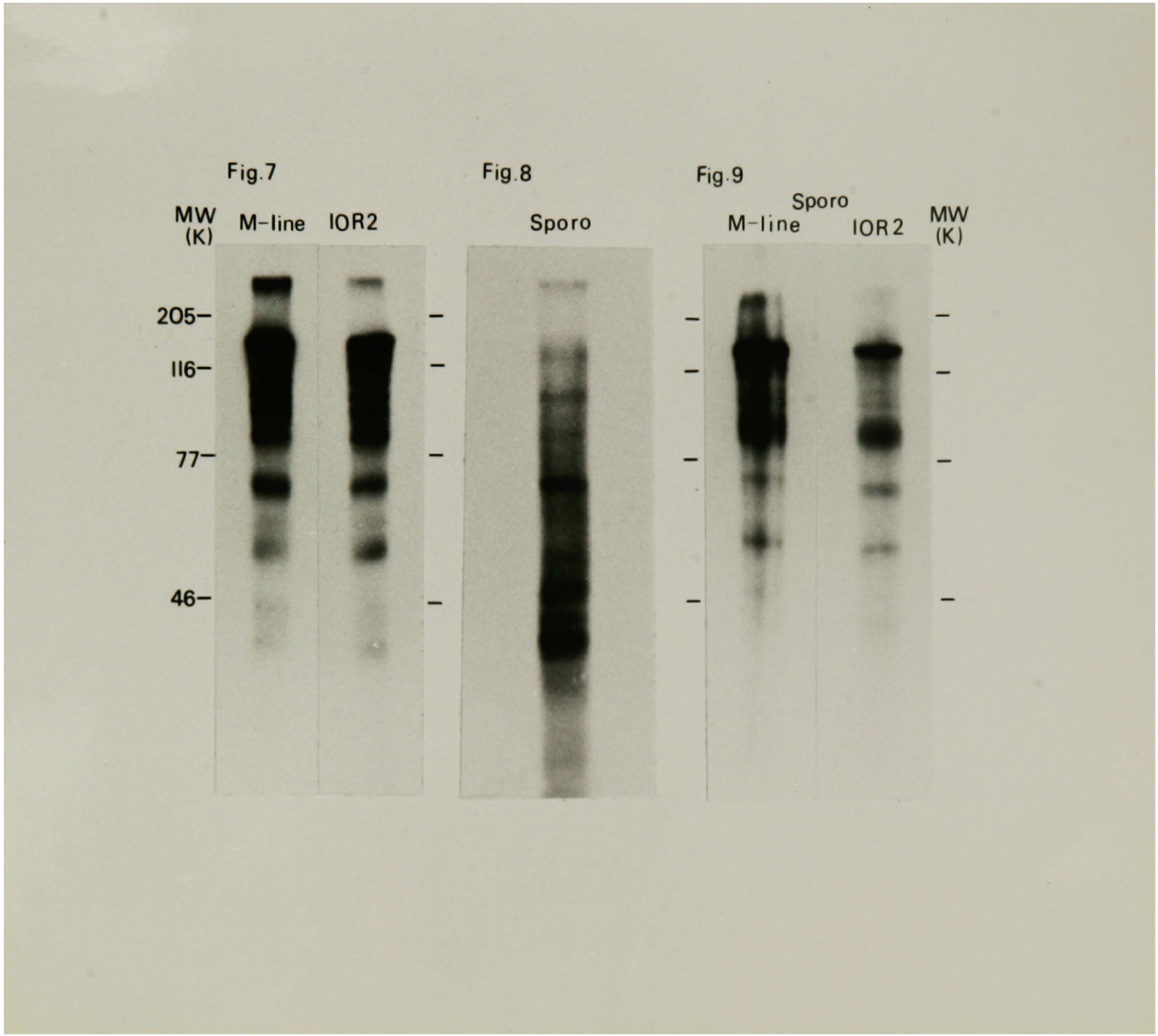


Fig.7
MW (K)
M-line
IOR2
205
116
77
46
Fig.8
Sporo
Fig.9
Sporo
M-line
IOR2
MW (K)

Incubation Experiments. The results of experiments designed to determine if B. glabrata serum can alter lectin binding to S. mansoni are shown in Figures 10, 11, and 12. The lectins used in this study were the same three lectins which identified serum glycoprotein differences between resistant and susceptible B. glabrata, namely Con A, soybean and wheat germ. Sporocysts were incubated in M-line serum (SPO+M-line), 10-R2 serum (SPO+10-R2), or in CBSS (SPO+CBSS). All three samples were adjusted so that the concentration equaled 1.1 mg of protein per ml.

Figure 10 shows electroblots of SDS-PAGE separated sporocysts incubated in either M-line or 10-R2 serum or CBSS, and then probed with Con A. Serum glycoproteins from both M-line and 10-R2 serum, which bound to the sporocysts exhibited similar electrophoretic profiles. However, serum proteins from schistosome-susceptible M-line and resistant 10-R2 snails which bound to the sporocysts exhibited differences in the binding intensity of lectin probes. For example, a serum glycoprotein at 70 kDa (*) reacted more intensely with the Con A probe when sporocysts had been incubated in 10-R2 serum, as compared to sporocysts incubated in M-line serum. Further, when the same sporocyst samples were probed with soybean lectin (Figure 11) a 50 kDa (*) snail serum glycoprotein reacted more intensely to the lectin probe, with sporocyst samples incubated in M-line serum as compared to parasites incubated

Figure 10. Electroblot of S. mansoni sporocysts incubated in CBSS (SPO+CBSS), in 10-R2 strain (SPO+10-R2), or M-line (SPO+M-line) B. glabrata serum. The electroblot was probed with the lectin Con A. A serum glycoprotein which reacted more intensely to the lectin probe when sporocysts were incubated in 10-R2 snail serum is shown (*). Molecular weight markers (MW) are in thousands of daltons (K).

Figure 11. Electroblot of S. mansoni sporocysts incubated in CBSS (SPO+CBSS), in 10-R2 strain (SPO+10-R2), or M-line (SPO+M-line) B. glabrata serum. The electroblot was probed with the lectin soybean. A serum glycoprotein which reacted more intensely to the lectin probe when sporocysts were incubated in M-line snail serum is shown (*). Molecular weight markers (MW) are in thousands of daltons (K).

Fig. 10

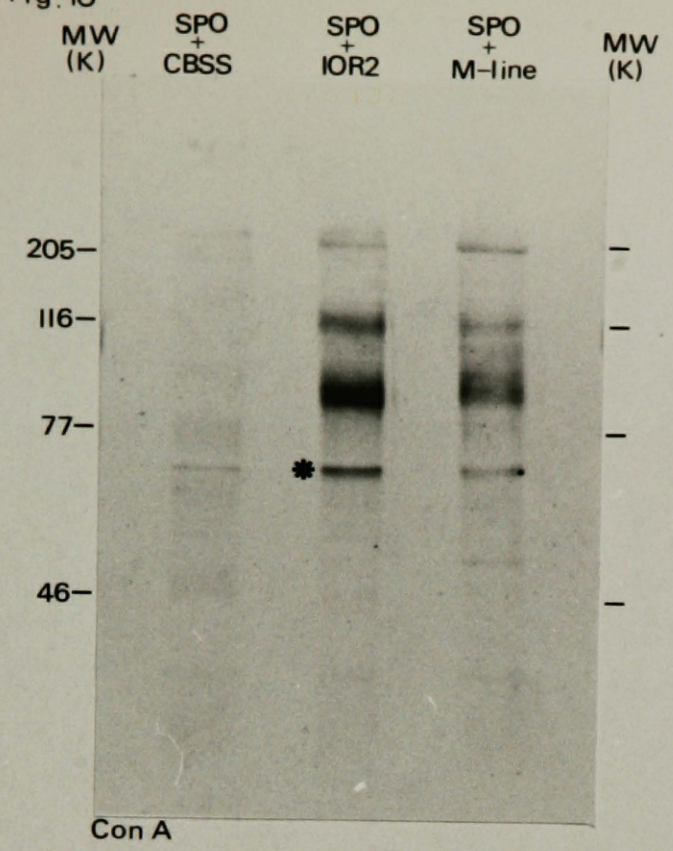
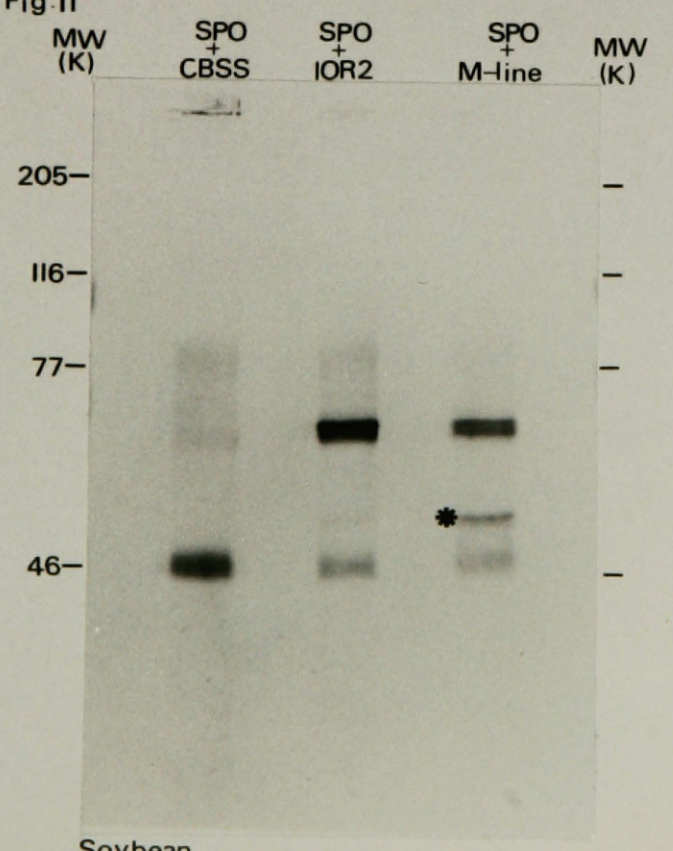

MW (K)
SPO + CBSS
SPO + IOR2
SPO + M-line
MW (K)
205
116
77
46
Con A

Fig. 11


MW (K)
SPO + CBSS
SPO + IOR2
SPO + M-line
MW (K)
205
116
77
46
Soybean

in 10-R2 serum. Likewise, when electroblots of the incubated sporocyst samples were probed with wheat germ lectin similar results were seen. Figure 12 demonstrates that a 70 kDa (*) serum glycoprotein reacted with greater intensity to the wheat germ probe, with sporocysts incubated in 10-R2 serum as compared to those incubated in M-line serum. Also, intensity differences in the 50 kDa (*) serum glycoprotein as seen previously in Figure 11, were also seen again when probed with wheat germ lectin (Figure 12).

Besides differences in the binding of snail serum glycoproteins to the sporocysts during this incubation study, two other glycoprotein differences between sporocysts incubated in M-line or 10-R2 serum were observed. A unique sporocyst associated glycoprotein was visible at 69 kDa (←) in only sporocysts that had been incubated in 10-R2 serum (Figure 12). Furthermore, sporocysts incubated in M-line serum lacked a sporocyst associated glycoprotein at about 55 kDa (←) which was present in sporocysts incubated in either 10-R2 serum or CBSS (Figure 12).

Sporocyst samples incubated in either M-line serum, 10-R2 serum or in CBSS, were also subjected to qualitative silver staining (Figure 13) and quantitative Commassie Brilliant Blue (CBB) staining (Figure 14). Silver stained electropherograms revealed more than 50 bands. Snail serum components which bound to the sporocysts during incubation were seen at approximately 210, 200, 116, 70, and 50 kDa (*)

Figure 12. Electroblot of S. mansoni sporocysts incubated in CBSS (SPO+CBSS), in 10-R2 strain (SPO+10-R2), or M-line (SPO+M-line) B. glabrata serum. The electroblot was probed with the lectin wheat germ. Intensity differences in snail serum glycoproteins (*) when sporocysts were incubated in 10-R2 or M-line snail serum as well as differences in native sporocyst glycoproteins (←) are shown. Molecular weight markers (MW) are in thousands of daltons (K).

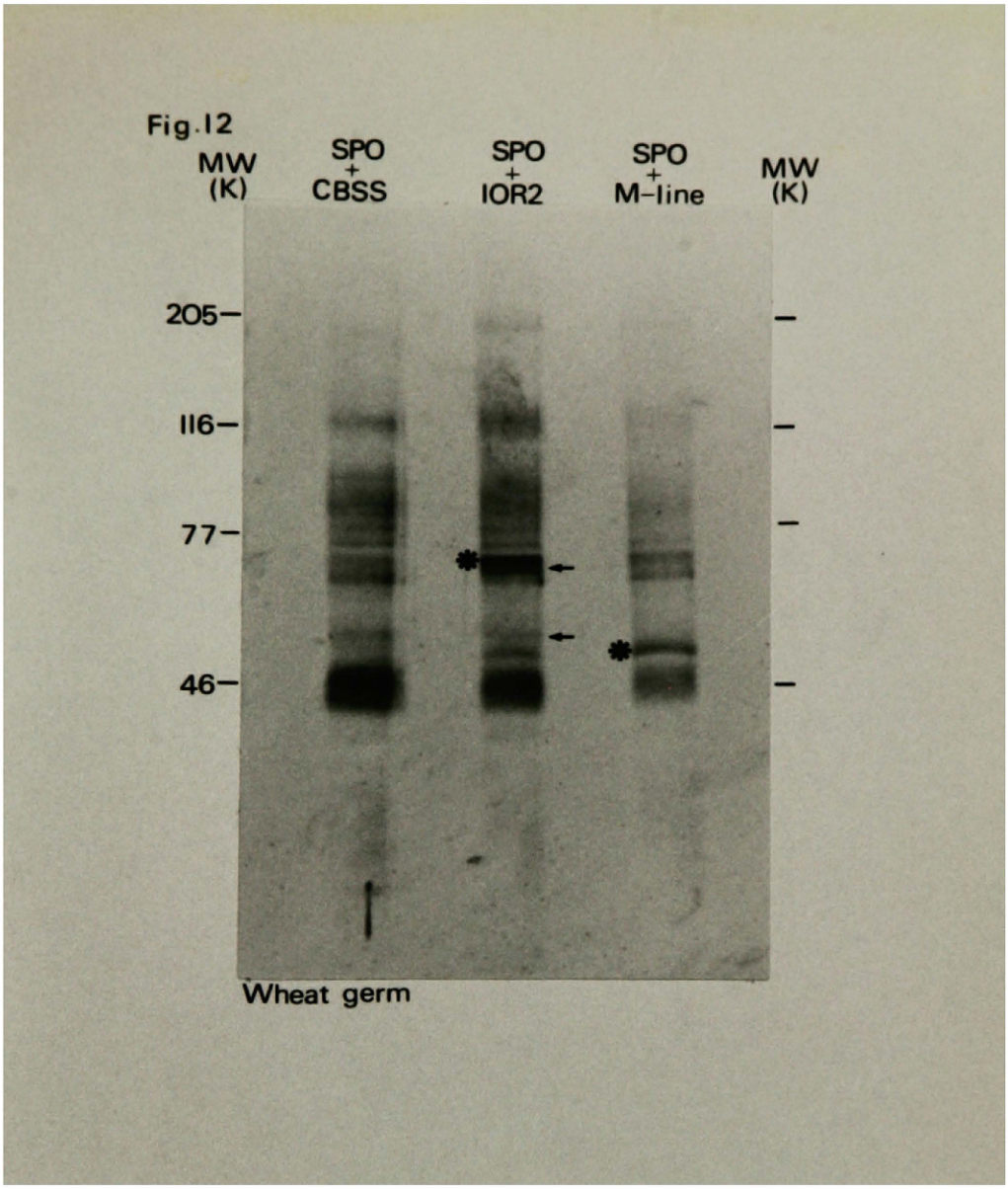


Fig.12
MW (K)
SPO + CBSS
SPO + IOR2
SPO + M-line
MW (K)
205
116
77
46
Wheat germ

Figure 13. SDS-PAGE (10%) polypeptide profile of sporocysts incubated in CBSS (SPO+CBSS), in 10-R2 strain (SPO+10-R2), or M-line (SPO+M-line) B. glabrata serum. This gel was stained with silver nitrate. Serum proteins which bound to the sporocysts after incubation can be seen (*). Molecular weight markers (MW) are in thousands of daltons (K).

Figure 14. SDS-PAGE (10%) polypeptide profile of sporocysts incubated in CBSS (SPO+CBSS), in 10-R2 strain (SPO+10-R2), or M-line (SPO+M-line) B. glabrata serum. The gel was stained with Coomassie Brilliant Blue. Serum proteins which bound to the sporocysts after incubation can be seen (*). Molecular weight markers (MW) are in thousands of daltons (K).

Fig. 13

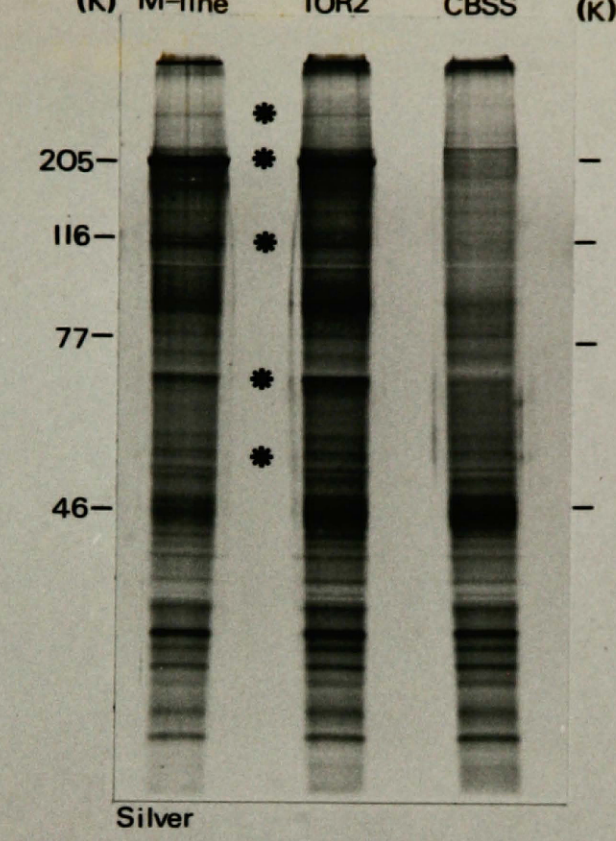
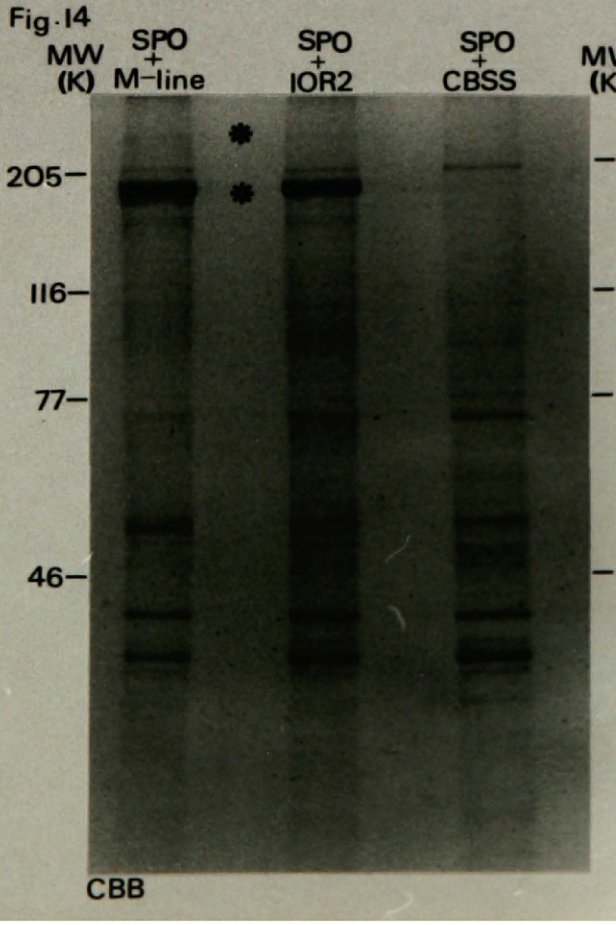

MW (K)
SPO + M-line
SPO + IOR2
SPO + CBSS
MW (K)
205
116
77
46
Silver

Fig. 14


MW (K)
SPO + M-line
SPO + IOR2
SPO + CBSS
MW (K)
205
116
77
46
CBB

(Figure 13). Sporocyst samples stained with CBB revealed fewer bands, reflecting the lower sensitivity of this stain. However, serum proteins which bound to the sporocysts during incubation could still be visualized at 210 and 200 kDa (*) (Figure 14).

Immunoblotting. In order to determine if B. glabrata serum possessed molecules with structural similarity to the well characterized plant lectins Con A, soybean and wheat germ, sera from snails were probed with anti-sera to these lectins. Figures 15, 16, and 17 depict whole snail serum (SER) and Hb-depleted serum (SUP) from M-line and 10-R2 snail strains probed with anti-sera to Con A, soybean, and wheat germ, respectively. Probing with anti-Con A revealed no differences between serum from M-line and 10-R2 snails (Figure 15), although broad cross reactivity was seen when whole serum samples were probed with anti-Con A. In Figure 16, M-line and 10-R2 serum samples were probed with anti-soybean sera. Very little cross reactivity with anti-soybean sera was seen. However, when the serum samples were probed with anti-wheat germ sera, several differences in the polypeptide profile were noted. Figure 17 demonstrates that two polypeptides at 80 and 40 kDa (*) were recognized in the Hb-depleted sample from 10-R2 serum but not in the M-line serum. Anti-wheat germ sera also showed broad cross reactivity to whole serum samples from both snail strains.

Figure 15. Immunoblot of whole serum (SER) and Hb-depleted supernatants (SUP) from 10-R2 and M-line strains of B. glabrata. The immunoblot was probed with anti-Con A sera. Polypeptides that were cross-reactived with anti-Con A are indicated (*). Molecular weight markers (MW) are in thousands of daltons (K).

Figure 16. Immunoblot of whole serum (SER) and Hb-depleted supernatants (SUP) from 10-R2 and M-line strains of B. glabrata. The immunoblot was probed with anti-soybean sera. Molecular weight markers (MW) are in thousands of daltons (K).

Fig. 15

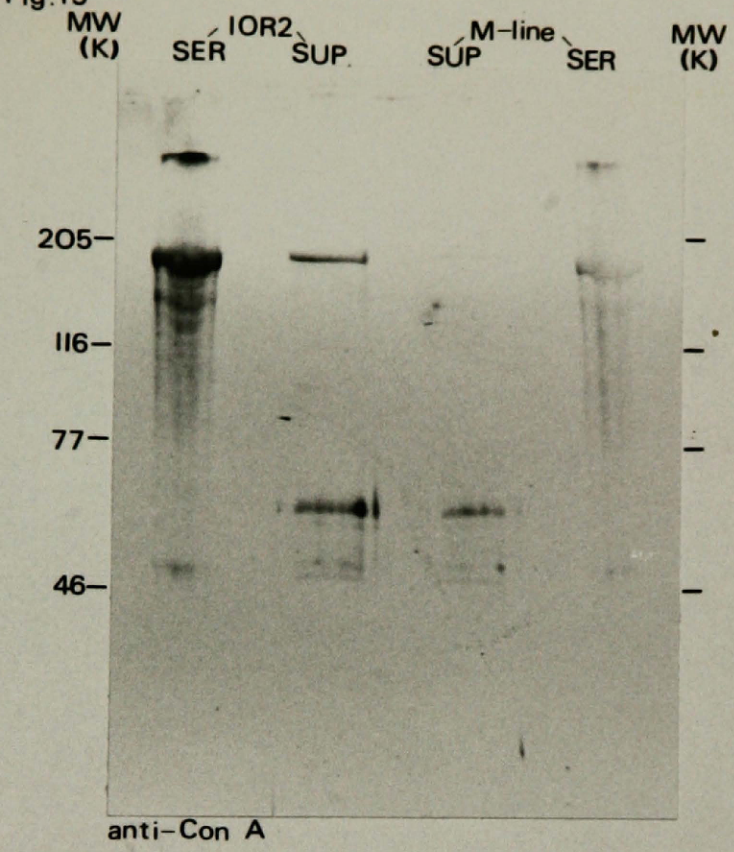

MW (K)
IOR2
SER
SUP
M-line
SUP
SER
MW (K)
205
116
77
46
anti-Con A

Fig. 16

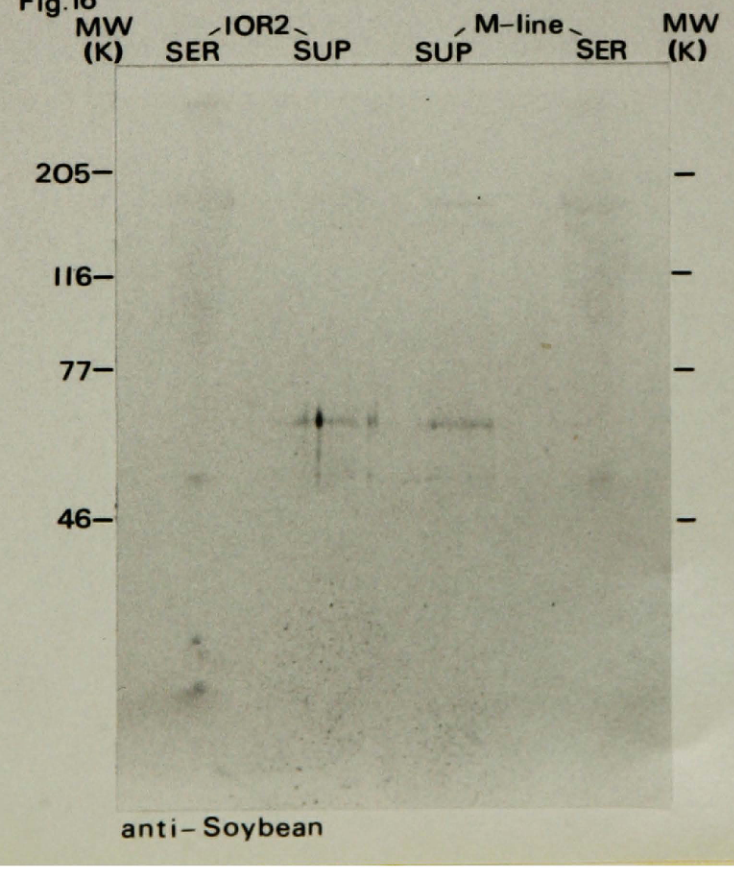

MW (K)
IOR2
SER
SUP
M-line
SUP
SER
MW (K)
205
116
77
46
anti-Soybean

Figure 17. Immunoblot of whole serum (SER) and Hb-depleted supernatants (SUP) from 10-R2 and M-line strains of B. glabrata. The immunoblot was probed with anti-wheat germ sera. Differences between the immunoblots of resistant and susceptible snail sera are indicated (*). Molecular weight markers (MW) are in thousands of daltons (K).

Figure 18. Immunoblot of whole serum (SER) and Hb-depleted supernatants (SUP) from 10-R2 and M-line strains of B. glabrata. The immunoblot was probed with normal rabbit serum (NRS). Molecular weight markers (MW) are in thousands of daltons (K).

Fig. 17

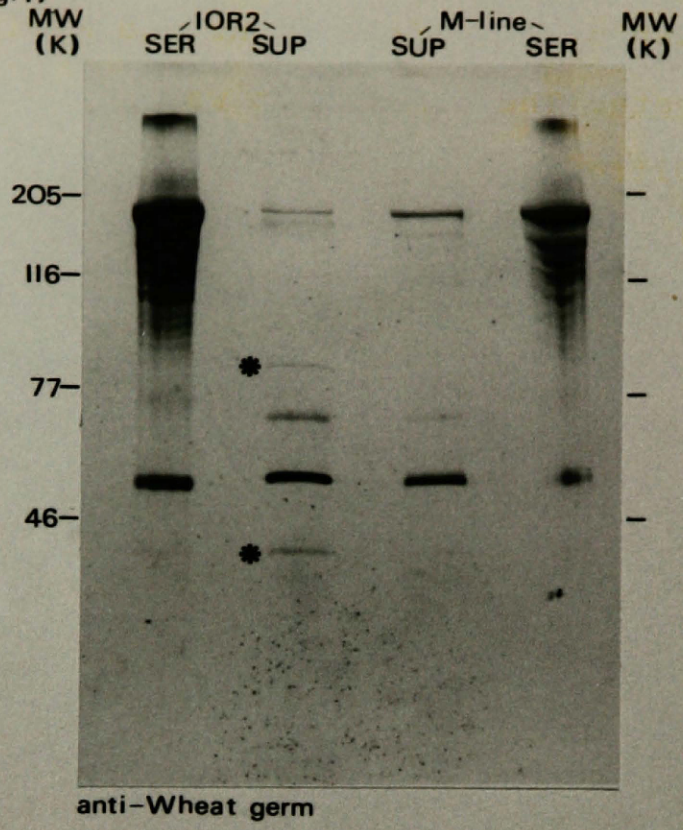
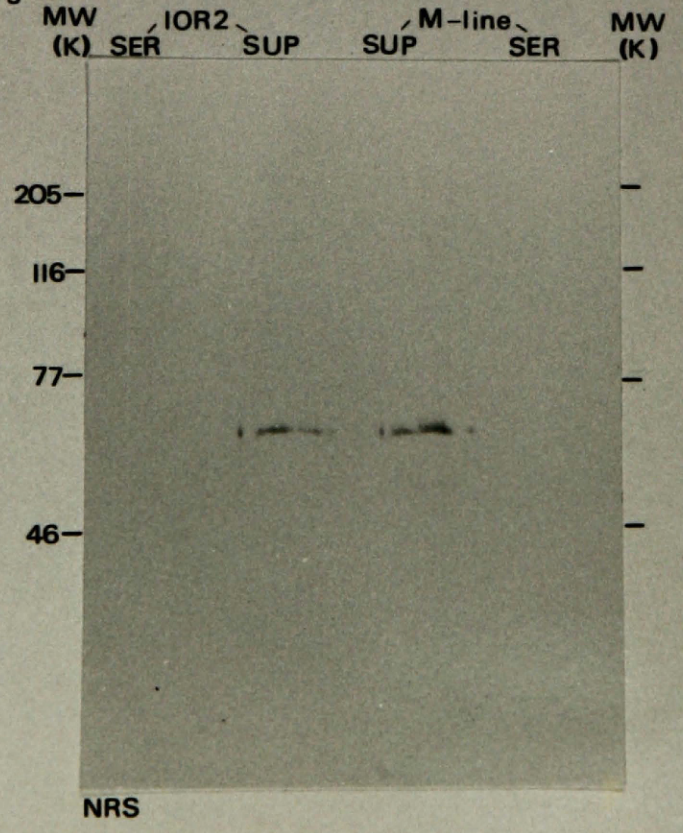

MW (K)
IOR2
SER
SUP
M-line
SUP
SER
MW (K)
205
116
77
46
anti-Wheat germ

Fig. 18

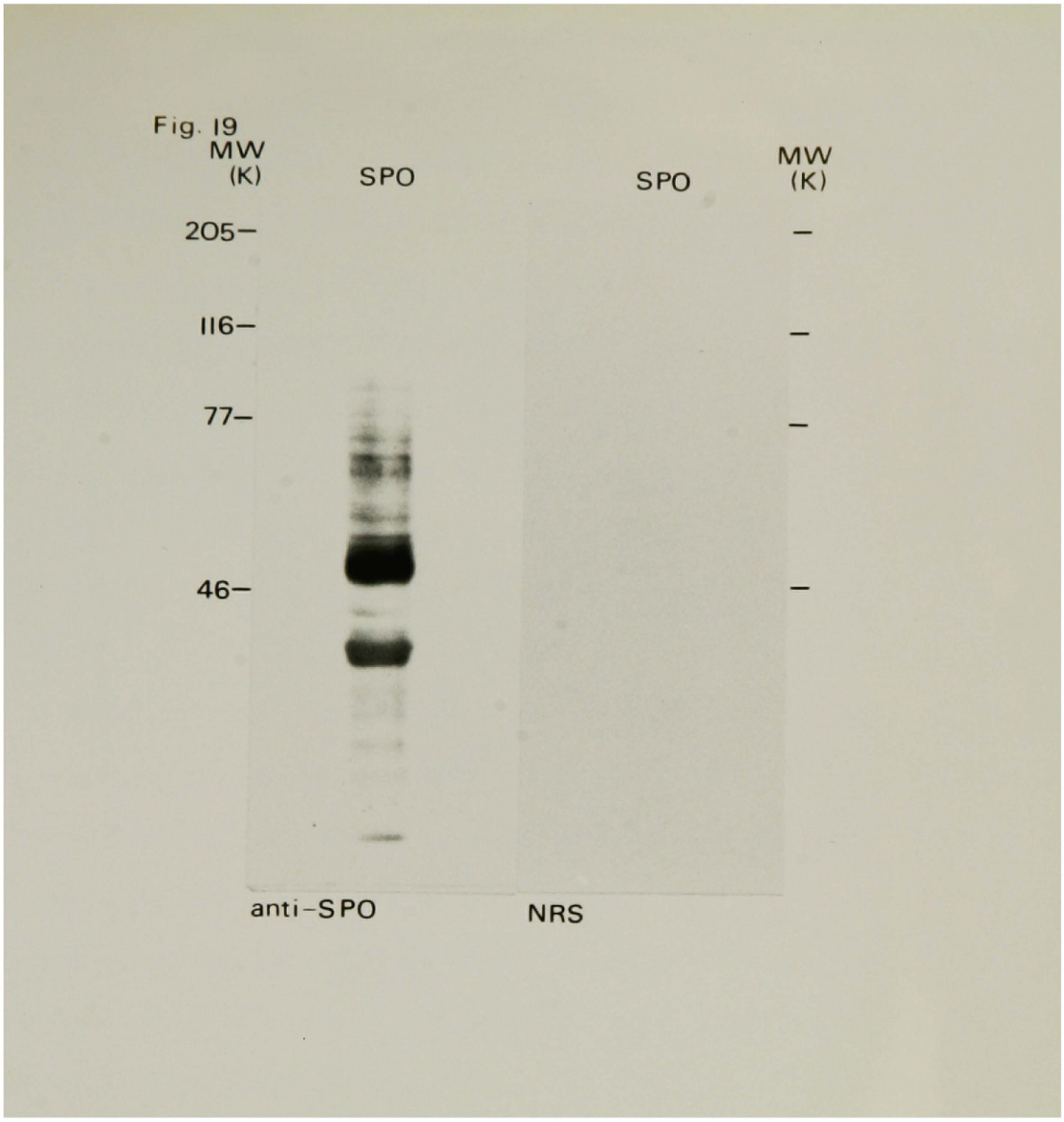

MW (K)
SER
IOR2
SUP
SUP
M-line
SER
MW (K)
205
116
77
46
NRS

To rule out non-specific binding of rabbit anti-sera to B. glabrata serum samples, normal rabbit serum (NRS) was used. Non-specific binding of NRS was observed in the Hb-depleted serum samples of both snail strains at approximately 66 kDa (ME band) (Figure 18).

Immunodominant surface epitopes of S. mansoni primary sporocysts are shown in Figure 19. Sporocyst samples separated by SDS-PAGE were probed with rabbit polyclonal anti-sera raised against whole sporocysts. Many polypeptides were visualized ranging in molecular weight from 150 kDa to less than 33 kDa. Again, NRS was used to rule out non-specific binding. NRS did not bind to SDS-PAGE separated sporocyst proteins (Figure 19).

Figure 19. Immunoblot of S. mansoni sporocysts probed with anti-sporocyst sera (anti-SPO) and normal rabbit serum (NRS). Molecular weight markers (MW) are in thousands of daltons (K).

Fig. 19



Discussion:

The comparison of serum glycoproteins from schistosome-susceptible and resistant B. glabrata is an important step in understanding which host factors influence snail susceptibility or resistance to schistosome infection. Specifically, the identification of snail serum glycoproteins, and determining if these molecules modified the parasite surface though the alteration of lectin binding during in vitro incubation, was examined in this thesis.

Results from this study, which used SDS-PAGE and a series of lectins with broad sugar specificities to identify glycoconjugates, indicated that serum glycoprotein differences between schistosome-susceptible and resistant B. glabrata do exist. Specifically, schistosome-resistant 10-R2 snails possessed at least three glycoproteins that were not present in schistosome-susceptible M-line snails. These three serum glycoproteins, found only in schistosome-resistant snails, consisted of a 205 kDa glycoprotein with either N-acetyl-D-glucosamine or N-acetyl-neuraminic acid residues, a 116 kDa glycoprotein with a N-acetyl-D-galactosamine sugar residue, and a 70 kDa glycoprotein with an α-D-mannose or α-D-glucose sugar.

These glycoproteins from resistant snail serum only became visible when ultracentrifuged serum was used; they were not observed in whole serum samples. Thus it appeared that these glycoproteins were not associated with Hb since

serum ultracentrifugation pelleted the snail Hb. However, previous studies of B. glabrata serum, have indicated that nearly all serum polypeptides are associated with heme, the major component of B. glabrata Hb. Those studies, which used immunoblot assays with anti-Hb antibodies as probes (Spray and Granath, 1988) and specific heme-staining procedures (Granath, 1988), demonstrated that ultracentrifuged serum samples still cross-reacted with antibodies raised against B. glabrata Hb. Therefore, although most of the Hb was pelleted during ultracentrifugation, Hb-associated protein fragments still remained in the supernatant. Since the Hb molecule is believed to be identical in both resistant and susceptible snail strains (Spray and Granath, 1988), the presence of these protein fragments with different patterns of glycosylation between resistant and susceptible snails is intriguing. Recent studies have indicated that the carbohydrate diversity of glycoproteins is biologically significant, since carbohydrates may modify the activities of proteins to which they are conjugated (Sharon and Lis, 1989). Although the function of low concentrations of glycoproteins present in the serum of B. glabrata is unknown, it is possible, by nature of their carbohydrate epitopes, that these molecules interact with receptors on the surface of S. mansoni. Thus, these molecules could be intimately involved with snail defense mechanisms.

In this study, both soybean and wheat germ lectin probes bound to the same 116 kDa glycoprotein, which was present only in the serum of schistosome-resistant B. glabrata. Likewise, both of these lectin probes bound to several other serum glycoproteins which did not differ between resistant and susceptible snails. The binding of two different lectin probes, each with a different specific sugar specificity to the same glycoprotein, suggested that these snail serum proteins may have multiple types of glycosylation patterns, and as a result are complex molecules.

Glycoprotein differences between schistosome-resistant and susceptible snails were still visualized when snail serum samples were adjusted to the same protein concentration. Since it is known that M-line snails have more total protein than 10-R2 snails, adjustment of serum samples to correct for this difference allowed for the identification of qualitative variations in glycoproteins. The identification of qualitative differences between schistosome-susceptible and resistant B. glabrata was an important consideration. That is, previous studies by Bayne et al. (1986) indicated that only a few consistent differences existed between the polypeptide profiles of serum antigens acquired by live sporocysts that were incubated in serum from resistant and susceptible snails. Furthermore, differences they reported in acquired serum

antigens between the snail strains appeared to be quantitative rather than qualitative in nature. Quantitative differences in serum antigens binding to the parasite surface are more difficult to analyze since slight variations in concentrations, of serum proteins both inter-strain as well as intra-strain, could lead to erroneous conclusions. However, since this study has identified qualitative differences in serum glycoproteins between resistant and susceptible snails, this study could address the question of differential binding of snail serum proteins to the parasite surface after *in vitro* incubation. Such knowledge is important since any alterations in the sporocyst's lectin binding sites, due to snail proteins, may mediate the binding of the punitive serum agglutinin responsible for hemocyte activation.

The fact that sporocysts rapidly acquire snail host antigens has been documented (Bayne *et al.*, 1986). This study also confirmed the rapid uptake of snail serum antigens by *S. mansoni* sporocysts during *in vitro* incubation. By the use of $^{125}I$ radiolabeling, many snail serum proteins were shown to have bound to the sporocyst surface. In fact, almost all serum proteins that were radiolabeled bound to the sporocyst after a two hour incubation period. Since sporocysts have no digestive tract, and nutritional uptake by the parasite occurs across the parasite tegumental surface (Bayne *et al.*, 1986), the

presence of large amounts of host protein on the parasite surface is not surprising. Indeed, host proteins on the surface of the parasite has been suggested as a mechanism by which the parasite avoids the host immune response. Thus, by bathing itself in host molecules, the parasite would appear as self and the snail would not mount a hemocyte-mediated cytotoxic response in an attempt to clear the foreign material.

The results of the studies in which sporocysts were incubated in susceptible serum, resistant serum, or in CBSS, indicated that serum glycoproteins had bound to the sporocyst surface and altered binding of the lectin probes. Altered binding to the parasite surface was evident with serum glycoproteins of low molecular weights. Specifically, a serum glycoprotein of 70 kDa was seen to react more intensely, with all three lectin probes, when sporocysts had been incubated in 10-R2 serum. Likewise, a serum glycoprotein of 50 kDa was seen to react more intensely to soybean and wheat germ lectin probes when sporocysts were incubated in M-line serum. Thus, sporocysts incubated in B. glabrata serum acquired snail serum glycoproteins on their surface. Furthermore, differences occurred between the glycoproteins that sporocysts acquired from the serum of schistosome-resistant or susceptible B. glabrata.

Interestingly, the 205 and 116 kDa glycoproteins present only in resistant snails, visualized by both soybean

and wheat germ probes, did not bind selectively to the sporocysts. In fact serum glycoproteins from resistant and susceptible snails (seen at 70 and 50 kDa respectively) both readily bound to the sporocysts and induced changes in lectin probe binding. This suggests that both resistant and susceptible snail serums each have the capacity to alter sporocyst tegumental antigens during incubation. This altered tegumental antigenicity is important since the resistant serum agglutinin responsible for hemocyte activation presumably must bind to the sporocyst surface. Although the role that these serum glycoproteins play in facilitating or impeding the binding of the resistant strain serum agglutinin remains to be elucidated, this study gives preliminary evidence that serum glycoproteins are immunologically important in snail defense mechanisms.

In addition to B. glabrata serum glycoproteins altering S. mansoni lectin binding sites by coating the parasite surface, it has also been suggested that snail serum could induce alterations in native sporocyst antigens as well (Bayne et al., 1986). A closer examination of the incubation experiments in this study also supports this contention. In the blot probed with wheat germ lectin (Figure 12) there exists a unique glycoprotein at about 69 kDa that was observed only when sporocysts were incubated in 10-R2 serum. This glycoprotein was not visualized in sporocysts incubated in M-line serum. Furthermore, this

glycoprotein also is not constitutively expressed by the sporocysts (visible in the CBSS control), nor is it a snail serum glycoprotein, all of which are visible when stained by silver and CBB. Thus, this glycoprotein represents a novel sporocyst protein which was induced when the sporocysts were in contact with 10-R2 snail serum during the incubation period. The function of this unique sporocyst glycoprotein is unknown. However, when sporocysts were probed with anti-sporocyst antibody and when they were examined by $^{125}I$ surface radiolabeling, a major sporocyst surface exposed polypeptide was revealed that had a molecular weight of about 70 kDa. Therefore, it is possible that the unique sporocyst glycoprotein induced during incubation with serum from resistant snails represents an antigenic change to a surface exposed immuno-dominant epitope.

Further evidence that B. glabrata serum induces changes in native S. mansoni glycoproteins can also be seen in Figure 12. A native sporocyst glycoprotein visible at about 55 kDa can be seen in sporocysts incubated in CBSS as well as those incubated in 10-R2 serum. However, when sporocysts were incubated in M-line serum, the glycoprotein was not observed, thus suggesting that B. glabrata serum down-regulated the expression of this native glycoprotein. Again, rabbit anti-sera derived against S. mansoni sporocysts as well as $^{125}I$ surface radiolabeling, revealed the existence of a surface exposed sporocyst polypeptide of

a similar molecular weight. Once again this suggests that these induced sporocyst glycoproteins are involved in alteration of lectin binding sites on the surface of S. mansoni.

Alterations in surface-exposed, native sporocyst glycoproteins induced by incubation in schistosome-resistant or susceptible B. glabrata serum, are not the only mechanisms whereby changes in sporocyst proteins could affect resistance or susceptibility in the snail host. Recent evidence also implicates soluble schistosome glycoproteins in influencing schistosome-snail interactions. Lodes and Yoshino (1989) demonstrated that S. mansoni sporocysts cultured in vitro synthesized and secreted a wide variety of glycoproteins. The quantity and type of these newly synthesized glycoproteins was dependent on the culture conditions. For example, sporocysts cultured in medium containing fetal bovine serum synthesized and released significantly more protein than sporocysts cultured in medium lacking serum. Noteworthy is that excretory-secretory products from other larval digeneans have been implicated in the interference of their host snail defense systems (van der Knaap and Meuleman, 1986) by specifically suppressing host hemocytes (Li et al., 1981). Since encapsulation of S. mansoni by B. glabrata hemocytes has been observed as early as three hours post-infection (Loker et al., 1982), these excretory-secretory products released

by larval schistosomes, in high concentrations during the first hours of culture, may be important in the interference of the defense system of B. glabrata.

Whether the alterations in sporocyst glycoproteins observed in the present study were excretory-secretory products released in response to incubation in snail serum, or whether they represent changes in structural proteins on the surface of S. mansoni remains unclear. Regardless, since these newly induced proteins were glycosylated and cross-reacted with the lectin probes, it is again likely that they affect lectin binding sites either on the parasite surface or by binding to serum agglutinins. This further implicates soluble lectins as hemocyte activating factors.

Other evidence also indicates that S. mansoni sporocysts actively interfere with the host defense system of B. glabrata. Recently, it has been reported that sporocysts had the ability to alter the phagocytosis rate of B. glabrata hemocytes. Studies by Fryer and Bayne (1990) showed that sporocysts incubated in schistosome-susceptible (M-line) serum reduced the level of in vitro phagocytosis of yeast cells by M-line hemocytes. In contrast, schistosome-resistant (13-16-R1) hemocytes showed higher rates of phagocytosis after 13-16-R1 serum was co-incubated with S. mansoni sporocysts. Likewise, hemocytes from M-line snails that had been exposed to S. mansoni miracidia for three hours, showed reduced levels of in vitro phagocytosis

of yeast cells compared to hemocytes from unexposed snails. In contrast hemocytes from 13-16-R1 snails exhibited increased levels of *in vitro* phagocytosis of yeast cells, provided the snails had been previously exposed to *S. mansoni* miracidia. Thus, these experiments present strong evidence that *S. mansoni* sporocysts alter the host snail defense system within hours of miracidial penetration. Fryer and Bayne (1990) further suggested that sporocyst mediated suppression or enhancement of *B. glabrata* hemocytes required that both hemocytes and serum be present at the time of parasite exposure. This suggested a complex relationship between sporocyst, hemocyte and snail serum.

In contrast to the above, the experiments reported here identified changes in native sporocyst glycoproteins which were induced solely by incubation in either susceptible or resistant snail serum. Whether or not these alterations in sporocyst glycoproteins also have a modulatory effect on phagocytosis by *B. glabrata* hemocytes is unknown. However, correlations between this study and the work of Fryer and Bayne (1990) is an intriguing possibility and deserves further consideration.

The results when serum from *B. glabrata* was probed with anti-lectin antibodies were interesting. Anti-wheat germ lectin recognized two polypeptides in the serum of resistant snails that were not present in the serum of susceptible *B. glabrata*. Since lectins, as a group, are heterogeneous

molecules with very different structures and functions, one would not expect similarities between invertebrate lectins and lectins found in plants. Moreover, even within the small group of invertebrate lectins which have been characterized to date, no universal homology has been observed (Olfason, 1986). However, results observed in this study suggest that both B. glabrata serum and wheat germ lectin share some similar epitopes. Whether these cross reactive epitopes represent homology in the binding sites between snail and plant lectins or homology in some other non-binding domain is unknown. Therefore, it is premature to suggest that these snail serum polypeptides, which cross-reacted with anti-wheat germ lectin, might be involved in schistosome-snail interactions. Further study, including lectin isolation and purification, needs to be conducted. Also it needs to be demonstrated that this epitope does recognize S. mansoni tegumental carbohydrate antigens and plays a role in hemocyte activation. Such studies are crucial in proving that serum lectins are involved in invertebrate defense mechanisms.

The exact role of serum glycoproteins in enhancing or impeding the binding of a hemocyte activating factor, specific to serum from schistosome-resistant B. glabrata, remains unknown. Although qualitative differences in glycoprotein composition between schistosome-susceptible and resistant B. glabrata were observed, only lower molecular

weight serum glycoproteins seemed to be involved in altering lectin binding sites on the parasite surface through differential binding. In addition, incubating sporocysts in resistant or susceptible snail sera induced changes in the expression of native sporocyst glycoproteins. It remains to be elucidated whether these induced sporocyst glycoproteins are alterations in surface exposed epitopes on the parasite tegument or whether they represent excretory-secretory glycoproteins which function in hemocyte modulation. Further work still needs to be undertaken in order to determine if the observed differential binding of snail serum glycoproteins, along with the altered native sporocyst glycoproteins observed after incubation in snail serum, are truly involved in mediating hemocyte activation. Such future experiments might include determining if sugars or glycoconjugates can block 10-R2 snail hemocytes from destroying larval S. mansoni. Chemical or enzymatic modification of S. mansoni sporocyst tegumental glycoproteins might provide additional clues to the functional role of serum glycoproteins. Furthermore, the actual serum molecule(s) that activates B. glabrata hemocytes to become cytotoxic must be isolated and purified. Finally, passive transfer of this molecule must allow susceptible snail strains to actively resist and destroy S. mansoni sporocysts in vivo, thus confirming this molecule's protective function. These objectives will

provide years of intriguing research for those who attempt to understand how invertebrates encapsulate and destroy larval parasitic trematodes.

References:

Anderson, R.S. 1980. Hemolysins and hemagglutinins in the coelomic fluid of a polychaete annelid, Glycera dirranchiata. Biol. Bull. 159:259-268.

Anderson, R.S. and R.A. Good. 1976. Opsonic involvement in phagocytosis by mollusk hemocytes. J. Invert. Pathol. 27:57-64.

Anderson, R.S., S.B. Holmes and R. Good. 1973. In vitro bactericidal capacity of Blaberus craniifer hemocytes. J. Invert. Pathol. 22:127-135.

Arimoto, R. and M.R. Tripp. 1977. Characterization of a bacterial agglutinin in the hemolymph of the hard clam Mercenaria mercenaria. J. Invertebr. Pathol. 30:406-413.

Bayne, C.J., C.A. Boswell, E.S. Loker and M.A. Yui. 1985. Plasma components which mediate cellular defences in the gastropod mollusc Biomphalaria glabrata. Dev. Comp. Immunol. 9:523-530.

Bayne, C.J., P.M. Buckley and P.C. DeWan. 1980a. Macrophagelike hemocytes of resistant Biomphalaria glabrata are cytotoxic for sporocysts of Schistosoma mansoni in vitro. J. Parasitol. 66:413-419.

Bayne, C.J., P.M. Buckley and P.C. DeWan. 1890b. Schistosoma mansoni: Cytotoxicity of hemocytes from susceptible snail hosts for sporocysts in plasma from resistant Biomphalaria glabrata. Exp. Parasitol. 50:409-416.

Bayne, C.J., E.S. Loker and M.A. Yui. 1986. Interactions between the plasma proteins of Biomphalaria glabrata (Gastropoda) and the sporocysts tegument of Schistosoma mansoni (Trematoda). Parasitology. 92:653-664.

Bayne, C.J., E.S. Loker, M.A. Yui and J.A. Stephens. 1984. Immune-recognition of Schistosoma mansoni primary sporocysts may require specific receptors on Biomphalaria glabrata hemocytes. Parasit. Immunol. 6:519-528.

Boswell, C.A. and C.J. Bayne. 1984. Isolation, characterization and functional assessment of a hemagglutinin of the plasma of Biomphalaria glabrata, intermediate host of Schistosoma mansoni. Dev. Comp. Immunol. 8:559-568.

Boswell, C.A. and C.J. Bayne. 1985. Lectin-dependent cytotoxicity of hemocytes from susceptible host snails, Biomphalaria glabrata. Exp. Parasitol. 60:113-138.

Boswell, C.A., T.P. Yoshino and T.S. Dunn. 1987. Analysis of tegumental surface proteins of Schistosoma mansoni primary sporocysts. J. Parasitol. 73:778-786.

Capron, A., J.P. Dessaint, M. Capron, J.H. Ouma and A.E. Butterworth. 1987. Immunity to schistosomes: Progress toward Vaccine. Science. 238:1065-1072.

Cheng, T.C. 1978. The role of lysosmal hydrolases in molluscan cellular response to immunologic challenge, In "Comparative Pathobiology". Vol.4. pp.59-71. Plenum Publishing Corp., New York, New York.

Cheng, T.C. 1979. Cellular immunity in molluscs: With emphasis on the intermediate host of human infecting schistosomes. Inst. Lab. Resour.News. 22:9-16.

Cheng, T.C. 1986. General Parasitology 2ed. Academic Press, Inc. Orlando, Florida. 827pp.

Cheng, T.C. and T.A. Garrabrant. 1977. Acid phosphatase in granulocytic capsules formed in strains of Biomphalaria glabrata totally and partially resistant to Schistosoma mansoni. Int. J. Parasitol. 7:467-472.

Cheng, T.C. and E. Rifkin. 1970. Cellular reactions in marine molluscs in response to helminth parasitism, In "A Symposium on Diseases of Fishes and Shellfishes". Am. Fisher. Soc. Symp. 5., S.F. Snieszko.(ed.) pp.443-496. Washington, D.C.

Chernin, E. 1963. Observation on hearts explanted in vitro from the snail Australorbis glabratus. J. Parasitol. 40:353-364.

Crichton, R. and K.J. Lafferty. 1975. The discriminatory capacity of phagocytic cells in the chiton (Liolophura gaimarda). Adv. Exp. Med. Biol. 64:89-99.

Coles, G.C., H.B. Jansson and B.M. Zuckerman. 1988. Lectin studies of surface carbohydrates and induction of gland secretion in the free-living stages of Schistosoma mansoni. Journal of Chemical Ecology. 14:691-700.

Dissous, C., J.M. Grzych and A. Capron. 1986. Schistosoma mansoni shares a protective oligosaccharide epitope with freshwater and marine snails. Nature. 323:443-445.

Fryer, S.E., C.J. Hull and C.J. Bayne. 1989. Phagocytosis of yeast by Biomphalaria glabrata: Carbohydrate specificity of hemocyte receptors and a plasma opsonin. Dev. Comp Immunol. 13:9-16.

Fryer, S.E. and C.J. Bayne. 1990. Schistosoma mansoni phagocytosis in Biomphalaria glabrata. J. Parasitol. 76:45-52.

Granath, W.O. 1988. Identification of hemeproteins in the hemolymph of Biomphalaria glabrata (Gastropoda) separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Journal of Invertebrate Pathology. 52:43-48.

Granath, W.O. Jr., F.J. Spray and R.C. Judd. 1987. Analysis of Biomphalaria glabrata (Gastropoda) hemolymph by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, high-performance liquid chromatography, and immunoblotting. J. Invertebr. Pathol. 49:198-208.

Granath, W.O. Jr. and T.P. Yoshino. 1984. Schistosoma mansoni: Passive transfer of resistance by serum in the vector snail, Biomphalaria glabrata. Exp. Parasitol. 58:188-193.

Hayunga, E.G. and M.P. Sumner. 1986a. Expression of lectin-binding surface glycoproteins during the development of Schistosoma mansoni schistosomula. J. Parasitol. 72:913-920.

Hayunga, E.G. and M.P. Sumner. 1986b. Characterization of surface glycoproteins on Schistosoma mansoni adult worms by lectin affinity chromatography. J. Parasit. 72:283-291.

Huber, H., M.J. Polley, W.D. Linscott, H.H. Fudenberg and H.J. Muller-Eberhart. 1968. Human monocytes: Distinct receptor sites for the 3rd component of complement and for immunoglobulin G. Science. 162:1281-1283.

Hulse, E.V. 1971. Joshua's curse and the abandomnent of ancient Jericho: schistosomiasis as a possible medical explanation. Med. Hist 15:376-386.

Jordan, P. and G. Webbe. 1982. Schistosomiasis Epidemiology, Treatment and Control. William Heinmann Medical Books Ltd. London pp.361

Jourdane, J. and A. Theron. 1987. Larval development: Eggs to cercariae. In "The biology ofschistosomes-from genes to latrines". Rollinson, D. and A. J. Simpson, (eds.), Academic Press, New York. pp.83-113.

Judd, R.C. 1982a. $^{125}$I-peptide mapping of protein III isolate from four strains of Neisseria gonorrhoeae. Infect. Immun. 37:622-631.

Judd, R.C. 1982b. Surface peptide mapping of protein I and protein III of four strains of Neisseria gonorrhoeae. Infect. Immun. 37:632-641.

Katz, M., Despommier, D.D. and R. Gwadz. 1989. Parasitic Diseases. 2nd ed. Springer-Verlag Inc., New York, New York. pp.94-108.

Lackie, A.M. 1980. Invertebrate Immunity. Parasitol. 40:393-412.

Laemili, U.K. 1970. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. Nature (London). 277:680-685.

Lie, K.J., K.H. Jeong and D. Heyneman. 1981. Selective interference with granulocyte function induced by Echinostoma paraensi (Trematoda) larvae in Biomphalaria glabrata (Mollusca). J. Parasitol. 67:790-796.

Lieper, R.T. 1915. Report on the results of the bilharzia mission in Egypt 1915. Journal of the Royal Army Medical Corps. 25:1-55, 147-192, 253-267.

Lodes, M.J. and T.P. Yoshino. 1989. Characterization of excretory-secretory proteins synthesized in vitro by Schistosoma mansoni primary sporocysts. J. Parasitol. 75:853-862.

Loker, E.S., J. Bayne, P.M. Buckley and K.T. Kruse. 1982. Ultrastructure of encapsulation of Schistosoma mansoni mother sporocysts by hemocytes of juveniles of the 10-R2 strain of Biomphalaria glabrata. J. Parasitol. 68:84-94.

Loker, E.S., M.A. Yui and C.J. Bayne. 1984. Schistosoma mansoni: Aggultination of sporocysts, and formation of gels on miracidia transforming in plasma of Biomphalaria glabrata. Exp. Parasitol. 58:56-62.

Macgregor, A.N., D.I. Stott and J.R. Kusel. 1985. Lectin binding to glycoproteins in the surface membrane of Schistosoma mansoni. Mol. Biochem. Parasitol. 16:163-172.

McLaren, D.J. and R.J.Terry. 1982. The protective role of acquired host antigens during schistosome maturation. Parasite Immunology. 4:129-135.

Mott, K.E. 1987. Schistosomiasis control. In "The biology of schistosomes-from genes to latrines". Rollinson, D. and A. J. Simpson, (eds.), Academic Press, New York. pp. 431-450.

Nappi, A.J. 1973. The role of melanization in the immune reaction of Drosophilia alonguin against Pseudocoila bochei. Parasitology. 66:23-32.

Noble, E.R., and G.A. Noble (eds.). 1982. Parasitology, The Biology of Animal Parasites. Lea and Febiger Pubs. Philadelphia, PA. 522p.

Olafsen, J.A. 1986. Invertebrate Lectins: Biochemical heterogeneity as a possible key to their biological function. In "Immunity in Invertebrates". Ch.8. M. Brehelin, (ed.), pp. 94-111. Springer-Verlag, Berlin.

Parrinello, N. and V. Arizza. 1988. D-galactose binding lectins from the tunicate Ascidia malaca: Subunit characterization and hemocyte surface distribution. Dev. Comp. Immunol. 12:495-507.

Prowse, R.E. and N.N. Tait. 1969. In vitro phagocytosis by amoebocytes form the hemolymph of Helix aspersa. Immunology. 17:437-443.

Renwrantz, L. 1983. Involvement of agglutinins (lectins) in invertebrate defense reactions: The immuno-biological importance of carbohydrate specific binding molecules. Dev. Comp. Immunol. 7:603-608.

Renwrantz, L.R. and T.C. Cheng. 1977. Identification of agglutinin receptors on hemocytes of Helix pomatia. J. Invertbr. Pathol. 29:88-96.

Renwrantz, L. and W. Mohr. 1978. Opsonizing effect of serum and albumin gland extracts on the elimination of human erythrocytes from the circulation of Helix pomatia. J. Invertbr. Pathol. 31:164-170.

Renwrantz, L.R. and A. Stahmer. 1983. Opsonizing properties of an isolated hemolymph agglutinin and demonstration of lectin-like recognition molecules from Mytilis edulis. J. Comp. Physiol. 149:535-546.

Renwrantz, L., W. Sch ncle, H. Harm, H. Erl, H. Liebsch, and J. Gercken. 1981. Discriminative ability and function of the immunobiological recognition system of the snail Helix pomatia. J.Comp. Physiol. 144:477-488.

Richards, C.S. 1972. Biomphalaria glabrata genetics: Pearl formation. J. Invertbr. Pathol. 20:37-40.

Richards, C.S. 1975a. Genetic studies of pathological conditions and susceptibility to infection in Biomphalaria glabrata. Ann. N.Y. Acad. Sci. 266: 394-410.

Richards, C.S. 1975b. Genetic factors in susceptibility of Biomphalaria glabrata for different strains of Schistosoma mansoni. Parasitology. 70:231-241.

Rollinson, D. and V.R. Southgate 1987. The genus Schistosoma: A taxonomic appraisal.In "The biology ofschistosomes-from genes to latrines". Rollinson, D. and A. J. Simpson, (eds.), Academic Press, New York. pp.1-49.

Rudiger, H. 1981. Lectins-an introduction. In "Lectins-Biology, Biochemistry, Clinical Biochemistry". Vol 1. T.C. Bog-Hansen, (ed). Proc. 3rd. Lectin Meeting, Copenhagen, June 1980. pp.1-7. Walter de Gruyter. Berlin. New York. 1981.

Schmidt, G.D. and L.S. Roberts. 1977. Foundations of Parasitology. The C.V. Mosby Company. Saint Louis, MI. 604p.

Schoenberg, D.A. and T.C. Cheng. 1980. Lectin-binding specificities of hemocytes from two strains of Biomphalaria glabrata as determined by microheamadsorption assays. Dev. Comp.Immunol. 4:617-628.

Scribner, D.J. and D. Fahrney. 1976. Neutrophil receptors for IgG and complement: Their roles in the attachment and ingestion phases of phagocytosis. J. Immunol. 116:892-897.

Sharon, N. 1984. Surface carbohydrates and surface lectins are recognition determinants in phagocytosis. Immunol. Today. 5:143-147.

Sharon, N. and H. Lis. 1989. Lectins as cell recognition molecules. Science. 246:227-246.

Sharon, N. and H. Lis. 1989. Lectins. Chapman and Hall London.

Sloan, B., C. Yocum and L.W. Clem. 1975. Recognition of self from non-self in crustaceans. Nature. 258:521-523.

Sminia, T. and L. Barendsen 1980. A comparative, morphological and enzyme histochemical study on blood cells of the freshwater snails Lymnaea stagnalis, Biomphalaria glabrata and Bulinis trucatus. J. Morphol. 165:31-39.

Siminia, T., W.P.W van der Knaap and F.G.M. Kroese. 1979. Fixed phagocytes in the freshwater snail Lymnaea stagnalis. Cell. Tissue. Res. 196:545-548.

Sminia, T., A.A. Winsemius and W.P.W. van der Knapp. 1981. Recognition of foreignness by blood cells of the freshwater snail Lymnaea stagnalis with special reference to the role and structure of the cell coat. J. Invertbr. Pathol. 38:175-183.

Spray, F.J. and W.O. Granath, Jr., 1988. Comparison of haemolymph proteins from Schistosoma mansoni (Trematoda)-susceptible and resistant Biomphalaria glabrata (Gastropoda). Comp. Biochem. Physiol. 91B:619-624.

Spray, F.J. and W.O. Granath, Jr., 1990. Differential binding of hemolymph proteins from schistosome-resistant and -susceptible Biomphalaria glabrata to Schistosoma mansoni sporocysts. J. Parasitol. 76:225-229.

Stoscheck, C.M. 1990. Quantitation of Protein. In "Methods in Enzymology (M.P. Deutscher, ed.) Vol 182:50-68. Academic Press, Inc.

Ulmer, M.J. 1970. Notes on rearing of snails in the laboratory. In "Experiments and Techniques in Parasitology". (A.J. MacInnis and M.Voge eds.) pp. 143-144. W.H. Freeman and Company, San Francisco.

van der Knaap, W.P.W., L.H. Boerrigter-Barendsen, D.S.P. van den Hoeven, and T. Sminia. 1981. Immunocytochemical demonstration of a humoral defense factor in blood cells (amoebocytes) of the pond snail, Lymnaea stagnalis. Cell. Tissue. Res. 219:291-296.

van der Knaap, W.P.W. and E.A. Meuleman. 1986. Interaction between the immune system of lymnaeid snails and trematode parasites In "Immune Mechanisms in Invertebrate Vectors". Ann M. Lackie, (ed.), Sypm. Zool. Soc. Lond. 56:179-198.

Vasta, G.R. and J.J. Marchalonis. 1985. Humoral and cell membrane-associated lectins from invertebrates and lower chordates: Specificity, molecular characterization and their structural relationships with putative recognition molecules from vertebrates. Dev. Comp. Immunol. 9:531-539.

Vasta, G.R., J.T. Sullivan, T.C. Cheng, J.J. Marchalonis and G.W. Warr. 1982. A cell membrane-associated lectin of the oyster hemocyte. J. Invertebr Pathol. 40:367-377.

von Lichtenberg, F. 1987. Consequences of infections with schistosomes. In "The biology ofschistosomes-from genes to latrines". Rollinson, D. and A. J. Simpson, (eds.), Academic Press, New York. pp.185-232.

Yoshino, T.P. 1981. Comparison of concanvalin A-reactive determinants on hemocytes of Biomphalaria glabrata snail stocks: Receptor binding and redistrubution. Dev. Comp. Immunol. 5:229-240.

Yoshino, T.P. 1983. Lectins and antibodies as molecular probes of molluscan hemocyte surface membranes. Dev. Comp. Immunol. 7:641-644.

Yoshino, T.P., T.C. Cheng and L.R. Renwrantz. 1977. Lectin and human blood group determinants of Schistosoma mansoni: Alteration following in vitro transformation of miracidium to mother sporoctyst. J. Parasitol. 63:818-824.

Yoshino, T.P. and W.O. Granath, Jr. 1983. Identification of antigenically distinct hemocyte subpopulations in Biomphalaria glabrata (Gastropoda) using monoclonal antibodies to surface membrane markers. Cell. Tissue Res. 232:553-564.