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FINAL REPORT

ON

IDENTIFICATION OF SURFACE ANTIGENS AND PRODUCTION OF MONOCLONAL ANTIBODIES IN *SCHISTOSOMA JAPONICUM*

A RESEARCH PROJECT

USAID/PSTC PROGRAM

GRANT NO. 936-5542-G-00-6070-00

SUBMITTED BY

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PROJECT PROFILE

COUNTRY :	Thailand
GRANT NO. :	936-5542-G-00-6076-00
PROGRAM :	Program on Science and Technology Co-operation
PROJECT TITLE :	Identification of surface antigens and production of monoclonal antibodies in *Schistosoma japonicum*
PROJECT LEADER :	Prasert Sobhon, Ph.D.
ORGANIZATION :	Faculty of Science Mahidol University Bangkok 10400, Thailand.
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PROJECT CONSULTANT :	-
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TOTAL PROJECT BUDGET:	US$150,000
PROJECT DURATION :	3 years
PERIOD COVERED :	August 1986 - December, 1989

1. BACKGROUND

The grant was awarded on August 11, 1986 per agreement signed on behalf of USAID by Dr. John R. Eriksson, Director of USAID THAILAND, accepted on behalf of the Royal Thai Government by Mr. Wanchai Sirirattna, Director-General, Department of Technical and Economic Co-operation, and acknowledged on behalf of Mahidol University by Dr. Natth Bhamarapravati, Rector, Mahidol University. The research was to be carried out from August 11, 1986 to December 31, 1989 with the total budget of US$150,000.

2. PROJECT OBJECTIVES

The overall objectives of this research project are as follows :

1. To identify and characterize antigens on the surface of the tegument of cercariae, schistosomula and adult *Schistosoma japonicum* (Chinese & Philippine), and to study cross-reactions with surface antigens of other schistosome species.
2. To produce monoclonal antibodies (MAB) against parasite surface antigens, identify MAB clones that can react with specific surface antigens and/or those that can kill schistosomula.

3. LITURATURE REVIEW

The chemistry of the schistosome surface membrane and its glycocalyx coating is being increasingly studied for the better understanding of the host-parasite interaction, and the mechanisms of the parasite evasion from hosts' immune responses. Stein and Lumsden (1973) have shown that the surface membrane of adult *Schistosoma mansoni* can be stained with colloidal iron, thereby demonstrating the presence of anionic groups on the surface. This is further confirmed by Wilson and Barnes (1977) who demonstrated the selective affinity of cationized ferritin to the outer bilayer of the heptalaminate surface membrane, and the work of McDiarmid and Podesta (1984) who showed the binding of ruthenium red to the external layer of this membrane. Further work by Murrell et al. (1978) showed that lectins, concanavalin A (Con A) and wheat germ agglutinin (WGA) bind to the surface of adult *S. mansoni* while *Ulex europaeus* (UEA) and soy-bean agglutinins (SBA) do not. In a similar study, Simpson and Smithers (1980) found very strong binding of Con A, *Ricinus communis*

agglutinin (RCA), WGA, limited binding of SBA and peanut agglutinin (PNA) but no binding of UEA. Moreover, Stein and Lumsden (1973) also showed that Con A binds to similar sites on the surface membrane of fixed worms as colloidal iron. Together these studies provide strong evidence that the membrane-bound anions are probably residing in surgar residues on the surface. From lectin studies, it can be further assumed that major sugar residues that are present on the surface membrane of adult *S. mansoni* are mannose, glucose, galactose, small amount of N-acetylgalactosamine and sialic acid, but probably few or no fucose. There are strong evidence indicating that these sugar residues are part of glycoproteins and/or glycolipid molecules that constitute the surface membrane (Simpson and Smithers, 1980). This is confirmed by Bennett and Seed (1977) who demonstrated that most carbohydrate residues reside in glycoproteins by using lectin affinity chromatography, and that the treatment of adult schistosomes with trypsin results in a 53% reduction in Con A binding. Caulfield et al. (1987), using phenol-water extraction and gel chromatography, reported that glycocalyx on the surface of *S. mansoni* cercaria is composed mostly of carbohydrates and proteins; and Hayunga et al. (1982, 1983, 1986a, b) also reported that major Con A-binding surface materials from adult *S mansoni* are also glycoproteins. From these studies, it is likely that the majority of sugar residues which are detected by lectins bindings are the carbohydrate components of glycoproteins. Biochemical studies have also revealed that glycoproteins are the major components of the tegument surface membrane. Employing lectin affinity chromatography, Bennett and Seed (1977) have found that immobilized Con A binds to three radio-iodinated surface membrane proteins of *S.mansoni*, and using similar technique Strand et al. (1982) and Aronstein and Strand (1984) have shown up to 35 bands of glycoproteins from adult tegument. More recently, Hayunga and Sumner (1986) using column chromatography containing Con A, Lentil lectin, WGA, SBA and RCA and *Helix promatia* agglutinin found 15 ^{125}I-labelled glycoproteins with molecular weights of > 300,000, 215,000, 168,000, 152,000, 134,000, 122,000, 108,000, 83,000, 58,000, 53,000, 46,000, 41,000, 34,000, 30,000 and 23,500, and many of these glycoproteins react with more than one lectins.

It has been demonstrated further that surface glycoproteins, especially their carbohydrate moieties, are major epitopes of schistosome antigens that can elicit antibody production in the naturally as well as artificially-infected hosts (Omer-Ali et al., 1986; Weiss et al., 1986; Grzych et al. (1987). However,

only the surface antigens of *S. mansoni* have been extensively characterized by many groups of investigators who obtained slightly different results. By radiolabelling surface proteins and glycoproteins with ^{125}I or ^{3}H and using SDS gel electrophoresis to separate them, most investigators generally agreed that there are between 7 to 15 bands of surface proteins and glycoproteins, ranging in molecular weights from 12,000 to 200,000 daltons. Most of these surface proteins and glycoproteins could be immunoprecipitated with antisera from patients and infected animals and the reported molecular weights of these antigens vary considerably (Ramasamy, 1979; Hayunga et al. 1979a, b; Snary et al. 1980; Taylor et al., 1981; Dissous et al., 1981; Dissous et al., 1982; Strand et al. 1982; Samuelson and Caulfield, 1982; Simpson et al., 1983; Taylor and Well, 1984). Taylor and Wells (1984), utilizing iodosulphanilic acid to label schistosomula, could precipitate polypeptides with MW 185,000; 68,000 and 24,000 daltons with infected human sera. Dissous et al. (1981), on the other hand, could immunoprecipitate polypeptides with MW 100.000; 40,000; 37,000; 32,000 and 20,000 to 25,000 with immune rat and human sera, following ^{125}I labelling of these schistosomula surface antigens by lactoperoxidase method. Using similar technique, Simpson et al. (1983) showed that there are seven major proteins with MW 94,000, 68,000, 45,000, 40,000 to 32,000, 22,000 and 16,000 on the surface of 3-hour schistosomula. On the other hand, Samuelson and Caulfield (1982) using galactose oxidase/Na $B^{3}H_{4}$ revealed 12 bands of major surface glycoproteins and seven glycolipids on newly transformed schistosomula. By contrast, Strand et al. (1982) could immunoprecipitate up to 15 bands of glycoproteins (MW 200,000 to 12,000) isolated from the surfaces of cercariae and adult parasites by Con A-affinity chromatography. Eventhough the surface morphology is different during various developmental stages from cercaria to adult, it was found that the surface antigens of these stages show marked similarity, and that most are glycoproteins. And despite slight variation in the number of bands and their molecular weights, recent studies revealed that schistosomula, lung-stage and adult parasites share some common surface antigens. Dissous et al., (1981) demonstrated a common antigenic determinant in the surface molecules with MW 38,000 daltons in newly transformed schistosomula and 115,000 daltons in adult parasites; Payares et al. (1985) also showed that schistosomula, lung stage and adults bear prominent common surface antigens with MW 32,000 to 38,000 and 20,000 daltons, whereas antigens with MW 25,000 daltons was found only in lung stage and adult worms. Furthermore, it was shown in these studies that antisera against the purified adults' membrane and monoclonal antibodies raised against adult surface antigens can readily kill

schistosomula *in vitro*. These evidence strongly support the concept of "concomittant immunity", and demonstrated the possibility that centain surface glycoprotein antigens may be selected as potential candidates for the ultimate production of vaccines.

In contrast to *S. mansoni*, up to now there is only limited information on the surface glycoproteins and antigens of *S. japonicum* and none on another Oriental schistosome, *S. mekongi*. Therefore, in this study we have: (1) investigated the types and the distribution of charged groups and associated sugar residues on the surface of these two species of Oriental schistosomes by using colloidal iron, ruthenium red, and lectin stains ; (2) identified the surface proteins and glycoproteins by radio-labelling with ^{125}I and lectin blotting; (3) characterized and localized glycoprotein antigens on the surface and in the tegument of *S. japonicum* and *S. mekongi* by immunoprecipitation, immunoblotting, immunofluorescence and immunoelectron microscopic techniques ; and (4) attempted to produce specific monoclonal antibodies against the tegument antigens.

4. MATERIALS AND METHODS

4.1 Maintenance of *Schistosoma japonicum* Life Cycles and Collection of Cercariae, Schistosomula and Adult Parasites

4.1.1 Collection of Cercariae *S. japonicum* cycles have been maintained at the Center for Applied Malacology and Entomology, Department of Biology, Mahidol University. Miracidia were obtained by hatching the eggs recovered from the liver of infected Swiss Albino mice. Specific snail hosts that were used consisted of *Oncomelania hupensis hupensis* (hosts for Chinese strain) and *Oncomelania hupensis quadrasi* (hosts for Philippine strain). The snails and schistosomes have been collected from fields, ie., Anhui Province, People's Republic of China and from Mindoro Island, The Philippines. Each snail is infected with approximately 6-8 miracidia. Eight to nine weeks after the infection, cercariae were shed naturally from the snails by placing them under warm light, for 2-3 hours, into aquarium water. Cercaria were then concentrated in a small volume of water and used for furtner experiments.

4.1.2 The Preparation of Schistosomula by Mechanical Transformation Cercariae were transformed into schistosomula *in vitro*, by physical technique as previously described (Ramalho-Pinto et al., 1974; McLaren & Hockley, 1976; Brink et al., 1977) and slightly modified by Lazdins et al. (1982).

Briefly, the procedure was as follows : the concentrated cercariae were immobilized by chilling in an ice-bath for 10 minutes and further concentrated by centrifugation at 2,000 rpm for 5 minutes, at 4°C. The supernatant fluid was discarded and the pellet gently resuspended in 2 ml of Earle's saline containing 0.5% lactalbumin hydrolysate, 100 ug/ml pennicilin and 100 units/ml streptomycin (ELAC), and then shaken vigorously for 10-15 minutes on a Vortex mixer to break the tails from the bodies. The preparation was resuspended in 10 ml ELAC and allowed to sediment for 10 minutes. The pellets containing mostly separated cercarial bodies were then resuspended and washed 3 times in 10 ml ELAC at room temperature. Before being used further they were incubated for at least 3 hours at 37°C in Eagles's Minimal Essential Medium (MEM) (GIBCO) supplemented with 10 mg/ml pennicilin, 100 units/ml streptomycin and 5% foetal calf serum, which was gassed with air containing 5% CO_2, at pH of 7.0.

4.1.3 Collection of Lung Stage and Adult Schistosomes Swiss albino mice were infected with approximately 20-30 cercariae per mouse by the intraperitoneal route. Young worms were recovered from the lung on day 5 by puncturing the left ventricle of the heart, and the lung was perfused with normal saline containing heparin via the right ventricle until all blood containing worms had been removed. Adult worms were obtained from Swiss albino mice, infected 8 week previously with cercariae, by portal perfusion with 0.15 M sodium chloride and 0.05 M sodium citrate. The worms were washed 5 times with Minimum Essential Medium (MEM) before used. In addition, adult *S. mekongi* and *S.mansoni* were collected from mice kept in cycle similar to *S. japonicum* for comparative studies.

4.2 Collection of antisera. Immune mouse antisera were obtained from Swiss albino mice, each infected with 30 cercariae for 8 weeks, by heart puncture. Animals were bled and the blood was allowed to clot at 25°C for 3 hours. The clot was removed and the antisera were centrifuged at 1,000 X g for 5 minutes to obtain clear supernatant. Normal mouse serum was obtained in a similar manner from uninfected mice. In addition, both pooled and individual human antisera from *S. japonicum* (Philippine)-infected patients were graciously supplied by Professor Edito Garcia, Institute of Public Health, University of the Philippine System, and Dr. Biyani Blas, Director, Schistosomiasis Control and Service Center, Ministry of Health, Manila, The Philippines.

4.3 Staining for charges and sugar moieties on the tegument surface. Colloidal iron and ruthenium red were employed for specific staining of charged groups on the surface according to the procedure of Gasic et al. (1966, 1968) and Shepard and Mitchell (1977). The sugar moieties were detected by avidin-biotin-peroxidase method as described by Guesdon et al. (1985), the lectins used were biotinylated Con A, WGA, RCA1, PNA, SBA and UEA1. For these three stainings all three stages of parasites· cercaria, schistosomule and adult were studied.

4.4 Labelling of surface proteins/glycoproteins with ^{125}I Adult male *S. japonicum* (Chinese & Philippine), *S. mekongi, S. mansoni* and *S. haematobium* were radiolabelled by ^{125}I according to Iodogen method of Fraker and Speck (1978): 200 uCi $Na^{125}I$, 100 ug Iodogen (Pierce Chemical Co.) were used per 100 adult male parasites. After 5 minutes the labelling was stopped and chased with 5 mMKI; labelled parasites were rapidly frozen and thawed in 50 ml Tris HCl, pH 7.4, 150 mM NaCl, 10 mM EDTA and 1 mM PMSF(TNEP buffer) for 10 times to obtain the labelled tegument antigens for immunoprecipitation and autoradiography. In addition, prepared schistosomula of *S. japonicum* (Chinese) were also labelled using similar technique but with approximately 100 uCi of $Na^{125}I$ per 3000 schistosomula.

4.5 EM autoradiography (EMARG) of labelled worms. In order to monitor the sites of ^{125}I labelling EMARG was performed. Another batch of worms were washed after ^{125}I labelling, fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer pH 7.2 for 18 hrs at 4°C. After rinsing 3 times in cacodylate buffer, the worms were postfixed in 1% OsO_4 in cacodylate buffer for 2 hrs and washed 3 times with cacodylate buffer. Following washings, worms were dehydrated and embedded in Araldite 502. Ultrathin sections were cut with Porter-Plum MT-2 ultramicrotome and coated with Ilford L-4 emulsion maintained at 45°C and diluted 1:1 (V/V) with H_2O. Autoradiographs were exposed, developed in undiluted Dektol developer (Eastman Kodak Company) for 2 minutes, fixed for 10 minutes, washed in water, dried and stained with lead citrate and uranyl acetate.

4.6 Identification of lipids on the surface of *S. japonicum*. The presence of lipids in the surface membrane and the tegument have been reported in various species of parasitic helminths, including *S. mansoni*. Some of the lipids have been demonstrated to be immunogenic in both animals or human infected by the parasites. In this experiment, living adult *S. japonicum*

(Philippines) were ^{125}I-labelled by a similar procedure as used in the protein labelling in section 4.4. Freeze-thawed tegument of parasites was prepared and lyophilized into dry powder and lipid components were extracted by hexane-isopropanol. The solvent could reproducibly recover the majority of the radioactively labelled compounds, whereas the precipitate (presumably proteins) always represented less than 20%, suggesting that lipids were major components of the *S. japonicum* tegument. The lipid fraction was further extracted in an aqueous system to separate polar lipids (glycolipids/lipoproteins) from non-polar lipids; and it was found that there were glycolipids about 3-4 times higher than non-glycolipids as determined from their radioactivities. The radioactively labelled lipids were observed to bind tightly to the Eppendorf plastic tubes leading to marked loss during the extraction. However, 10^5 - 10^6 cpm of the ^{125}I-glycolipids were usually obtained following the extraction. Analysis of labelled lipids were performed on thin layer chromatography, and revealed by autoradiography in comparison with standard lipids.

4.7 Immunoprecipitation of ^{125}I surface antigens by mouse and human immune sera. Immunoprecipitations were performed using labelled surface antigens from freeze-thawed fractions of tegument in 100 ul (106 count/min) per aliquot, and these were clarified by incubation with 10 ul of corresponding normal serum. After 10 minutes the complexes were precipitated by the addition of 100 ul of 10% protein-A Sepharose and centrifuged at 2000 X g for 10 minutes at 4°C. Supernatants were incubated with mouse anti-*S. japonicum* immune sera as well as mouse immune sera against other schistosoma and human immune sera from patients infected with *S. japonicum* (Philippine), for 30 minutes at 37°C, and kept further overnight (~ 18 hrs) at 4°C. The antigen-antibody complexes were then precipitated with protein-A sepharose. The immunoprecipitates were centrifuged at 2000 X g for 10 minutes at 4°C to obtain pellets which were washed 3 times in phosphate buffer saline pH 7.4. Each of the pellet was then treated with 50 ul of sample buffer (62.5 mM Tris-HCl, pH 6.8; 2% SDS; 5% B-Mercaptoethanol and 10% Glycerol) and boiled for 2 minutes to complete the dissolution.

Electrophoresis and autoradiography. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the method of Laemmli (1970). Electrophoresis was performed on 12.5% gels, and the voltage was set at 100 volts, and gel run for 4½ hrs. After drying, the gels were exposed to X-ray film (Kodak XO mat RP film) using intensifying screens. Autoradiographs were developed in Kodak GBX for 2 minutes.

4.8 Identification and comparison of tegument antigens of adult *S. japonicum, S. mekongi* and *S. mansoni* by immunoblotting. In this experiment, the tegument antigens of parasites were obtained as follows : the parasites' tegument were extracted by freeze-thawing on dried ice in Tris-buffer for 20 cycles, and the shed pieces of tegument in the supernatant were collected after centrifugation of the mixture at 100 X g for 5 minutes. The supernatant was solubilized in the SDS-PAGE sample buffer and applied onto 12.5% SDS-PAGE gels. Then, electrophoresis was performed at 100 volts for 4 hours, and the proteins on gel were blotted onto nitrocellulose (NC) paper using blotting apparatus with setting at 0.5 mA for 1 hour. Antigenic proteins on the nitrocellulose paper were detected by incubating NC strips in mouse immune sera and followed by rabbit anti-mouse Ig labelled with horseradish peroxidase (HRP). The antigen-antibody complexes that bind with anti-mouse Ig-HRP were visualized by incubating the nitrocellulose papers in 3,3'-diaminobenzidine (DAB) and H_2O_2. In addition, similar nitrocellulose strips are also stained with biotinylated lectins as mentioned in section 4.3 in order to identify glycoprotein bands.

4.9 Preparation of monoclonal antibodies (MAB) against tegument antigens of *S. japonicum*

4.9.1 Immunization

Six week old BALB/c strain mice were used for immunization with tegument antigens of the Chinese and Philippine strains *S. japonicum*. Ten mice were used for each immunization schedule. Mice were first given 200 micrograms of antigens mixed with an equal amount of complete Freunds' adjuvant innoculated at 4 sites subcutaneously. The second and third immunizations were given at two week interval on the same route with the same amount of antigens but with incomplete Freunds' adjuvant. The fourth immunization was given intravenously 2 weeks after the third with 800 micrograms of the antigens. The antibody titers of each animal were checked by ELISA assay after 4-6 days of each immunization. Only mice found to have antibody titer of over 1 in 400 were used for cell fusion.

4.9.2 Preparation of monoclonal antibodies

P3U1 myeloma cells cultured in 25 cm^2 flasks in exponential phase of growth were used for fusion with the spleen cells from 5-6 hyperimmune mice killed at 4 days after the last boosting. The hybrid cells that survived in HAT medium were tested for production of antibody by ELISA assay. The

antibody-producing cells were expanded in culture and clonning was performed by limiting dilution method. The cloned cell was expanded slowly *in vitro* by transferring to 24-well plates, and cm^2 flasks, respectively, while the cell density was maintained between 10^5-10^6 cell/ml.

4.10 Testing of monoclonal antibodies

4.10.1 Immunoblotting assay

The adult parasites tegument was extracted by freeze-thawing and the protein fractions were separated by SDS-PAGE and blotted onto nitrocellular paper (NC) in the same manner as mentioned in section 4.8. Instead of using immune sera, the NC strips were incubated in solutions of monoclonal antibodies (MAB) collected either from ascitic fluid or from cultures. The positive MAB that react with tegument proteins were detected by incubation with rabbit anti-mouse Ig-HRP and visualized by further incubation in DAB and H_2O_2.

4.10.2 Indirect immunofluorescence assays

Freshly-obtained adult worms were fixed in 2% paraformaldehyde in 0.1 M PBS, pH 7.4, for 2 hours at 4°C. The fixed worms were washed with the same buffer, then they were frozen and cut into 4-6 um thick sections in a cryostat. These frozen sections were picked up on gelatin coated slides and allowed to thaw at room temperature for 30 minutes. The sections were covered with 10% normal sheep serum in 0.1 M PBS, for 30 minut s and followed with the appropriate dilution of monoclonal antibodies (MAB) for 2 hours at room temperature. After washing with PBS buffer, the sections were incubated with secondary antibody (sheep anti-mouse IgG conjugated with FITC) for 1 hour at room temperature. At the end of incubation period, the sections were washed with double distilled water 3 times for 10 minutes at each washing. The sections on slides were mounted in buffered glycerol and covered with glass coverslips. The completely stained sections were examined by UV light using the incidence illumination in Leitz Orthoplan microscope. The positive and negative controls were carried out simultaneously by replacing the monoclonal antibodies with an immune mouse serum and PBS, respectively. In addition, mechanically transformed schistosomula were also used in the assay, and both experimental and positive and negative controls were performed as for adult parasites.

5. RESULTS AND DISCUSSION

Wherever it is possible both strains of *Schistosomo japonicum* and *Schistosomo mekongi,* another species of Oriental schistosome, were studied and compared.

5.1 Charges groups on the tegument surface

5.1.1 Positive colloidal iron staining

Positive colloidal iron was employed for specific staining of negatively charged groups on acidic glycoproteins, and negative colloidal iron for positively charged groups, such as amino groups of amino sugars (Curren et al., 1965; Gasic et al., 1966). The procedure was performed by fixing the freshly-collected parasites in 2.5% glutaraldehyde in 0.15 M sodium cacodylate buffer, pH 7.4, at 4°C, overnight. The parasites were stained with positive or negative colloidal iron according to the procedure of Gasic et al. (1968). The positive colloid was non-dialyzed and used at pH 1.8 in order to stabilize the charge of the colloid. The negative colloid was dialyzed and used at pH 6.0 for the same reason. After staining, the parasites treated with positive colloid were washed with 12% acetic acid. Then, both groups of specimens were washed with distilled water and processed for TEM. Unstained sections were examined by TEM for the deposit of iron particles, which appeared as fine dense granular materials of ferric oxide on the sites of charged groups.

In cercariae of both *Schistosomo japonicum* and *Schistosomo mekongi*, positive colloidal iron binds strongly throughout the thickness of the glycocalyx, which appears as interlacing network of fine filaments on which black granules are deposited (Fig. 1 A,B). In ½- to 1-hour schistosomula of both species, the staining appears on the external surface of the outer membrane, on the shed glycocalyx and those that still surround blebs, microvilli nd membrane puffs (Fig. 1 C,D). In 3- to 6-hour schistosomula, the amount and intensity of staining is much decreased, and the stain is limited mostly to the external surface of the outer membrane and its vicinity. In *S. japonicum,* only small amount of shed glycocalyx is still stained, whereas in *S. mekongi,* some areas of the tegument still show intense staining, particularly on the remaining glycocalyx, which indicates that in this species the glycocalyx is shed at a slower rate than in *S. japonicum.* At high magnification, the stain on the membrane appears as a coat of fine particles that is uneven in thickness. In 12-hour schistosomula of both species, the staining is decreased even more than in 6-hour stage, the tegument is generally cleared of glycocalyx, and the

staining is confined only to the external surface of the outer membrane (Fig. 1 E,F).

In adult males of both species, the pattern of staining with positive colloidal iron is quite similar. At low magnification, the staining appears evenly distributed over the entire dorsal and lateral surface of the tegument (Fig. 2 A,C); however, at high magnification it is revealed that the iron particles bind heavily to the surface membrane of ridges (Fig. 2 B,D,E). In the pits, the staining is fairly intense on the membrane covering the neck region close to the ridge, whereas it decreases towards the bottom part. The lowest part of pits, which in cross section appears as small lumen, is usually not stained. The surface of the gynaecophoral canal also exhibits very little or no staining at all eventhough the colloid has ready access to the canal, which in all preparations is widely opened and devoided of female parasite.

5.1.2 Negative colloidal iron staining

In cercariae, the staining occurs only on the glycocalyx lying at a distance away from the tegument surface, which may represent the outermost part of the thick glycocalyx. Little staining is found on the inner part of the glycocalyx and on the surface membrane itself. Thus, there is a clear zone between the aggregates of dense particles on the periphery of glycocalyx and the surface membrane. Furthermore, the staining is not even and some areas are not stained at all. In ½- to 12-hour schistosomula of both species, the intensity of the staining of the glycocalyx drastically decreases, and there is no staining on the outer membrane. In adult stage, the negative colloidal iron does not stain the surface membrane of *S. japonicum* at all, while it shows only very little and spotty staining on the surface of *S. mekongi*.

5.1.3 Ruthenium red staining

This stain can be used to detect negative charges due to carboxyl groups of N-acetyl neuraminic (sialic) acid; while other negative charges groups, such as sulphate and phosphate groups can be prevented from binding to this stain by using proper concentration and time of incubation (Shepard and Mitchell, 1977). In our study, the procedure was performed by fixing parasite specimens in 2.5% glutaraldehyde and 0.2% ruthenium red in 0.15 M sodium cocadylate buffer for one to two hours at room temperature. After washing three times in the same buffer, the specimens were incubated with 2.0% osmium tetroxide and 0.2% ruthenium red in 0.15 M sodium cacodylate buffer, pH 7.4, for three hours at room temperature. After

washing thoroughly, they were dehydrated through increased concentrations of alcohol and processed for TEM. The sites that carry negative charges appeared as homogeneously black areas that contained very fine grain of ruthenium red. It is expected that this technique complements the staining by colloidal iron which tends to bind non-selectively to all negatively-charged groups.

In cercariae of both species, ruthenium red staining appears on the whole thickness of the glycocalyx coating, and in contrast to colloidal iron, ruthenium red also stains the surface membrane of the tegument (Fig. 3 A,B). The glycocalyx appears as tightly woven filaments on which very fine black particles are deposited. These particles are much finer and appear more homogeneous than colloidal iron; therefore, they can penetrate down to the level of the surface membrane. In ½- to 1-hour schistosomula, the staining of ruthenium red starts to decrease due to the shedding of glycocalyx by microvilli and membrane puffs; however, the surface membrane is still intensely stained (Fig. 3 C,D). The interior of the tegument is not stained, but some intracytoplasmic membrane sheets and Mb granules close to the surface are lightly stained (Fig. 3 C,D). In 3-hour schistosomula, the staining on glycocalyx decreases further, but very intense staining remains on the surface membrane. Some staining is observed around blebs and microvilli, which presumably is glycocalyx that still adheres to these to be casted off structures. The intracytoplasmic membrane sheets and Mb granules close to the surface are also stained. In 6- to 12-hour schistosomula of both species, there is only a sparse staining on the remaining glycocalyx, while the membrane of the tegument is still intensely stained (Fig. 3 E,F).

In adult parasites, ruthenium red stains only the surface membrane, and *S. mekongi* is generally more intensely stained than *S. japonicum*. The pattern of membrane staining is like that of positive colloidal iron, in which the staining is intense on the membrane of ridges but progressively decreases towards the bottom of pits (Fig. 4 A to D), and the surface membrane of the gynaecophoral canal is only lightly stained.

From the results of colloidal iron and ruthenium red stainings, it can be interpreted that the cercarial glycocalyx of both species of Oriental schistosomes is highly negatively charged. After the transformation, the degree of staining of the glycocalyx decreases drastically during 6- to 12-hour schistosomula of both species. This may be due primarily to the shedding of the glycocalyx by the formation and later the detachment of

blebs, microvilli and membrane puffs from the surface. In *S. mansoni*, the cercarial glycocalyx is believed to be composed mainly of acidic and neutral mucopolysaccharides that can bind to colloidal iron (Stein and Lumsden, 1973; Stirewalt and Walters, 1973; Kemp et al., 1973). The decreased binding of colloidal iron is also observed in *S. mansoni* schistosomula after the transformation (Stein and Lumsden, 1973). In late schistosomula and adults of *S. japonicum* and *S. mekongi*, even after the complete shedding of glycocalyx, the surface membrane still bears substantial amount of negative charges as shown by an intense binding of positive colloidal iron and ruthenium red. In adult parasites, the staining also exhibits distinctive pattern in which the staining of ridges' membrane is intense and it decreases progressively towards the bottom of the pits.

The chemical groups responsible for the negative charges on the schistosomes' surface have not yet been conclusively identified. However, on the basis of the present cytochemical information and previous studies on *S. mansoni*, it is likely that they are associated with acidic mucopolysaccharides, glycolipids or glycoproteins which are the normal constituents of the glycocalyx in cercariae (Clegg, 1972; Stein and Lumsden, 1973), and the parasites' membrane. At proper pH ($\sim$ 1.8), positive colloidal iron can selectively demonstrate acid polysaccharides or acidic glycoproteins that contain sulphate, carboxyl and sialic acid residues, and that the negative colloidal iron at approximately pH 6.0 is specific for amino groups of amino sugars (Curren et al., 1965; Gasic et al., 1966). Furthermore, it is suggested that ruthenium red can be used to detect the negatively-charged sites due to carboxyl groups of N-acetyl neuraminic (sialic) acid (Shepard and Mitchell, 1977) and also sulphate and phosphate groups of polysaccharides (Mcdiarmid and Podesta, 1984). Therefore, it is plausible to conclude that most of the negative charges in the cercarial glycocalyx and on the schistosomulum's and adult's membranes are probably contributed by carboxyl, phosphate, sulphate groups, and sialic acid which in most eucaryotic cell is the terminal residues of the oligosaccharide chains of glycoproteins. The intensity of staining on the membrane over the ridges and in the pits of the adult tegument is not the same probably due to the different quantity of negatively-charged groups. The newly synthesized membrane in the pits may not yet have these negatively-charged terminal residues. As the membrane "flows up" along the sides of the pits and later the ridges, more negatively-charged groups (acidic sugar residues) may be added. On the other hand, the gynaecophoral canal is relatively

unexposed to the hostile hosts' immune attack; therefore, it does not need to bear a large quantity of negatively-charged glycoconjugates as the lateral and dorsal surfaces.

5.2 Sugar moieties on the tegument surface

The presence and distribution of sugar residues in glycoproteins which are the major constituents of the surface membrane and associated glycocalyx can be investigated by staining with lectins that are marked by microscopically visible tracers. Generally, the binding of lectins to the surface membrane can be demonstrated in two principal ways : firstly, in the direct technique lectins are conjugated to labels such as fluorochrome dyes, an enzyme such as horseradish peroxidase (HRP), gold particles, or ferritin. The latter three can also be visualized under the electron microscope. Secondly. in the indirect technique the surface bound lectins are localized either with labelled antibodies directed against the lectins, or by avidin-biotin reaction. Direct technique is the most convenient but lacks sensitivity unless a high concentration of lectin is used. By contrast, the indirect technique has better sensitivity since the amplification of the lectin binding sites can be enhanced by using lectin specific antibodies, or biotinylated lectins followed with avidin coupled to the marker for the final visualization. The application of avidin-biotin-peroxidase method which involves the use of biotinylated lectins followed by an avidin-biotin-peroxidase complex (ABC) shows at least eight times more sensitivity and is less time consuming than the normal indirect method (Hsu and Raine, 1982).

In our study, avidin-biotin-peroxidase method was used to investigate the types and the distribution of sugar moieties present on the surface of *S. japonicum* and *S. mekongi*. Lectins that were used to study the surface of cercariae, schistosomula and adults were concanavalin A (Con A), wheat germ agglutin (WGA). In addition, *Ricinus communis* agglutinin (RCAI), *Arachis hypogaea*-peanut agglutinin (PNA), *Glycine maximus* - soybean agglutinin (SBA) and *Ulex europaeus*-gorse seed agglutinin (UEA I), whose sugar specificities as shown in Table 1, were also used to study the surface of adult schistosomes. In order to stabilize the surface membrane, freshly collected cercariae, schistosomula and adult worms were fixed in 2.5% glutaraldehyde in 0.15 M sodium cacodylate buffer, pH 7.2, containing calcium ions, at 4°C, for four hours or overnight. After throughout washing in PBS, the specimens were treated with 50 mM ammonium chloride in the same buffer for overnight at 4°C in order to block any residual

aldehyde groups. Specimens were processed by Avidin-Biotin-Peroxidase method (Guesdon et al., 1985) as follows : the parasites were treated with 3% hydrogen peroxide in PBS at 4°C for five minutes to block any endogenous peroxidase activity, then rinsed in cold PBS. Specimens were later immersed in 1% BSA in PBS at 4°C for 30 minutes to reduce the nonspecific background staining, and then rinsed in PBS. They were incubated further in the working dilutions of each biotinylated lectin for one hour at room temperature. The various dilutions of each biotinylated lectins in 10 mM PBS solution containing 0.1 mM $CaCl_2$ and 0.02% sodium azide were as follows : Con A 20 to 500 ug/ml; WGA 10 to 50 ug/ml; RCA I 2 to 100 ug/ml; and 50 to 500 ug/ml for PNA, SBA and UEA I. Each lectin has its "minimal concentration" where the staining first appears on the membrane of adult parasites as revealed by TEM (shown in Table 1). After incubation in lectin solutions, specimens were rinsed in PBS, then incubated in PBS solution containing 50 ug/ml of avidin-HRP for one hour at room temperature, after which they were rinsed several times in PBS and stained for 15 to 20 minutes at room temperature with a solution containing 15 mg of 3,3' - diaminobenzidine tetrahydrochloride (DAB) in 50 mM Tris-HCl buffer, pH 7.4, and 0.01% H_2O_2. This was followed by an extensive rinsing in Tris-HCl buffer pH 7.4, and then they were processed for TEM. The following controls were employed to ensure that the bindings of lectins were specific : (1) the omission of biotinylated lectins in the incubation sequence; (2) the blockade of biotinylated lectins with their specific hapten sugars; in this group, each biotinylated lectin was first incubated with 0.2 M of its hapten sugar for one hour at room temperature before being applied to the sample; and (3) the samples were incubated in DAB and H_2O_2 only to detect whether there is any endogenous peroxidase.

TABLE 1

COMPARISON OF THE DISTRIBUTION AND STAINING INTENSITY DUE TO THE BINDINGS OF BIOTINYLATED LECTINS-AVIDIN-HRP TO THE TEGUMENTAL SURFACE OF *SCHISTOSOMA JAPONICUM* (CHINESE AND PHILIPPINE STRAINS) AND *SCHISTOSOMA MEKONGI* AS REVEALED BY TRANSMISSION ELECTRON MICROSCOPY

The number of "+" indicates relative degree of staining intensity as follows :
"+++" = high intensity, "++" = moderate intensity, "+" = low intensity and "-" = no staining
"Minimal concentration of lectin" is the concentration where staining is first observed on the membrane of ridges

Biotinylated lectins	Major sugar specificities	Minimal concentration of lectin (ug/ml)	Ridges		Pits		Gynaecophoral canal
			Upper half	Lower half	Upper part	Lower part	
Con A	D-mannose, D-glucose	30	+++	+++	+++	-	+
WGA	N-Ac-D-glucosamine, sialic acid	10	+++	+++	++	-	+
RCA I	D-galactose	2	+++	++	++	-	++
PNA	D-galactose-N-Ac-D-galactosamine	250	+++	++	+	-	+
SBA	N-Ac-D-galactosamine	250	+++	+	+	-	+
UEA I	L-fucose	250	+++	+	+	-	-

The HRP-catalysed reaction product will be termed "lectin staining" or "staining product" which would be used interchangeably in this discussion. Usually, the staining product appears in either a dense homogeneous or a granular form. The dense homogeneous form may indicate that there are abundant lectin-binding or receptor sites which probably are closely spaced, so much that HRP-catalysed staining product from each lectin receptor complex can coalesce together, and appears as a homogeneous dense layer. In contrast, the granular form may be resulted from the wider spacing of lectin-receptor complexes on the membrane, hence it may represent less abundant lectin-binding sites than the homogeneous form. Another interesting characteristic of lectin binding to the membrane and the appearance of the staining product is the exhibition of saturation, in which at low concentration of lectins the staining product could hardly be discernible. The staining by each lectin was first observed when the "minimal concentration" as defined in Table 1, was applied. When the lectin concentration was increased beyond this point, the staining was not significantly more intense. This implies that receptors on the membrane of parasites, once being saturated, cannot bind any more lectin molecules. The distribution of lectin binding sites and their abundance can, therefore, be semi-quantitatively estimated by observing the pattern and intensity of the staining product.

Concanavalin A (Con A)

In cercariae of both species, Con A staining is most intense in comparison to other stages, and appears as a thick layer of dense product on the glycocalyx outside the trilaminate surface membrane. The thickness of the staining is even throughout, which implies that the whole width of the glycocalyx is stained, and at high magnification the staining appears as beaded fibers (Fig. 5 A,B).

In schistosomula, the early stages (½ to 3 hours) of both species are stained more intensely than adults, and the staining is thick and even on the surface of the tegument. The staining also occurs around microvilli and blebs, where it appears as short beaded lines radiating out from these structures. The outer membrane itself is intensely stained, while the intracytoplasmic membrane sheets and Mb granules near the surface are only lightly stained (Fig. 5 C, D). In 6- to 12- hour schistosomula of both species, the pattern of binding is similar, and the intensity of staining on the remaining glycocalyx becomes progressively decreased, until practically all glycocalyx is shed by 12 hours. In contrast, the surface membrane is always intensely stained (Fig. 5 E, F).

Therefore, it appears that cercariae of both species bind Con A very intensely than other stages. The binding occurs throughout the whole thickness of the glycocalyx and evenly on the external face of the surface membrane. After the transformation, the binding of Con A decreases quickly with the age of the schistosomula due to the shedding of glycocalyx, and eventually a constant amount of binding on the surface membrane is observed from 12-hour schistosomula onwards to adult stage. In contrast to cercariae of Oriental schistosomes, cercariae of *S. mansoni* exhibited only weak binding of Con A and WGA, while the early stages ($\frac{1}{2}$ to 1-hour) schistosomula of *S. mansoni* bind this lectin very intensely. Later, the binding decreases in older schistosomula (Murrell et al., 1978; Pearce et al., 1986).

In adults, Con A binding pattern appears generally similar in both species at both light and electron microscopic levels (Fig. 6). As inferred from the intensity of staining product, the binding of this lectin is high on the dorsal and lateral aspects of the parasite's body (Fig. 6 C,E), particularly on the membrane covering the sides and the tips of ridges, while that of the gynaecophoral canal shows only light binding (Fig. 6 B,D,F). On the former, the staining intensity of the ridge membrane and the neck region of pits is high and usually appears in homogeneous form, and it progressively decreases towards the bottom of the pits, where the staining appears in either granular form, or the membrane may not be stained at all (Fig. 6 C,E). In all control experiments where either biotinylated Con A is omitted or the parasites are incubated only with DAB + H_2O_2, or the incubation of Con A is carried out in the presence of its specific sugar inhibitor, the reaction product is not observed on the tegument (Fig. 6 A). This confirms that the binding is the result of specific interaction between Con A and sugar receptor sites on the surface membrane of adult worms. Furthermore, neither endogeneous peroxidase activities on the surface membrane nor in the tegument are observed when the parasites were pretreated with 3% H_2O_2 as suggested by Benerjee and Pettit (1984). Con A is known to bind specifically to alpha-D-mansosyl, glucosyl and glucosaminyl residues (Lis and Sharon, 1973), which in mammalian cells mostly serve as "core" or "internal" sugars of oligosaccharide chains of glycoproteins (Johnson and Cross, 1977). Our study may, therefore, indicate that these sugar residues are extensively distributed over all areas of the surface membrane of Oriental schistosomes, except in the regions where the membrane may be newly synthesized, i.e., in the lower and bottom parts of the pits, and in the gynaecophoral canal where the surface of the parasite is not directly exposed to the host blood, and therefore may have a much thinner glycoprotein coating.

Wheat germ agglutinin (WGA)

In cercariae of both species, the binding of WGA is very strong and has similar pattern to Con A binding. The glycocalyx is heavily stained, while the surface membrane is not intensely stained (Fig. 7 A,B). In early schistosomula ($\frac{1}{2}$ to 3 hours) of both species, the binding of WGA is more intense than in adult. The staining appears as a thick layer on the external surface of the membrane, around microvilli and blebs that project out from the surface (Fig. 7 C,D). In 6- to 12-hour schistosolmula, the staining of glycocalyx decreases in intensity but the surface membrane is evenly and intensely stained (Fig. 7 E,F).

In adults of both species, the pattern of WGA binding is similar to Con A. The binding is strong on the dorsal and lateral surface, while the gynaecophoral canal has only light staining (Fig. 8 A to F). On the former, the binding is confined mostly to the ridge membrane, while the pit membrane is only lightly stained (Fig. 8 B,C,E,F). The control groups do not show any dense products on the surface membrane.

Since WGA is believed to bind specifically to both the internal N-acetyl-D-glucosamine, and the terminal N-acetyl-neuraminic (sialic) acid residues in carbohydrate portion of glycoproteins (Lis and Sharon, 1973), it is, therefore, interpreted that these sugar residues may also be present in a substantial quantity on the ridge membrane but may be less aboundant on the membrane covering the pit when compared to mannose and glucose residues as detected by Con A.

Galactose-binding lectins (RCA I, PNA and SBA)

In addition to Con A and WGA, galactose binding lectins, RCA I, PNA and SBA, can also bind to the surface membrane in adults of both species of Oriental schistosomes. These lectins show a similar staining pattern, and *S. japonicum* is generally more intensely stained than *S. mekongi* at both LM and TEM levels. The staining intensity is generally high in the case of RCA I, and progressively decreases in PNA and SBA (Fig. 9, Table 1). The staining resulted from these lectin bindings appears most intense on the upper half of the ridge membrane, while the lower half and the pits membrane show lighter staining than Con A and WGA (Fig. 10). Interestingly, the bindings of these lectins, particularly RCA I, to the membrane of the gynaecophoral canal are more intense than Con A and WGA (Fig. 10A and F inset). RCA I binds to D-galactose and SBA binds to N-acetyl-D-galactosamine (Lis et al., 1970; Pereira and Kabat, 1974), while PNA binds more readily to the terminal galactose residue that is conjugated to N-acetyl-D-galactosamine (Lotan et al., 1975). Therefore, the more intense staining of

RCA than PNA and SBA may indicate the presence of higher amount of D-galatose than D-galactose conjugated to N-acetyl-D-galactosamine and N-acetyl-D-galactosamine residues on the surface membrane of Oriental schistosomes. Furthermore, the surface of adult *S. japonicum* may also bear a larger amount of these sugar residues than that of *S. mekongi*. This observation is in agreement with the result reported in *S. mansoni* by Simpson and Smithers (1980), who demonstrated the binding of RCA, SBA and PNA to the adult tegument, and that the bindings of SBA and PNA could be increased when the parasites were treated with neuraminidase enzyme. Consequently, they suggested that oligosaccharides on the membrane of adult *S. mansoni* is terminated not only in D-galactose but also in N-acetyl-D-galactosamine-D-galactose-sialic acid sequence. In Oriental schistosomes, it appears that these sugar residues are confined mostly to the upper half of the ridge membrane, and therefore they may be added to the membrane glycoproteins later than mannose, glucose and glucosamine residues. Another distinctive characteristic is that the membrane of the gynaecophoral canal may contain a large amount of D-galactose as reflected by RCA I staining.

Ulex europeus agglutin UEA I

The binding of UEA I to the surface membrane of adult Oriental schistosomes exhibits similar pattern in both species, and it appears patchy throughout the dorsal-lateral aspects (Fig. 11 A,D). Under TEM, the binding is confined mostly to the upper half of the ridges' membrane, while the pits' membrane and the membrane of the gynaecophoral canal show very light staining or none at all (Fig. 11 B,C,E). UEA I is known to bind specifically to L-fucose residue, which usually occupies the terminal sites of mammalian cell membrane's glycoproteins, hence the presence of L-fucose on the surface of adult Oriental schistosomes is implied, but it may be in limited amount as well as in sporadic distribution in comparison to other sugars. The patchy pattern of UEA binding is different from the uniform staining of other lectins, and this raises the question whether fucose-bearing glycoconjugates are of parasite origin. Several previous studies in *S. mansoni* indicated that the surface of this schistosome can absorb host molecules; for example, immunoglobulins and activated complement C_3 (Tarlaton and Kemp, 1981), red blood cell antigens (Smithers et al., 1969; Coelho et al., 1980; Goldring et al., 1976), which may serve to disguise and giving protection against the host immune responses (Clegg, 1974). It was, therefore, possible that the fucose residues and its associated glycoconjugates detected on the parasite surface may be from the adsorption of host-derived components.

It is not possible to pin point the precise location of the binding sites or receptors of these lectins on the surface membrane as pointed out by Wilson and Barnes (1977), who demonstrated the difficulty in determining whether Con A binding in adult *S. monsoni* occurred on the glycoprotein coating, which was termed membranocalyx, or on the heptalaminate membrane. In our study, the staining product derived from the bindings of most lectins (Con A, RCA I, PNA, SBA, UEA I), as revealed under high resolution TEM, appears as a row of dense granules depositing on the external surface of the heptalaminate membrane (Fig. 11F). This implies that most of the sugar receptors for these lectins are located on the thin coating of the surface membranes. However, clumps of electron dense material in the space assumed to be between the two lipid bilayers are also observed in the case of SBA binding (Fig. 11G). The clumpings are also observed in *S. monsoni* membrane and was suggested to represent either the porous structure or the transfer of lectins across the two bilayers rather than being merely an artifact (McDiarmid and Podesta, 1984).

Overall, the results obtained in our study indicate the presence of D-mannose and /or D-glucose, N-acetyl-D-glucosamine, D-galactose, N-acetyl-D-galactosamine, sialic acid as well as L-fucose on the surface of the adult Oriental schistosomes. The different intensity of staining on the membrane of pits and ridges indicate two possibilities concerning the synthesis of the membrane glycoconjugates. Firstly, the glycosylation of these sugar residues may occur mostly at the surface membrane level, beginning in the regions where the membrane is newly synthesized, ie., membrane sheets and the bottom part of pits. The sugar residues which are the core or internal sugars (D-mannose, D-glucose, N-acetyl-D-glucosamine) may be added to the glycoconjugates first since the staining attributed to these sugars is more extensively distributed on the pits' and ridges' membranes. The terminal sugar residues (D-galactose, N-acetyl-D-galactosamine, sialic acid, fucose) are probably added at later stage since the staining for these sugars is confined chiefly on the upper half of ridges. Alternatively, some sugar residues may be glycosylated to the completely assembled membrane in the subtegumental cell then packaged in Mb granules, which are translocated to the tegument, where they release the new membrane at the base of the pits. The data from our study do not support this possibility because the staining resulted from lectin bindings appears only external to the heptalaminate membrane, while most membranous bodies are not stained. However, this observation may be limited by the application of the pre-embedding technique, whereby the lectins may penetrate the parasite membrane poorly because of their large molecular sizes. In contrast to our observation, Reisig (1970) has demonstrated the presence of carbohydrate in the membrane bound

granules in the sub-tegumental cell of adult *S. mansoni* and suggested its possible involvement in the production of the tegument glycocalyx. The controversy regarding the first site of glycosylation of surface membrane glycoproteins in the tegument of schistosomes can only be settled by using post-embedding technique, either with cryosectioning method, or by using non-denaturing water-miscible plastics such as Lowicryl for embedding.

.3 Tegument glycoproteins in adult *Schistosoma japonicum* (Chinese and Philippine strains) in comparison to *S. mekongi* and *S. mansoni* as detected by electro-blotting and lectin staining.

In *S. mansoni*, several previous studies have shown the presence of specific carbohydrate moieties in the side chains of tegument glycoproteins. Rumjanek et. al., (1979) demonstrated that the major monosaccharide components in the freeze-thaw isolated tegument of adult worms are mannose, glucose and galactose residues. And by using radiolabelled hexose precursors to metabolically labelled glycoproteins, Hayunga and Sumner (1986b) found that the radiolabelled saccharides were incoporated into at least 10 tegumental glycoproteins of *S. mansoni* with MW ranging from 30,000 to < 300,000. Furthermore, by employing direct radioiodination of adult surface membrane and lectin affinity chromatography with Con A, lentil lectin, WGA, SBA, RCA I & II and *Helix pomatia,* to fractionate membrane proteins, Hayunga and Sumner (1986a) also revealed 14 glycoproteins in the eluated fractions from these lectin columns. From these studies, it is suggested that D-mannose, D-glucose, N-acetyl-D-glucosamine, D-galactose, D-galactose-N-acetyl-D-galactosamine conjugate, and L-fucose residues are the major carbohydrate moieties of the adult *S. mansoni* tegument glycoproteins.

In our study, the tegument of adult *S. japonicum* (Chinese and Philippine strains), *S. mekongi* and also *S. mansoni* were obtained by repeated freeze-thawing of the adult parasites. This treatment usually resulted in a fairly clean separation of the tegument from the parasites' bodies, leaving the basement membrane and the underlying connective tissue and muscle layers intact (Fig. 12). Hence it is believed that there is very little leakage of the content from tissues underneath the basement membrane. The shed tegument was then solubilized and its protein and glycoprotein contents were separated in 12.5% SDS-PAGE and stained with Coomassie blue. In general, the number and pattern of protein bands in all three species are quite similar with molecular weight (MW) ranging from 300,000 to 14,000 daltons. There are nine regions of major bands at MW 205,000; 97,000 and 90,000; 68,000, 64,000, 58,000, 56,000 and 54,000; 52,000; 48,000; 46,000, and 45,000; 42,000, 38,000 and 37,000; 33,000, 32,000, 30,000, 29,000, 28,000 and 27,000; 14,000.

S. japonicum has a total of 42 bands, while *S. mekongi* and *S. mansoni* have 36 and 29 bands, respectively (Table 2; Fig. 13). As revealed under TEM, it is believed that since the basement membrane is still intact after the freeze-thaw, most of glycoproteins shown in the gels are probably derived from the parasite tegument with little contamination of proteins from tissues lying underneath the basement membrane. The number of resolved tegument proteins in 12.5% SDS-PAGE were further characterized by being electrophoretically transferred onto nitrocellulose sheets and then stained with biotinylated lectins (Con A, WGA, RCA I, PNA, SBA and UEA I). The results show that most bands bind to at least two lectins except bands at MW <25,000 which only bind weakly to SBA, and therefore most if not all bands are glycoproteins, and these positive bands are more likely to be glycoproteins than glycolipids because the latter are usually lost very rapidly from the gel due to their fast migration as they have much smaller molecular weight.

Based on the staining intensities, the bindings of lectins to tegumental glycoproteins in all three species of adult schistosomes appear to have four different patterns : Con A uniformly stains most bands in all three species with fairly high intensity, except in the regions between MW 42,000 to 32,000 and 28,000 to 26,000 where there appear to be more bands in *S. japonicum* that are intensely stained by Con A than in other species (Fig. 14 A; Table 2). WGA and UEA show the weak staining of most bands (Fig. 14 B,C; Table 2). RCA I shows moderate staining of most bands, but in *S. japonicum* it shows two regions of very intense staining at MW of 56,000 to 86,000 and 32,000 to 42,000 (Fig. 14 D; Table 2). PNA shows moderate staining of most bands but in *S. japonicum* it shows very intense staining at MW 56,000 to 86,000, while in *S. mekongi* it shows very intense staining at MW 56,000 to 64,000. SBA also stains most band with moderate intensity, but in Oriental schistosomes it also shows two regions of fairly intense staining like RCA at MW 32,000 to 42,000 and 56,000 to 68,000 (Fig. 14F, Table 2). These staining patterns imply that the tegument glycoproteins in all three species contain similar basic sugar residues: ie., D-mannose/D-glucose (Con A), N-acetyl-D-glucosamine/sialic acid (WGA), D-galactose (RCA), D-galactose-N-acetyl-D-galactosamine (PNA & SBA) and L-fucose (UEA), but probably with slightly different quantity as indicated by the varied staining intensity; the most striking of which are in *S. japonicum* which exhibit heavy bindings of RCA I and PNA and SBA to glycoproteins in two regions at MW of 56,000 to 86,000 and 32,000 to 42,000, and in *S. mekongi* which exhibits heavy binding to PNA at MW 56,000 to 64,000 (Fig. 14 E; Table 2). When compared to Oriental schistosomes

S. mansoni shows only weak binding to both RCA and PNA in these regions. These results confirm the surface-labelling studies in TEM which show that the surface membranes of both Oriental schistosomes are more intensely stained by RCA than PNA, and therefore may contain D-galatose in larger amount than D-galactose-N-acetyl-D-galactosamine residues, and that the tegument of *S. japonicum* may also contain larger amount of galactose-bearing glycoproteins than *S. mekongi* and *S. mansoni*.

5.4 Immunogenic glycoproteins in the tegument of adult *Schistosoma japonicum* (Chinese and Philippine strains), *S. mekongi* and *S. mansoni*

The immunogenic glycoproteins in the tegument of Oriental schistosomes were characterized and compared to those of *S. mansoni* by immunoblotting technique in which proteins/glycoproteins in the freeze-thawed tegument were separated in 12.5% SDS-PAGE, electroblotted onto nitrocellulose (NC) papers. Then, NC strips were incubated sequentially in mouse antisera against *S. japonicum* (Chinese strain) - ISCH, against *S. japonicum* (Philippine strain) -ISPH, against *S. mekongi* - ISME, against *S. mansoni* (ISMA), and human antisera from patients infected with *S. japonicum* (Philippine) - IHPH. The immunogenic bands that bind to these antisera were detected with rabbit-antimouse Ig- and goat antihuman Ig-labelled with HRP. To verify which immunogenic bands are glycoproteins, the nitrocellulose strips of electroblotted tegument proteins were also stained with biotinylated lectins (Con A, WGA, RCA I, PNA, SBA and UEA I) and avidin-HRP.

5.4.1 Immunoblotting by mouse antisera

Both strains of *S. japonicum* show almost identical patterns of immunogenic bands against all antisera being used, but with slight difference in staining intensity of some bands. Against ISCH and ISPH there are at least fifteen major common antigens in both strains that show comparable intensity: the most intense bands are at MW 205,000, 116,000, 110,000, 97,000, 68,000, 47,000, 45,000, 28,000, 27,000, 26,000, the moderately intense bands are at MW 86,000, 64,000, 56,000, 54,000 and 38,000 ;while major antigens at 105,000, 76,000, 50,000, 46,000 and 25,000 appear to be more intense in ISPH than ISCH treatment (Table 3; Fig. 15 A,B). In addition there are at least 24 faintly stained minor bands (Table 3). The treatment of *S. japonicum* antigens with ISME shows intense staining of most major bands except at MW 205,000, 28,000, 27,000 and 26,000 to 19,000. On the other hand ISMA reacts weakly with most major bands of *S. japonicum* except at MW 116,000; 105,000; 97,000; 86,000; 54,000; 45,000; 38,000 where the cross reactions are strong ,furthermore it shows particularly strong cross reaction to

MW 76,000, 37,000 and 32,000 which are only faint bands when treated with ISCH and ISPH (Table 3, Fig. 15 C,D). When compared to lectin stainings of freeze-thawed tegument proteins, all immunogenic bands except the lower MW at 27,000, 25,000, 19,000 and 14,000 are labelled with at least two lectins. However, another prominent group of immunogenic bands at MW 97,000, 68,000 and 64,000 are stained intensely by RCA I, PNA, and SBA but not with Con A and WGA; therefore, these glycoproteins may contain especially large amount of galactose residues (Table 5).

In adult *S. mekongi*, when tegument proteins/glycoproteins react with ISME there appear to be at least fifteen major immunogenic bands: the most intense are at MW 97,000; 86,000; 68,000; 64,000 and 43,000, and the moderately intense are at MW 116,000; 105,000; 76,000; 56,000; 52,000; 50,000; 47,000; 45,000; 38,000 and 30,000. Bands 43,000 appears to be particularly intense in *S. mekongi* while it is only a faint band in *S. japonicum* and *S. mansoni* (Table 3; Fig. 15 C). In addition, there are about 21 minor bands which are mostly similar to those of *S. japonicum* (Table 3). When reacted against ISCH and ISPH the immunogenic bands of adult *S. mekongi* show similar pattern to those of *S. japonicum*, except band 43,000 which is particularly intense when treated with ISPH (Table 3, Fig. 15 A,B). ISMA also reacts with most major immunogenic bands of *S. mekongi* except bands 68,000; 64,000; 56,000, 52,000; 50,000 and 47,000 which become very weak (Table 3, Fig. 15 D). When compared to lectin stainings, all immunogenic bands of *S. mekongi* are labelled by at least two lectins except the major band at 47,000 which binds to only one lectin (PNA). And like *S. japonicum*, a group of major antigens at 97,000; 68,000; 64,000; 52,000 and 47,000 are stained by RCA I, PNA, SBA and UEA I but not Con A and WGA, eventhough the staining is not as intense as in *S. japonicum* (Table 7). Therefore, it can be concluded that all tegument antigens of *S. mekongi* are glycoproteins, and that the glycoproteins at MW 98,000, 68,000, 64,000, 52,000, 47,000 may contain especially large amount of D-galactose, D-galactose-N-acetyl-D-galactosamine, N-acetyl-D-galactosamine and L-fucose residues.

In *S. mansoni* the treatment of tegument proteins/glycoproteins with ISMA reveals seventeen immunogenic bands: the most intense are at MW 76,000, 68,000; 56,000; 45,000; 37,000; and the moderately intense bands are at MW 105,000; 97,000; 80,000; 64,000; 52,000; 47,000; 38,000; 34,000; 32,000; 30,000; 28,000 and 27,000. The bands at MW37,000; 34,000 and 32,000 appear to be particularly intense in *S. mansoni*, while they are either absent or very faint in

S. japonicum and *S. mekongi*. In addition, bands at MW 28,000 and 27,000 also appear intense though not as much as in *S. japonicum* while they are absent in *S. mekongi* (Table 3, Fig. 15 D). ISCH and ISPH react with most major immunogenic bands of *S. mansoni* but usually with lower intensity than corresponding bands in *S. japonicum* ; however, a band at MW 56,000 exhibits higher intensity than in *S. japonicum* (Table 3, Fig. 15 A,B). ISME reacts only nine major immunogenic bands while the remaining appear very faint (Fig. 15 C, Table 3). Despite a slight shift in molecular weights, most of the major immunogenic bands in *S. mansoni* appear to correspond to those reported by Hayunga et al. (1979 a, 1979 b), who used immuno-precipitation to reveal surface antigens at MW 110,000, 78,000, 68,000, 60,000, 43,000, 36,000, 30,000, 26,000 and 12,000. When compared with lectin-binding patterns, almost all immunogenic bands of *S. mansoni* are also labelled by at least two lectins. Like *S. japonicum* and *S. mekongi* , bands at MW 97,000, 86,000, 76,000, 68,000, 56,000 and 47,000, 45,000 are stained by RCA I, PNA, SBA, UEA I but not Con A and WGA; however, the staining is weaker than in Oriental schistosomes. Even the band at 68,000 which shows the most intense staining with RCA I and PNA is rather weak when compared to the same band in Oriental Schistosomes (Table 8).

5.4.2 Immunoblotting by human antisera

In this experiment both pooled and individual antisera from patients infected with *S. japonicum* (Philippine) were used. Immunoblotting patterns of tegument antigens of both Chinese and Philippine strains of *S. japonicum* vary slightly from one antiserum to another (Fig. 16 A to E). However, when results from all individual antiserum are compiled together ($IHPH_2$) and when the pooled antiserum ($IHPH_1$) was used to detect antigens (Table 4), positive bands correspond in range and molecular weights with those detected by mouse antisera already presented in Table 3. Both strains of *S. japonicum* show similar immunoblot pattern against $IHPH_1$ and individual antisera; when all results are compiled together there appears to be 22 bands that are consistently detected at MW 180,000; 150,000; 116,000; 97,000; 68,000; 64,000; 62,000; 58,000; 54,000; 52,000; 50,000; 48,000; 47,000; 45,000; 43,000; 39,000; 38,000; 28,000; 27,000; 25,000; 23,000 and 19,000. Among these the most prominent bands are 116,000; 97,000; 68,000; 64,000; 62,000; 50,000 and 45,000; 28,000 and 27,000 (Table 4, Fig. 16 A to F). Fewer *S. mekongi* antigens are detected by IHPH and the prominent ones are at MW 116,000; 97,000; 68,000; 64,000

62,000; 48,000; 45,000; 38,000. *S. mansoni* generally shows more positive bands against IHPH than *S. mekongi* but not as many as those of *S. japonicum* ; and the major bands appear at MW 97,000; 68,000; 64,000; 52,000; 48,000; 39,000; 38,000, 37,000 and 27,000 (Fig. 16 A to F, Table 4).

5.5 Tegument antigens in adult *Schistosoma japonicum* (Chinese and Philippine strains) in comparison to *S. mekongi* and *S. mansoni* as detected by the labelling surface with ^{125}I and by immunoprecipitation

5.5.1 Identification of surface proteins from adult *S. japonicum* (Chinese & Philippine), *S. mekongi* and *S. mansoni* by labelling with ^{125}I

Proteins and glycoproteins that are located on the surface of the outer plasma membrane were identified by labelling with ^{125}I using iodogen method (Fraker & Speck, 1978). It was found that the optimal labelling condition was using about 2 uCi of Na ^{125}I per worm for 5 minutes; hence this condition was used throughout the study. EM autoradiographic studies of ^{125}I labelled adult worms revealed that over 95% of the silver grains appear on the plasma membrane covering the ridges and the pits of the tegument (Fig. 17). Hence it is quite certain that most of labelled molecules displayed in gel electrophoreses are from the outer plasma membrane. In subsequent experiments, following the labelling of adult male schistosomes with ^{125}I, the tegument was extracted with 0.2% Triton X-100 for 1 hour at 4°C. The extracted surface components were solubilized and run in 12.5% SDS-PAGE and autoradiograms developed. It was found that for *S. japonicum* (Chinese & Philippine), there are 5 major and 13 minor bands of proteins being labelled, ranging in molecular weight from 14,000 to 205,000 daltons. The heaviest labels are at the regions of MW 205,000, 180,000, 160,000, 128,000 and 14,000 daltons (Tables 9, 10; Fig. 18 A to D).

For *S. mekongi* there are 6 major bands and 10 minor bands: the heavy labellings were at MW 185,000; 155,000; 94,000; 86,000 and 43,000 and 14,000 daltons (Table 11, Fig. 19 A). For *S. mansoni* the ^{125}I labelled surface proteins consist of 15 bands: 7 major bands at MW 64,000; 58,000; 43,000; 30,000; 27,000; 19,000; 14,000 and 8 minor bands (Table 12, Fig. 19 B,C).

5.5.2 Immunoprecipitation of surface antigens from adult *S. japonicum* (Chinese & Philippine)

The pattern of immunoprecipitation of surface antigens by mouse immune sera against *S. japonicum* (Chinese & Philippine - ISCH & ISPH), *S. mekongi* (ISME) *S. haematobium* (ISHE), *S. mansoni* (ISMA) and human immune serum against *S. japonicum* (Philippine - IHPH) are similar in both strains. Eleven major bands precipitated by ISCH, ISPH and IHPH are at MW 205,000; 180,000; 160,000; 128,000; 45,000; 38,000; 34,000; 30,000; 26,000; 23,000 and 14,000; and five minor

bands at MW 116,000; 86,000; 68,000; 62,000; and 19,000 (Tables 9 & 10; Fig. 18 A to D). Two minor bands at MW 56,000 and 48,000 are not precipitated even though they are revealed in immunoblotting (Table 3). The most intense band that stands out in contrast to others is 128,000, and it is not precipitated by ISME, ISHE and ISMA. However, in immunoblotting (Table 3, Fig. 15) this band is present only in *S. japonicum,* eventhough it is faint; therefore, this band may be unique and specific antigen in *S. japonicum.* In addition, band 86,000, though appears as a minor band when reacted with ISCH and ISPH, is heavily precipitated by ISME and ISHE. The remaining bands of *S. japonicum* antigens show light to moderate cross-reactions with ISME, ISHE and ISMA. However, band 14,000 is probably non-specifically precipitated, since it is always heavily precipitated by all antisera as well as non immune serum (Tables 9, 10; Fig. 18 A to D).

Immunoblotting of *S. japonicum* antigens (Table 3, Fig. 15 A to D) reveals more bands than immunoprecipitation, which is not surprising, since the former detects all antigens from the tegument, whereas the latter detects antigens that are exposed on the external surface of the outer membrane, particularly those that are specifically labelled with ^{125}I which has affinity for tyrosine residues. The major and minor bands as defined by the two techniques may not necessary coincide : the only antigens shown to be major bands by the two methods are at MW 205,000; 45,000 and 38,000, while major bands in immunoprecipitation at MW 180,000; 160,000; 128,000; 34,000 and 30,000 appear as minor bands in immunoblotting and vice versa for minor bands at MW 116,000; 86,000; 68,000 and 26,000. Furthermore, some bands at MW 97,000; 64,000; 56,000; 47,000; 28,000 and 27,000 which are not detected by immunoprecipitation are actually shown to be major antigens in immunoblotting. This discrepancy may be attributed to different systems of detection by ^{125}I labelling versus immunoenzyme labelling and the accessibility of antigens to the labelling. By checking that the bands appear in both methods with at least moderate (plus two) and faint staining (plus one) (see Table 3), it is probably save to conclude that there are fourteen major antigens of *S. japonicum* that appear in both methods of detection at MW 205,000; 180,000; 160,000; 128,000; 116,000; 86,000; 68,000; 45,000; 38,000; 34,000; 30,000; 26,000; 23,000; and 19,000. Most of these surface antigens are probably exposed on the external surface of the outer membrane. Major tegument antigens that appear only in immunoblotting are at MW 110,000; 97,000; 64,000; 56,000; 47,000; 28,000; 27,000 and 25,000. Most bands show weak to moderate cross reactions with immune sera against ISME and ISMA except band 128,000 which is the only antigen that does not cross-react with other antisera, and therefore may be specific to *S. japonicum.*

Band 14,000 may be non specific small lipoprotein or lipid-rich fraction that are always present on the surface membrane of all schistosoma species, as it is extracted away by lipid solvent (Section 5.6) and is barely discernible in immunoblotting.

5.5.3 Immunoprecipitation of surface antigens from adult *S. mekongi* (Table 11; Fig. 19 A).

The labelled surface antigens of *S. mekongi* that are heavily precipitated by ISME are at MW 185,000; 155,000; 94,000; 86,000; 68,000 and 14,000, while those with moderate precipitation are at MW 54,000; 43,000; 32,000; 26,000 and those that are lightly precipitated are 64,000; 39,000 and 35,000. Most bands except 43,000; 39,000 and 35,000 show moderate to weak cross reactions with ISCH, ISPH and ISMA. Interestingly, the pattern of immunoprecipitate by ISHE is quite similar to that of ISME, thus implying strong cross reactions between surface antigens of the two species, particularly at MW 94,000; 86,000 and 68,000. Band 14,000, like in other schistosoma, is non specific since it is precipitated by all immune and non-immune sera.

In immunoblotting (Table 3, Fig. 15) more immunogenic bands, including those appearing in immunoprecipitation, are detected, perhaps for the same reasons as mentioned in *S. joponicum*. By using similar criterion as in *S.japonicum* (Table 3), it can be concluded that there are ten major bands that appear in both immunoprecipitation and immunoblotting at MW 185,000; 155,000; 94,000; 86,000; 68,000; 64,000; 54,000; 43,000; 32,000; 26,000 and these antigens are probably exposed on the external surface of the outer membrane. In addition, other major tegument antigens that appear only in immunoblotting are at MW 116,000; 105,000; 76,000; 56,000; 52,000; 50,000; 47,000; 45,000; 38,000 and 30,000. All major antigens except band 43,000 show weak to moderate cross reaction with ISCH, ISPH and ISMA.

5.5.4 Immunoprecipitation of surface antigens from *S. mansoni*

Fourteen bands of ^{125}I labelled surface proteins/glycoproteins are precipitated by mouse immune serum against *S. mansoni* (ISMA), with heaviest precipitation at MW 30,000; 27,000 and 14,000 and moderate precipitation at MW 205,000; 160,000; 105,000; 64,000; 58,000; 43,000 and 19,000. There are also four faint bands at MW 80,000; 37,000; 34,000 and 23,000 (Table 12; Fig. 19 C, D). ISCH, ISPH, ISME and ISHE all show cross-reactions with most bands except at 80,000; 37,000, and particularly strong cross-reaction is detected at band 30,000 and 27,000. As in other schistosoma band 14,000 is non-specific since it can be precipitated by all immune sera as well as non-immune serum.

Immunoblotting (Table 3, Fig. 15) can detect more tegument antigens, including those that appear in immunoprecipitation. Based on the same criterion as in *S. japonicum*, there are 11 major bands that appear in both methods at MW 205,000; 160,000; 105,000; 64,000; 58,000; 43,000; 37,000; 34,000; 32,000; 30,000 and 27,000; these antigens are probably exposed on the surface membrane. Furthermore, there are tegument antigens that appear only in immunoblotting at MW 105,000; 97,000; 86,000; 76,000; 68,000; 56,000; 52,000; 47,000; 45,000; 38,000; 28,000; 27,000; and 26,000. Most bands of antigens except at 37,000 show weak to moderate cross reaction with all immune sera being used. Band 43,000 shows strong cross-reaction only with ISME, while band 28,000; 27,000 and 26,000 show strong cross-reaction with ISCH and ISPH.

5.6 Identification of lipid components on the surface of *S. japonicum*

Extraction of the tegument using hexane-isopropanol (3:2, V/V) reproducibly recovered majority of the radioactively labelled material in the solvent. The remaining precipitate, presumably proteins, represented about 16% of the total radioactivity (Table 13), indicating that most of the ^{125}I was incorporated into the lipid components of the *S. japonicum* tegument. After removal of the isopropanol by evaporation, the hexane containing lipid fraction was further extracted by aqueous system to separate polar lipids (27.9%) from non-polar lipids (4.5%).

The presence of large proportion of lipid component in *S. japonicum* tegument was also supported by indirect evidence obtained from SDS-polyacrylamide electrophoresis. It was found that, of total radioactively labelled tegument applied onto the gel, only 17% was precipitated on the gel after the electrophoresis and staining-destaining in acetic acid-methanol solution. The amount of macromolecule remained on the gel was similar to that found in the protein precipitate during lipid extraction which is about 16.1% (Table 13).

Both polar and non-polar lipid fractions were analyzed on thin-layer chromatography of Silica Gel G (F 1500/LS254, 100 um thick) for 2 hours using butanol-acetic acid-water (3:1:1). The TLC plate was dried and the radioactivity detected by autoradiography. Major components in both fractions were indistinguishable under experimental condition (Figure 20). However, the aqueous fraction (Figure 20, lane 1) appeared to contain some components not observed in the hexane (Figure 20, lane 2). Modification of the chromatographic solvent system is evidently required to obtain better resolution as well as identification of the extracted lipids in comparison with standard lipids, this will provide useful information for future studies.

Antigenic property of the extracted lipids was also preliminarily investigated. Due to material availability, the polar lipids (aqueous fraction) was immunoprecipitated. Starting from 7.2 x 10^4 cpm of the polar lipids, about 74% (5.3 x 10^4 cpm) was recovered following preabsorption by normal mouse serum and protein A-Sepharose. Following precipitation by *S. japonicum* mouse immune serum and successive washes, 205 cpm from 5.3 x 10^4 cpm (0.4%) was found in the final precipitate. Whether this amount significantly represented the specific immunoprecipitable lipid(s) of *S. japonicum* tegument should be investigated further.

5.7 Detection of schistosomula antigens

5.7.1 Immunoblotting of whole body proteins from cercariae and 6 hour-schistosomula of *S. japonicum* (Chinese) and *S. mansoni* (Table 14; Fig. 21).

Cercaria and mechanically-transformed schistosomula were collected and the latter were incubated in MEM medium for 6 hours before use. Subsequently, both types of specimens were solubilized in SDS-sample buffer, and their protein and glycoprotein components were separated in 12.5% SDS-PAGE, electroblotted onto nitrocellulose (NC) sheets and treated with ISCH, ISPH, ISME and ISMA, and immunogenic bands detected as mentioned in adult. In general, the immunoblot patterns of schistosomula and cercariae are similar except there are fewer and less intense immunogenic bands in the latter. In *S. japonicum* schistosomula treated with ISCH and ISPH there are eight major bands at MW 205,000; 97,000; 76,000; 68,000; 64,000; 58,000; 45,000; 39,000 and nineteen minor bands at MW 128,000; 120,000; 116,000; 105,000; 86,000; 62,000; 56,000; 52,000; 50,000; 48,000; 47,000; 46,000; 39,000; 34,000; 32,000; 30,000; 28,000; 26,000 and 25,000. Cercariae show fewer immunogenic band which are usually confined to the range between 97,000 to 25,000. In comparison to adult there are very few bands (except band 205,000) at MW higher than 97,000, whilst between bands 97,000 and 58,000 the immunogenic bands are as intense as those in adult. In cercariae and schistosomula of *S. mansoni* the major bands are also confined to the middle range at MW 68,000 to 58,000. Below and above this range the positive bands are fewer, and when present are much weaker than those in adult.

5.7.2 Immunoprecipitation of antigens from 6-hour schistosomula of *S. japonicum* (Chinese) (Table 14; Fig. 22).

Mechanically-transformed schistosomula were collected and kept in MEM medium for 6 hours to complete its heptalaminate membrane replacement. Then they were labelled with ^{125}I using iodogen technique; after which they were

solubilized in sample buffer and immunoprecipitated by ISCH as mentioned in adult. It was found that the number of precipitated bands are fewer than those of adult parasites, and those that appear have similar molecular weight. There are only six major bands at MW 68,000; 62,000; 46,000; 45,000; 26,000; 25,000 and six minor bands at MW 160,000; 128,000; 97,000; 86,000; 38,000; 34,000. The band at 14,000, eventhough appears intense as in adult, is probably not specific for the same reason as mentioned previously.

On comparing the results of immunoblotting and immunoprecipitation, using the same criterion as applied previously, it can be concluded that the major schistosomula antigens are at MW 205,000; 97,000; 76,000; 68,000; 64,000; 62,000; 58,000; 46,000; 45,000; 26,000 and 25,000, which are confined mostly between the middle range to lower molecular weights.

5.8 Localization of surface antigens

Attempts have been made to localize Oriental schistosomes' antigens by using naturally infected mouse antisera against *S. japonicum* and *S. mekongi* by immunofluorescence and immunoelectron microscopic methods.

Indirect Immunofluorescence

Cercariae, schistosomula, and adult parasites of both species were collected and prepared as described previously. After the collections, specimens were fixed as soon as possible in 2% paraformaldehyde in 0.1 M PBS pH 7.4 for overnight at 4°C, then washed thoroughly with PBS. The fixed cercariae and schistosomula were studied in whole mount, while the adult parasites were cut into 4 to 6 um thick sections in a cryostat. These frozen sections were picked up on gelatin coated slides and air-dried for 30 minutes. The sections were washed with PBS before incubated in 1% BSA in 0.1 M PBS for two hours to prevent non-specific adsorption and then followed with mouse antisera against *S. japonicum* (Chinese strain - ISCH), or against *S. mekongi* (ISME) for overnight at 4°C. After washing with PBS, the sections were incubated in secondary antibody, sheep anti-mouse IgG conjugated to FITC, for one hour at room temperature. Finally, the sections were washed with PBS, mounted in buffered glycerol and then examined in a fluorescence microscope with incidence illumination. The controls were carried out simultaneously by replacing the mouse antisera with normal mouse sera.

Cercariae and schistosomula

Control cercariae and schistosomula treated with normal mouse sera do not show any autofluorescence (Fig. 23 A,B). When treated with immune sera, cercariae of both species show fluorescence on all the surface of the head, the body, and the tail; however, fluorescence on the dorsal surface of the head and

the body, which appears convex, is stronger than on the ventral surface, which will become the gynaecophoral canal in adult parasites. The fluorescence over the proximal part of the tail also appears stronger than the distal part and the furculae (Fig. 23 C, E). In schistosomula of both species, the fluorescence appears over the whole surface, but is relatively stronger over the postero-dorsal aspect (Fig. 23 D, F).

Adult *S. japonicum* and *S. mekongi*

In control adult specimens, the autofluorescence is strong in the gut lumen and possibly also the gut epithelium. The tegument and muscle layers do not fluoresce but the interior of the parasite bodies show small weak fluorescent spots that scatter all over (Fig. 24 A,B). In adult male *S. japonicum* (Chinese strain) treated with ISCH, apart from the autofluorescence gut, strong fluorescence due to specific binding of antibodies is present over the whole thickness of the tegument of the dorsal and lateral aspects, but only weak fluorescence is observed on the tegument of the gynaecophoral canal (Fig. 24 C,D). At high magnification, the whole width of the tegument from the ridges down to the level of the basement membrane shows strong fluorescence, while the muscle layer is completely black. However, in some areas underneath the muscle layer a row of fluorescent tegument cells can also be observed. Each cell has fluorescent cytoplasm surrounding a completely black nucleus (Fig. 24D). In adult male, *S. mekongi* treated with ISME, the pattern of fluorescence is similar to that of *S. japonicum;* however, in and around bubble-like structure which may be pleomorphic papillae the fluorescence appears weaker than the rest of the tegument (Fig. 24 E,F).

Adult *S. mansoni*

When sections of *S. mansoni* were treated with ISMA, very intense fluorescence occurs over the outer surface and the entire thickness of the tegument (Fig. 25 C). Specially bright fluorescence also appears within the elevations which may be the spine-bearing tubercles, whilst the spines themselves also show very bright fluorescence (Fig. 25 D). The interior of the parasites' bodies show uniform weak fluorescence (Fig. 25 C, D) in contrast to that of the control specimens in which autofluorescence appears only over the gut (Fig. 25 A, B).

Cross reaction

Both ISCH and ISMA show strong cross reaction when tested against parasites of opposite species. When *S. japonicum* (Chinese) is treated with ISMA strong fluorescence appears over the entire width of the tegument and the thin

streaks between muscle cells (Fig. 26 A,B), while the interior of the parasites' bodies show even but weak fluorescence. In *S. mansoni* treated with ISCH strong fluorescence also appears over the surface, the tegument, and to some extent on the tubercles and spines which is some what weaker in comparison to the treatment with ISMA. The interior of the parasites' bodies also shows weak but even fluorescence throughout (Fig. 26 C, D). Therefore, it can be concluded that antisera of Oriental schistosomes and *S. mansoni* have fairly strong cross reaction which supports the results obtained in immunoblotting (Section 5.4).

Immunoelectron microscopy

Cercariae, schistosomula and adults of both species were briefly fixed with 2.5% glutaraldehyde in 0.5 M cacodylate buffer at 4°C for 10 minutes. They were then rinsed three times with the buffer and incubated in 0.1% $NaBH_4$ for 30 minutes to block free aldehyde groups. The specimens were pre-incubated in normal mouse serum and then in ISCH or ISME overnight. Subsequently, they were washed thoroughly and incubated further in second antibody (rabbit anti-mouse Ig) labelled with horseradish peroxidase enzyme (HRP). The antibody binding sites on the tegument were demonstrated by incubation with DAB and H_2O_2 for 10 minutes, which resulted in the formation of dark precipitate. The control specimens were incubated sequentially in normal mouse serum and HRP before immersing in the substrates. Finally, specimens were prepared for TEM by conventional method and viewed without electron staining.

All control sections from cercariae, schistosomula and adult do not exhibit any black reaction products on the tegument surface (Fig. 27 A). By contrast, in experimental samples the black reaction product deposits on the whole thickness of cercaria's glycocalyx, while the cercaria's membrane is lightly stained (Fig. 27 B). In schistosomula of both species, the reaction product deposits on the remaining glycocalyx around blebs and microvilli, and the surface membrane itself is intensely stained (Fig. 27 C, D). Membranous bodies and intracytoplasmic sheets are not stained, probably because primary antibodies cannot penetrate into the interior of the tegument. The staining of the adult surface membrane of both species is similar : it is intense on the membrane covering the ridges and decreases gradually towards the pits. The membrane of the bottom half of the pits is usually unstained (Fig. 27 E,F). This pattern is quite similar to lectin stains, especially that of Con A and WGA (see section 5-2).

Therefore, in this study it is demonstrated that antisera from natural infection with both species of Oriental schistosomes contain principally antibodies

against antigens residing in cercarial glycocalyx, and the surface membrane of schistosomula and adult tegument, and possibly also in the tegument cell as shown by immunofluorescence study. At TEM level, the binding of antibodies in both schistosomula and adults appears only on the surface membrane, and no reaction product is detected within the tegument cytoplasm or tegumental bodies, but this could be due to the limited penetration of primary and secondary antibodies in the pre-embedding technique. Therefore, the post-embedding technique should also be tried to verify the immunofluorescence study whether membrane antigens are already present in Mb granules, or they become "completed" only at the surface membrane level. The similarity between the binding of lectins and the binding of natural antibodies to the surface of the tegument in both schistosomula and adults suggests that the receptor sites for these two groups of ligands may be the same. This together with evidence from immunoblotting strongly suggest that the lectin receptor sites are antigenic glycoproteins which constitute major portion of cercarial glycocalyx and the surface membrane of schistosomula and adult parasites.

In Oriental schistosomes, it is possible to correlate the results from TEM and immunoblotting studies. The surface labelling with various lectins reveals that the terminal sugar residues, i.e., D-galactose, N-acetyl-D-galactosmine, L-fucose (detected by RCA I, PNA, SBA and UEA I) are confined mainly on the upper half of ridges membrane, and the immunoblotting shows that the most immunogenic glycoprotein bands, at MW 98,000, 68,000, which also contain especially large amount of these sugar residues, are the major antigens of adult parasites. It is, therefore, plausible to suggest that these major antigens may be confined to the upper half of ridges, while the antigens that contain internal sugar residues, as revealed by Con A and WGA bindings, may be spread out all over the ridge and the upper half of pits. These major antigens may be continuously released from the adult Oriental schistosomes' membrane, so that antibody formation in hosts against them are more pronounced than other surface glycoproteins.

5.9 The release of surface membrane proteins in adult *S. japonicum* (Chinese) *in vitro*

5.9.1 Detection of released proteins by ^{125}I labelling

In order to verify and estimate the rate of release of membrane proteins and glycoproteins from the parasites, 100 adult males *S. japonicum* were labelled with 100 uCi of Na ^{125}I for 5 minutes using iodogen technique. Following the labelling, worms were transferred to 4 ml of MEM solution, periodically flushed with air and kept at 37°C. Samples of the culture medium (0.5 ml each) were taken for radio-activity counting at 5, 10, 30 minutes, 1, 3, 6, 12 and 24 hours. Finally, the worms were briefly washed and radioactivity remaining on

their surface were counted. Total radioactivity released at each interval was calculated from the samples, and percentages of release were calculated against the total radioactivity (= radioactivities in all samples + radioactivity remaining on the worms). The experiment was repeated thrice. The kenetics of radioactivity release from the parasites was biphasic, that is, about half of the radioactivity was released within 3 hours; thereafter, the release was very slow (Fig. 28 A). The electrophoretic patterns of concentrated culture medium from various time intervals reveal that the radioactivities reside in the bands corresponding to the ^{125}I labelled surface molecules as obtained from the extraction of the tegument immediately following the radio-labelling. Hence, there is very little break down of the released molecules in the solution even after 24 hours of incubation (Table 15 A; Fig. 28 B,C). Interestingly, the band that appears in the medium first is 14,000, and second are bands 160,000, 128,000, 68,000 and 30,000. All these bands, except 68,000, are major surface proteins that are revealed by ^{125}I labelling. On the other hand, band 68,000, though not labelled readily by ^{125}I, could be a membrane protein of substantial quantity as it is prominent in immunoblotting.

5.9.2 Detection of released antigens from adult male *S. japonicum* (Chinese) incubated *in vitro* in MEM by immunoblotting

Approximately 500 adult male *S. japonicum* (Chinese) worms, freshly perfused from mice, were incubated in 1 ml of Minimal Essential Medium (MEM) at 37°C. The incubating medium was collected and 1 ml new MEM medium was added at 0, 1, 3, 9 and 20 hours. Each collection was concentrated by using aquacide 11, and protein fractions separated in 12.5% SDS-PAGE system using 100 V for 4 hours. After electrophoresis, the gel was electroblotted onto nitrocellulose paper (NCP) for 1 hour at 0.5 A. The NCP was soaked in 1% BSA for 1 hour at room temperature, and then reacted with *S. japonicum* (Chinese) infected mouse serum-ISCH (1:50 dilution) for overnight at 4°C. The immune complexes on NCP were detected by reacting with peroxidase-conjugated rabbit anti-mouse antibody (1:200 dilution) for 2 hours at room temperature and further incubated in 0.3 mg/ml DAB + 0.01% H_2O_2 for 10 minutes at room temperature to visualize the brown colored bands on the NCP strips.

Tegument antigens that are released during the course of 20 hours are shown in Table 15 B, Figure 29. The major bands that are released early and in large quantity throughout the incubation period are at MW 205,000; 68,000 and 66,000. In addition other bands at MW 105,000; 97,000; 86,000; 76,000; 54,000; 50,000; 45,000; 38,000; 30,000 are also continuously released in small amount. Later, bands with small molecular weight at 26,000 is also released in small quantity.

From these two experiments it is suggestive that tegument antigens, particularly those on the surface membrane are continuously released from the adult parasites. It is possible that a similar situation may occur *in vivo* , so that these released antigens can continuously elicit the production of antibodies in hosts.

5.10 Production and testing of monoclonal antibodies (MAB) against tegument antigens of *S. japonicum*

5.10.1 Detection of MAB reactivities against tegument proteins by immunoblotting

There are 37 clones of hybridoma cells that have been tested to be positive by ELIZA assay. These clones were propagated further to obtain sizable volume of culture fluid which have been used to test the reaction of MAB against tegument antigens by immunoblotting, and for determining the antibody class and subisotype by ELIZA assay using Hybridoma Subisotyping Kit for mouse monoclonal antibodies (CALBIOCHEM)

For immunoblotting, freeze-thawed adult *S. japonicum* (Chinese) tegument was used as immobilized antigens that were separated by SDS-PAGE and electroblotted onto nitrocellulose (NC) strips. The screening was performed by incubating NC strips in culture fluid obtained from various clones. The positive MAB was detected by HRP-conjugated rabbit anti-mouse Ig. Based on the reactivities with tegument antigens, the positive clones can be divided into 4 groups (Table 16, Figs. 30 & 31). Group 1 are clones with code names of 6C4-1, 6C4-2, 6C4-4, 6C7-10, 6F11-1, 8D6-4, 8G11-10 and 9C3, which produce MAB that react against 3 regular bands of antigens at MW 62,000; 56,000; 52,000 and sporadic bands at 116,000; 105,000; 97,000; 86,000; 47,000; 43,000; 37,000 and 34,000. The second group are clones 2B4, 2D4, 8F2, 8F5, 8G3-3, 8G3-4, 8G9-5, 8G11-2 which consistently produce MAB against band 54,000. Clones 2B4, 2D4, 8F2 produce only a single MAB against band 54,000, while other clones also produce MAB against other sporadic bands at 116,000; 105,000; 97,000; 64,000; 50,000; 47,000; 45,000 and 30,000. It is possible that the clones which contain more than one MAB may have more than one cell line, or another possibility is that a single MAB reacts with a common epitope that may be present on different bands of tegument antigens. The third group consists of clone 9E7 which produce a single MAB against the antigen at 54,000, and other clones, 2G4-4, 8C9, 8D6-1, 8E2-1, 8G9-6 which produce MAB against band 54,000 as well as other sporadic bands at MW97,000; 86,000; 80,000; 68,000; 50,000; 45,000 and 43,000. The fourth group are clones 8D6, 8D6-2, 8D6-3, 8D6-7 which produce MAB against 3 bands at MW 47,000, 43,000 and 34,000. Based on the single MAB against a single antigen at 54,000

clones 2B4, 2D4, 8F2 and 9E7 have been given priority in selection for further propagation. These clones are also quite stable and the MAB is IgG 2a. In addition to band 54,000 other bands of tegument antigens that can elicit MAB production in more than half of the positive clones are band 97,000 and 47,000. Clones 2B4, 2D4, 8F2 and 9E7 will be propagated in large quantity so that their MAB could be used further in devising a method for immunodiagnosis.

5.10.2 Immunofluorescence assay of MAB

Adult parasites Culture fluid from MAB clones which show positive immunoblotting were tested for MAB bindings to frozen sections of adult *S. japonicum* and *S. mansoni* (Figs. 32, 33, 34, 35) and to whole mount schistosomula of *S. japonicum* (Figs. 36,37).

In adult parasites, the negative control sections which were stained with normal mouse serum in place of primary antibody, autofluorescence occurs only in the lumen and to a lesser degree in the gut epithelium. Small fluorescent spots are also scatterred throughout the interior of the parasite's body, which may represent the neurochemicals in nerve processes (Fig. 32 A,B). In positive control section, in which the primary antibody is mouse antiserum against *S. japonicum*, very intense fluorescence occurs over the whole thickness of the tegument, whilst the interior of the parasites' bodies is only lightly fluoresce and the muscle layer totally lacks fluorescence (Fig. 32 C, D).

When culture fluid containing MAB from various clones (as shown in Table 16) were tested using frozen sections of adult *S. japonicum* (Chinese), it was found that there are 3 patterns of fluorescence stainings. The first is represented by MAB from clones 8F2 and 8F5, which produce MAB against a single tegument antigen at MW 54,000 : fairly intense fluorescence occurs over the whole width of the tegument, and a row of large bright spots underneath the muscle layer that may represent the tegument cells. The muscle layer itself does not fluoresce (Fig. 33). The second pattern is represented by clone 8G9-5 which produces more than one MAB against multiple tegument antigens (Table 16). In this group the fluorescence also occurs over the whole thickness of the tegument, and in most regions there are fine fluoresce granules dispersed throughout. The fluorescence also appears intense over the tegument cell cytoplasm that surround a dark round nucleus (Fig. 34 A,B). The third pattern is represented by clone 9E7 which also produces MAB against a single antigen at 54,000. MAB from this clone appears to bind to the periphery and the outer part of the tegument, and in some areas the fluorescence may be brighter than other (Fig. 34 C, D). The fourth pattern is represented by clone 8G11-2 which also produces MAB against multiple antigens. The

fluorescence appears patchy on the tegument of *S. japonicum* (Fig. 35 A,B), and when it is tested against *S. mansoni* the fluorescence is particularly bright over areas that appear to be tubercles with spines (Fig. 35 C, D).

In conclusion, it is apparent that most of MAB produced are addressed against tegument structure. MAB from clones 9E7, may bind preferentially to the surface membrane which covers the outer part of the tegument, while MAB from clones 8F2, 8F5 and 8G95 may also bind to the intrategument structures which could be tegument granules that are present in abundance, and thus giving a granulated form of fluorescence over the whole width of the tegument. In addition these MAB also bind to antigens inside the tegument cell cytoplasm, which indicates that antigens may already be imparted with their haptenic groups even at the early stage of synthesis within the cell. Clone 8G11-2 may contain MAB that binds to the spines in addition to the general structure of the tegument since the spine-bearing tubercles are especially bright in *S. mansoni* treated with this MAB.

Schistosomula MAB of some of the positive clones were also tested against whole mount living schistosomula (Figs. 36, 37). In negative control specimens, fluorescence is very faint (Fig. 36 A, B), except on area which may have autofluorescence like histiolytic gland in the head and the gut content (Fig. 37 C, D). In positive control specimens treated with ISCH strong and even fluorescence appears over the whole surface of schistosomula, and their edges appear very bright especially towards the anterior part (Fig. 36 C). MAB from clones 8F2 binds strongly to the surface of schistosomula (Fig. 36 D), however the fluorescence is not even as in the positive control specimens. MAB from 9E also shows strong binding over the whole surface of schistosomula and tend to show stronger and more even fluorescence (Fig. 37 A, B). MAB from clone 8G9-5 shows more even but weaker fluorescence (Fig. 37 C, D). By and large, it could be concluded that most MAB produced against adult tegument antigens also bind strongly to the surface of schistosomula.

6. CONCLUSION AND SUMMARY

The following conclusions have been reached from our studies.

1. The surface membrane of Oriental schistosomes has abundant negatively-charged groups that probably reside on carbohydrate constituents of the membrane as revealed by colloidal iron, ruthenium red stainings, and lectin bindings. Cercariae of both species show the strongest binding to positive colloidal iron and ruthenium red, and both stains exhibit strong intensity on the whole thickness of glycocalyx. After transformation, the intensity of stain is significantly decreased and remains only on blebs, microvilli and membrane

puffs, the structures that are responsible for the shedding of the cercarial trilaminate membrane. The surface membrane of cercariae and schistosomula are stained by ruthenium red but not by positive colloidal iron, perhaps due to different degree of penetration of the dye molecules through the layer of glycocalyx. In adult of both species, the staining of positive colloidal iron and ruthenium red is evenly distributed over the entire dorsal and lateral aspects of the surface of the parasites' bodies, while the surface of the gynaecophoral canal exhibits very little or no staining. On dorsal and lateral aspects, both stains are most intense on the membrane of ridges, and the intensity decreases towards the pits, where the neck region is stained but the bottom part is not. By contrast, there is no staining on the surface membrane and glycocalyx of cercariae and schistosomula and adults by the negative colloidal iron, which indicates the lack of positively-charged groups on the tegument. These results suggest that the glycocalyx and the surface membrane of cercariae, and early schistosomula are highly-negatively charged, and when the original cercarial glycocalyx is completely shed, the surface of late schistosomula and adult membrane still retains a fairly high but constant amount of negative charges.

2. When using lectin stains, the cercariae of both species bind concanavalin A (Con A) and wheat germ agglutinin (WGA) very intensely throughout the whole thickness of the glycocalyx and on the surface membrane. In schistosomula of both species, the bindings of these lectins to glycocalyx are significantly decreased and remain only around blebs, microvilli and membrane puffs, while the surface membrane is intensely stained. In adults of both species, the bindings of lectins occur only on the surface membrane. Based on the staining intensity and the minimal concentration of each lectin that must be used to render positive staining, the abundance of binding sites for each lectin can be semi-quantitatively estimated. The binding sites for Con A, WGA and RCA I are present in relative large amount, in comparison to those of PNA, SBA and UEA I. From sugar specificities of lectins, it can, therefore, be concluded that there are D-mannose/D-glucose, N-acetyl-D-glucosamine/sialic acid, D-galactose in substantial amount, and D-galactose-N-acetyl-D-galactosamine, N-acetyl-D-galactosamine and L-fucose residues in lesser amount on the surface of adult Oriental schistosomes.

3. In adult parasites, the distribution and intensity of the staining resulted from lectin bindings vary : Con A and WGA, which bind primarily to the "core" or "internal" sugar residues of oligosaccharides in glycoconjugates (D-mannose/D-glucose, N-acetyl-D-glucosamine), bind extensively to pits' and

ridges' membranes. In contrast, RCA I, PNA, SBA and UEA I, which bind to the "terminal" sugar residues (D-galactose, D-galactose-N-acetyl-D-galactosamine, D-galactosamine, L-fucose) bind mostly to the membrane of the upper half of ridges. By contrast, the membrane of the gynaecophoral canal is either sparingly stained by most lectins except RCA or not stained at all.

4. The tegument glycoproteins of adult *S. japonicum, S. mekongi* and *S. mansoni* were separated by SDS-PAGE and electroblotting, and stained with biotinylated lectins. The binding patterns and the numbers of positive bands shown by Con A, WGA, SBA and UEA I are generally similar, but with slightly different staining intensity: the intensity is strongest in Con A, moderate in SBA and UEA I, and weakest in WGA. This may imply that most tegument glycoproteins contain similar proportion of D-mannose/D-glucose, N-acetyl-D-glucosamine/sialic acid, N-acetyl-D-galactosamine and L-fucose, but probably in slightly different quantity. By contrast, there are significant difference in the binding patterns of RCA I and PNA among the three species of schistosomes. Both strains of *S. japonicum* (Chinese and Philippine strains) appear to be more intensely stained than *S. mekongi* and *S. mansoni,* and the binding of RCA I is most intense on bands at MW 86,000 to 56,000 and 42,000 to 32,000. Thus, it can be concluded that both strains of *S. japonicum* probably contain much larger amount of D-galactose and D-galactose-N-acetyl-D-galactosamine residues in their tegument glycoproteins than other species, particularly in the glycoproteins within the above-mentioned ranges.

5. Treatments of freeze-thawed tegument proteins separated by SDS-PAGE and electroblotting with mouse antisera against *S. japonicum* (Chinese strain-ISCH; Philippine strain - ISPH), *S. mekongi* (ISME) and *S. mansoni* (ISMA) and human antisera against *S. japonicum* (Philippine-IHPH) reveal similar number of major immunogenic bands among all three species of adult schistosomes. In both strains of *S. japonicum* there are at least fifteen major antigens at MW 205,000; 116,000; 110,000; 105,000; 97,000; 76,000; 68,000; 50,000; 47,000; 46,000; 45,000; 28,000; 27,000; 26,000 and 25,000. The immunogenic bands at 28,000, 27,000 and 26,000 are prominent only in *S. japonicum* and may be abundant antigens in this species. There are also fifteen major immunogenic bands in *S. mekongi* at MW 116,000; 105,000; 97,000; 86,000; 76,000; 68,000; 64,000; 56,000; 52,000; 50,000; 47,000; 45,000; 43,000; 38,000 and 30,000. The band at MW 43,000 appears to be very prominent in *S. mekongi* eventhough it is also lightly stained with ISPH. In *S. mansoni* there are eighteen major immunogenic

bands at MW 105,000; 97,000; 90,000; 80,000; 76,000; 68,000, 64,000; 56,000; 52,000; 47,000; 45,000; 38,000; 37,000; 34,000; 32,000; 30,000; 28,000 and 27,000, and that the bands 37,000, 34,000, 32,000 appear to be specific antigens for this species. In all three species of schistosomes, the immunogenic bands at MW 98,000 and 68,000 show strongest staining intensities and, therefore, are the most prominent common antigens of these parasites.

6. The comparison of immunogenic bands from immunoblotting and positive bands revealed by lectin-bindings indicate: that almost all bands are labelled by at least two lectins, and thus they are probably all glycoproteins. In all three species of schistosomes, there appear to be two groups of immunogenic bands as judged from the lectin binding patterns. Firstly, most glycoprotein bands which are labelled by Con A, WGA, RCA I, PNA, SBA and UEA I; these bands may contain both internal (D-mannose/D-glucose, N-acetyl-D-glucosamine) and terminal sugar residues (D-galactose, N-acetyl-D-galactosamine, L-fucose) in their molecules. The second group of immunogenic bands, especially the bands at 97,000 and 68,000, are labelled intensely by RCA I, PNA, SBA as well as UEA I but not by Con A and WGA. These major immunogenic bands of the adult surface may, therefore, contain especially large amount of the terminal sugar residues (D-galactose, N-acetyl-D- galactosamine and L-fucose).

7. From the findings in our study, it is possible to suggest a hypothesis that the "core" or "internal" sugar residues may be inserted to glycoproteins first probably at the regions where the new membrane is added to the surface at the bottom part of pits, since the staining product resulted from Con A and WGA bindings are extensively distributed on the membrane of pits and ridges of adult schistosomes, while the "terminal" sugar residues may be inserted at later stage, as the corresponding lectins bind mostly to the membrane of the upper half of ridges. Furthermore, the most prominent immunogenic bands at MW 97,000 and 68,000 may concentrate mostly on the membrane of the upper half of ridges, the region where the membrane turn over in adult occurs. Consequently, these bands may comprise the major portion of released antigens that stimulate the greatest amount of antibody formation in hosts. Hence, in the immunoblotting patterns, these highly immunogenic glycoproteins are always very prominent in comparison to other antigens.

8. The patterns of ^{125}I-labelled surface proteins and immunoprecipitations by mouse antisera aganist *S. japonicum* (Chinese) (ISCH), against *S. japonicum* (Philippine) (ISPH), against *S. mansoni* (ISMA) and human immune serum against *S. japonicum* (Philippine) (IHPH) are identical for adults of both

strains of *S. japonicum*. The ^{125}I-labelled surface proteins consist of 18 bands, with the heaviest labelling at bands 180,000; 160,000; 128,000 and 14,000. Immunoprecipitations by ISCH, ISPH, IHPH result in similar pattern The most intense band that stands out in contrast to others is 128,000, which is not precipitated by ISMA, mouse antiserum against *S. mekongi* (ISME), nor mouse antiserum against *S. hematobium* (ISHE); thus it is the most uinque and specific antigen detected only in *S. japonicum*. Other bands with moderate precipitation against ISCH, ISPH and IHPH are 205,000; 180,000; 160,000; 45,000; 38,000; 34,000; 30,000; 26,000 and 23,000. It should be noted that mouse and human antisera produce identical patterns and that these bands show slight to moderate cross-reactions with ISMA. Band 14,000 is probably non-specifically precipitated, since it is always intense against all antisera as well as non-immune serum. In addition, it was found that band 86,000 is specifically and heavily precipitated by ISME and ISHE. The rest shows similar and light cross-reactions to ISME and ISHE as against ISMA. In conclusion, adults of *S. japonicum* (Chinese & Philippine) show major and non-cross reacting surface antigens against ISMA, ISME and ISHE at 128,000 and 160,000, and specifically cross-reacting antigen against ISME and ISHE at 86,000. The rest of the surface proteins show light to moderate cross-reactions against ISMA, ISME and ISHE.

In adult *S. mekongi* the ^{125}I-labelled major surface antigens precipitated by ISME are at MW 185,000; 155,000; 94,000; 86,000; 68,000; 54,000; 43,000; 32,000 and 26,000. Most bands, except 43,000; 39,000 and 35,000, show cross-reaction with ISCH, ISPH and ISMA. In *S. mansoni* the major surface antigens precipitated by ISMA are at MW 205,000; 160,000; 105,000; 80,000; 64,000; 58,000; 43,000; 37,000; 34,000; 27,000 and 23,000. ISCH, ISPH, ISME and ISHE react with most bands except 80,000 and 37,000.

9. Major antigens of *S. japonicum* schistosomula revealed in immunoblotting are at MW 205,000; 97,000; 76,000; 68,000; 64,000; 58,000; 45,000; 39,000, and in immunoprecipitation the major bands are at MW 65,000; 62,000; 46,000; 45,000; 26,000 and 25,000. Most of these bands are confined to the middle and lower range molecular weights as compared to the full set of adult antigens.

10. From immunofluorescence and immunoelectron microscopic studies, it is shown that mouse antisera from natural infection contain principally antibodies against antigens residing in cercarial glycocalyx, schistosomule and adult surface membrane. These antigens may be mostly glycoproteins and/or other carbohydrate bearing glycoconjugates in the surface membrane, as judged

from the similarity between antibody and lectin bindings.

It is further shown by immunoprecipitation and immunoblotting that these antigens are released into the culture medium *in vitro* , which indirectly indicates that the antigens and surface membrane of *S. japonicum* may also be turned over continuously *in vivo*. The released antigens may be the major challenge that elicit immune responses in hosts.

11. In *S. japonicum* (Chinese), as revealed by immunoblotting assay, mouse monoclonal antibodies (MAB) can be consistently produced against surface glycoproteins at MW 97,000, 54,000 and 47,000. From the testing of Ig-type it was found that most of the clones are unstable and generate antibodies of IgM and IgG_3 classes. The only stable and reproducible clone is producing MAB against antigen at 54,000 and the antibody is IgG_{2a} class. From immunofluorescence asay most antibodies, including anti-54,000, bind preferentially to the tegument and surface membrane of adult parasites and schistosomula.

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TABLE 2 MOLECULAR WEIGHT AND STAINING INTENSITY OF PROTEINS DERIVED FROM THE FREEZE-THAW ADULT TEGUMENT OF *SCHISTOSOMA JAPONICUM* (CHINESE – CH; PHILIPPINE – PH), *SCHISTOSOMA MEKONGI* (ME), AND *SCHISTOSOMA MANSONI* (MA) AS SEPARATED IN 12.5% SDS-PAGE, BLOTTED ONTO NITROCELLULOSE SHEETS AND BOUND TO VARIOUS BIOTINYLATED LECTIN USING AVIDIN-BIOTINYLATED HORSERADISH PEROXIDASE (HRP) AS INDICATED

Protein Bands	MW (Kdal)	Bands of Proteins in SDS-PAGE				Con A				WGA				RCA I				PNA				SBA				UEA I			
		CH	PH	ME	MA	CH	PH	ME	MA	CH	PH	ME	MA	CH	PH	ME	MA	CH	PH	ME	MA	CH	PH	ME	MA	CH	PH	ME	MA
1	300	+	+			++	+	+		+	+	+	–	+		+		+++	+++			–	+	+			+		
2	290	+	+	++	+		+	+	+++	+		+	++	+	+	+	+++	+++	+	+++	++			+++	++	+	+	+	+
3	270	+	+	+	+	+	+	+		+	+			+++	+++	++	+	+++	+++	+++	++	+++	+++					+	+
4	245	+	+			+	+		++	+++	+++			+	+	+	+	+	+	+		+							
5	230	+	+	+		+++	+++	+++				+		+		+		+	+	+						+	+		
6	205	++	++	++	++	+++	+++	+++	+++	+	+	+	+	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+	+	+
7	190	+	+	+		+++	+++		+++	+	+						+++	+	+	+		+	+	+		+	+	+	
8	170	+	+	+		+++	+++	+++	+++	++	++	+	+			+	+	–	+		+	+	+	+		+	+	+	+
9	145	+	+	+	+	+++	+++	+++	+++	+	+	+		+++	+++		++	+	+	+++	+	+	+	+	+			++	+
10	120	+	+	+		+++	+++	+++	+++	+	+	+						+	+	+++	++	+	+	+	+				
11	116	+	+	+	+	+++	+++	+++	+	+	+	++	++	+	+	+	+	+++	+++	+++	+	+	+	+	+	+	+	+	+
12	110	+	+		+	++	++	++		+	+	+	+	+	+			+	+	+		+	+	+					+
13	105	+	+	+		++	++	++	+									+	+	++	+					+	+	+	
14	97	+++	+++	+++	+++									++	++		++	++	++	+	+	+	+	+					
15	90	+++	+++	+++		++	++			++	++			+	+		+	+	+	+	+		+	+	+	+	+		+
16	86	+	+	+	+	+++	+++	+++	++	+	+	+	+	++	++	+	+	++	++	++	+	+	+	+	+	+	+	+	
17	84	+	+											+++	+++			+++	+++	+						+	+	+	
18	82				++				+												+								+
19	76	++	++	+		++	++	++		++	++	+		+++	+++			+++	+++			+	+	+	+	+	+	+	+
20	75	+	+	+	++	+	+	+	+	++	++	+	+	+++	+++		+	+++	+++			+	+	+					
21	68	+++	+++	+++	++									+++	+++	+	+	+++	+++			++	++	++	+++	++	++	++	+++
22	64	++	++	+	+				+++				+	+++	+++		+	+++	+++	++	+	+	+			++	++	+	
23	58	++	++	+++		+++	+++	+++		++	++	++	+	+++	+			+++	+++	+++	+	+++	+++	+++	+				
24	56	+++	+++	+++		+++	+++	+++		+	+	+		+++	++	++	++	+++	++	+++	+	++	++	+		++	++	+	+++
25	54	++	++	+	+++	+	+	+	+++	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+				
26	52	++	++	+++					+++					+	+	++	++	++	++	+++	+	+	+	+					
27	50	+	+	+	+++	+++	+++	++	+++	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+				
28	48	+++	+++	+++		++	++	++		+	+			+	+	++		+++	++	+++		+++	+++	+++	+++	+++	+++	+++	+
29	47	+	+															+	+	+		+	+		+				+
30	46	+++	+++	+	+++	+	+	+++	+	+	+	+		+	+			+	+	+						+	+	+	++
31	45	+++	+++	+++		+	+	+		+	+	+	+	+	+			+	+	+		+	+	+		+++	+++	+++	+++
32	43				+++	++	++			+	+			+	+			+	+										
33	42	+++	+++	+	+	+++	+++	+	+	+	+	+	+	+++	+++			+	+	+	+	+	+	+					
34	38	+++	+++	+++		+++	++	+	+	+	+	+	+	+++	+++	+		+	+	+	+	++	++	++	++	++	++	++	++
35	37	+++	+++		+++	+++	++							+++	++							++	+++	++	+++	++	++	++	++
36	34	+	+		+++	+++	+		++	+	+		++	+++	++		++				++				++				++
37	33	++	++	++	+	+++								+++	++														
38	32	+++	+++	+++		+++	++	++				+		+++	++	+		+	+	+	+	+	+	+	+	++	++	++	
39	30	+++	+++	+	+				++				++												++				+
40	29	+++	+++	+	+				++			+	++	+		+	+	+	+	+	+	+	+	+	++			+	+
41	28	+++	+++	+	++	+++	++	+	++				++				+	+	+	+	+	++	++	+	++	+	+	+	+
42	27	++	++	+	++	+++	+		+								+												
43	26	+	+	+++	+	+++	+										+					++	+	+					
44	25	+	+	+																		+	+	+	+				
45	23	+	+		+																								

Protein Bands	MW (Kdal)	Bands of Proteins in SDS-PAGE				Con A				[illegible]A				RCA I				PNA				SBA				UEA I			
		CH	PH	ME	MA	CH	PH	ME	MA	CH	PH	ME	MA	CH	PH	ME	MA	CH	PH	ME	MA	CH	PH	ME	MA	CH	PH	ME	MA
46	20	+	+	+	+																	+	+	+	+				
47	19	+	+	+	+																	+	+	+	+				
48	14	+++	+++	+++	+++																	+	+	+					
Total Protein Bands		42	42	36	29	29	29	24	22	21	21	20	15	30	27	16	19	32	33	28	21	33	33	31	23	21	22	22	21

"+" = low intensity, "++" = moderate intensity, "+++" = high intensity

TABLE 3 IMMUNOBLOTTING OF ANTIGENS FROM FREEZE-THAW ADULT TEGUMENT OF *S. JAPONICUM* (CHINESE - CH), *S. JAPONICUM* (PHILIPPINE - PH), *S. MEKONGI* (ME) AND *S. MANSONI* (MA) STAINED WITH MOUSE ANTISERA AGAINST *S. JAPONICUM* (CHINESE - ISCH), *S. JAPONICUM* (PHILIPPINE - ISPH), *S. MEKONGI* (ISME) AND *S. MANSONI* (ISMA). THE CORRESPONDING SURFACE ANTIGENS DETECTED BY IMMUNOPRECIPITATION (CH*, PH*, ME*, MA*) ARE ALSO SHOWN ON THE LEFT HAND COLUMNS FOR COMPARISON

MW	ISCH	ISPH	ISME	ISMA	ISCH				ISPH				ISME				ISMA			
(Kdal)	CH*	PH*	ME*	MA*	CH	PH	ME	MA	CH	PH	ME	MA	CH	PH	ME	MA	CH	PH	ME	MA
270					+	+	+		+	+	+									
245					+	+	+		+	+	+									
230					+				+	+	+	+								
205	++	++		++	+++	+++	+++	++	++	++	++	+	+	+	+	+	+	+	+	+
190					+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
180	++	++	+++(185)		+	+			+	+					+	+	+	+	+	+
170					+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
160	++	++		++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
150			+++(155)		+	+	+	+	+	+	+	+	+	+	+	++			+	+
145					+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
128	+++	+++			+	+			+	+										
120					+	+	+	+	+	+	+	+	+	+	+	+				+
116	+	+			+++	+++	++	+	++	++	+	+	++	++	++	+	++	++	++	+
110					++	+++	++	++	++	++	+	+	++	++	+	+	+	+	+	+
105				++	+	+	+	+	++	++	+++	++	++	++	++		++	++	++	++
97			+++(94)		+++	+++	+++	+++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++
86	+	+	+++		++	++	++	++	+++	+++	+++	+++	++	++	+++	+	++	++	++	+
80				+				+								+				++
76					+	+	+		++	++	+	+	++	++	++		+++	+++	+++	+++
68	+	+	+++		+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	++	+	+	+	+++
64			+	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+	+	+	++
62	+	+			+	+	+	+	+	+	+	+	+	+	+	+			+	+
58				++	+	+	+	+	+	++	++	+	+	+	+	+	+	+	+	+
56					++	++	+	+++	++	++	+	+++	++	+	++	+++	+	+	+	+++
54			++		++	++	+	+	++	++	+	+	+	+	+	+	++	++	+	+
52					+	+	+	+	+	+	+	+	++	+	++	++				++
50				+	+	+	+	+	++	++	++	+	++	++	++	++	+	+	+	+
48					+	+	+	+	+	+	+		+	+	+		+	+	+	
47					+++	+++	++	+	++	++	++		++	++	++	++			+	++
46					+	+		++	++	+++		+	+++	+++		+				
45	++	++			+++	+++	+++	+++	+++	+++	++	++	+++	+++	++	+++	+++	+++	+++	+++
43			++	++	+	+	+	+	+	+	+++	+	+	+	+++	+	+	+	++	+
39			+		+	+		+	+	+			+	+	+		+	++	++	+
38	++	++			++	++	++	+	++	++	++	+	+	+	++	+	++	++	+++	++
37				+				+	+	+	+	+		+	+	+	+++	+++	+++	+++
36	++	++	+(35)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	++
32			++	++	+	+	+	+	+	+	+	+	+	+	+	+	++	+	++	++
30	++	++		++	+	+	+	+	+	+	+	+	+	+	++	+	+	+	+	++
28					+++	++	+	+	+++	++	+	+	+	+	+	+	+	+	+	++
27				+++	+++	++	+	+	+++	++		+	+	+	+	+	+	+	+	++
26	+	++	++		+++	++	+	+	+++	++	+	+	+	+	+	+	+	+	+	+
25					++	+			++	++			+	+			+	+	++	
23	+	++		+	+	+			+	+										
19	+	+		++	++	+			+	+										
< 14	+++	+++	+++	+++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE 4 IMMUNOBLOTTING OF ANTIGENS FROM FREEZE-THAW ADULT TEGUMENT OF *S. JAPONICUM* (CHINESE - CH), *S. JAPONICUM* (PHILIPPINE - PH), *S. MEKONGI* (ME) AND *S. MANSONI* (MA) STAINED WITH HUMAN ANTISERA AGAINST *S. JAPONICUM* (PHILIPPINE - IHPH). $IHPH_1$ IS THE FIRST LOT OF POOLED SERUM, $IHPH_2$ IS THE COMPILATION OF RESULTS FROM INDIVIDUAL ANTISERUM FROM 5 PATIENTS

MW (Kdal)	$IHPH_1$ CH	$IHPH_1$ PH*	$IHPH_1$ ME	$IHPH_1$ MA	$IHPH_2$ CH	$IHPH_2$ PH*	$IHPH_2$ ME	$IHPH_2$ MA
270					++	+		+
245								+
230				+				
205	+	+		+	+	+		+
190					+	+		+
180	+	+		+	+	++		+
170					+	+	+	+
165					++	+		+
160				+	+	+	+	+
150	++	+			++	+		
145				+	+	+		+
128					+	+	+	+
120	+	+			+	+		+
116	++	++	++	++	++	++	+	+
110					++	+	+	+
105					+	+	+	
97	+++	+++	++	+++	+++	+++	+++	+++
86		+			+	+	+	+
80					+	+	+	+
76	++	+		+				
68	++	++	++	++	+++	+++	+++	+++
64					+++	+++	+++	+++
62	+	+	++		+++	+++	++	+
58	++	++		++	+	+	+	+
56	+	+	++	++	+	+	-	+
54		+			+++	+++	++	+
52			+	++	++	++	++	++
50	+++	+++			++	++	+	+
48	+	+	++	++	++	++	++	++
47				+	++	++	++	+
46	+	+	+	+	+	+	++	+
45	++	++	++	+	+++	+++	++	+
43	+	++	++		+	+	+	
39	+	+	+		+	++	++	+++
38	+	+	++	++	++	+	+++	++
37			+	+	+	+	+	++
34	+	+	+		+	+	+	+
32			+		+	+	+	+
30	+	+			+	+	+	+
28	+	+	+	++	+++	+++	+	+
27	+	+	+	++	++	++	++	++
26	+	+			+	+	+	+
25	++	++			++	++	+	
23	++	+			++	++		
19	+	++	++	++	++	++	+	+
< 14	+	+	+	+	+	+		

$IHPH_2$ antisera were kindly given by Dr. Biyani Blas, Director, Schistosomiasis Control and Service Center, Ministry of Public Health, Manila, The Philippines.

TABLE 5 COMPARISON OF *SCHISTOSOMA JAPONICUM* (CHINESE STRAIN) TEGUMENT ANTIGENS AS DETECTED BY IMMUNOBLOTTING AND THE TEGUMENT GLYCOPROTEINS AS REVEALED BY LECTIN BINDINGS

MW of immunogenic bands (Kdal) (detected by ISCH)	Biotinylated lectins					
	Con A	WGA	RCA_1	PNA	SBA	UEA_1
270	+	+	+++	+++	+++	
245	+	+++	+	+	+	
230	+++		+	+		+
205(•)	+++	+	+++	+++	+++	+
190	+++	+		+	+	+
170	+++	++		+	+	+
145	+++	+	+++	+	+	
128						
120	+++	+		+	+	
116(•)	+++	+	+	+++	+	+
110(•)	++	+	+	+	+	
105	++			+		+
97(•)			++	++	+	
86(•)	+++	+	++	++	+	+
76	++	++	+++	+++	+	+
68(•)			+++	+++	++	++
64(•)			+++	+++	+	++
56(•)	+++	+	+++	+++	++	++
54(•)	+	+	+	+	+	+
52			+	++	+	
48	++	+	+	+++	+++	+++
47(•)				+	+	
46	+	+	+	+		+
45(•)	+	+	+	+	+	+++
43	+++	+	+++		+	
37	+++		+++		++	++
32	+++		+++	+	+	++
28(•)(≠)	+++			+	++	+
27(•)(≠)	+++					
26(•)(≠)	+++				++	
25(•)(≠)					+	
19					+	
< 14					+	
Major bands (15)						
Minor bands (18)						

The relative intensity of staining is arbitrarily graded by "+" sign :

"+++" = high intensity, "++" = moderate intensity, "+" = low intensity, and (•) indicates major bands, and (≠) indicates bands that are notably more intense in comparison to the other two species. ISCH = mouse anti-*S. japonicum* (Chinese strain).

TABLE 6 COMPARISON OF *SCHISTOSOMA JAPONICUM* (PHILIPPINE STRAIN) TEGUMENT ANTIGENS AS DETECTED BY IMMUNOBLOTTING AND THE TEGUMENT GLYCOPROTEINS AS REVEALED BY LECTIN BINDINGS

MW of immunogenic bands (Kdal) (detected by ISPH)	Biotinylated lectins					
	Con A	WGA	RCA_1	PNA	SBA	UEA_1
270	+	+	+++	+++	+++	
245	+	+++	+	+		
230	+++			+		+
205(•)	+++	+	+++	+++	+++	+
190	+++	+		+	+	+
170	+++	++		+	+	+
145	+++	+	+++	+	+	
128						
120	+++	+		+	+	
116(•)	+++	+	+	+++	+	+
110(•)	++	+	+	+	+	
105(•)	++			+		+
97(•)			+	+	+	
86(•)	+++	+	++	++	+	+
76(•)	++	++	+++	+++	+	+
68(•)			+++	+++	++	++
64(•)			+++	+++	+	++
56(•)	+++	+	++	++	++	++
54(•)	+	+	+	+	+	+
52			+	++	+	
48	++	+	+	++	+++	+++
47(•)				+	+	
46(•)	+	+	+	+		+
45(•)	+	+	+	+	+	+++
43	+++	+	+++	+	+	
37	++		++		+++	++
32	++		++	+	+	++
28(•) (≠)	++			+	++	+
27(•) (≠)	+				+	
26(•) (≠)	+				+	
25(•) (≠)					+	
19					+	
< 14					+	
Major bands (18)						
Minor bands (15)						

The relative intensity of staining is arbitrarily graded by "+" sign :

"+++" = high intensity, "++" = moderate intensity, "+" = low intensity, and (•) indicates major bands. ISPH = mouse anti-*S. japonicum* (Philippine strain).

TABLE 7 COMPARISON OF *SCHISTOSOMA MEKONGI* TEGUMENT ANTIGENS DETECTED BY IMMUNOBLOTTING AND TEGUMENT GLYCOPROTEINS AS REVEALED BY LECTIN BINDINGS

MW of immunogenic bands (Kdal) (detected by ISME)	Biotinylated lectins					
	Con A	WGA	RCA_1	PNA	SBA	UEA_1
205	+++	+	+++	+++	+++	+
190				+	+	+
170	+++	+	+		+	+
145	+++	+		+++	+	++
120	+++	+	+++	+++	+++	+
116(*)	+++	++	+	+++	+	+
110	++	+		+	+	
105(*)	++			++		+
97(*)				+	+	
86(*)(≠)	++	+	++	++	+	+
76(*)	++	+			+	+
68(*)			+		++	++
64(*)			++	++	+	++
56(*)	+++	+	++	+++	+	+
52(*)			++	+++	+	
50(*)	++	+	+	+	+	
48(*)	++		++	+++	+++	+++
47(*)				+		
45(*)	+	+		+	+	+++
43(*)(≠)	+	+		+	+	
38(*)	+	+	+	+	++	++
Major bands (15)						
Minor bands (6)						

The relative intensity of staining is arbitrarily graded by "+" sign :

"+++" = high intensity, "+" = moderate intensity, "+" = low intensity, and (*) indicates major bands, and (≠) indicates bands that are notably more intense in comparison to the other two species. ISME = Mouse anti-*S. mekongi*.

TABLE 8 COMPARISON OF *SCHISTOSOMA MANSONI* TEGUMENT ANTIGENS DETECTED BY IMMUNOBLOTTING AND TEGUMENT GLYCOPROTEINS AS REVEALED BY LECTIN BINDINGS

MW of immunogenic bands (Kdal) (detected by ISMA)	Biotinylated lectins					
	Con A	WGA	RCA_1	PNA	SBA	UEA_1
205	+++	+	+++	+++	+++	+
170	+++	+	+	+		+
145	+++		++	+	+	+
120	+++			++	+	
116	+	++	+	+	+	+
105(*)	+			++		
97(*)			++	+		
86(*)				+	+	+
80(*)(≠)	+		+	+		+
76(*)					+	+
68(*)			+	+	+++	+++
64(*)			++	++	+	++
56(*)			++	+		+++
52(*)	+++		++	+		
47(*)					+	+
45(*)		+				+++
43						
38(*)	+	+	+	+	+	+
37(*)(≠)	+	+	+	+	+	+
34(*)(≠)	++	++	++	++	+	+
32(*)(≠)	++	++			++	+
30(*)(≠)	++	++	+	+	++	+
28(*)	++	++	+	+	+	+
27(*)	+		+	+	+	
Major bands (17)						
Minor bands (6)						

The relative intensity of staining is arbitrarily graded by "+" sign :

"+++" = high intensity, "++" = moserate intensity, "+" = low intensity, and (*) indicates major bands, and (≠) indicates bands that are notably more intense in comparison to the other two species.
ISMA = Mouse anti- *S. mansoni*.

TABLE 9 IMMUNOPRECIPITATIONS OF ^{125}I LABELLED SURFACE PROTEINS OF ADULT MALE *S. JAPONICUM* (CHINESE) BY MOUSE ANTISERA AGAINST *S. JAPONICUM* (CHINESE & PHILIPPINE - ISCH & ISPH), POOLED HUMAN ANTISERA AGAINST *S.JAPONICUM* (PHILIPPINE - $IHPH_1$) , MOUSE ANTISERA AGAINST *S. MEKONGI* (ISME), *S. MANSONI* (ISMA) AND *S. HEMATOBIUM* (ISHE). ALL SAMPLES WERE PRE-ABSORBED WITH NORMAL MOUSE OR NORMAL HUMAN SERA

^{125}I Labelled Surface Proteins (MW X 10^{-3})	Immunoprecipitated by ISCH,ISPH,$IHPH_1$ (MW X 10^{-3})	Immunoprecipitated by ISME,ISHE (MW X 10^{-3})	Immunoprecipitated BY ISMA (MW X 10^{-3})
205 (+)	++	+	+
180 (++)	++	+	+
160 (++)	++		
128 (++)	+++		
116 (+)	+	+	+
86 (+)	+	++	
68 (+)	+	+	+
62 (+)	+	+	+
56 (+)			
48 (+)			
45 (+)	++	+	+
38 (+)	++	+	+
34 (+)	++	+	+
30 (+)	++	+	+
26 (+)	+	+	+
23 (+)	+	+	+
19 (+)	+	+	+
14 (+++)	+++	+++	+++
(18 bands)			

"+" sign indicates relative intensity; "++" = light; "++" = moderate; "+++" = intense.

$IHPH_1$ - kindly supplied by Prof. E. Garcia, Institute of Public Health, University of the Philippine System, Manila.

TABLE 10 IMMUNOPRECIPITATIONS OF ^{125}I LABELLED SURFACE PROTEINS OF ADULT MALE *S. JAPONICUM* (PHILIPPINE) BY MOUSE ANTISERA AGAINST *S. JAPONICUM* (CHINESE & PHILIPPINE - ISCH & ISPH), HUMAN ANTISERA AGAINST *S. JAPONICUM* (PHILIPPINE - $IHPH_1$), AND MOUSE ANTISERA AGAINST *S. MANSONI* (ISMA). ALL SAMPLES WERE PRE-ABSORBED WITH NORMAL MOUSE OR NORMAL HUMAN SERA.

^{125}I labelled Surface Proteins (MW X 10^{-3})	Immunoprecipitated by ISPH, ISCH, $IHPH_1$ (MW X 10^{-3})	Immunoprecipitated by ISMA (MW X 10^{-3})
205 (++)	++	++
180 (++)	++	++
160 (++)	++	
128 (++)	+++	
116 (+)	+	+
86 (+)	+	+
68 (+)	+	
62 (+)	+	+
56 (+)		
48 (+)		
45 (+)	++	++
38 (+)	++	++
34 (+)	++	+
30 (+)	++	+
26 (+)	++	+
23 (+)	++	+
19 (+)	+	
14 (+++)	+++	+++
(18 bands)		

TABLE 11 IMMUNOPRECIPITATIONS OF ^{125}I LABELLED SURFACE PROTEINS OF ADULT MALE *S. MEKONGI* BY MOUSE ANTISERA AGAINST *S.MEKONGI* (ISME), *S. JAPONICUM* (CHINESE & PHILIPPINE - ISCH & $ISPH_1$), *S. MANSONI* (ISMA) AND *S. HAEMATOBIUM* (ISHE). ALL SAMPLES WERE PRE-ABSORBED WITH NORMAL MOUSE SERA

^{125}I Labelled Surface Proteins	Immunoprecipitated by ISME	Immunoprecipitated by ISCH,ISPH ,ISMA	Immunoprecipitated by ISHE
(MW X 10^{-3})	(MW X 10^{-3})	(MW X 10^{-3})	(MW X 10^{-3})
185 (++)	+++	++	++
155 (+++)	+++	++	++
120 (+)			
110 (+)			
94 (++)	+++	++	+++
86 (++)	+++	+	+++
68 (+)	+++	+	+++
64 (+)	+	+	+
54 (+)	++	++	++
43 (++)	++		+
39 (+)	+		+
35 (+)	+		
32 (+)	++	+	++
28 (+)			
26 (+)	++	++	++
14 (+++)	+++	+++	+++
(16 bands)			

TABLE 12 IMMUNOPRECIPITATIONS OF ^{125}I LABELLED SURFACE PROTEINS OF ADULT MALE *S. MANSONI* BY MOUSE ANTISERA AGAINST *S. MANSONI* (ISMA), AGAINST *S. JAPONICUM* (CHINESE & PHILIPPINE - ISCH & ISPH), AGAINST *S. MEKONGI* (ISME) AND AGAINST *S. HEMATOBIUM* (ISHE), AND COMPARISON TO THE RESULTS ON ADULT *S. MANSONI* AS REPORTED BY SHAH AND RAMASAMY (1982)[1] AND HAYUNGA (1979 a, b)[2]

Immunoprecipitations of adult *S. monsoni* ^{125}I labelled surface proteins as reported by		^{125}I labelled surface proteins of *S. monsoni*	Immunoprecipitations of adult male *S.monsoni* ^{125}I labelled surface proteins by				
Shah & Ramasamy (1982)*	Hayunga et al 1979(a,b)**		ISMA	ISCH	ISPH	ISME	ISHE
(MW X 10^{-3})	(MW X 10^{-3})	(MW X 10^{-3})	(MW X 10^{-3})				
-	-	> 205 (+)	++	+	+	+	+
> 150	-	160 (+)	++	+	+	+	+
102	100	105 (+)	++	+	+	+	+
78	78	80 (+)	+				
-	68	64(++)	++	+	+	+	+
59	60	58(++)	++				
-	-	50 (+)					
45	43	43(++)	++	+	+	+	+
-	36	37 (+)	+				
35	-	34 (+)	+	+	+	+	+
-	30	30(++)	+++	++	++	++	++
-	26	27(+++)	+++	++	++	++	++
22	20	23	+	+	+	+	+
-	-	19(++)	++	+	+	+	+
-	12	14(+++)	+++	+++	+++	+++	+++
		(15 bands)					

"+" sign indicates relative intensity of the protein bands; "+" = light; "++" = moderate ; "+++" = intense

* Shah, J. & Ramasamy, K. (1982) *Int. J. Parasitol,* 12, 451-461.

** Hayunga, E.G., Murrell, K.D., Taylor, D.W. & Vannier, W.E. (1979 a) *J. Parasitol.* 65, 488-496.

** Hayunga, E.G., Murrell, K.D., Taylor, D.W. & Vannier, W.E. (1979 b) *J. Parasitol.* 65, 497-506.

TABLE 13 EXTRACTION OF LIPID COMPONENTS FROM *SCHISTOSOMA JAPONICUM TEGUMENT*

Fractions	^{125}I-cpm	% recovery
1. Freezed-thawed tegument	2.24×10^7	100
2. Hexane-isopropanol extraction :		
2.1 Precipitate	0.36×10^7	16.1
2.2 Supernatant	1.0×10^7	44.6
2.2.1 Aqueous	6.25×10^6	27.9
2.2.2 Hexane	1.0×10^6	4.5

TABLE 14 IMMUNOBLOTTING OF ANTIGENS FROM SCHISTOSOMULA AND CERCARIAE OF *S. JAPONICUM* (CHINESE -CCH & SCH) AND *S. MANSONI* (CMA & SMA) IN COMPARISON TO ADULT ANTIGENS (CH, PH, ME, MA) WHEN STAINED WITH MOUSE ANTISERA AGAINST *S. JAPONICUM* (ISCH & ISPH), *S. MEKONGI* (ISME) AND *S. MANSONI* (ISMA)

MW		ISCH	ISCH								ISPH								ISME								ISMA							
(Kdal)	SCH*	SCH*	CCH	SCH	CH	PH	ME	CMA	SMA	MA	CCH	SCH	CH	PH	ME	CMA	SMA	MA	CCH	SCH	CH	PH	ME	CMA	SMA	MA	CCH	SCH	CH	PH	ME	CMA	SMA	MA
270																																		
245																																		
230																																		
205				++	++	+	++	+	+	+			+	+	+			+			+	+	+			+	+	+	+	++	+	+	+
190					+	+	+			+			+	+	+			+			+	+	+			+			+	+				+
180					+	+						+	+	+				+											+	+				++
170					+	+	+	+	+	+			+	+	+			+			+	+	+			+	+	+	+					+
160	+	+			+	+	+			+			+	+	+			+			+	+	+			+			+	+				
150					+	+	+			+			+	+	+			+			+	+	+			+	+	+	+	+	+	+	+	+
145					++	+	++			+		+	+	+	+			+			+	+	+			+			+	+				+
128	+	+			+	+							+	+							+	+	+			+	+	+	+	+	+	+	+	+
120				+	+	+	+						+	+															+	+	+			+
116				+	++	++	+	+	+	+			+	+				+									+	+	+					+
110					+	+	+			+			++	++	+			+			+	+				+	+	+	+					+
105				+	++	+	+			+		+	+	+	+			+			+	+	+			+			+	+				+
97	+	+	+++	+++	+++	+++	+++	+	+	+++	+	+	+	+	+			+			+	+	+			+			+	+	+	+	+	+
86	+	+	+	+	+	+	+	+	+		++	++	++	++	++		+	++			+	+	+			+	+	+	+		+	+	+	+
80											+	+	+	+	+	+	+	+			+	+	+			+	+	+	+	+				+
76			++	++	+	+		++	++	+											+	+	+			+	+	+	+	++				+
68	+++	+++	+	++	++	++	++	+	+	+	+	+	+	+		+	+	+																
64			+	++	+++	+++	+++	+	+	+	+	+	+++	+++	+++	+	+	+	+	+	++	++	++		+	++	+	+	+	+	+	+	+	+
62	++	++	+	+	++	+		+	+	++	+	+	+	+	+			+			+	+	+			+			+	+	+	+	+	+
58			++	++	+	+		++	++	+									+	+	++	++	+++						+	+				++
56			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
54					+	+			+	+			+	+							+	+	+			+			+	+			+	+
52	++		+	+	+	+	+	+	+				+	+	++				+	+	+	+	+			+			+	+				+
50	+		+	+	+	+				++			+	+	+			+			+	+	+			+	+	+	+	+	+	+	+	+
48			+	+	+	+	+++	+	+	+			+	+	+	+	+	+			+	+	+			+	+	+	+	+	+	+	+	+
47			+	+	+	+		+	+	+			+	+				+			+	+	+			+	+	+	+	+	+	+	+	+
46	+	+++	+	+	+	+		+	+	+			+	+				+			+	+	+			+	+	+	+	+				+
45	+	+++	++	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+	+	+			+	+	+	+	+	++	++	+	+
43																					+	+	+			+	+	+	+					+
39			+	++	+	+	+		+	+	+	++	+	+						+	+	+	+	+	+	+	++	++	+	+		+	+	+
38	+	+			+	+	+						+	+					+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+++
37					+								+	+							+	+	+			+	+	+	+					
34	+	+	+		+	+	+	+	+	+			+	+	+			+		+	+	+	+			+	+	+	+					+
32			+	+	+	+		+	+	+			+	+				+			+	+	+			+			+	+	+	+	+	+
30			+	+	+	+		+	+	+			+	+	+		+	+	+	+	+	+	+			+	+	+	+	+	+	+	+	+
28			+	+	+	+	+	+	+	+			+	+	+			+			+	+	+			+			+	+	+	+	+	++
27			+	+	+	+	+	+	+	+	+	+	+	+				+			+	+	+			+			+	+			+	+
26	++	++	+	+	+	+		+	+	+			+	+				+			+	+	+			+			+	+			+	+
25	+	++	+	+	++	++							+	+							+	+	+			+			+	+				+
23					++	++																												
19													++	++																				
14	+++	+++																					+						+	+				

TABLE 15A THE RELEASE OF ^{125}I LABELLED PROTEINS FROM THE SURFACE OF ADULT MALE *S. JAPONICUM* AT VARIOUS TIME INTERVALS INTO THE MEM MEDIUM DURING THE *IN VITRO* CULTURE OF THE PARASITES

^{125}I Labelled Surface Proteins (MW X 10^{-3})	Time in MEM Culture (Hours) 1	3	6	12	24
205 (+)				+	++
180 (+)				+	++
160 (++)	+	+	++	++	++
128 (++)	+	+	++	++	++
116 (+)					
86 (+)			+	+	+
68 (+)	+	+	++	++	++
62 (+)		+	++	++	++
56 (+)		+	++	++	++
48 (+)					+
45 (+)					
38 (+)					
34 (+)				+	++
30 (+)	+	+	++	++	+++
26 (+)				+	+
23 (+)				+	+
19 (+)				+	+
14 (+++)	+++	+++	+++	+++	+++
(18 bands)					

TABLE 15B RELEASED ANTIGENS OF *S. JAPONICUM* INCUBATED *IN VITRO* IN MEM MEDIUM AS DETECTED BY IMMUNOBLOTTING : The released antigens collected from MEM culture fluid were detected at various time intervals from 1 to 20 hours by separating the antigens in SDS-PAGE and blotted onto NC strips, which are in turn reacted with mouse antisera against *S. japonicum* (Chinese).

Protein bands (MW X 10^{-3})	Time in MEM Culture (Hours) 1	3	9	20	CH+ISCH
< 205		+	+	+	+
205	+	++	++	++	+
180				++	+
160				+	+
116					+
105	+	+	+	+	+
97	+	+	+	+	+
86	+	+	+	+	+
76	+	+	+	+	+
68	++	++	++	++	+++
64	+	+	+	++	+++
54	+	+	+	+	+
50	+	+	+	+	+
45	+	+	+	+	+
38	+	+	+	+	+
30	+	+	+	+	+
26			+	+	+
(17 bands)					

+ = low intensity, ++ = moderate intensity, +++ = high intensity
CH+ISCH - antigens from freeze-thawed tegument that appear in immunoblot

TABLE 16 IMMUNOBLOTTING ASSAYS FOR MOUSE MONOCLONAL ANTIBODIES (MAB) AGAINST *S. JAPONICUM* (CHINESE) ANTIGENS WHICH ARE DERIVED FROM FREEZE-THAWED ADULT TEGUMENT. MAB WERE DERIVED FROM CULTURE FLUIDS OF VARIOUS CLONES OF MAB-PRODUCING CELLS. THE SECOND ANTIBODY USED TO DETECT THE BINDING OF MAB TO TEGUMENT ANTIGENS IS RABBIT ANTI-MOUSE Ig LABELLED WITH HRP.

MW (Kdal) \ Clones	GROUP 1									GROUP 2								GROUP 3						GROUP 4			
	6C4-1	6C4-2	6C4-4	6C4-7	6C7-10	6F11-1	8D6-4	8G11-10	9C3	2B4*	2D4*	8F2*	8F5	8G3-3	8G3-4	8G9-5	8G11-2	2G4-4	8C9	8D6-1	8E2-1	8G9-6	9E7*	8D6	8D6-2	8D6-3	8D6-7
116	+	+	+	+	+									+	+	+											
110																											
105						+								+	+	+											
97*	+	+	+	+		+								+	+	+	+	+	+	+	+	+					
86					+	+		+										+	+	+	+	+					
80																		+	+	+	+	+					
76																											
68	+				+	+												+				+					
64														+	+	+											
62	+	+	+	+	+	+	+	+	+																		
58																											
56	+	+	+	+	+	+	+	+	+																		
54*										+	+	+	+	+	+	+	+	+	+	+	+	+	+				
52	+	+	+	+	+	+	+	+	+																		
50													+	+	+	+	+					+					
48																											
47*	+	+	+	+	+	+		+						+	+	+								+		+	+
46																											
45													+	+	+	+		+	+			+					
43						+																+					
39																								+	+	+	+
38																											
37						+																					
34	+	+	+			+	+																	+	+	+	+
32																											
30														+	+												
O.D. (ELIZA)	0.413	0.376	0.324	0.379	2.528	0.615	0.220	0.224	0.266	0.184	0.430	0.200	0.145	0.360	2.611	0.236	0.504	0.128	0.209	0.146	0.304	0.358	0.258	0.085	0.073	0.125	0.099
Ig	IgM	IgM	IgM+IgG$_3$	IgM	IgM	IgG$_3$	IgG$_3$	IgM	IgG$_{2a}$	IgM	IgG2a	IgG$_{2b}$	IgG$_{2b}$	IgM+IgG$_{2b}$	IgM	IgM	IgM	IgM	IgM	IgM	IgM	IgM	IgG$_{2a}$	IgG$_3$	IgG$_3$	IgG$_3$	IgG$_3$

*Major antigens that can be detected by more than half of the positive MAB clones.
O.D. (control) = 0.086

FIGURE LEGENDS

FIGURE 1

TEM OF COLLOIDAL IRON STAINING OF CERCARIAE AND SCHISTOSOMULA OF *S. JAPONICUM* (CHINESE) AND *S. MEKONGI*

A , B) *S. japonicum* (A) and *S. mekongi* (B) cercariae : the staining occurs over the whole thickness of glycocalyx (Gy) and appears as black granular deposit on the interlacing strands of glycocalyx. The surface membrane of the tegument (Te) is not stained A X 9,400; A inset, X 27,000; B X 13,500; B inset, X 20,200.

C , D) *S. japonicum* (C) and *S. mekongi* (D) 1-hour schistosomula : the staining occurs over a thin layer of remaining glycocalyx (Gy) coating the surface membrane, around blebs and microvilli. At high magnification (insets) the stain appears as aggregates of black dots. C, X 22,600; C inset X 33,900; D, X 22,600; D inset, X 33,900.

E , F) *S. japonicum* (E) and *S. mekongi*(F) 12-hour schistosomula : the staining occurs on the surface membrane of the tegument (Te), and appears as a row of black dots. E, E inset, F, X 22,600.

FIGURE 1

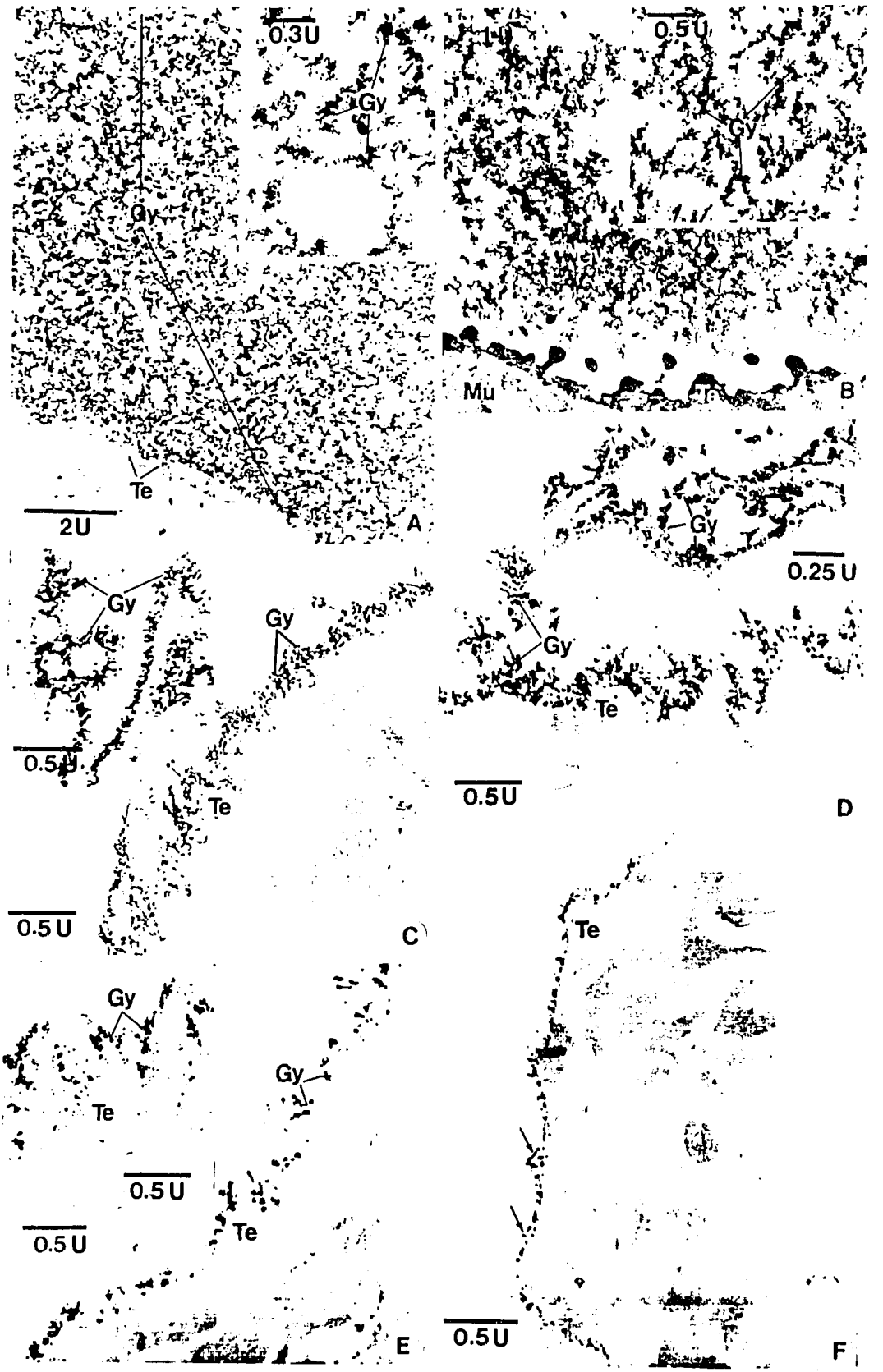

0.3U
Gy
Gy
Te
2U
A
1U
0.5U
Gy
Mu
B
Gy
0.25 U
Gy
Te
0.5U
D
Gy
Gy
Te
0.5U
0.5 U
C
Gy
Gy
Te
0.5 U
0.5 U
Te
E
Te
0.5U
F

FIGURE 2

TEM OF COLLOIDAL IRON STAINING OF ADULT *S. JAPONICUM* (CHINESE) AND *S. MEKONGI*

A, B) *S. japonicum* : the staining is intense on the membrane (arrows) of ridges (Ri), and decreases progressively towards the membrane of pits (Pi). In the middle part of the pits (*) the membrane is only lightly stained, while in the bottom part (*) the membrane is not stained at all. A, X 12,000; B, X 25,000.

C, D) *S. mekongi* : the staining has a similar pattern as in *S. japonicum*. C, X 12,000; D, X 25,000.

E) At high magnification, the staining appears as a granulated layer of dense dots on the surface of the membrane (arrows). X 49,000.

F) The membrane of the gynecophoral canal is barely stained. X 19,900.

FIGURE 2

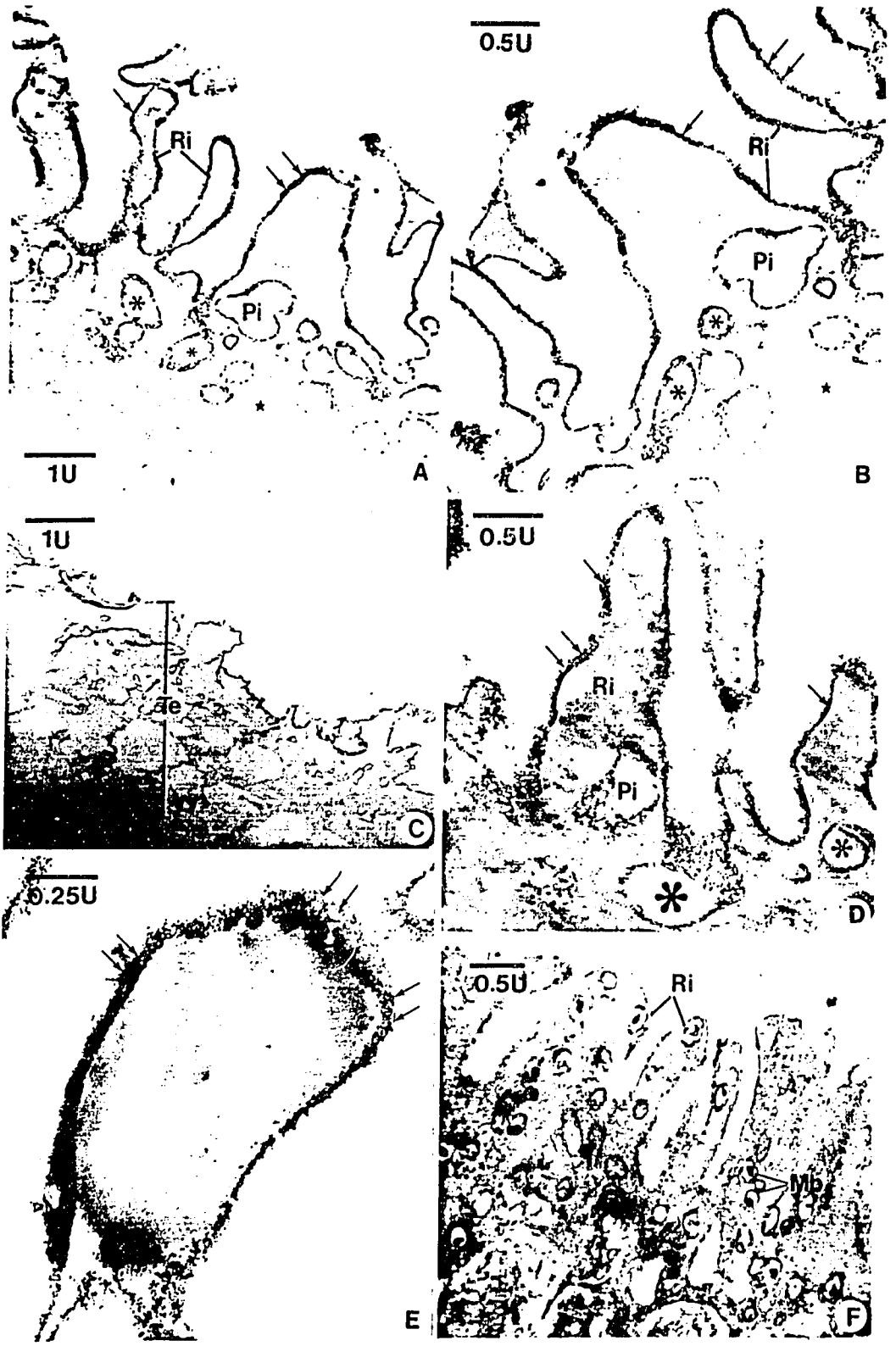


FIGURE 3

TEM OF RUTHENIUM RED STAINING OF CERCARIAE AND SCHISTOSOMULA OF *S. JAPONICUM* (CHINESE) AND *S. MEKONGI*

A , B) *S. japonicum* (A) and *S. mekongi* (B) cercariae : intense staining occurs over the whole width of glycocalyx (Gy), and light staining occurs over the membraneous bodies (Mb) close to the surface. The staining appears as very fine electron-dense deposit on the interlacing strands of the glycocalyx. A, B, X 27,000.

C , D) *S. japonicum* (C) and *S. mekongi* (D) 1-hour schistosomula : intense staining occurs over the remaining thin layer of the glycocalyx (Gy) coating the surface membrane, around microvilli (Mv) and membrane puffs (Mp), and the membrane itself is also intensely stained, while light staining occurs over the membrane sheet or channels(Mc) and membraneous bodies (Mb) close to the surface. C, X 27,000; C inset X 33,900; D, X 33,900.

E , F) *S. japonicum* (E) and *S. mekongi* (F) 12-hour schistosomula : only a thin layer of stained glycocalyx (Gy) remains and appears as an irregular granular layer, while the surface membrane itself is also intensely stained and appears as a black continuous line (arrows). E, X 33,900; F, X 27,000; F inset X 33,900.

FIGURE 3

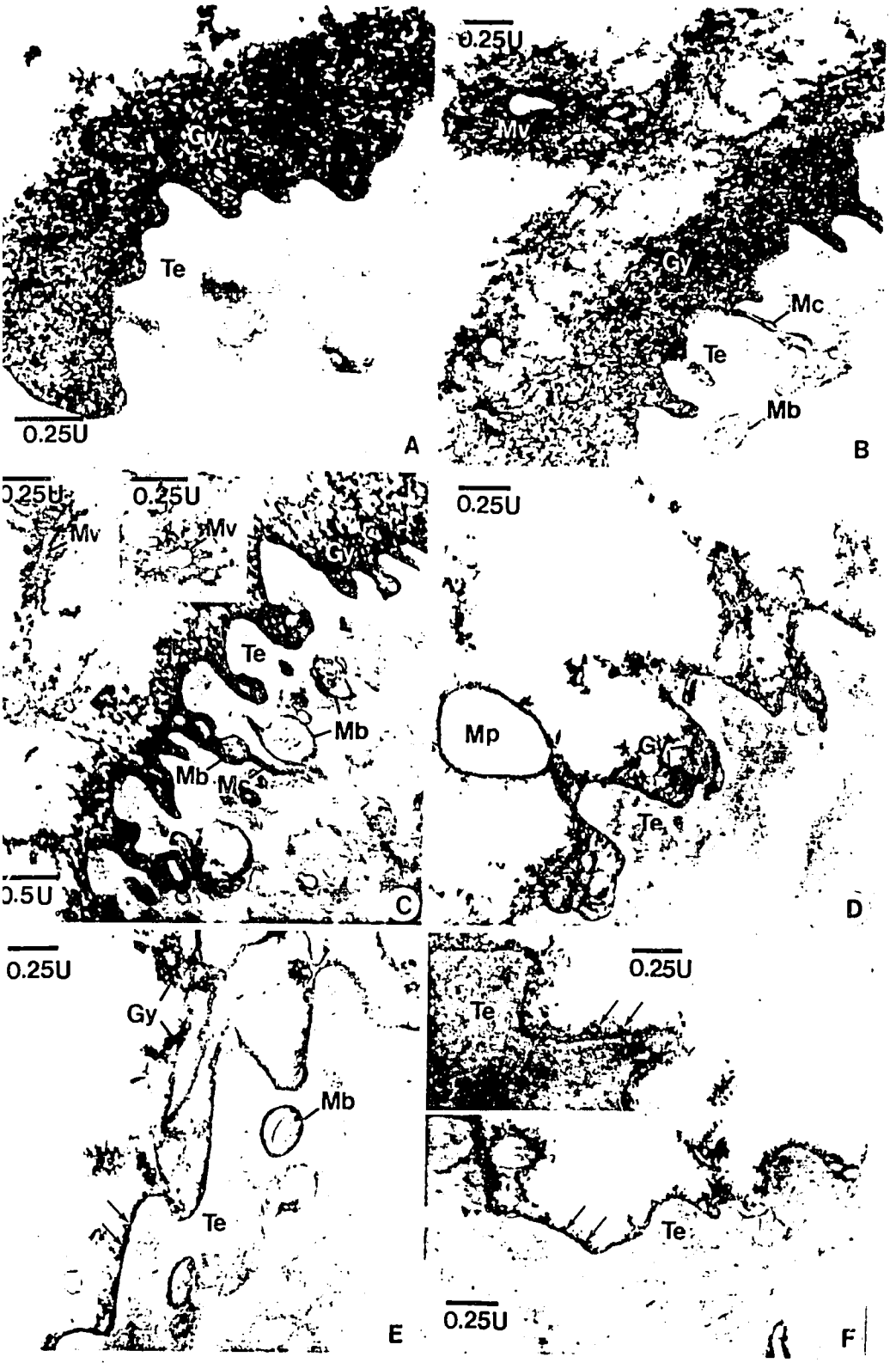

Gy
Te
0.25U
A
0.25U
Mv
Gy
Mc
Te
Mb
B
0.25U
0.25U
Mv
Mv
Gy
Te
Mb
Mb
Mc
0.5U
C
0.25U
Mp
Gy
Te
D
0.25U
Gy
Mb
Te
E
0.25U
Te
Te
0.25U
F

FIGURE 4

TEM OF RUTHENIUM RED STAINING OF ADULTS OF *S. JAPONICUM* (CHINESE) AND *S. MEKONGI*

A,B,C) *S. japonicum* : the staining appears on the surface membrane as a thin line of fine black granules (arrows). The membrane of ridges (Ri) is fairly intense, while the membrane of the upper and middle parts (*) of pits (Pi) is lightly stained, and the membrane of the bottom part (*) is not stained at all. A, X 33,900; B, X 28,600; C, X 33,900.

D) *S. japonicum* : the membrane of the gynecophoral canal is only lightly stained (arrows). X 22,600.

E, F) *S. japonicum* the staining has a similar pattern as that of *S. japonicum* as shown in A & B. E & F, X 22,600.

FIGURE 4

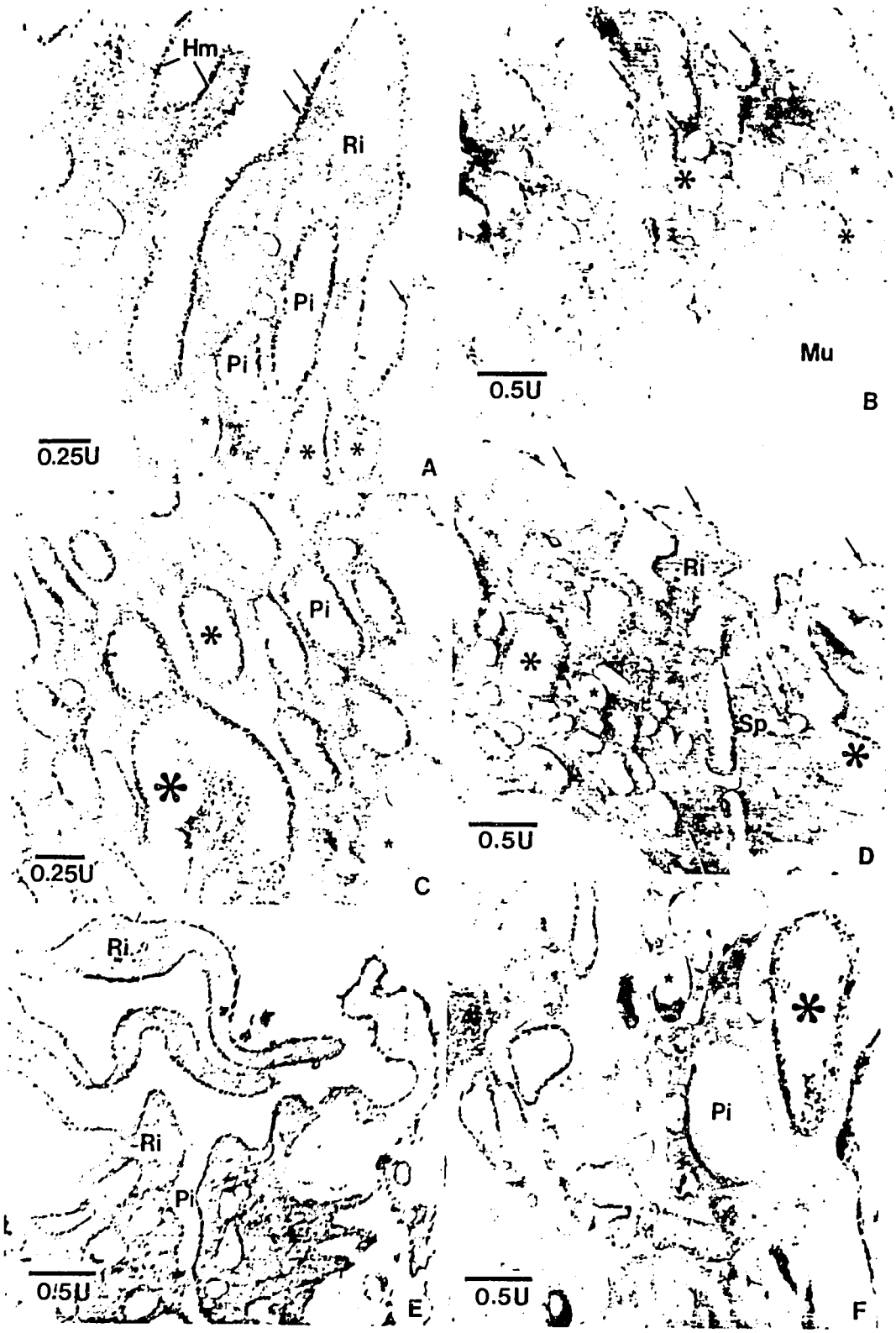

Hm
Ri
Pi
Pi
0.25U
A
Mu
0.5U
B
Pi
0.25U
C
Ri
Sp
0.5U
D
Ri
Ri
Pi
0.5U
E
Pi
0.5U
F

FIGURE 5

TEM OF CON A-HRP STAINING OF CERCARIAE AND SCHISTOSOMULA OF *S. JAPONICUM* (CHINESE) AND *S. MEKONGI*

A, B) *S. japonicum* (A) and *S. mekongi* (B) cercariae : intense staining occurs over the whole thickness of the glycocalyx (Gy) and appears as interlacing strands with dense dots. The surface membrane (Tm) is also intensely stained and appears as a thin black continuous line. A, X 11,300; A inset, X 33,900; B, X 22,600; B inset, X 33,900.

C, D) *S. japonicum* (C) and *S. mekongi* (D) 1-hour schistosomula : the staining of glycocalyx (Gy) appears as beaded lines that project out from the microvilli (Mv) and the surface membrane. The latter and closely associated membrane channels (Mc) are also intensely stained. C, X 18,000; C inset, X 33,900; D, X 16,900; D inset X 33,900.

E, F) *S. japonicum* (E) and *S. mekongi* (F) 12-hour schistosomula : only a thin irregular layer of stained glycocalyx remains, while the surface membrane (Hm) is intensely stained and appears as a black continuous line. E, X 22,600; F, X 22,600; F inset, X 33,900.

FIGURE 5

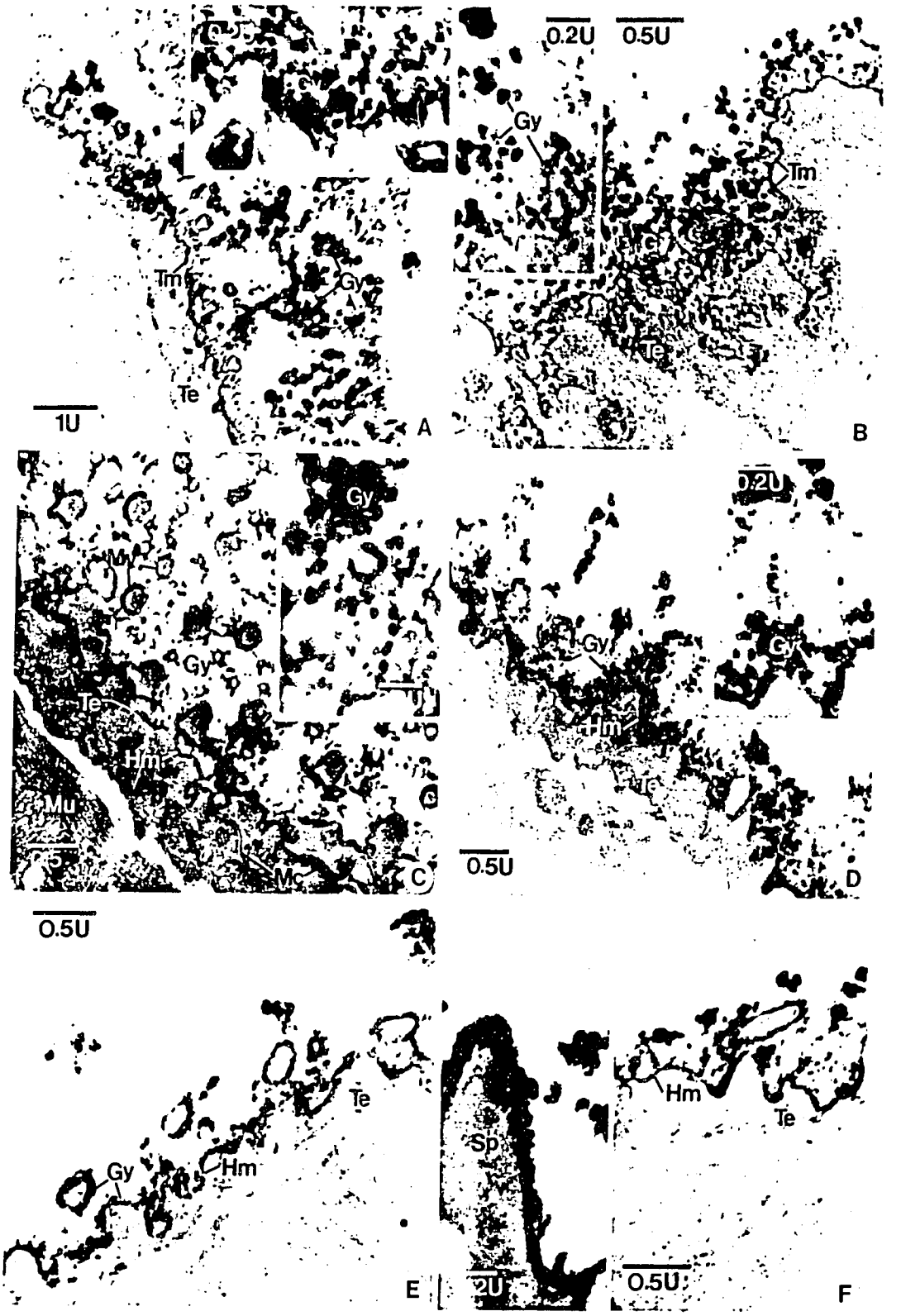

0.2U
0.5U
Gy
Tm
Tm
Gy
Te
Te
1U
A
B
Gy
Mu
Gy
Te
Hm
Mu
Mc
C
0.5U
0.2U
Gy
Gy
Hm
Te
0.5U
D
0.5U
Te
Gy
Hm
E
Sp
2U
Hm
Te
0.5U
F

FIGURE 6

LM AND TEM OF CON A-HRP STAINING OF ADULT *S. JAPONICUM* (CHINESE) (A,B,C,D) AND *S. MEKONGI* (E, F)

A) Control section : there is neither staining on the surface of the tegument (Te) at LM level nor the surface membrane of ridges (Ri) and pits (Pi) at TEM level. A, X 1,000, A inset X 13,300.

B) Con A-HRP stain : the staining is very intense over the tegument of the dorsal and lateral aspects (Do), while very light or non-existent on the tegument of the gynecophoral canal (Gn).B X 1,000.

C) At high magnification, the staining of the membrane (Hm) appears as a continuously black line which may actually be a row of closely-packed dense dots (arrows). X 26,600.

D) The membrane of the gynecophoral canal is only sparingly stained (Hm - arrows). X 13,300.

E) Con A-HRP : the staining is intense on the surface membrane (Hm) of ridges (Ri) and decreases toward pits (Pi). The membrane of the middle part of pits (*) is stained while that of the bottom part (*) is not. The staining appears as a dense line of closely packed dots (arrows). X 13,300.

F) Con A-HRP : the membrane of the gynecophoral canal is only lightly stained. X 13,300.

FIGURE 6

0.5U
Mu
Pi
Mu
Do
Te
A
Gn
B
1U
Ri
Hm
Hm
Pi
Pi
0.25U
Mu
C
D
Hm
Mu
Pi
E
Ri
Pi
Mu
F

FIGURE 7

TEM OF WGA-HRP STAINING OF CERCARIAE AND SCHISTOSOMULA OF *S. JAPONICUM* (CHINESE) AND *S. MEKONGI*

A, B) *S. japonicum* (A) and *S. mekongi* (B) cercariae : the staining is intense on the whole width of glycocalyx (Gy) particularly on the peripheral part (in B), and appears as strands of dense dots. The surface membrane ((Tm) is also lightly stained. A, 27,000; A inset, X 33,900; B X 17,000; B inset X 33,900.

C, D) *S. japonicum* (C) and *S. mekongi*(B) 1-hour schistosomula : a thin layer of intensely stained glycocalyx (Gy) remains around microvilli (Mv) and on the surface membrane. The membrane itself is also intensely stained. C X 17,000; C inset, X 33,900; D, 17,000; D inset, X 17,000.

E, F) *S. japonicum* (E) and *S. mekongi* (F) 12-hour schistosomula : in *S.japonicum* only a thin continuous line of surface membrane (Hm) is stained, while in *S. mekongi* a thin irregular layer of glycocalyx (Gy) remains while the surface membrane (Hm) is also intensely stained. E, X 22,600; F, X 17,000; F inset, X 33,900.

FIGURE 7

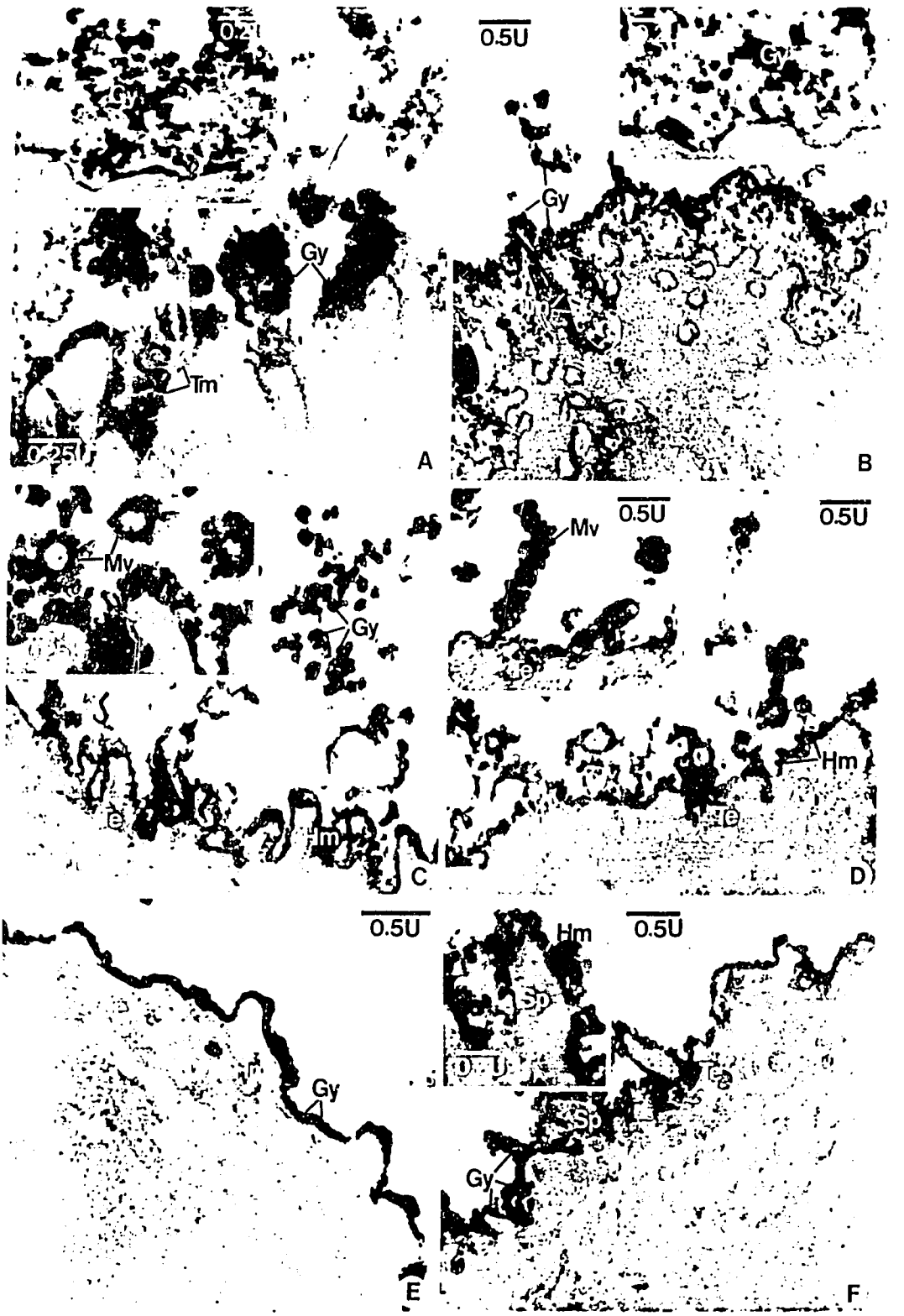


FIGURE 8

LM AND TEM OF WGA-HRP STAINING OF ADULT *S. JAPONICUM* (CHINESE) (A,B,C) AND *S. MEKONGI* (D,E,F)

A) At LM level : the staining is very intense on the tegument (Te) of dorsal-lateral aspects (Do), while that of the gynecophoral canal (Gn) is very lightly stained. A, X 400; A inset, X 1,000.

B) At TEM level : the staining is intense on the membrane of ridges (Ri) and progressively decreases on the membrane of pits (Pi). At high magnification (in D and E) the stain appears as a homogeneous black line on the ridges (arrows), and as granulated line in the pits (*). X13,300.

C) The membrane of the gynecophoral canal is only lightly stained. X19,900.

D) At LM level : the staining is intense on the tegument (Te) of the dorsal-lateral aspects (Do) but is very light on that of the gynecophoral canal (Gn). A, X 200; inset X 1,000.

E) At TEM level : the staining is very intense on the membrane over ridges (Ri) and pleomorphic papillae (Pp), and appears as homogeneously black layer. The intensity of stain decreases progressively towards the middle (*) and is absent on the bottom (*) parts of pits (Pi). X 13,300.

F) The staining of the membrane of the gynecophoral canal is much less intense than that of ridges, but appears to be slightly more than in *S. japonicum* X 13,300.

FIGURE 8

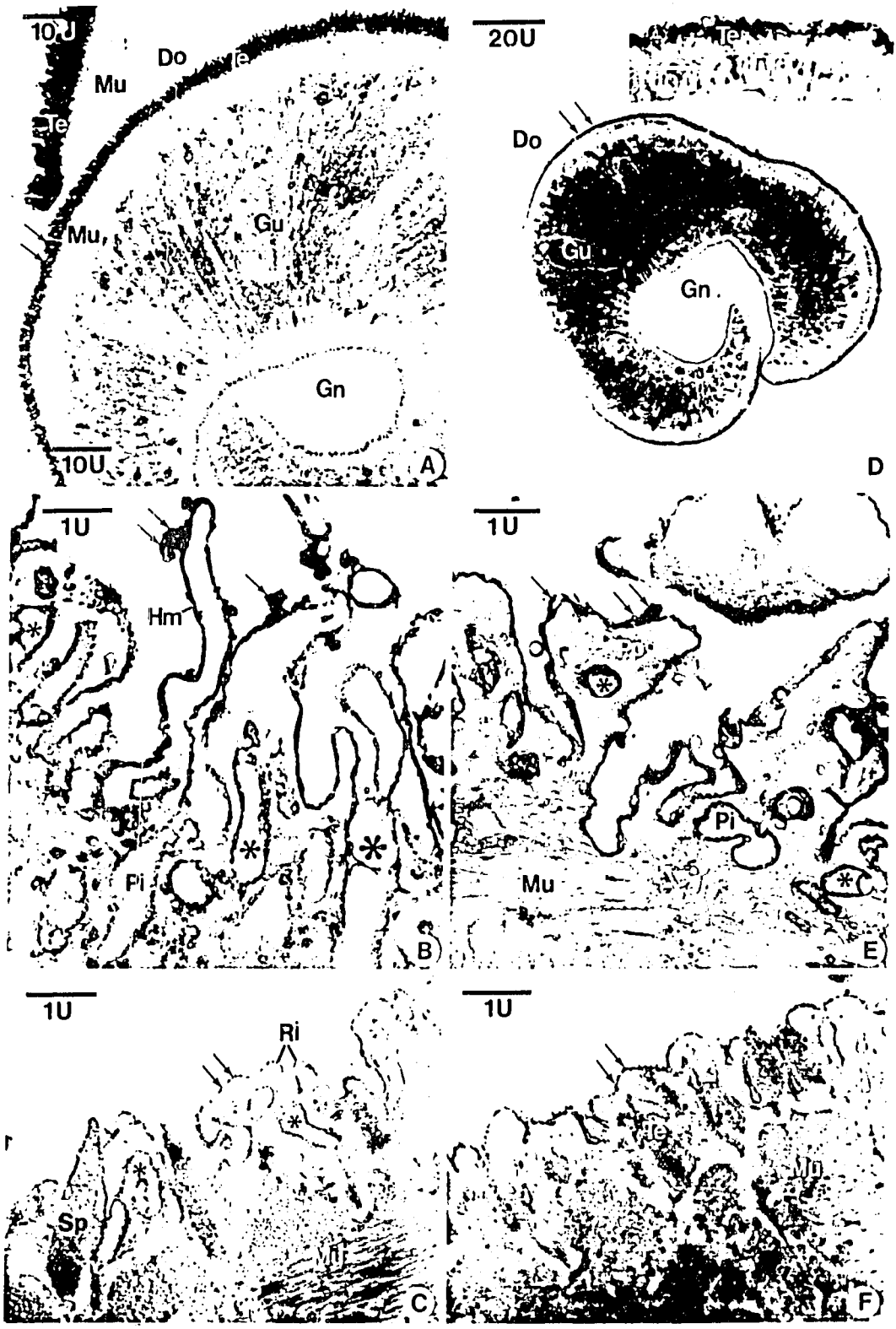

10U
Do
Te
Mu
Te
Mu
Gu
Gn
10U
A
20U
Te
Do
Gu
Gn
D
1U
Hm
Pi
B
1U
Pi
Mu
E
1U
Ri
Sp
C
1U
Te
F

FIGURE 9

LM OF RCA I-, PNA-, AND SBA-HRP STAININGS OF ADULT *S. JAPONICUM* (CHINESE) AND *S. MEKONGI*

A, B, G, H) RCA I -HRP staining of *S. japonicum* (A, B) and *S. mekongi* (G, H): the staining is very intense on the tegument (Te) of the dorsal-lateral aspects (Do), while that of the gynecophoral canal of *S. japonicum* (in A) is fairly intensely stained but that of *S. mekongi* (in G) is not. A, X 200; B, X 400; G, X 400; H, X 1,000.

C, D, I, J) PNA-HRP staining of *S. japonicum* (C, D) and *S. mekongi* (I,J): the staining is very intense on the tegument (Te) of the dorsal-lateral aspects (Do), while that of the gynecophoral canal in *S. japonicum* is lighter stained than RCA I, and that of *S. mekongi* is barely stained. C, X 200; D, X 1,000; I, X 1,000; J, X 400.

E, F, K) SBA-HRP staining of *S. japonicum* (E, F) and *S. mekongi* (K): the staining pattern is similar to PNA in both species. E, X 400; F, X 1,000; K, X 1,000.

FIGURE 9

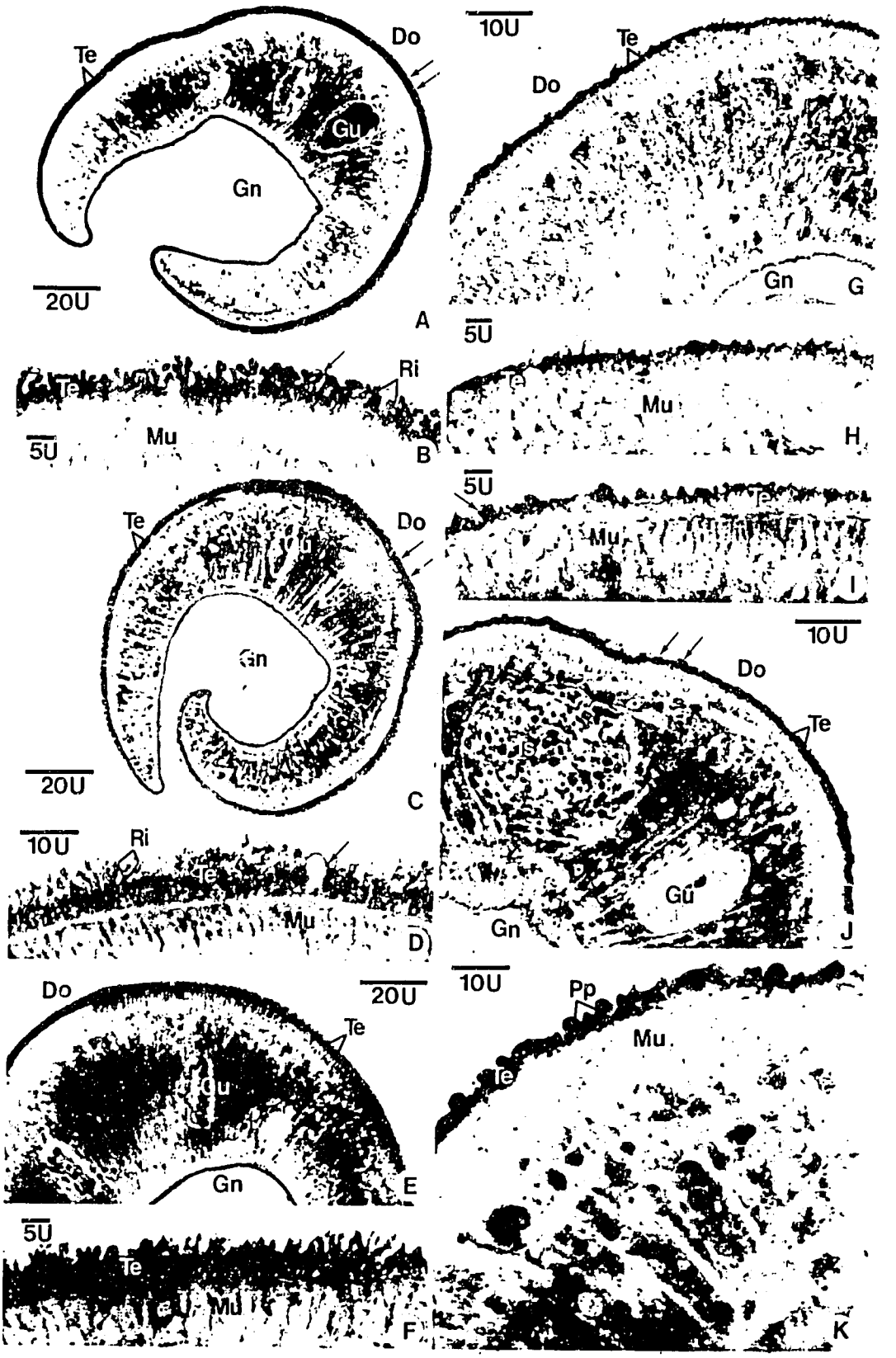

Te
Do
Gu
Gn
20U
A
10U
Te
Do
Gn
G
5U
Ri
Te
Mu
5U
B
Te
Mu
H
5U
Te
Mu
I
Te
Do
Gn
20U
C
10U
Do
Te
Gu
Gn
J
10U
Ri
Te
Mu
D
Do
20U
Te
Gu
Gn
E
10U
Pp
Mu
Te
5U
Te
Mu
F
K

FIGURE 10

TEM OF RCA I-, PNA-, AND SBA-HRP STAININGS OF ADULT *S. JAPONICUM* (A,B,C) AND *S. MEKONGI* (D,E,F)

A) RCA-HRP : in A the staining is very intense on the membrane of ridges, while the membrane of pits (*) is very lightly stained or not at all (*). Inset : the membrane of the gynecophoral canal is more intensely stained than other lectins. A, X 13,300; Inset, X 9,300

B) PNA-HRP : in B the staining of the ridges' membrane is much less intense than RCA I while the pits' membrane is barely stained (*) or not at all (*). Inset : the staining of the membrane of the gynecophoral canal is much less intense than RCA I. B X 9,300; Inset, X 13,300.

C) SBA-HRP : in C the staining of the ridges membrane is much less intense than RCA I and PNA, while the pits' membrane (*), is barely stained. At high magnification (inset) the staining on the ridges' membrane (Hm) appears as a granular layer (arrows). C & Inset, X 19,900.

D) RCA I - HRP : in D the staining appears intense on the membrane over ridges (Ri) and pleomorphic papillae (Pp), while the pits' membrane is lightly stained (*) or not at all (*). Inset : the membrane of the gynecophoral canal is also fairly intensely stained as in *S. japonicum*. A, X 13,300; Inset X 19,900.

E) PNA-HRP : in E the staining of the membrane over ridges (Ri) and pleomorphic papillae (Pp) is less intense than RCA I, while the pits' membrane is barely stained. Inset: the staining of gynecophoral canal's membrane is very light in comparison to RCA I. E & Inset X 13,300.

F) SBA-HRP : in F the staining of the membrane over ridges and pleomorphic papillae is similar to PNA, but the pit membrane (Pi) is very lightly stained in comparison to PNA and RCA I.
Inset : The staining intensity of the gynaecophoral canal membrane is comparable to that of RCA I; F, X 26,000; inset : 13,300.

FIGURE 10

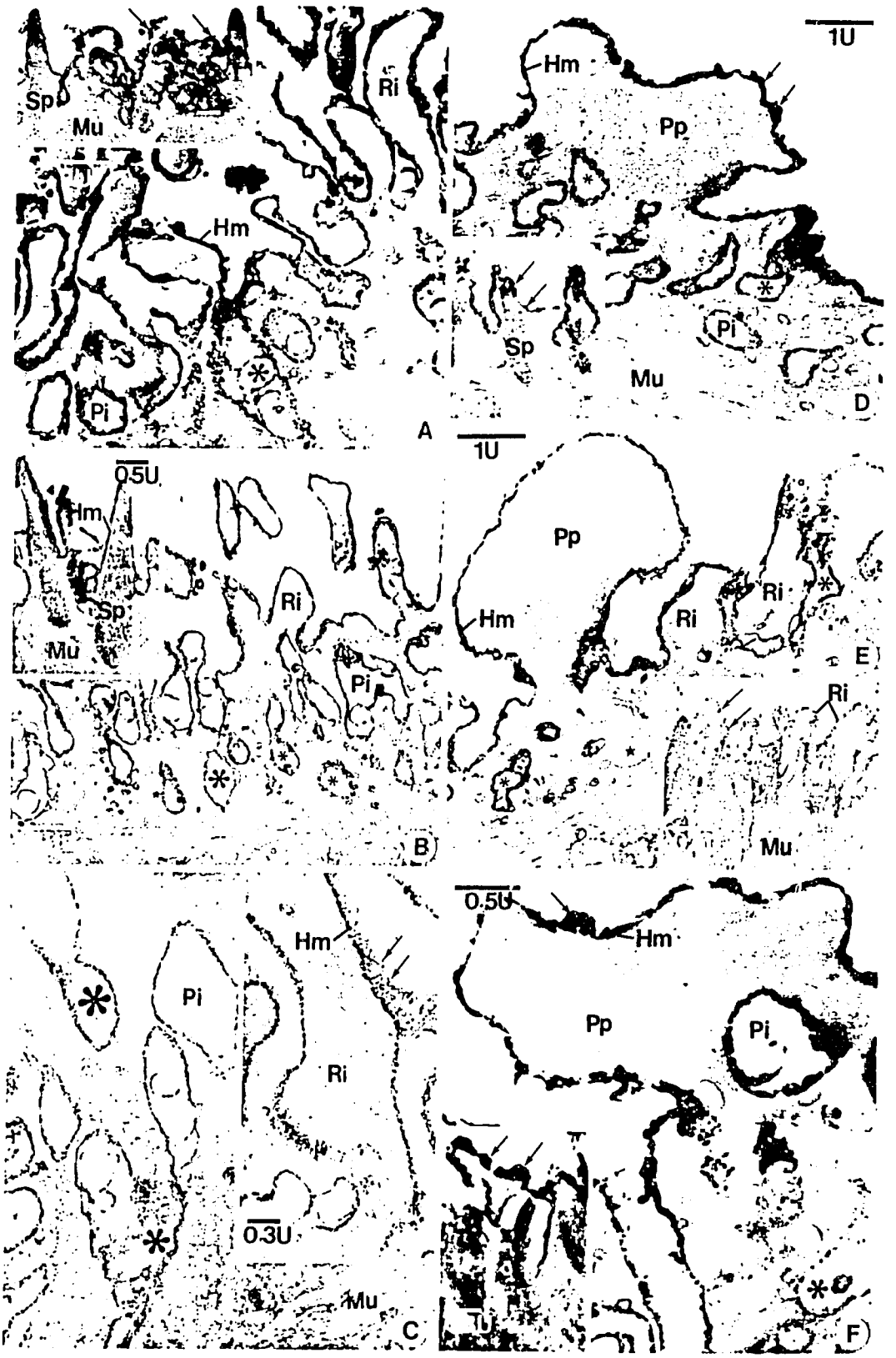

1U
Sp
Mu
Ri
Hm
Pi
A
Hm
Pp
Pi
Sp
Mu
D
1U
0.5U
Hm
Sp
Mu
Ri
Pi
B
Pp
Hm
Ri
Ri
E
Ri
Mu
Hm
Pi
Ri
0.3U
Mu
C
0.5U
Hm
Pp
Pi
F

FIGURE 11

LM AND TEM OF UEA I-HRP STAINING OF ADULT *S. JAPONICUM* AND *S. MEKONGI* AND HIGH RESOLUTION OF LECTIN STAININGS

A, D) At LM level : irregular and patchy staining occurs on the tegument of the dorsal-lateral aspects (Do) and none on the gynecophoral canal (Gn) of both *S. japonicum* (in A) and *S. mekongi* (in D). A, X 400, A inset, X 1000; D X 400, D inset X 1000.

B, C) *S. japonicum* (TEM) : in positive areas the staining occurs only on the membrane of the upper half of ridges, while the rest of the surface membrane is very lightly and not stained at all. B, X 9,300. C, X 19,900.

E) *S. mekongi* (TEM):the staining pattern is similar to *S. japonicum*. E, X 13,300.

F, G) The high resolution of Con A-HRP (F) and SBA-HRP (G) stainings : in F,the staining consists of dense globules disposed in a single row on the external surface of the outer membrane, while in G the dense spots intercalate the whole width of the outer membrane. F & G, X 48,000.

FIGURE 11

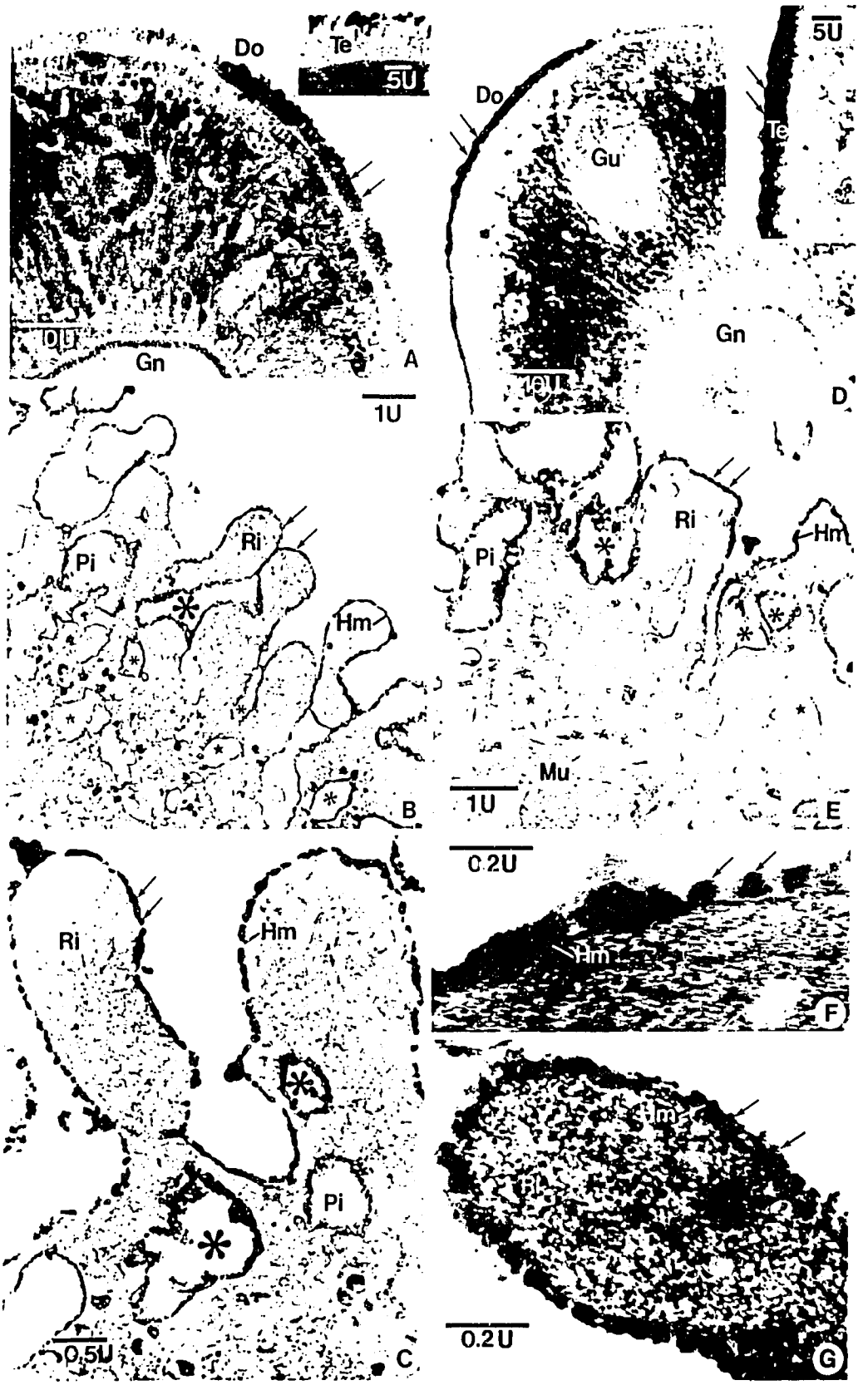

Do
Te
5U
0.1U
Gn
A
1U
Pi
Ri
Hm
B
Do
Gu
Te
5U
Gn
10U
D
Pi
Ri
Hm
Mu
1U
E
Ri
Hm
Pi
0.5U
C
0.2U
Hm
F
Hm
0.2U
G

FIGURE 12

TEM OF FREEZE-THAWED ADULT *S. JAPONICUM* (CHINESE) (A,B) AND *S. MEKONGI* (C,D)

A to D) In freeze-thawed adult parasites the tegument can be detached down to the level of the basement membrane (Bm) on the dorsal-lateral aspects, while leaving the underlying connective tissue and muscular (Mu) layers largely intact. The tegument of the gynecophoral canal can also be detached but not as cleanly as that on the dorso-lateral aspects. A & C, X 15,000; B & D, X 20,000.

FIGURE 12

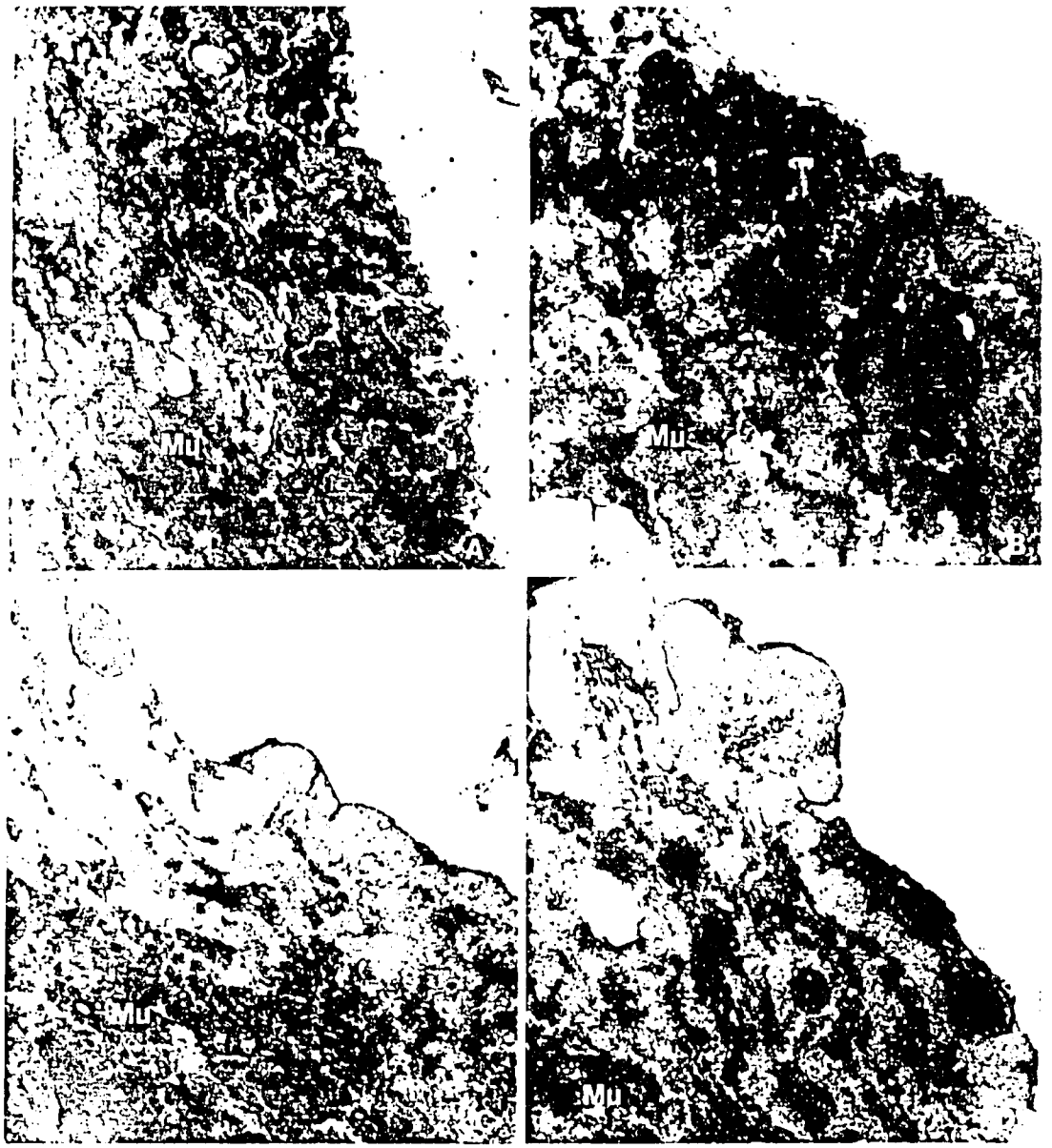
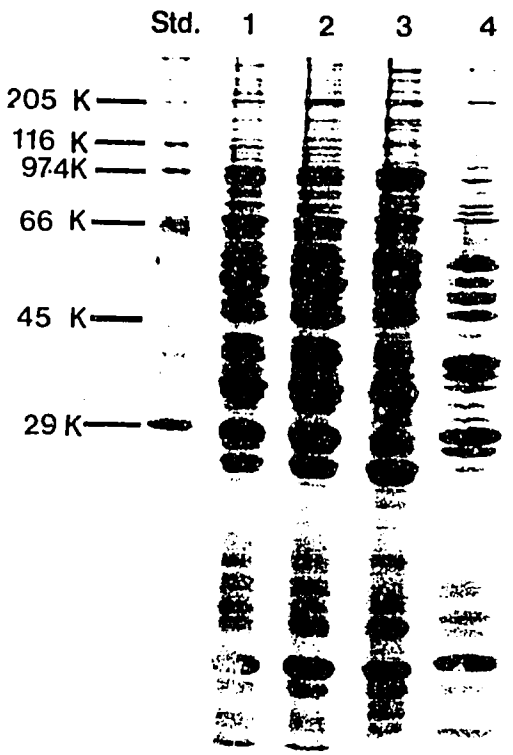


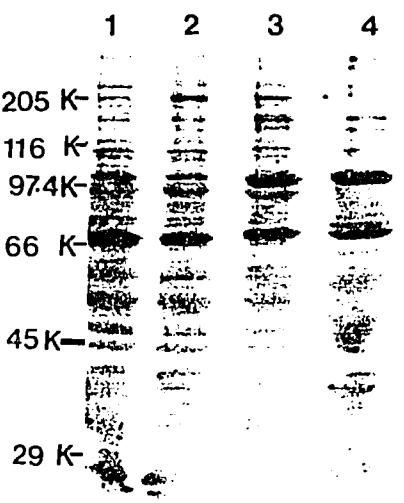
FIGURE 13

A,B) The SDS-PAGE-separated (A) and electroblotted (B) tegument proteins/glycoproteins from freeze-thawed parasites stained with Coomassie Blue: Std - Standard proteins; lane 1 - tegument from *S. japonicum* (Chinese); lane 2 - tegument from *S. japonicum* (Philippine); lane 3 - tegument from *S. mekongi*; lane 4 - tegument from *S. mansoni*.

FIGURE 13


Std.
1
2
3
4
205 K
116 K
97.4K
66 K
45 K
29 K

A


1
2
3
4
205 K
116 K
97.4K
66 K
45 K
29 K

B

FIGURE 14

Glycoproteins from freeze-thawed tegument separated by 12.5% SDS-PAGE electroblotted onto nitrocellulose sheets and stained with lectins-HRP: A , Con A-HRP; B , WGA-HRP; C , UEA-HRP; D , RCA I-HRP; E , PNA-HRP; F , SBA-HRP; lane 1 - tegument of *S. japonicum* (Chinese), lane 2 - *S. japonicum* (Philippine), lane 3 - *S. mekongi*, lane 4 - *S. mansoni*.

FIGURE 14

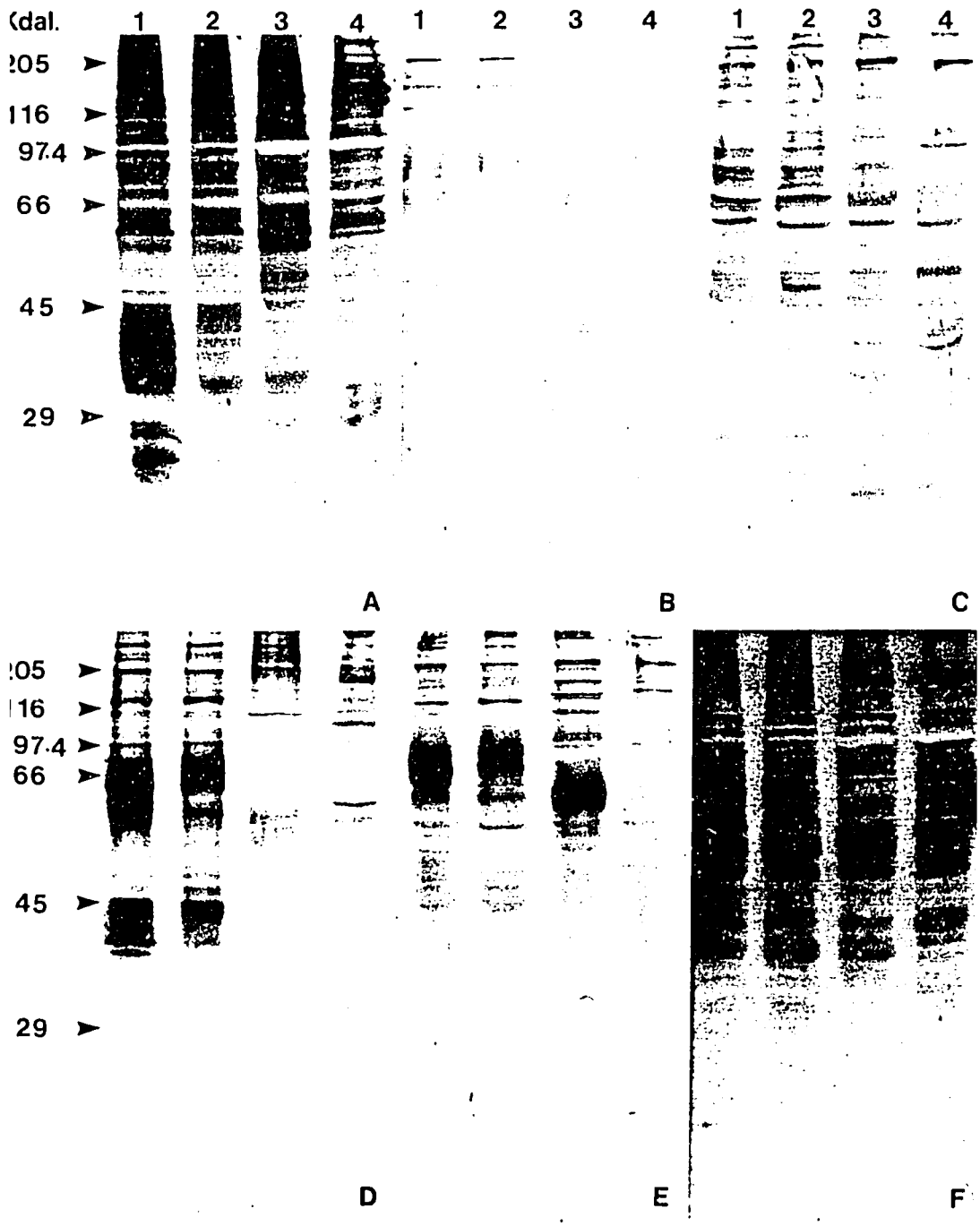


FIGURE 15

Immunoblots of freeze-thawed tegument antigens detected by staining with primary mouse immune sera against (A) *S. japonicum* (Chinese), (B) *S. japonicum* (Philippine), (C) *S. mekongi* and (D) *S. mansoni*, and secondary rabbit anti mouse Ig-HRP. Lanes 1, 2, 3, 4 are antigens derived from the freeze-thawed tegument of *S. japonicum* (Chinese), *S. japonicum* (Philippine), *S. mekongi* and *S. mansoni*, respectively.

FIGURE 15

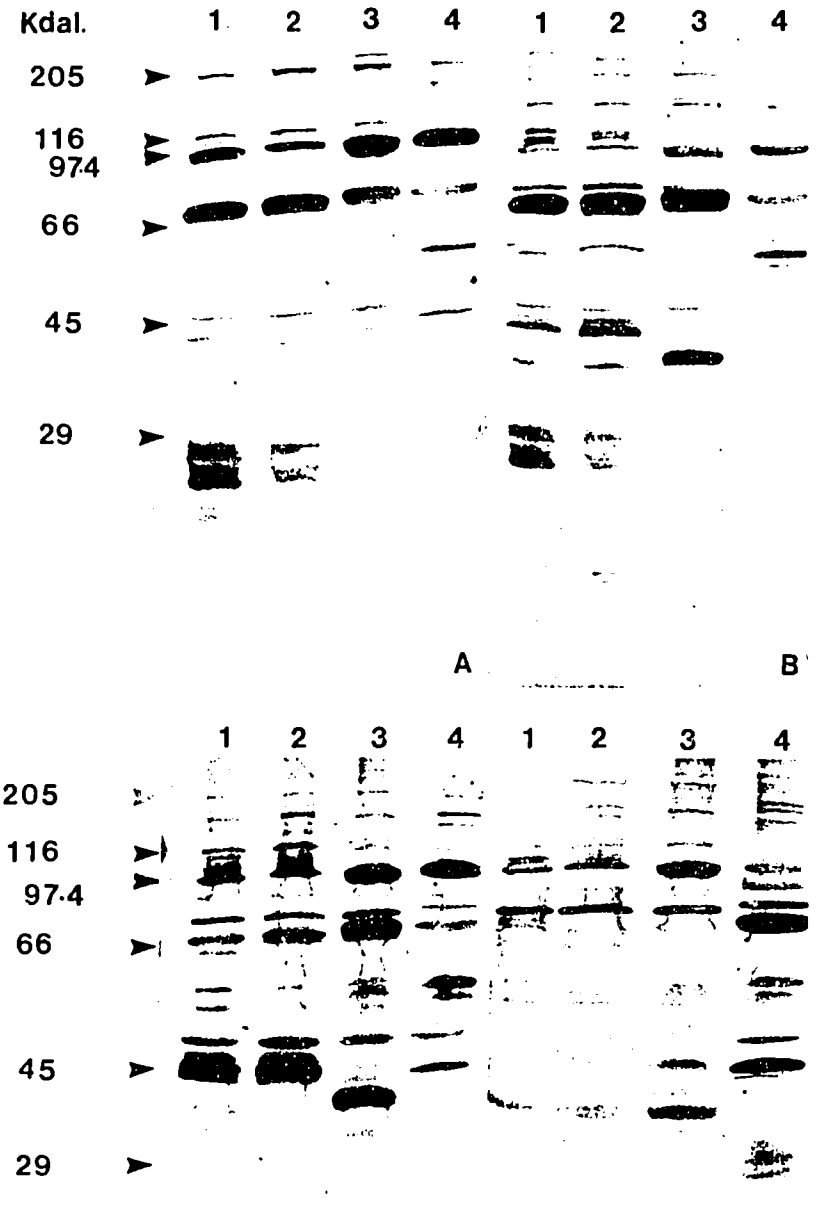

Kdal.
1
2
3
4
1
2
3
4
205
116
97.4
66
45
29
A
B
1
2
3
4
1
2
3
4
205
116
97.4
66
45
29

FIGURE 16

IMMUNOBLOTTING OF FREEZE-THAWED TEGUMENT ANTIGENS FROM *S. JAPONICUM* (CHINESE) - CH, *S. JAPONICUM* (PHILIPPINE) - PH, *S. MEKONGI* - ME AND *S. MANSONI* - MA, DETECTED BY HUMAN IMMUNE SERA AGAINST *S. JAPONICUM* (PHILIPPINE)

$IHPH_1$ - pooled human immune serum

$IHPH_{2-1 \text{ to } 2-5}$ - individual patient immune sera

Std - Standard molecular weights.

FIGURE 16

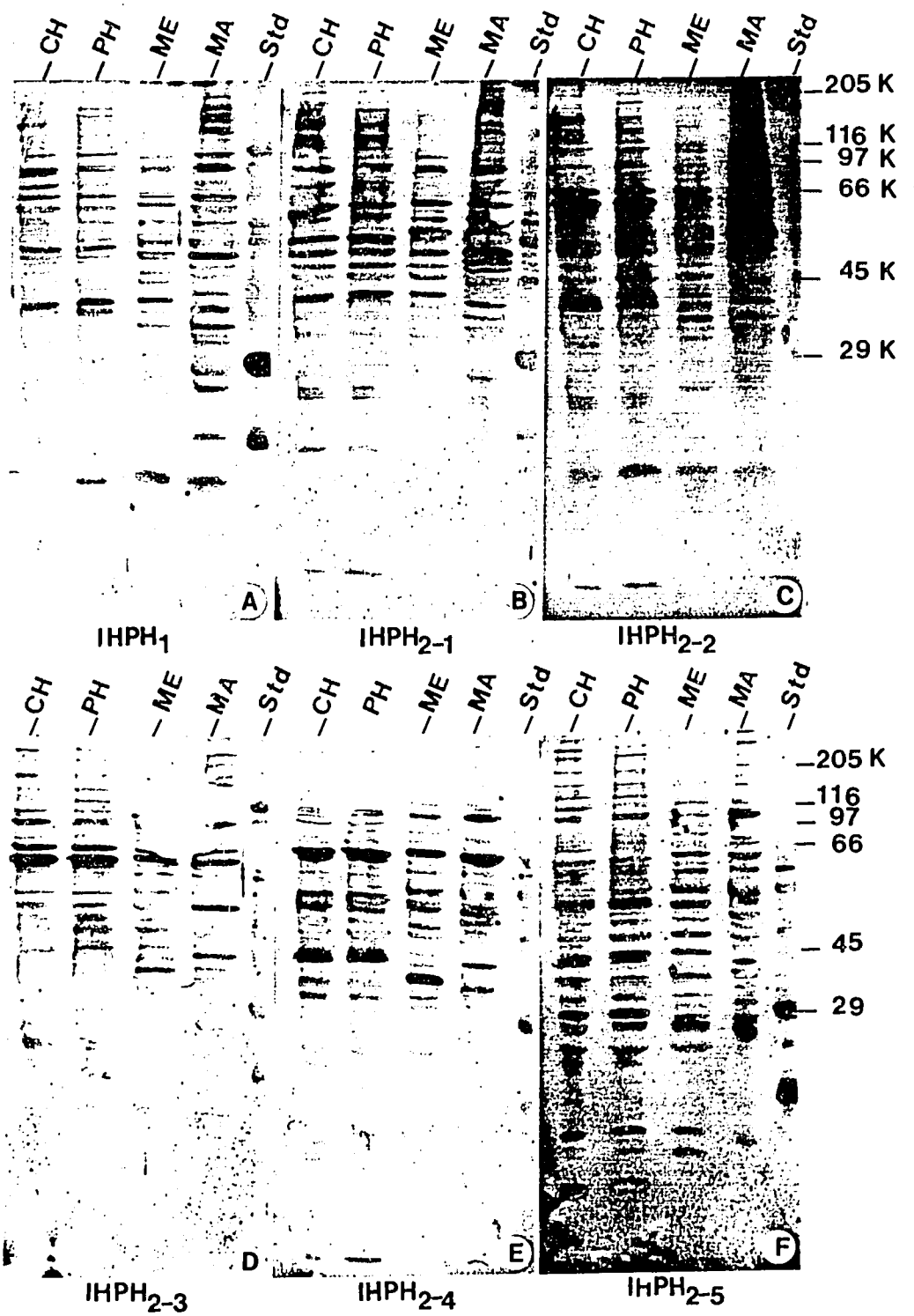

CH
PH
ME
MA
Std
205 K
116 K
97 K
66 K
45 K
29 K
A
B
C
IHPH1
IHPH2-1
IHPH2-2
205 K
116
97
66
45
29
D
E
F
IHPH2-3
IHPH2-4
IHPH2-5

FIGURE 17

Electron microscopic autoradiography (EM-ARG) to detect the ^{125}I-labelling sites on the tegument of *S. japonicum* (Chinese & Philippine) following iodination by iodogen method.

A & B) EM-ARG of the tegument of an adult male *S. japonicum* (Chinese). Notice that most silver grains (arrow heads) lie either just outside or on the membrane of pits (Pt) and ridges (Ri). X 20,000.

FIGURE 17

FIGURE 18

IMMUNOPRECIPITATION PATTERNS OF ^{125}I-LABELLED SURFACE PROTEINS FROM ADULT MALE *S. JAPONICUM* (CHINESE - CH) BY MOUSE IMMUNE SERA AGAINST *S. MANSONI* (ISMA), *S. JAPONICUM* (CHINESE & PHILIPPINE - ISCH & ISPH), *S. HEMATOBIUM* (ISHE), *S. MEKONGI* (ISME), AND HUMAN IMMUNE SERA AGAINST *S. JAPONICUM* (PHILIPPINE - IHPH). THE GELS USED IN ALL CASES WERE 12.5% SDS-PAGE, AND ALL SAMPLES WERE PREABSORBED WITH NON-IMMUNE MOUSE SERUM (NMS).

A & B) Duplicate experiments on *S. japonicum* (Chinese), in "B" different lots of mouse antisera (1, 2, 3, 4) were used.

C & D) *S. japonicum* (Chinese) for comparison with *S. japonicum* (Philippine).

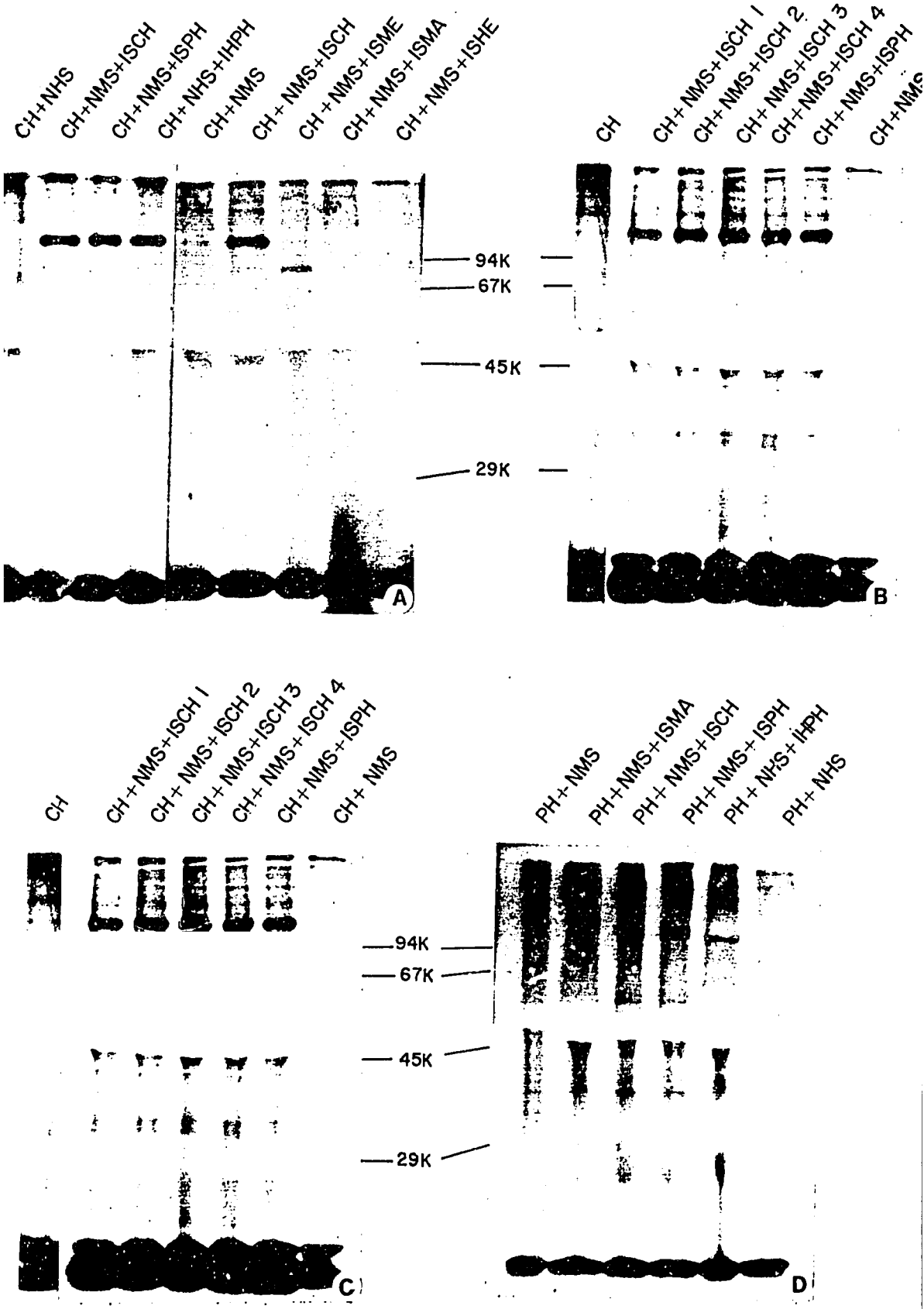
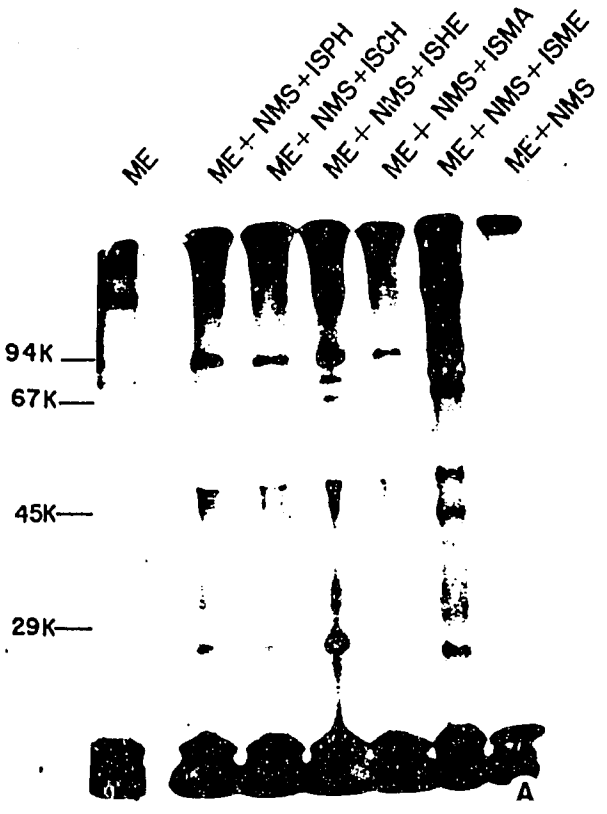

CH+NHS
CH+NMS+ISCH
CH+NMS+ISPH
CH+NHS+IHPH
CH+NMS
CH+NMS+ISCH
CH+NMS+ISME
CH+NMS+ISMA
CH+NMS+ISHE
94K
67K
45K
29K
A
CH
CH+NMS+ISCH 1
CH+NMS+ISCH 2
CH+NMS+ISCH 3
CH+NMS+ISCH 4
CH+NMS+ISPH
B
CH
CH+NMS+ISCH 1
CH+NMS+ISCH 2
CH+NMS+ISCH 3
CH+NMS+ISCH 4
CH+NMS+ISPH
CH+NMS
C
PH+NMS
PH+NMS+ISMA
PH+NMS+ISCH
PH+NMS+ISPH
PH+NHS+IHPH
PH+NHS
D

FIGURE 18

FIGURE 19

IMMUNOPRECIPITATIONS OF ^{125}I-LABELLED SURFACE PROTEINS FROM ADULT MALE : A - *S. MEKONGI* (ME), AND B & C - *S. MANSONI* (MA) BY ISMA, ISCH, ISPH, ISME, ISHE AND IHPH. ALL SAMPLES WERE PREABSORBED WITH NORMAL MOUSE SERUM (NMS), AND THE GELS USED WERE 12.5% SDS-PAGE.

FIGURE 19


ME
ME+NMS+ISPH
ME+NMS+ISCH
ME+NMS+ISHE
ME+NMS+ISMA
ME+NMS+ISME
ME+NMS
94K
67K
45K
29K
A

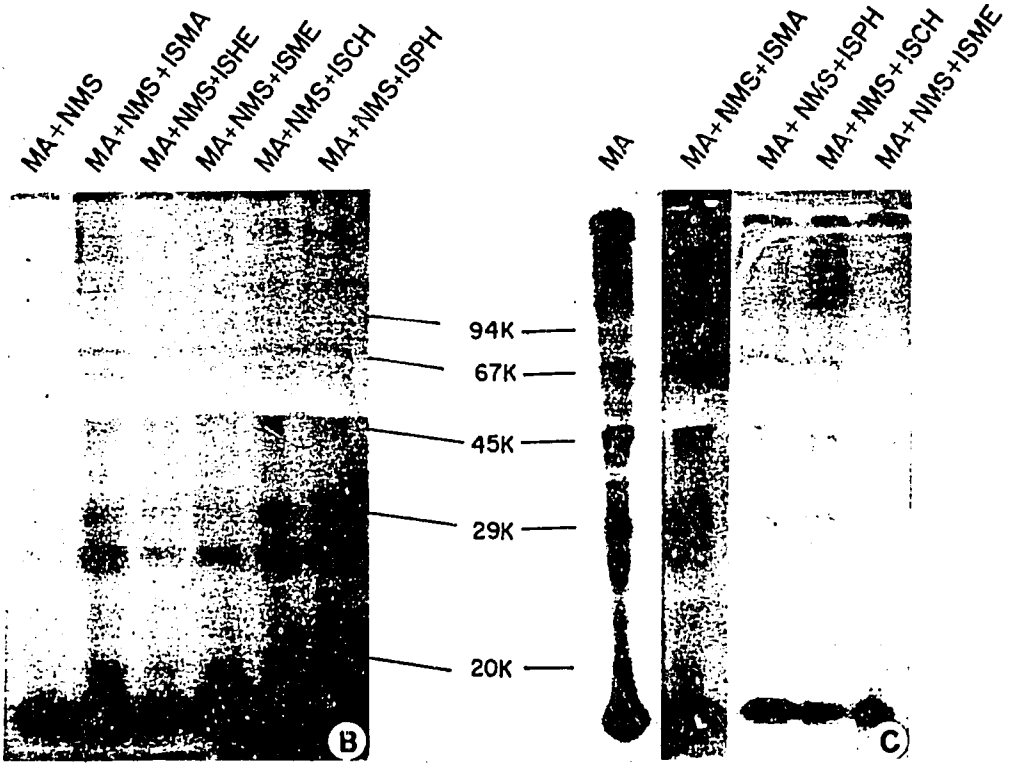

MA+NMS
MA+NMS+ISMA
MA+NMS+ISHE
MA+NMS+ISME
MA+NMS+ISCH
MA+NMS+ISPH
MA
MA+NMS+ISMA
MA+NMS+ISPH
MA+NMS+ISCH
MA+NMS+ISME
94K
67K
45K
29K
20K
B
C

FIGURE 20

AUTORADIOGRAM OF TLC-ANALYSIS OF POLAR AND NON-POLAR LIPIDS EXTRACTED FROM THE FREEZE-THAWED TEGUMENT OF *S. JAPONICUM* (CHINESE)

Lane 1 - polar lipid (aqueous extract)

Lane 2 - non-polar lipid (hexane extract)

FIGURE 20

1 2

FIGURE 21

IMMUNOBLOTTING OF ANTIGENS DERIVED FROM CERCARIAE (CCH), SCHISTOSOMULA (SCH) AND ADULT TEGUMENT (CH) OF *S. JAPONICUM* (CHINESE) AND OF *S. MANSONI* (CMA, SMA AND MA), DETECTED BY MOUSE IMMUNE SERA AGAINST *S. JAPONICUM* (CHINESE) - ISCH, *S. JAPONICUM* (PHILIPPINE) - ISPH, *S. MEKONGI* - ISME AND *S. MANSONI* - ISMA.

FIGURE 21

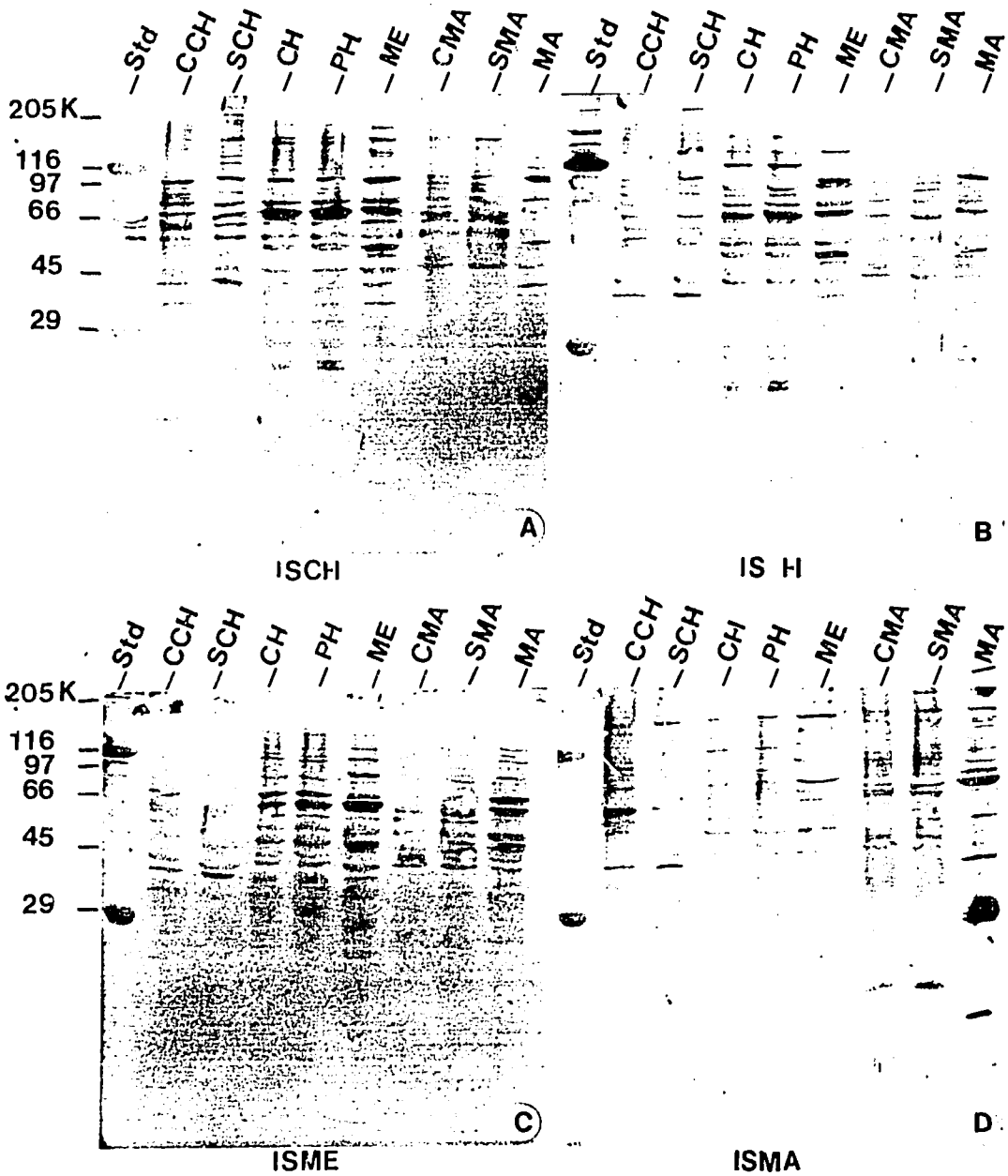

Std
CCH
SCH
CH
PH
ME
CMA
SMA
MA
205K
116
97
66
45
29
A
B
ISCH
IS H
C
D
ISME
ISMA

FIGURE 22

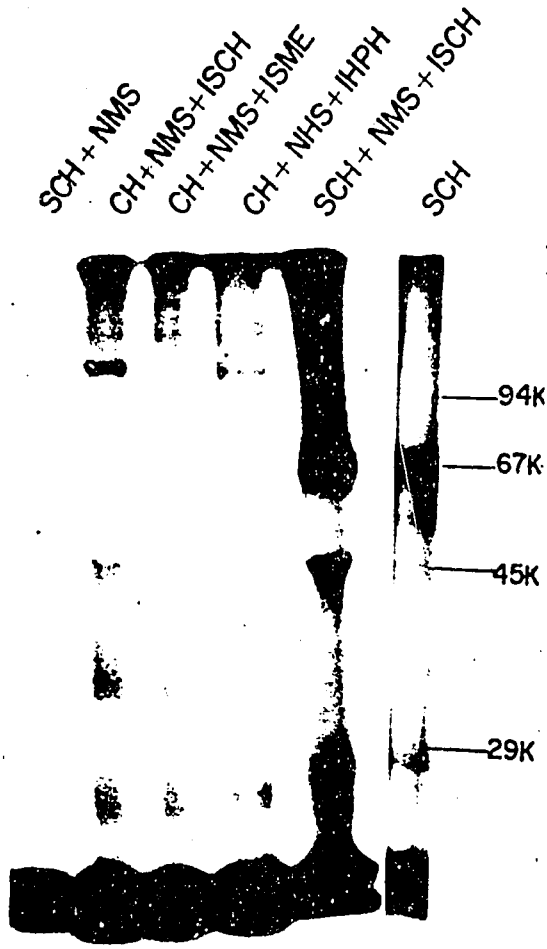
IMMUNOPRECIPITATION OF ^{125}I-LABELLED SURFACE ANTIGENS OF SCHISTOSOMULA OF *S. JAPONICUM* (CHINESE) TREATED WITH MOUSE IMMUNE SERUM AGAINST *S. JAPONICUM* (CHINESE) - ISCH AND HUMAN IMMUNE SERUM AGAINST *S. JAPONICUM* (PHILIPPINE)

CH - ^{125}I-labelled antigens from freeze-thawed tegument of adult *S. japonicum* (Chinese)

SCH - ^{125}I-labelled antigens from schistosomula

NMS - Normal mouse serum

FIGURE 22


SCH + NMS
CH+NMS+ISCH
CH+NMS+ISME
CH + NHS+IHPH
SCH + NMS + ISCH
SCH
94K
67K
45K
29K

A

FIGURE 23

IMMUNOFLUORESCENCE OF CERCARIAE AND 6-HOUR SCHISTOSOMULA OF *S. JAPONICUM* (CHINESE) AND *S. MEKONGI* STAINED WITH PRIMARY MOUSE IMMUNE SERA AGAINST CORRESPONDING SPECIES, AND SECONDARY RABBIT ANTI MOUSE Ig-FITC

A, B) Negative control in cercariae (A) and schistosomula (B) of *S. japonicum* : the background fluorescence is very light. A & B, X 660; A inset & B inset, X 330.

C, D) *S. mekongi* stained with mouse anti *S. mekongi* : in C strong fluorescence is present over the whole surface of cercaria head (Hd) and body (Bo) and becomes more intense in schistosomula (in D), particularly on the dorso-lateral aspect The surface of the tail is only lightly fluorescent. C & D, X 660; D inset X 330.

E, F) *S. japonicum* stained with mouse anti *S. japonicum* : strong fluorescence is present over the surface of dorso-lateral aspect of the head (Hd) and body of cercariae (in E) and schistosomula (in F), while the surface of the tail (Ta) is lightly fluorescent. E & F, X 1,320; E inset X 330.

FIGURE 23

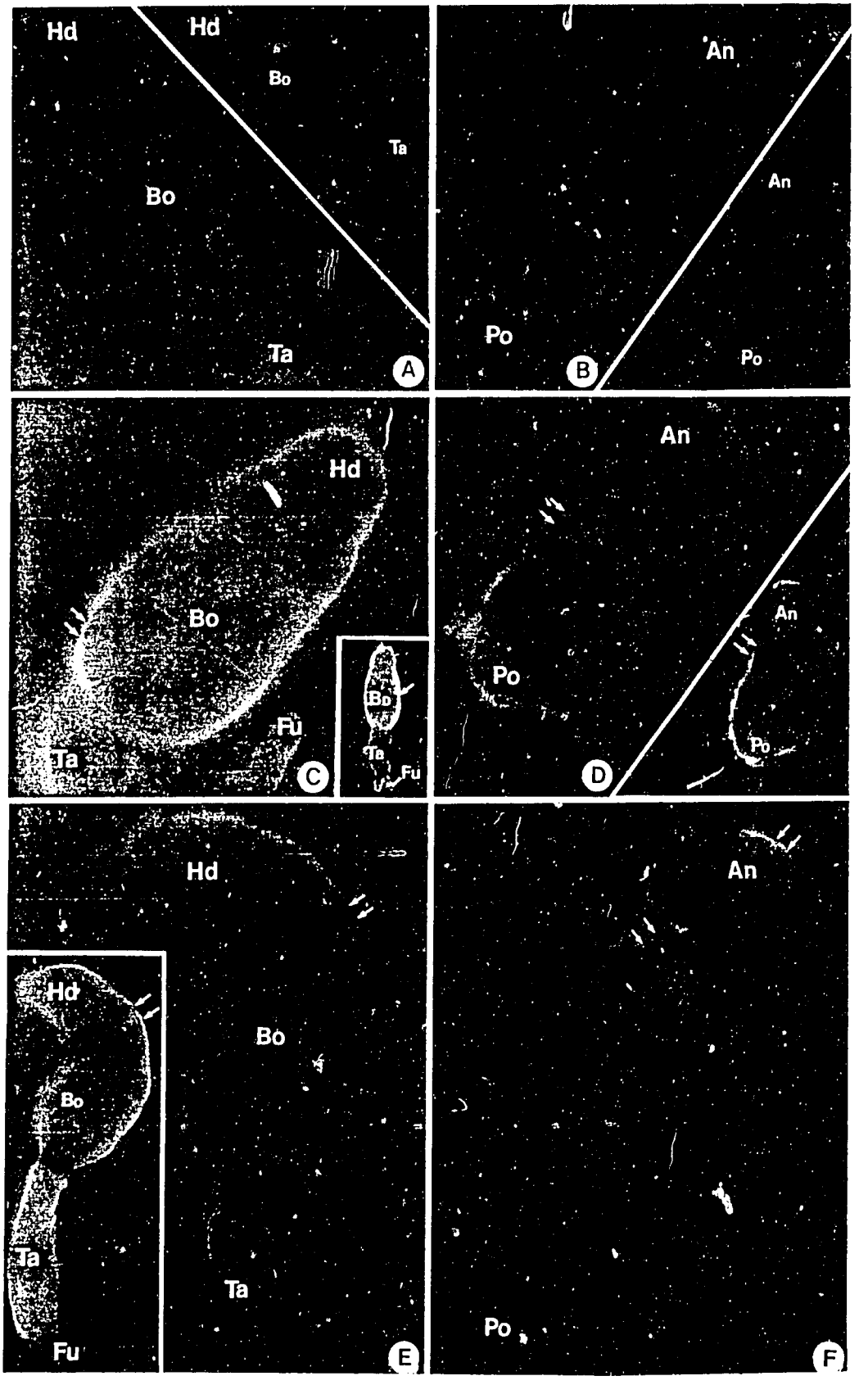

Hd
Hd
Bo
Ta
Bo
Ta
A
An
An
Po
B
Po
Hd
Bo
Fu
Ta
C
Bo
Ta
Fu
An
An
Po
Po
D
Hd
Hd
Bo
Bo
Ta
Ta
Fu
E
An
Po
F

FIGURE 24

IMMUNOFLUORESCENCE OF ADULT *S. JAPONICUM* (CHINESE) AND *S. MEKONGI* STAINED WITH PRIMARY MOUSE IMMUNE SERA AGAINST CORRESPONDING SPECIES AND SECONDARY RABBIT ANTI-MOUSE Ig-FITC

A, B) Nagative control : intense autofluorescence is present only over the gut (Gu) and light fluorescence light fluorescence appears as small dots throughout the stroma, but none on the tegument (Te). A, X 330; B, X 660.

C, D) *S. japonicum* stained with mouse anti- *S. japonicum* : the fluorescence is intense on the tegument (Te) of the dorsal-lateral aspects (Do) and less intense on that of the gynecophoral canal (Gn), and in the cytoplasm of a few tegument cells (Tc). At high magnification (in D) the ridges appear to be intensely stained. C, X 660; D & D inset X 1,320.

E, F) *S. mekongi* stained with mouse anti- *S. mekongi* : the fluorescence is strong on the tegument of the dorsal-lateral aspects (Do) and weaker on that of the gynecophoral canal (Gn). At high magnification (in F) both ridges (Ri) and pleomorphic papilla (Pp) appear to be intensely stained. E, X 330; F, X 1320.

FIGURE 24

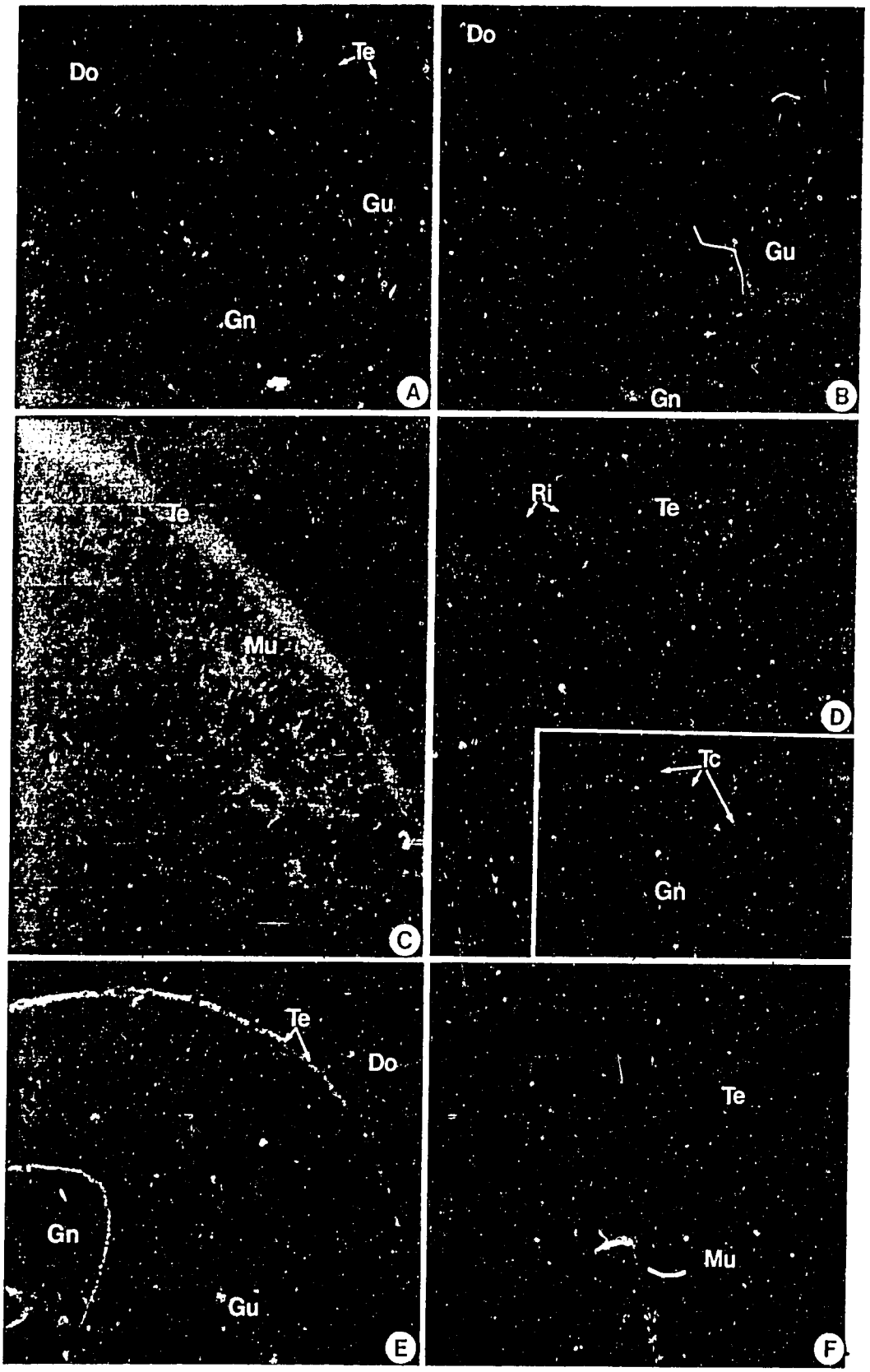


FIGURE 25

IMMUNOFLUORESCENCE OF ADULT *S. MANSONI* STAINED WITH PRIMARY MOUSE IMMUNE SERUM AGAINST *S. MANSONI* AND SECONDARY RABBIT ANTI-MOUSE Ig-FITC.

A,B) Negative control in which normal mouse serum replaces primary antibody : intense fluorescence is present only over the gut epithelium (Gu), and weak fluorescence appears as small dots scatterred throughout the stroma. The tegument (Te) is devoid of fluorescence. A, X 330; B, X 1320.

C,D) *S. mansoni* male stained with mouse anti- *S. mansoni* : the fluorescence is intense over the tegument (Te) of the dorso-lateral aspects (Do), and less intense on that of the gynaecophoral canal (Gn). At high magnification (D) the tubercles (Tb) bearing spines are intensely fluorescent. C, X 330; D, X 1320.

FIGURE 25

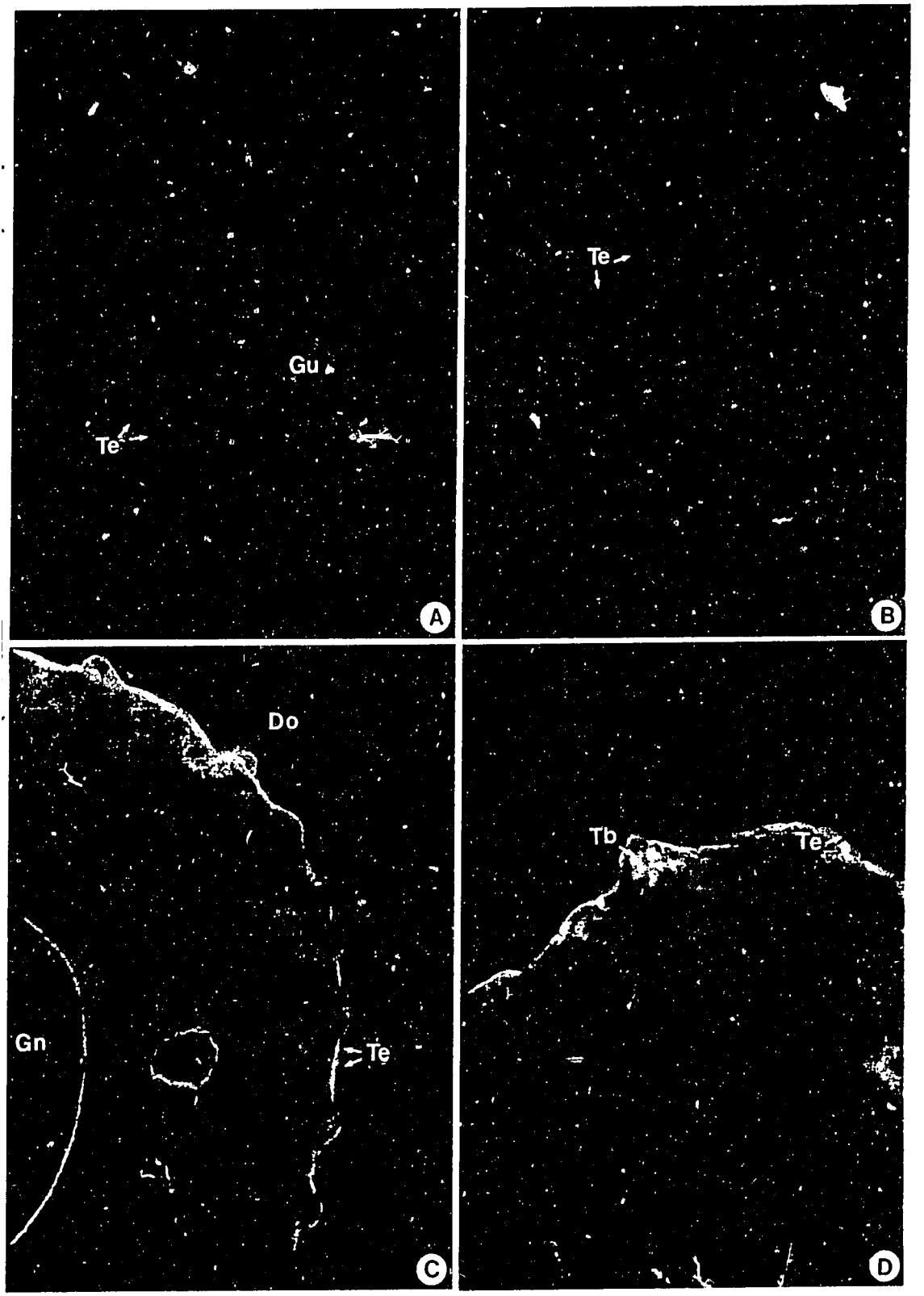


FIGURE 26

IMMUNOFLUORESCENCE OF ADULT *S. JAPONICUM* (CHINESE) (A, B) STAINED WITH PRIMARY MOUSE IMMUNE SERUM AGAINST *S. MANSONI*-ISMA, AND ADULT *S. MANSONI* (C, D) STAINED WITH PRIMARY MOUSE IMMUNE SERUM AGAINST *S. JAPONICUM* (CHINESE)-ISCH

A, B) *S. japonicum* (Chinese) : Intense fluorescence appears over the tegument (Te) on the dorso-lateral surfaces and irregular streaks between muscle cells (arrows). Weaker fluorescence appears on the tegument of the gynaecophoral canal (Gn). A, X 330; B, X1320.

C, D) *S. mansoni* : Moderately intense fluorescence appears over the tegument (Te) the tubercles (Tb) and spines of the dorso-lateral aspects, while only weak fluorescence occurs on the tegument of the gynaecophoral canal (Gn). In general, the fluorescence is weaker than when treated with ISMA. C, X 330; D, X 1320.

FIGURE 26

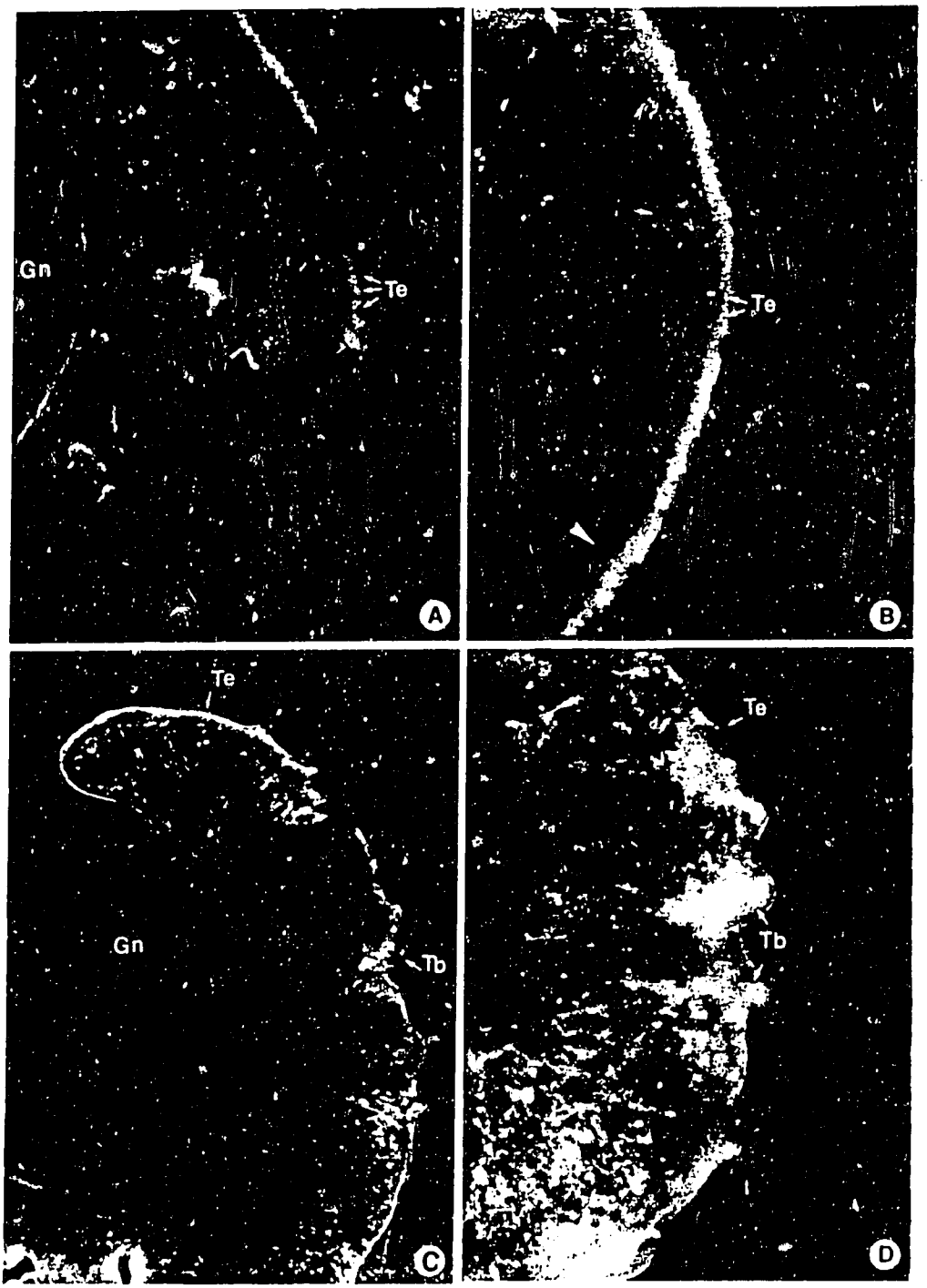


FIGURE 27

IMMUNOELECTRON MICROSCOPY OF THE CERCARIA, 1-HOUR AND 6-HOUR SCHISTOSOMULA, AND ADULT *S.JAPONICUM* (CHINESE) STAINED WITH PRIMARY MOUSE IMMUNE SERUM AGAINST *S.JAPONICUM* AND SECONDARY RABBIT ANTI-MOUSE-Ig-HRP

A) Negative control : 1-hour schistosomule shows no staining. X 19,900.

B) Cercaria : the intense staining occurs on the strands of glycocalyx (Gy), and the tegument surface membrane is only lightly stained. X 19,900.

C) 1-hour schistosomule : the intense staining occurs on both the remaining glyco alyx (Gy) and on the surface membrane (arrows). X 19,900.

D) 6-hour schistosomule : the intense staining occurs on the surface membrane (arrows) and on the very thin layer of glycocalyx (Gy) that still remains on the surface. X 19,900.

E, F) Adult : the staining is intense on the ridges' membrane (Ri - arrows) and decreases progressively towards the pits (Pi,*) while the bottom part of pits (*) may not be stained at all. E, X 20,200 ; F, X 27,000.

FIGURE 27

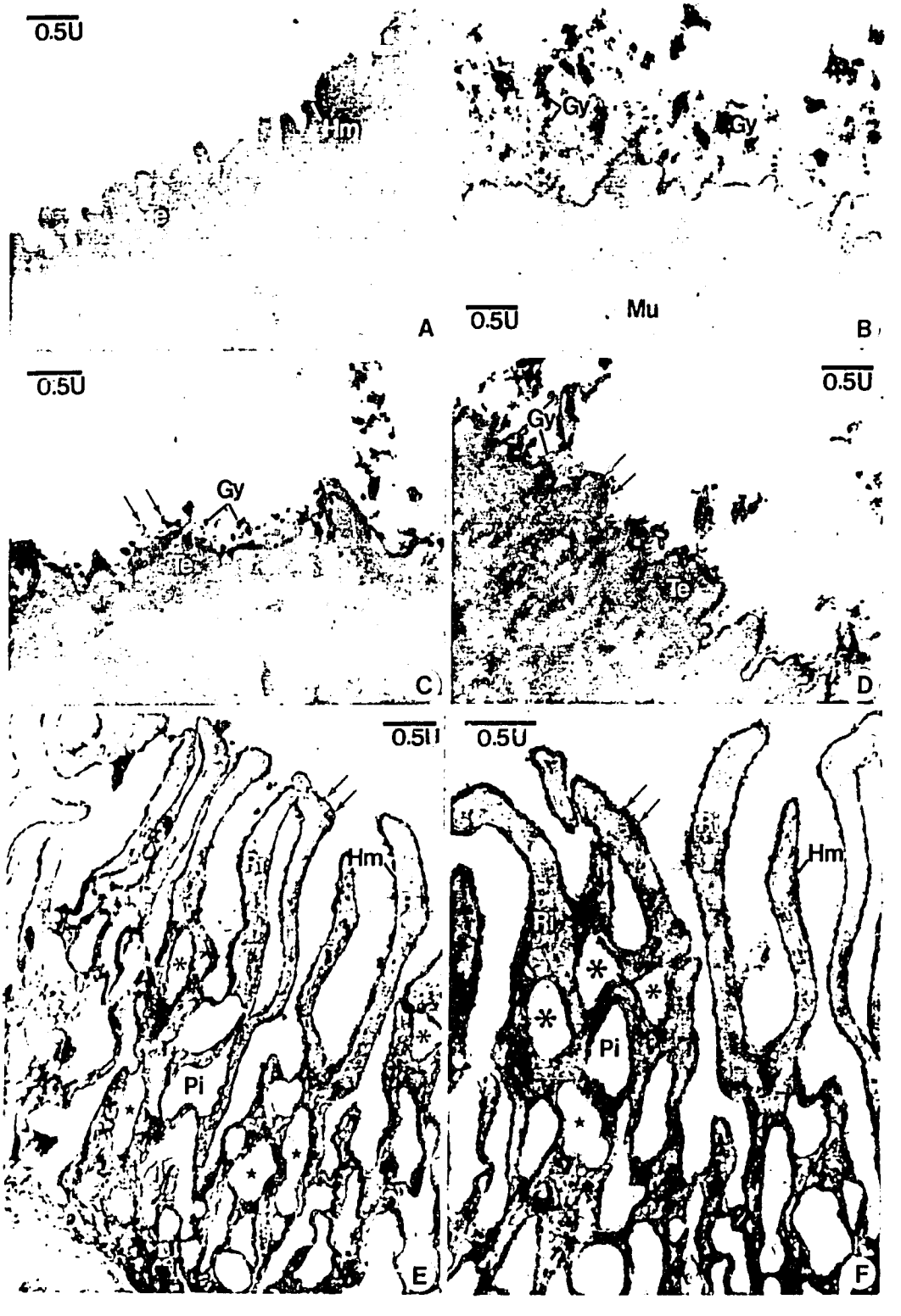
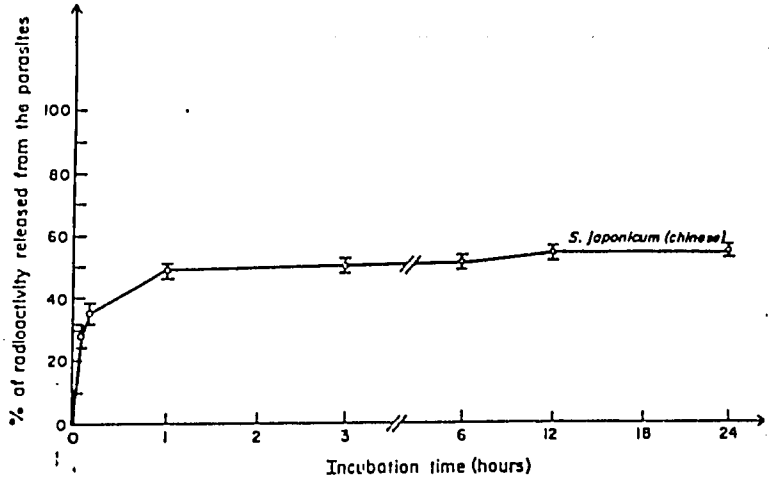

0.5U
Hm
Gy
Gy
A
0.5U
Mu
B
0.5U
Gy
Te
C
0.5U
Gy
Te
D
0.5U
Hm
Ri
Pi
E
0.5U
Hm
Ri
Pi
F

FIGURE 28

A) The release of ^{125}I-labelled surface proteins from adult *S. japonicum* (Chinese) that had been incubated in MEM medium at various time intervals up to 24 hours.

B, C) Autoradiogram of SDS-PAGE of ^{125}I-labelled surface proteins that are released from adult *S. japonicum* (Chinese) into MEM medium from 5 minutes to 24 hours and after.

FIGURE 28


% of radioactivity released from the parasites
100
80
60
40
20
0
S. japonicum (chinese)
0
1
2
3
6
12
18
24
Incubation time (hours)

A

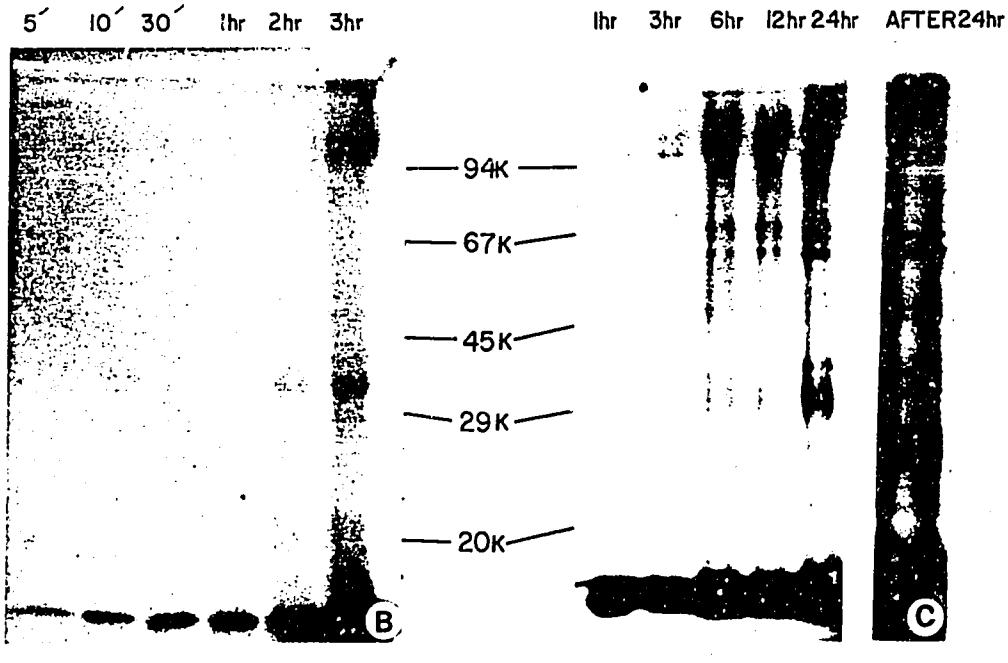

5′
10′
30′
1hr
2hr
3hr
1hr
3hr
6hr
12hr
24hr
AFTER24hr
94K
67K
45K
29K
20K
B
C

FIGURE 29

IMMUNOBLOTTING OF ANTIGENS THAT ARE RELEASED INTO THE MEM MEDIUM FROM ADULT *S. JAPONICUM* (CHINESE) THAT HAD BEEN INCUBATED *IN VITRO* FROM 1 TO 20 HOURS. "CH" ARE THE FREEZE-THAWED TEGUMENT PROTEINS, AND "CH + ISCH" ARE TEGUMENT ANTIGENS THAT ARE DETECTED BY MOUSE IMMUNE SERUM AGAINST *S. JAPONICUM* (CHINESE).

FIGURE 29

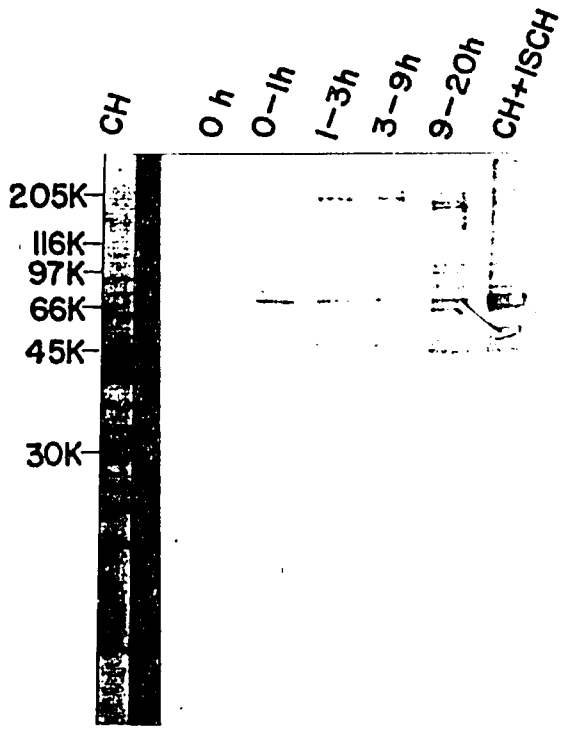
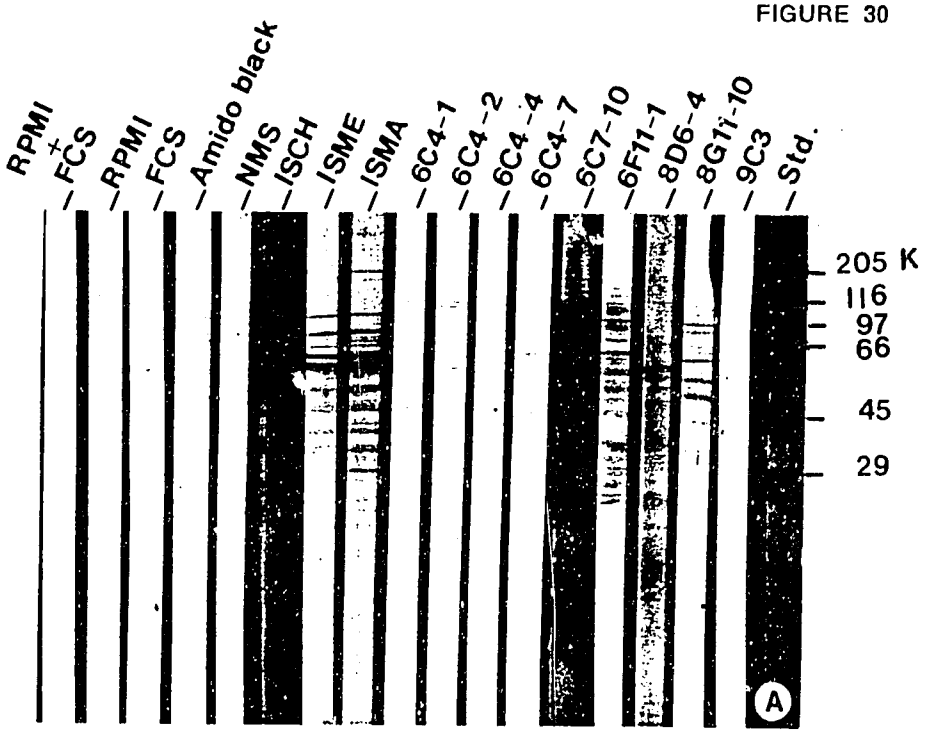


FIGURE 30

IMMUNOBLOTTING OF ANTIGENS FROM FREEZE-THAWED TEGUMENT OF ADULT *S. JAPONICUM* (CHINESE) DETECTED BY MONOCLONAL ANTIBODIES (MAB) FROM GROUPS 1 AND 2 CLONES (CODE NAMES), IN COMPARISON TO THE TEGUMENT ANTIGENS DETECTED BY MOUSE IMMUNE SERA AGAINST *S. JAPONICUM* (CHINESE-ISCH), *S. MEKONGI* - ISME, AND *S. MANSONI* - ISMA

FIGURE 30


RPMI + FCS
RPMI FCS
Amido black
NMS
ISCH
ISME
ISMA
6C4-1
6C4-2
6C4-4
6C4-7
6C7-10
6F11-1
8D6-4
8G11-10
9C3
Std.
205 K
116
97
66
45
29
A

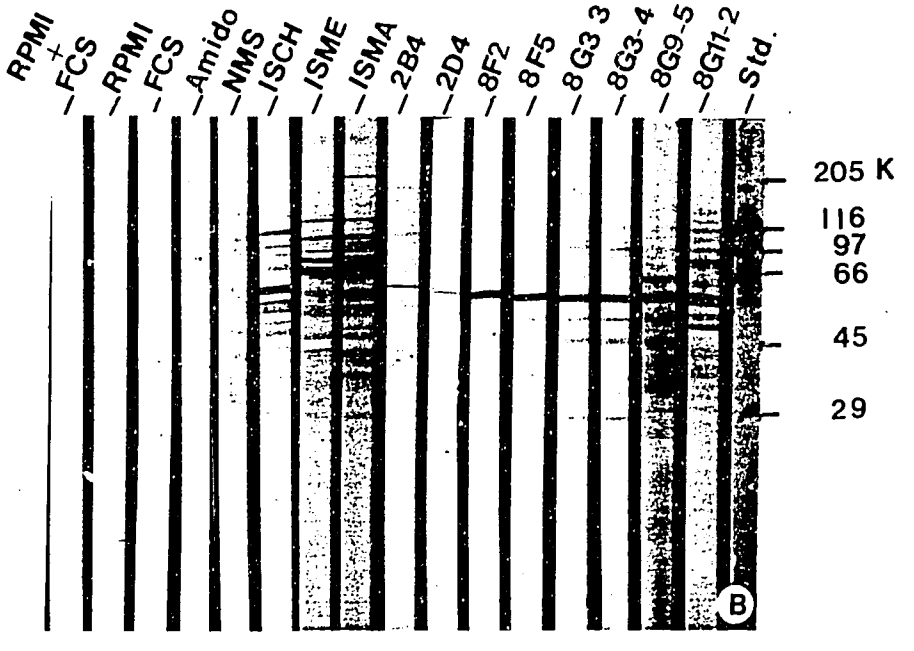
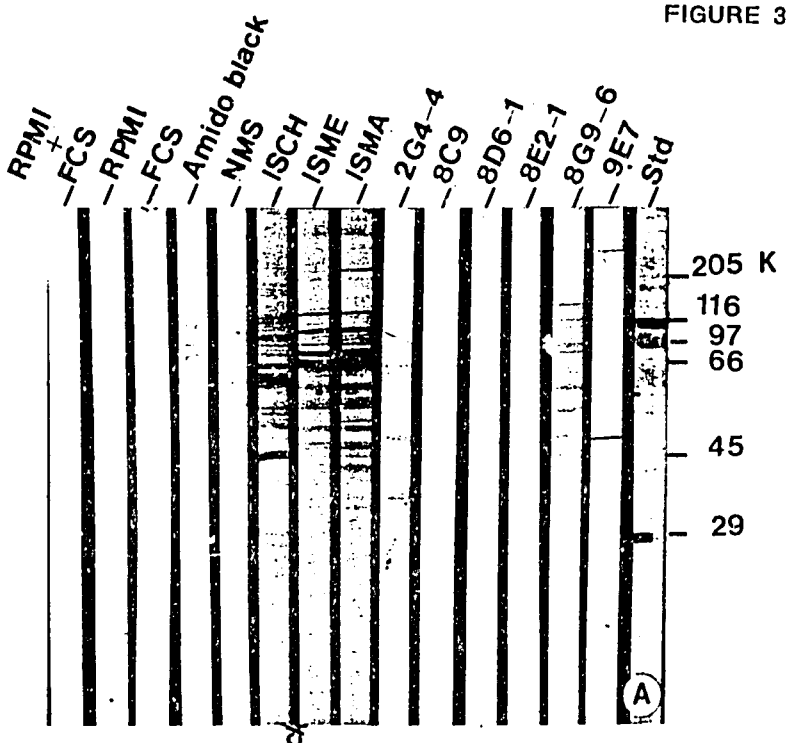

RPMI + FCS
RPMI FCS
Amido
NMS
ISCH
ISME
ISMA
2B4
2D4
8F2
8F5
8G3 3
8G3-4
8G9-5
8G11-2
Std.
205 K
116
97
66
45
29
B

FIGURE 31

IMMUNOBLOTTING OF ANTIGENS FROM FREEZE-THAWED TEGUMENT OF ADULT *S. JAPONICUM* (CHINESE) DETECTED BY MAB FROM GROUPS 3 AND 4 CLONES (CODE NAMES), IN COMPARISON TO THE TEGUMENT ANTIGENS DETECTED BY MOUSE IMMUNE SERA-ISCH, ISME AND ISMA.

FIGURE 31


RPMI + FCS
RPMI
FCS
Amido black
NMS
ISCH
ISME
ISMA
2G4-4
8C9
8D6-1
8E2-1
8G9-6
9E7
Std
205 K
116
97
66
45
29
A

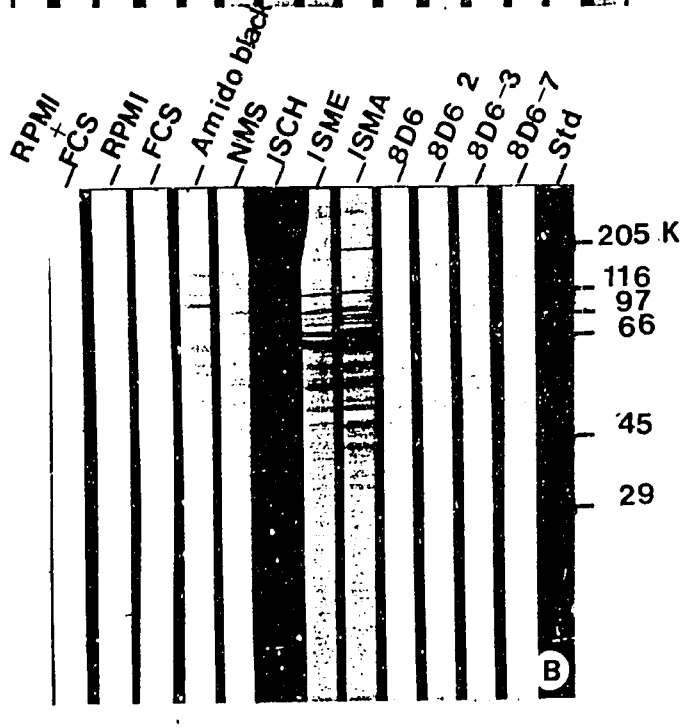

RPMI + FCS
RPMI
FCS
Amido black
NMS
ISCH
ISME
ISMA
8D6
8D6 2
8D6-3
8D6-7
Std
205 K
116
97
66
45
29
B

FIGURE 32

NEGATIVE AND POSITIVE CONTROLS OF IMMUNOFLUORESCENCE OF ADULT *S. JAPONICUM* (CHINESE) STAINED WITH NORMAL MOUSE SERUM (A, B) AND PRIMARY MOUSE IMMUNE SERUM AGAINST *S. JAPONICUM* (CHINESE -ISCH) (C, D), DETECTED WITH SECONDARY RABBIT ANTI-MOUSE IG - FITC.

A, B) Autofluorescence appears over the gut and minute spots that are scatterred throughout the stroma of the parasites' bodies

C, D) Intense fluorescence due to specific binding of ISCH occurs over the whole width of the tegument (Te).

FIGURE 32

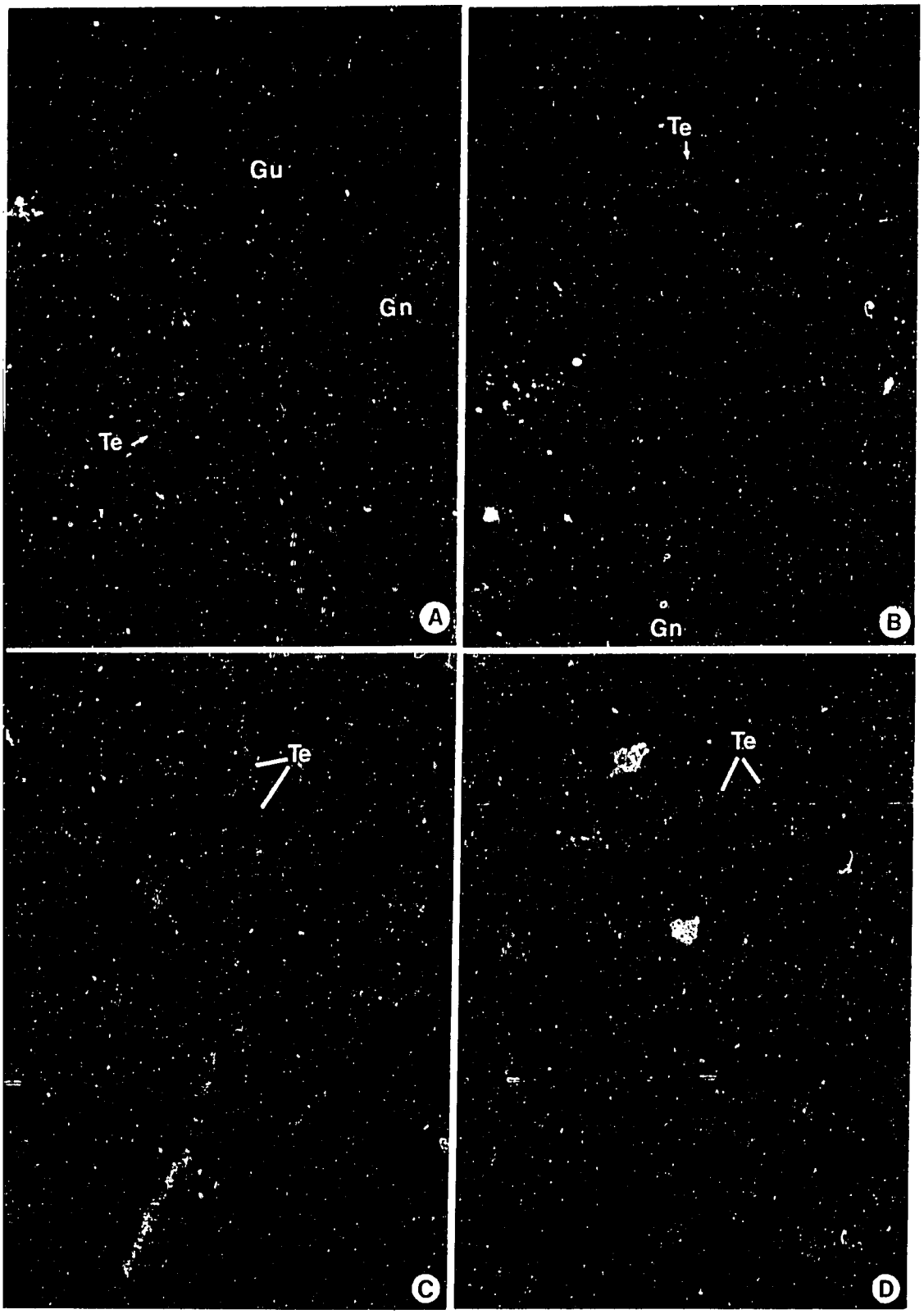


FIGURE 33

IMMUNOFLUORESCENCE OF ADULT *S. JAPONICUM* (CHINESE) STAINED WITH MOUSE MONOCLONAL ANTIBODIES (MAB) AGAINST *S. JAPONICUM* (CHINESE), AND DETECTED BY RABBIT ANTI MOUSE IG-FITC

A, B) Sections stained with MAB in culture fluid from clone 8F2 : specific intense fluorescence appears over the tegument (Te) of the dorso-lateral aspects, while on that of the gynaecophoral canal (Gn) and the cytoplasm of tegument cell (Tc) the fluorescence appears weaker. Minute bright spots (arrows) are scatterred throughout the tegument. A, B X 660.

C, D) Sections stained with MAB in culture fluid from clone 8F5 : the fluorescence exhibits similar pattern as in A and B. C, X 330; D, X 660.

FIGURE 33

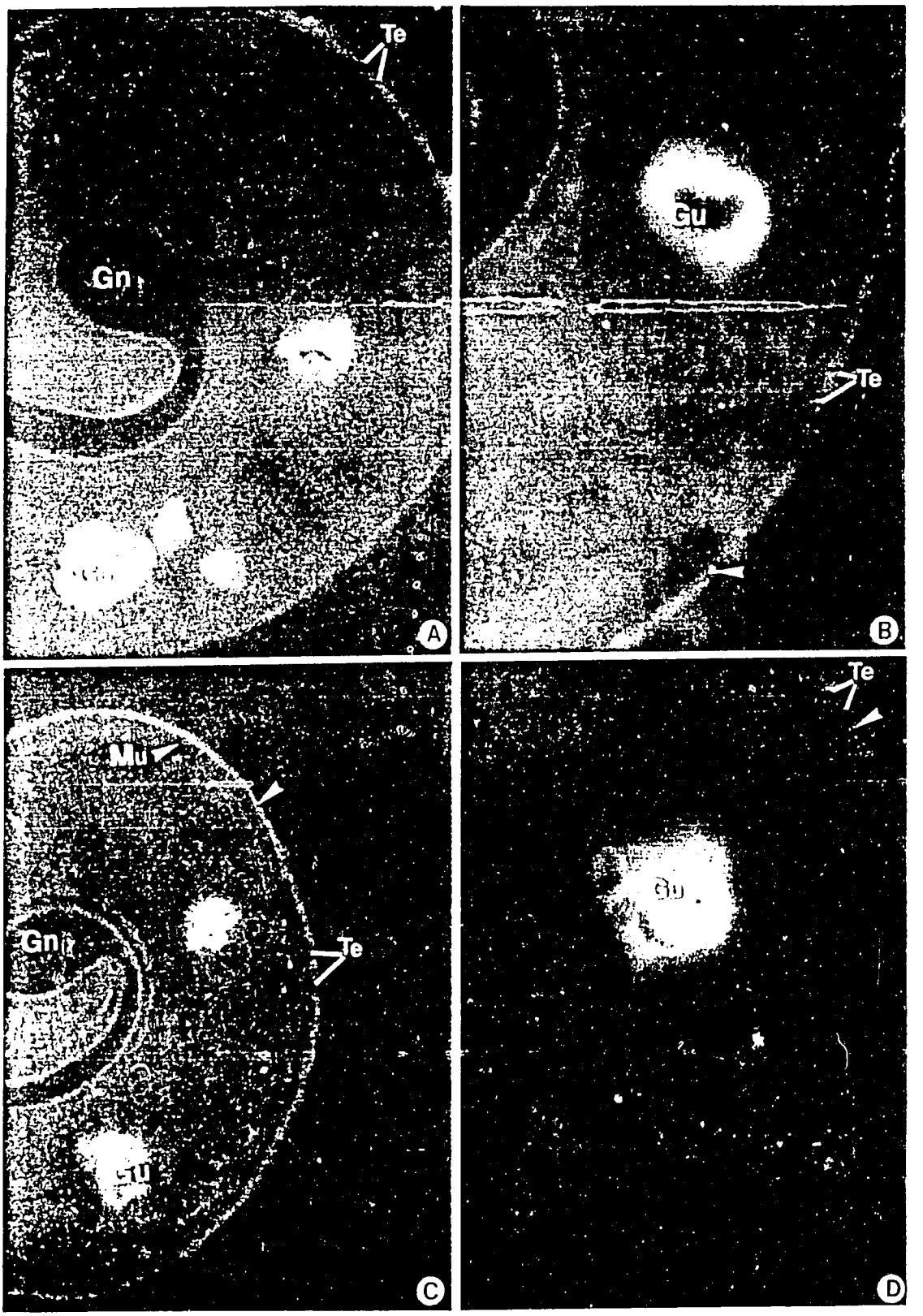


FIGURE 34

IMMUNOFLUORESCENCE OF ADULT *S. JAPONICUM* (CHINESE) STAINED WITH MOUSE MAB AGAINST *S. JAPONICUM* (CHINESE) AND DETECTED BY RABBIT ANTI-MOUSE Ig-FITC

A, B) Sections stained with MAB in culture fluid from clone 8G9-5 : intense fluorescence appears over the tegument (Te) of the dorso-lateral aspects (Do), the gynaecophoral canal (Gn), and in the cytoplasm of tegument cells (Te). Bright granules (arrows) are also numerous in the tegument. A, X 330; B, X 660.

C, D) Sections stained with MAB in culture fluid from clone 9E-7 : intense fluorescence appears on the outer portion of the tegument (Te), and in some areas the fluorescence is brighter than in other (arrows) and appears granulated. C, X 660; D, X 1320.

FIGURE 34

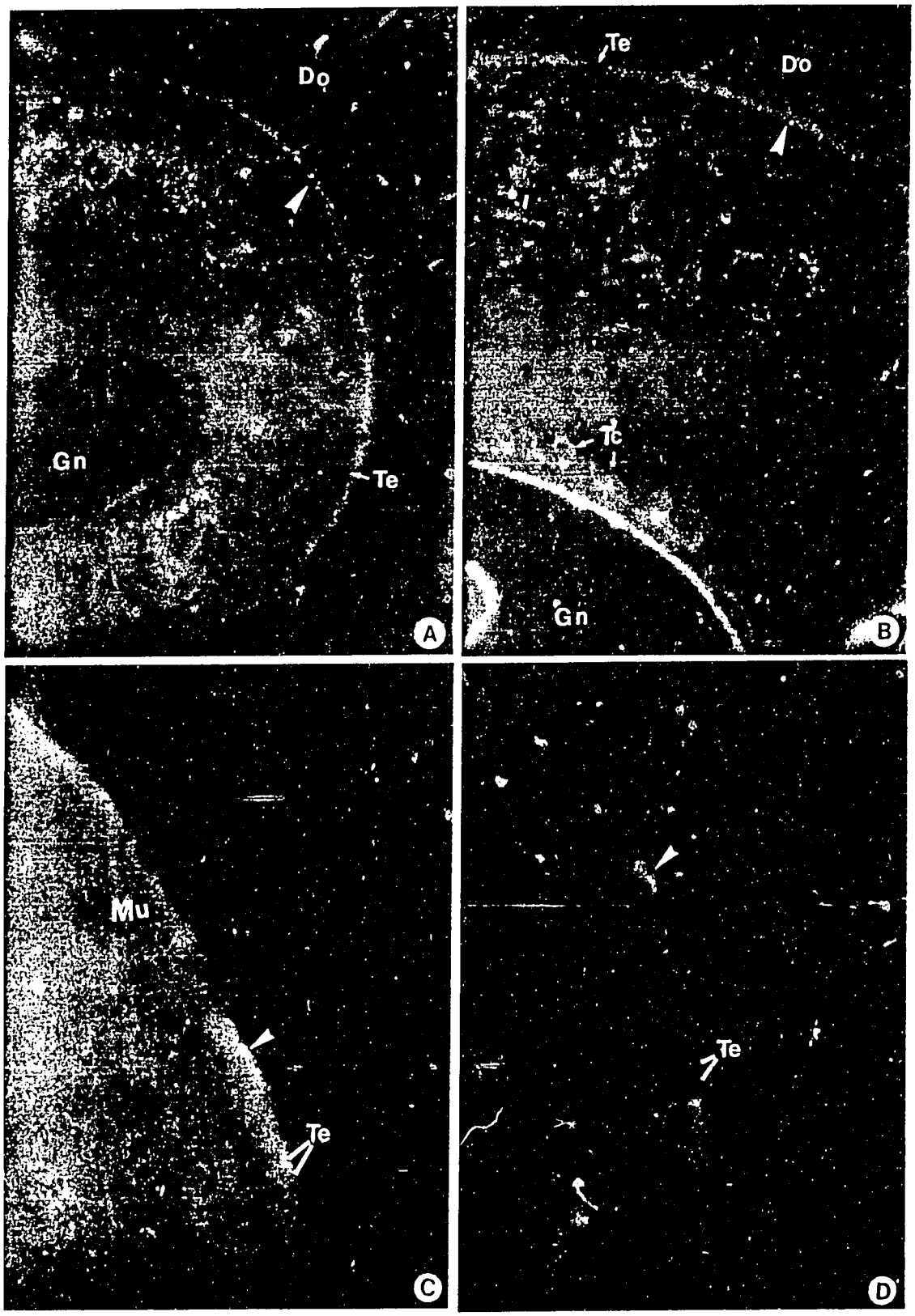


FIGURE 35

IMMUNOFLUORESCENCE OF ADULT *S. JAPONICUM* (CHINESE) (A, B) AND *S. MANSONI* (C, D) STAINED WITH MOUSE MAB AGAINST *S. JAPONICUM* (CHINESE), AND DETECTED BY RABBIT ANTI-MOUSE Ig-FITC.

A, B) Sections of *S. japonicum* stained with MAB in culture fluid from clone 8G11-2 : the fluorescence on the tegument appears weak and patchy (arrows). A, X 660; B, X 1320.

C, D) Sections of *S. mansoni* stained with MAB in culture fluid from clone 8F-2 : moderately intense fluorescence appears on the tubercles (Tb) of the tegument (Te) and the associated spines. C, X 330; D, X 1320.

FIGURE 35

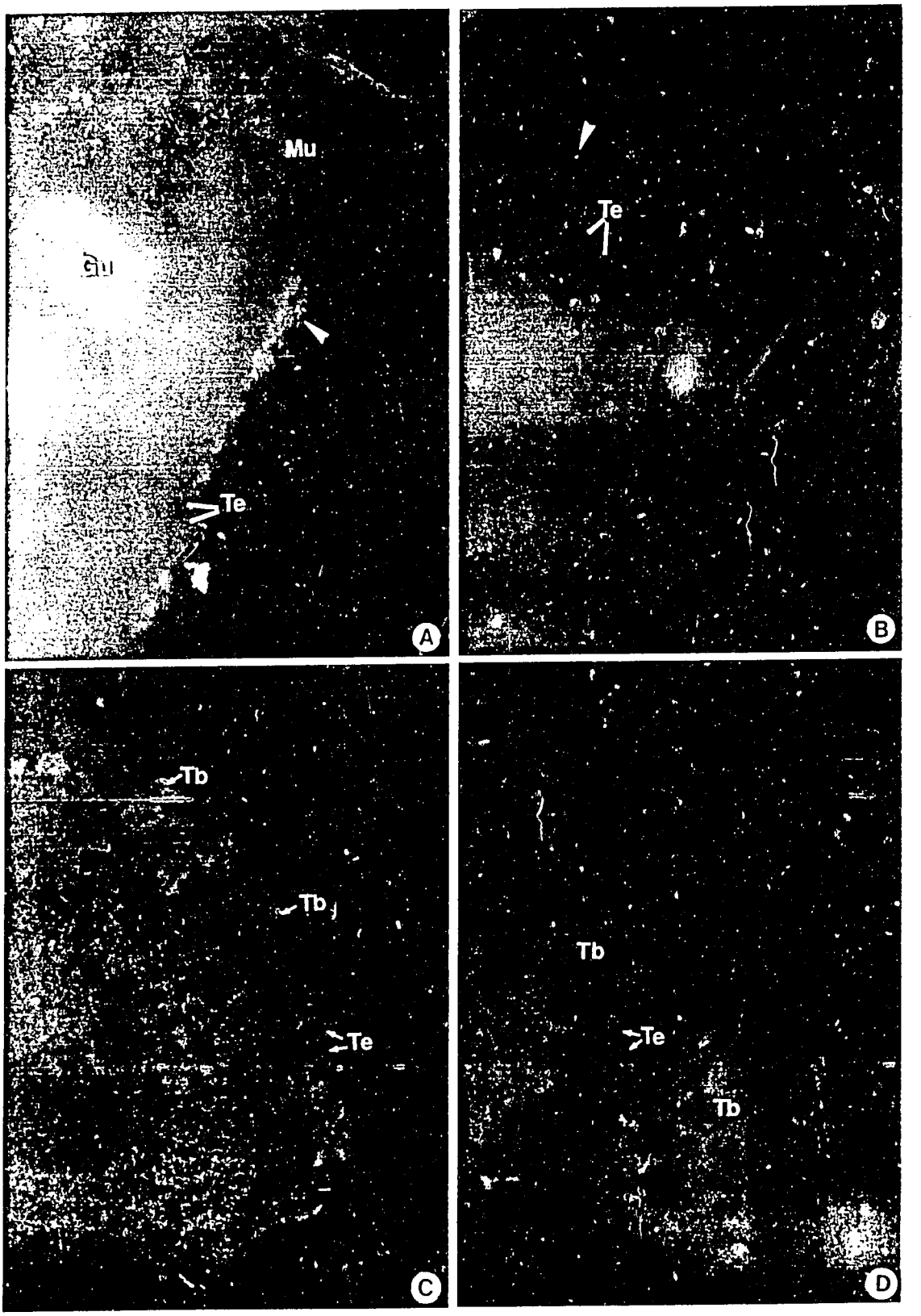


FIGURE 36

IMMUNOFLUORESCENCE OF SCHISTOSOMULA OF *S. JAPONICUM* (CHINESE) STAINED WITH PRIMARY MOUSE IMMUNE SERUM AGAINST *S. JAPONICUM* (CHINESE)-ISCH, DETECTED BY SECONDARY RABBIT ANTI-MOUSE Ig-FITC

A, B) Negative controls where normal mouse serum replaces primary antibody : the fluorescence is absent except in the area at the junction between the head and the body (arrow) where there is a faint autofluorescence. a, x 330; B, X 1320.

C, D) Positive control in which the primary antibody is ISCH : the fluorescence is intense over the whole surface of schistosomula, particularly on the most anterior and posterior ends (arrows). C, X 330; B, X 1320.

FIGURE 36

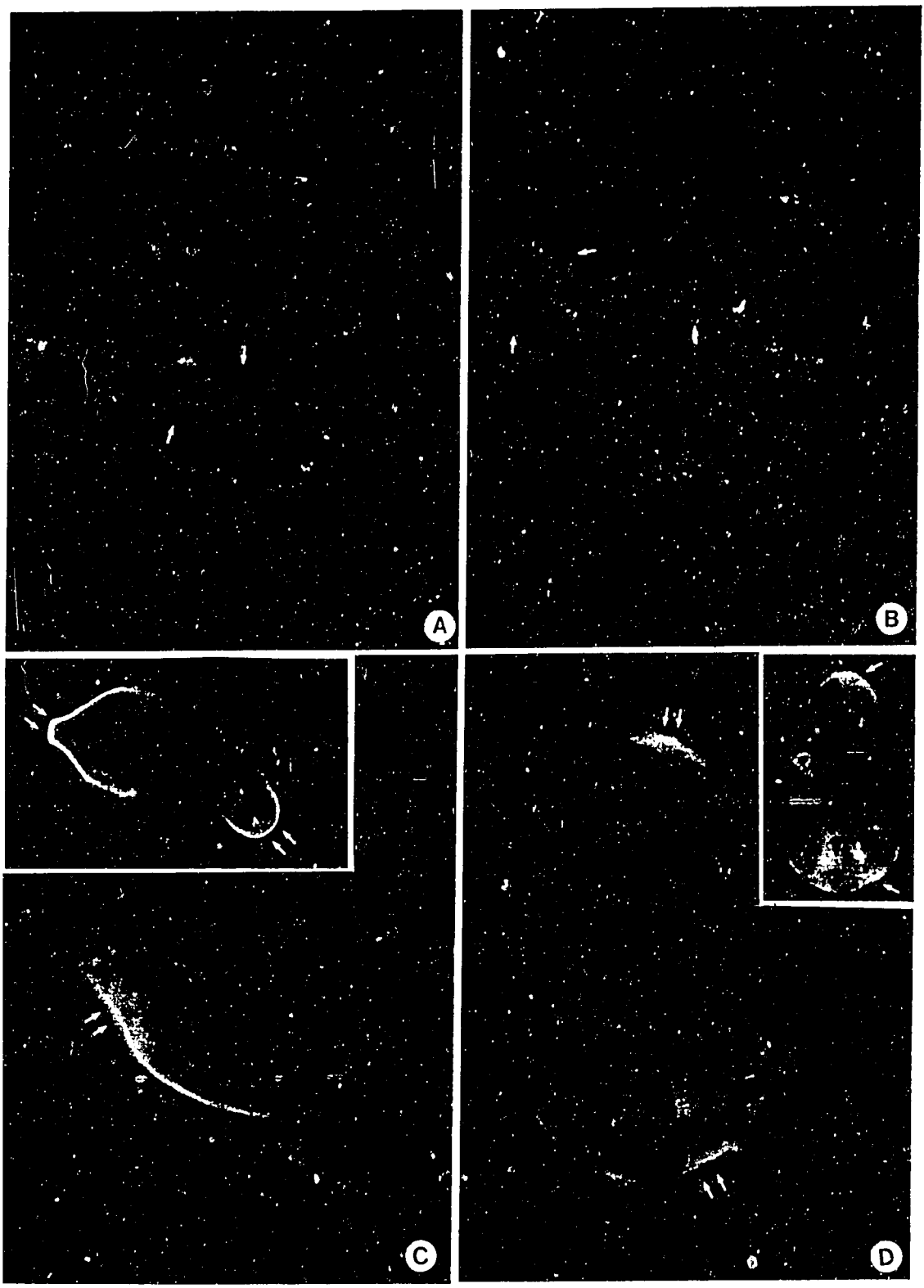


FIGURE 37

IMMUNOFLUORESCENCE OF SCHISTOSOMULA OF *S. JAPONICUM* (CHINESE) STAINED WITH MOUSE MONOCLONAL ANTIBODIES (MAB) AGAINST *S. JAPONICUM* (CHINESE), DETECTED BY RABBIT ANTI-MOUSE Ig-FITC

A, B) Schistosomula stained with MAB from clone 9 E7 : intense fluorescence appears over the entire surface eventhough it is uneven. Particularly strong fluorescence also occurs at the most anterior ends (arrows). A, X 330; B, X 1320.

C, D) Schistosomula stained with MAB from clone 8G9-5 : strong fluo esce occurs only at the anterior end (arrow), while the whole surface exhibits weak fluorescence. Autofluorescence also occurs in an area that may be histolytic gland (Gl). C, X 330;: B, X 1320.

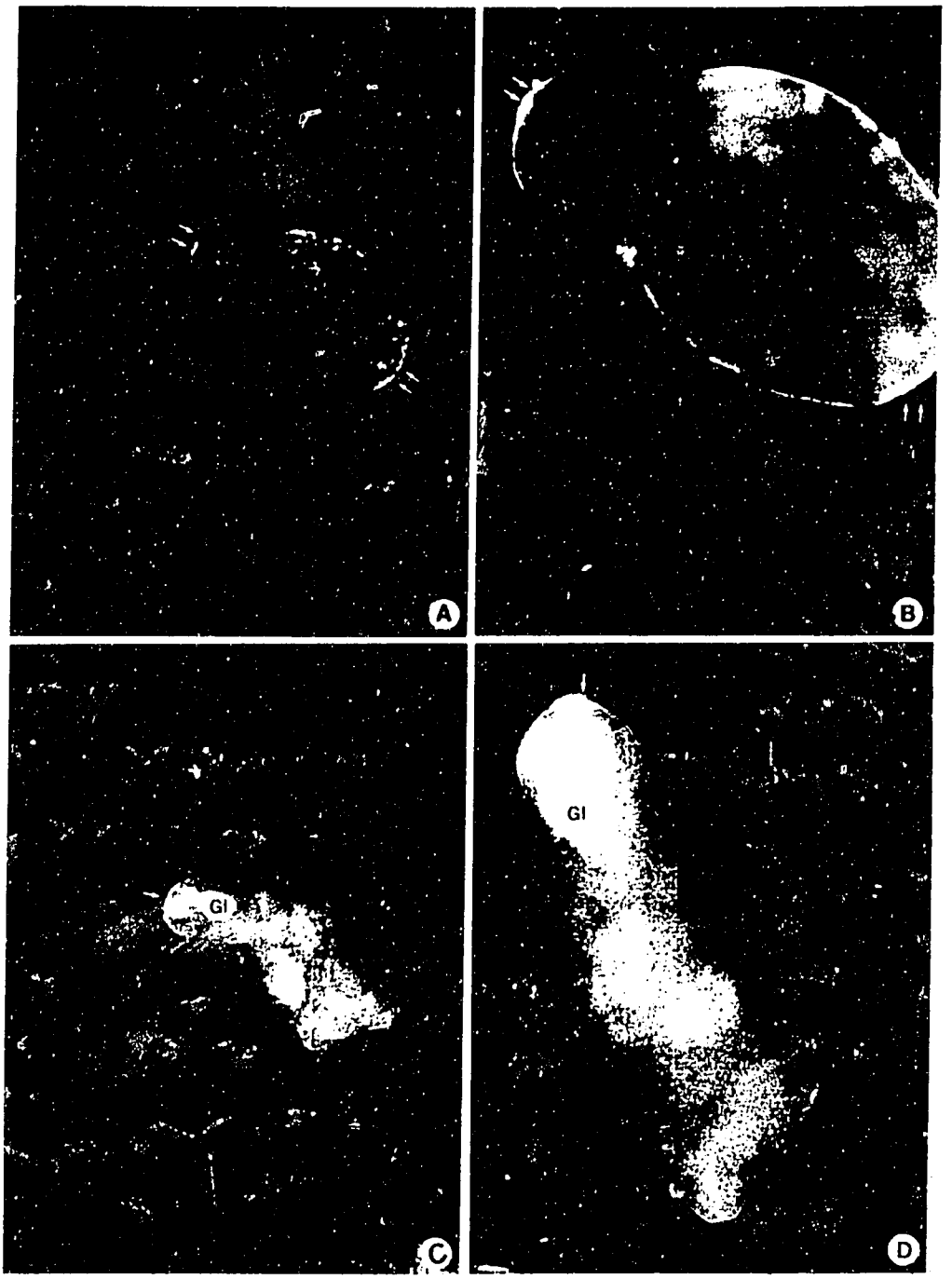



FIGURE 37