

# Human Erythrocytes Adhering to Schistosomula of *Schistosoma mansoni* Lyse and Fail to Transfer Membrane Components to the Parasite

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**ABSTRACT** We studied the adherence of human erythrocytes to larvae of the intravascular parasite *Schistosoma mansoni* by transmission microscopy, freeze fracture, and fluorescence techniques. In addition, we used the adherent cells to investigate the problem of host antigen acquisition. Schistosomula were cultured for from 24 to 48 h after transformation in order to clear the remnants of the cercarial glycocalyx. In some cases, the worms were preincubated with wheat germ agglutinin to promote adherence of the erythrocytes. The results were similar with and without the lectin except that more cells attached to the lectin-coated parasites. Erythrocytes adhered within a few hours and, unlike neutrophils, did not fuse with the parasite. A layer of 10–20-nm electron dense material separated the outer leaflets of the tegumental and plasma membranes. In addition, many deformed and lysed cells were seen on the parasite surface. The ability of the worm to acquire erythrocyte membrane constituents was tested with carbocyanine dyes, fluorescein covalently conjugated to glycophorin, monoclonal antibodies against B and H blood group glycolipids, and rabbit  $\alpha$ -human erythrocyte IgG. In summary, glycophorin, erythrocyte proteins, and glycolipids were not transferred to the parasite membrane within 48 h. Carbocyanine dyes were rapidly transferred to the parasite with or without lectin preincubation. Thus, the dye in the worm membrane came from both adherent and nonadherent cells. These studies suggest that, in the absence of membrane fusion, the parasite may acquire some lipid molecules similar in structure to host membrane glycolipids by simple transfer through the medium but that B and H glycolipids and erythrocyte membrane proteins are not transferred from adhering cells to the worm.

The human parasite *Schistosoma mansoni* lives for years within the circulation while paradoxically avoiding immune recognition and destruction. One of the mechanisms of immune evasion may be the acquisition of host antigens so that the parasite becomes a wolf in sheep's clothing (27). Supporting this theory, various host molecules have been found on the parasite, namely, Forssman antigens (7), ABH blood group glycolipids (6, 12), murine histocompatibility antigens (9, 10, 23–25), complement and IgG receptors (16, 27), and various serum proteins (14, 28). Although the presence of these antigens has not been demonstrated to protect against immune recognition, the hypothesis is an attractive one and

the methods by which these antigens are acquired merit further study.

In the present work we have focused on the interaction between human erythrocytes and schistosomula. Our overall goals were to promote adherence of the cells to the worms, to characterize the adherence ultrastructurally by both transmission electron microscopy and freeze fracture, and to test whether erythrocyte membrane components were transferred to the parasite from the adhering cells. The membrane components tested were glycophorin, which was labeled covalently with fluorescein; B and H blood group glycolipids, which were detected with rhodamine-conjugated monoclonal antibodies;

and miscellaneous erythrocyte (RBC)<sup>1</sup> membrane proteins, which were detected with a rabbit antiserum against human RBC membranes. In addition, carbocyanine dyes were used as test molecules for transfer since they fluoresce only when in a membrane and are held in membranes by long 14 to 18 carbon aliphatic side chains in a manner similar to glycolipids (26). We chose this stage of the parasite because the worms do not eat RBCs, unlike lung-, liver- and adult-stage organisms. Therefore, the presence of RBC membrane components on the parasite must be due to interaction between the surface membranes rather than biosynthetic incorporation of ingested RBC membranes. In these experiments we examined times from 15 min to 48 h since it has been shown that the schistosomula membrane is shed with a half-time of ~13 h (20), and thus any significant acquisition of antigen should occur within a short time.

## MATERIALS AND METHODS

### Reagents

Bovine serum albumin (BSA) and sodium borohydride (NaBH<sub>4</sub>) were purchased from Sigma Chemical Co. (St. Louis, MO); wheat germ agglutinin (WGA) and rhodamine-conjugated wheat germ agglutinin (Rh-WGA) from Vector Laboratories (Burlingame, CA); carbocyanine dyes and fluorescein-5-thiosemicarbazide (Fl-TSC) from Molecular Probes, Inc. (Junction City, OR); sodium metaperiodate (NaIO<sub>4</sub>) from Fisher Scientific Co. (Fair Lawn, NJ); rabbit anti-human RBC IgG (R $\alpha$ HRBC), rhodamine-conjugated rabbit anti-human RBC IgG (Rh-R $\alpha$ HRBC), and rhodamine-conjugated goat anti-mouse IgG (Rh-G $\alpha$ MlgG) from Cappel Laboratories (West Chester, PA); colloidal gold goat anti-rabbit IgG (Au-G $\alpha$ RlgG) from E.Y. Laboratories, Inc. (San Mateo, CA); mouse monoclonal antibody to blood antigen H ( $\alpha$ H) and antigen B ( $\alpha$ B) from Dako Corp. (Santa Barbara, CA); minimal essential medium (MEM) and RPMI-1640 from Gibco Laboratories (Grand Island, NY); and Percoll from Pharmacia Fine Chemicals (Piscataway, NJ).

### Preparation of Schistosomula

A Puerto Rican strain of *S. mansoni* was maintained by passage through outbred mice and *Biomphalaria glabrata* snails. We prepared schistosomula, the larval stage of the organisms, by a modification of the method of Ramalho-Pinto et al. (19). Cercariae were cooled to 4°C in an ice bath, passed through a metal screen to remove snail debris, concentrated by low speed centrifugation, and placed in cold MEM. The cercariae, 10–50 × 10<sup>3</sup> organisms in 2 ml MEM in 15-ml Falcon culture tubes (Falcon Labware, Div. Becton-Dickinson & Co., Oxnard, CA), were vortexed for 1 min at full speed to break the organisms into bodies and tails. The vortexed material was layered onto 60% Percoll in cold MEM and centrifuged at 600 g for 10 min (15). Tails, dead organisms, and snail debris remained on the top of the Percoll and were discarded. The pellet contained cercarial bodies which were washed three times in MEM to remove Percoll and incubated for 1–3 h at 37°C in RPMI-1640 to allow the bodies to complete their transformation into schistosomula. 0–2% of the organisms had tails and <2% were dead. 10,000–50,000 schistosomula were washed six times in RPMI-1640 with 0.1% BSA under sterile conditions. After every second wash, they were transferred to a fresh 15-cc centrifuge tube and finally to a sterile 50-cc culture flask which was placed in a 37°C 5% CO<sub>2</sub> incubator for from 16 h to 3 d. After culture, >95% of the schistosomula were viable (21).

### Preparation of RBCs

3 cc of human blood were drawn into a 5-cc heparinized syringe, transferred to plastic tubes, and centrifuged for 30 s in a Beckman Microfuge B (Beckman Instruments Inc., Palo Alto, CA). Serum and white blood cells were aspirated

<sup>1</sup> Abbreviations used in this paper: Au-G $\alpha$ RlgG, colloidal gold goat anti-rabbit IgG; Fl-TSC, fluorescein-5-thiosemicarbazide; MEM, minimum essential medium; R $\alpha$ HRBC, rabbit anti-human RBC IgG; Rh-G $\alpha$ MlgG, rhodamine-conjugated goat anti-mouse IgG; Rh-R $\alpha$ HRBC, rhodamine-conjugated goat anti-mouse IgG; RBC, erythrocyte; Rh-WGA, rhodamine-conjugated WGA; RT, room temperature; WGA, wheat germ agglutinin;  $\alpha$ B and  $\alpha$ H, mouse monoclonal antibody to blood antigen B and antigen H, respectively.

off and the RBC pellet was washed three times with phosphate-buffered saline (PBS). Packed RBCs were diluted 1:1 with PBS before use.

### Culture of Worms and Cells

For fluorescence experiments, 1,000 cultured worms were mixed with 2  $\mu$ l RBCs in 100–500  $\mu$ l of RPMI + BSA and incubated at 37°C for 5 min to 48 h. Before fluorescence microscopy and photography, we treated samples with 10<sup>-2</sup> M eserine sulfate to immobilize the worms. For transmission microscopy, 3,000–5,000 cultured worms and 6–10  $\mu$ l RBCs in 1 ml of RPMI + BSA were incubated from 30 min to 24 h at 37°C. In some experiments, to promote adherence, worms were incubated with WGA, 50  $\mu$ g/ml, for 30 min at room temperature (RT) then washed three times with RPMI + BSA before the addition of cells.

### Fluorescent Labeling

**RH-WGA:** Cultured worms were incubated with 50  $\mu$ g/ml Rh-WGA for 30 min at RT, washed three times in RPMI + BSA, and then incubated with cells.

**Fl-TSC:** Cells were labeled according to a protocol in which 90% of the incorporated fluorescein is attached to glycophorin (11). Cells were incubated in fresh 2 mM NaIO<sub>4</sub> in PBS, pH 7 at 4°C for 10 min. After four washes in PBS, an equal volume of Fl-TSC at 0.2 mg/ml was added to the cells for 30 min at RT. We tested specificity of the labeling by treating cells with Fl-TSC without prior NaIO<sub>4</sub> treatment. Labeled cells were incubated with worms with or without WGA.

### Carbocyanine Dyes

We used the following dyes: DiIC<sub>18</sub> (5), DiIC<sub>18</sub> (3), DiIC<sub>14</sub> (3), DiOC<sub>14</sub> (3), and DiIC<sub>10</sub> (3). 1  $\mu$ l of stock solution containing 1 mg dye/ml ETOH was added to 20  $\mu$ l RBCs and 80  $\mu$ l PBS for 20 min at RT. After three or four washes in PBS, labeled cells were incubated with schistosomula with or without WGA.

### Rh-R $\alpha$ HRBC

100  $\mu$ l of Rh-R $\alpha$ HRBC diluted 1/40 in RPMI + BSA was added to incubations of worms with or without WGA and cells that had been washed once to remove nonadherent RBCs. After 20 min, samples were washed four times in RPMI + BSA and examined. On Western blots this antibody specifically reacted with at least 12 bands from electrophoresed human RBC ghosts.

### Monoclonals $\alpha$ H and $\alpha$ B

Worms with or without WGA were incubated with RBCs from either a type H or a type B donor for from 1 h to 2 d. Samples were washed once, then the appropriate antiserum, diluted 1:2 with PBS for  $\alpha$ H and 1:1 for  $\alpha$ B, was added for 20 min at RT. The worms and cells were washed twice in RPMI + BSA, incubated at RT in 100  $\mu$ l Rh-G $\alpha$ MlgG diluted 1:20 with PBS, washed three times and examined by fluorescence microscopy. Controls omitting the monoclonals were prepared to test Rh-G $\alpha$ MlgG specificity.

### Transmission Electron Microscopy

We prepared cells and schistosomula for transmission microscopy as previously described (2). In brief, we fixed cells and parasites in suspension in an equal volume of Karnovsky's aldehyde fixative (13) for 15–30 min at RT or overnight at 4°C. The suspension was centrifuged in a microfuge, resuspended and postfixed in acetate veronal buffered 1% OsO<sub>4</sub> for 90 min at 4°C, and stained en bloc in buffered 0.5% uranyl acetate. Dehydration and Spurr's or Epon embedding were routine.

Thick sections (0.3  $\mu$ m) were cut with glass knives and stained with azure II-methylene blue. Thin sections with silver to silver-gold interference colors were cut with a diamond knife, picked up on naked or Formvar-carbon-coated copper grids, and stained on grid for 5–10 min in uranyl acetate and for 10–30 s in lead citrate. Grids were examined in a JEOL 100C electron microscope.

### R $\alpha$ HRBC and Au-G $\alpha$ RlgG

After incubation together, somules and cells were washed once then incubated for 20 min at RT with 100  $\mu$ l of 1/40 or 1/320 diluted R $\alpha$ HRBC. Three washes in RPMI + BSA were done. Then the samples were fixed 1:1 in Karnovsky's for 2 min at RT, washed three times in PBS, treated with an equal volume of 100 mM NaBH<sub>4</sub> for 10 min at RT, and washed twice with PBS. 25

TABLE 1. Number of Experiments

	Fluorescence microscopy	Transmission electron microscopy	Freeze fracture
Rh-WGA	4*	12	4
Fluorescinglycophorin	11	2	—
$\alpha$ B or $\alpha$ H	3	—	—
Rh-R $\alpha$ HRBC	6	—	—
Au-G $\alpha$ RlgG + R $\alpha$ HRBC	—	4	—

\* Each number represents a different day on which experiments were examined by the stated technique. Multiple samples taken at different times were examined on each experimental day.

$\mu$ l Au-G $\alpha$ RlgG at 30  $\mu$ g/ml was added and incubated for 20 min at RT. As a control, the R $\alpha$ HRBC was omitted. One wash with PBS then three with 0.1 M cacodylate buffer preceded additional fixation for 2 min at RT in Karnovsky's. Postfixation and subsequent steps were as above.

### Freeze Fracture

Somules with or without WGA and RBCs were fixed for 15 min at RT in Karnovsky's, washed in 0.1 M cacodylate buffer, and glycerinated for 2.5–3 h at RT or overnight at 4°C in 25% glycerol in 0.1 M cacodylate. A drop of sample was frozen between gold or copper support plates in either Freon or liquid propane cooled by liquid nitrogen. Samples were stored in liquid nitrogen until they were fractured in a Balzers 300 freeze fracture device (Balzers, Hudson, NH).

### Repetition of Experiments

Table I shows the number of times experiments were done.

## RESULTS

### Light Microscopy

RBCs adhered to WGA-coated parasites within 15 min, and by 3 h there were usually 20–30 cells/schistosomulum. By interference contrast microscopy the adherent cells were often elongated and anchored to the parasite by “tethers” (Fig. 1). When the same preparations were viewed by fluorescence microscopy to demonstrate fluorescein-glycophorin, fragments of RBC membrane and ghosts were seen on the parasite in addition to the distorted intact cells (Figs. 1 and 2). Distortion, lysis, and fragmentation were also seen with carbocyanine labeled adherent cells (Figs. 3 and 4). Similar changes occurred in RBCs adherent to parasites that had not been preincubated in WGA, but there were fewer adherent RBCs.

We used several fluorescent labeling procedures to test whether RBC membrane components were transferred to the worm from the adherent cells. First, carbocyanine dyes were rapidly transferred beginning within 30 min so that by 3 h the entire parasite tegumental membrane was fluorescent (Fig. 4). Earlier, fluorescence in the parasite membrane was limited to the areas where cells were attached (Fig. 5). The transfer of

these dyes was also dependent on the lectin coating of the worm in that worms incubated with WGA appeared to acquire more dye more quickly. However, organisms not treated with WGA also had carbocyanines in their membrane after 3 h of incubation with labeled cells.

The second set of fluorescent labels centered on RBC membrane components per se. Glycophorin was covalently labeled with fluorescein but no transfer to the parasite was seen at up to 48 h (Fig. 2). The cells were incubated with WGA-labeled parasites and the distribution of multiple RBC membrane proteins was demonstrated with Rh-R $\alpha$ HRBC. The cells were labeled and there were a few fluorescent patches on the membrane, but there was no generalized labeling of the parasite (Fig. 6). The distribution of B and H blood group glycolipids was also tested after 24-h incubations with indirect immunofluorescence and was the same as seen with Rh-R $\alpha$ HRBC, i.e., only the cells and a few fluorescent patches were labeled (Fig. 7). On parasites not pretreated with lectins, all three labels were confined to intact cells and membrane fragments, although there were fewer cells and fragments than appeared on lectin coated parasites. Finally, we examined the distribution of the lectin itself by incubating the parasites with Rh-WGA before adding the cells. Before cells were added, Rh-WGA was uniformly distributed over the parasite surface. Within 2 h the Rh-WGA was found over the entire surface of the adherent RBCs (Fig. 8).

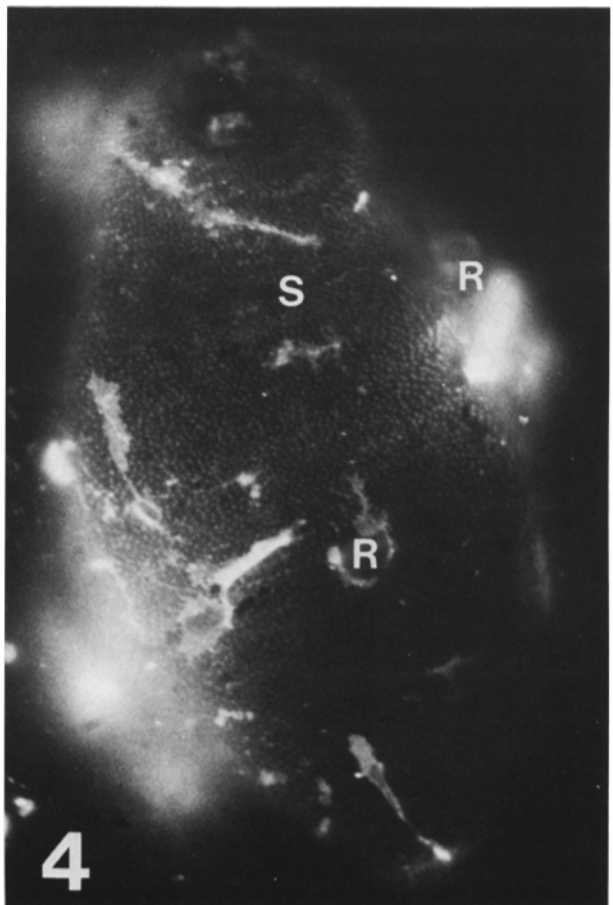
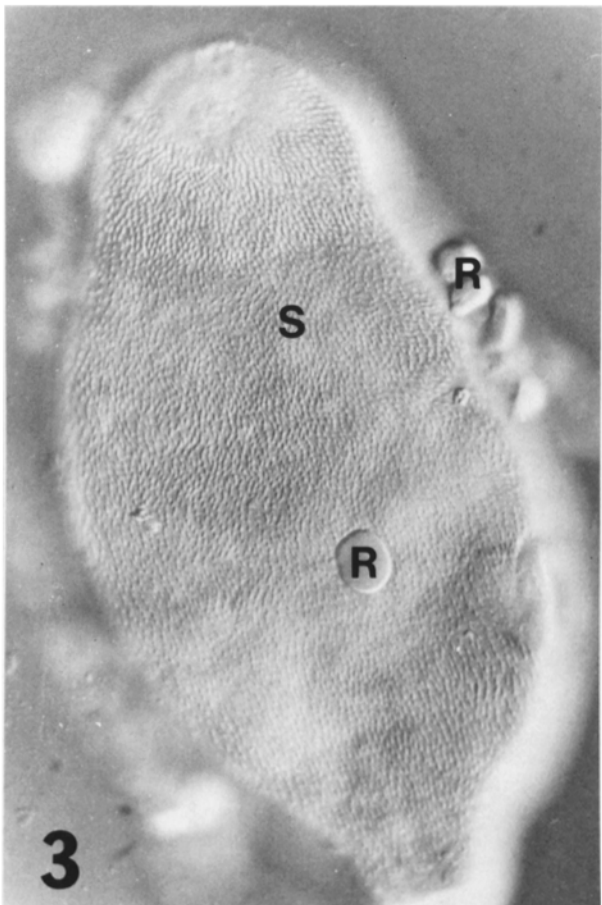
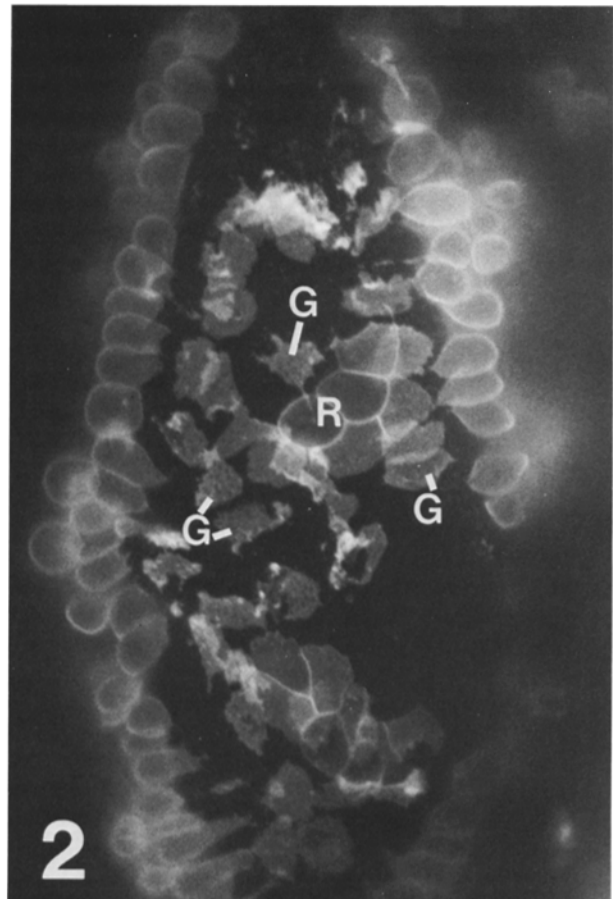
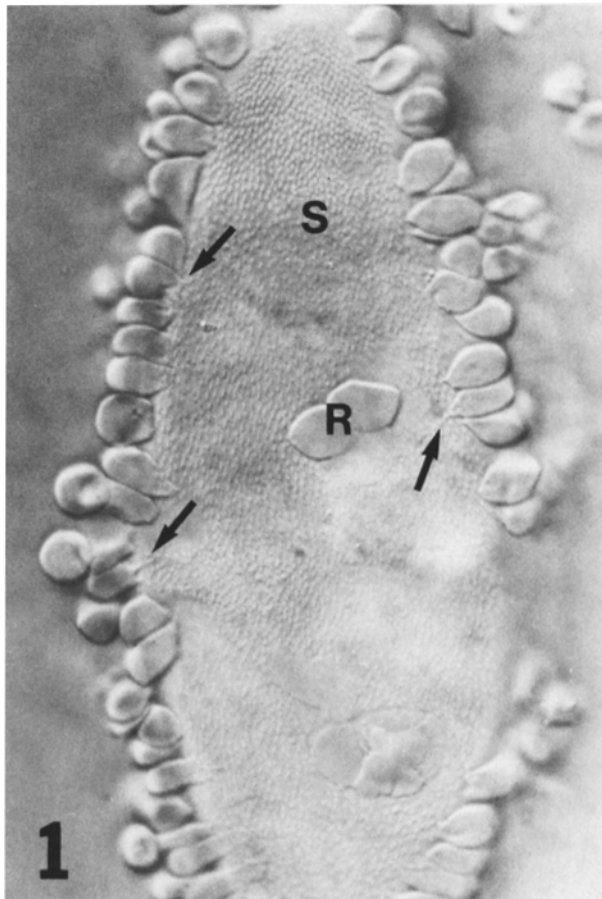
### Electron Microscopy

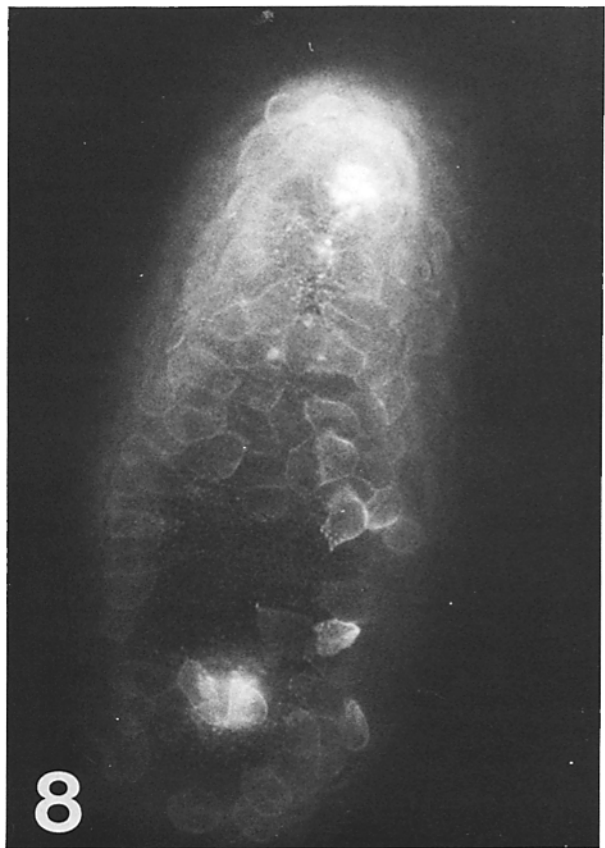
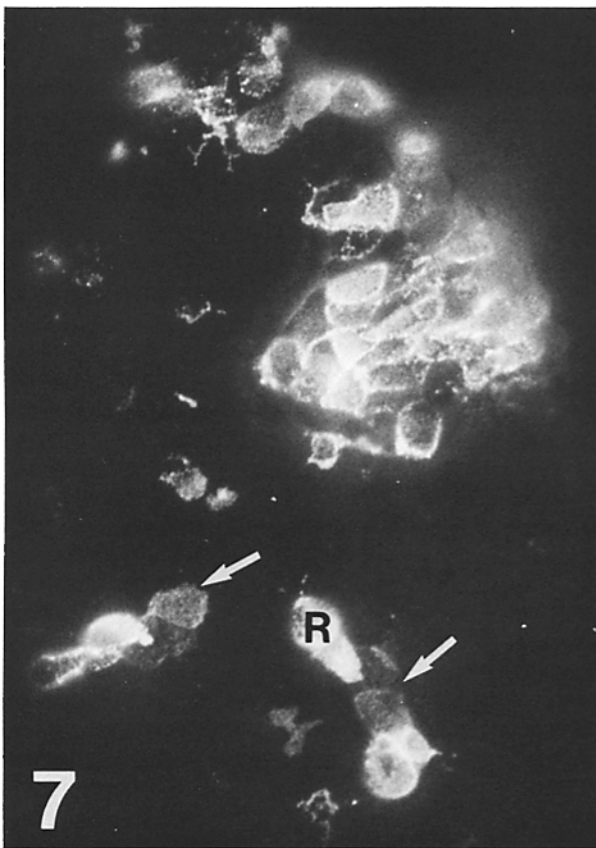
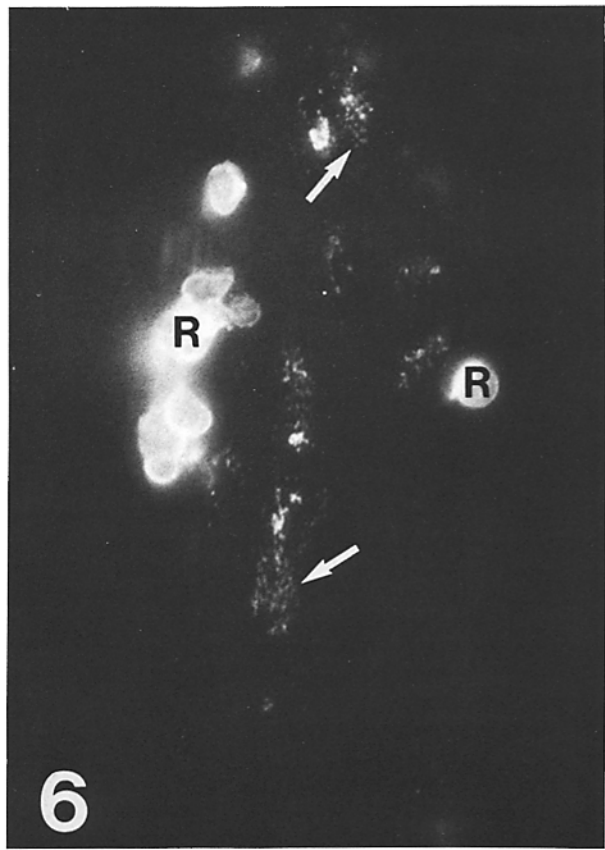
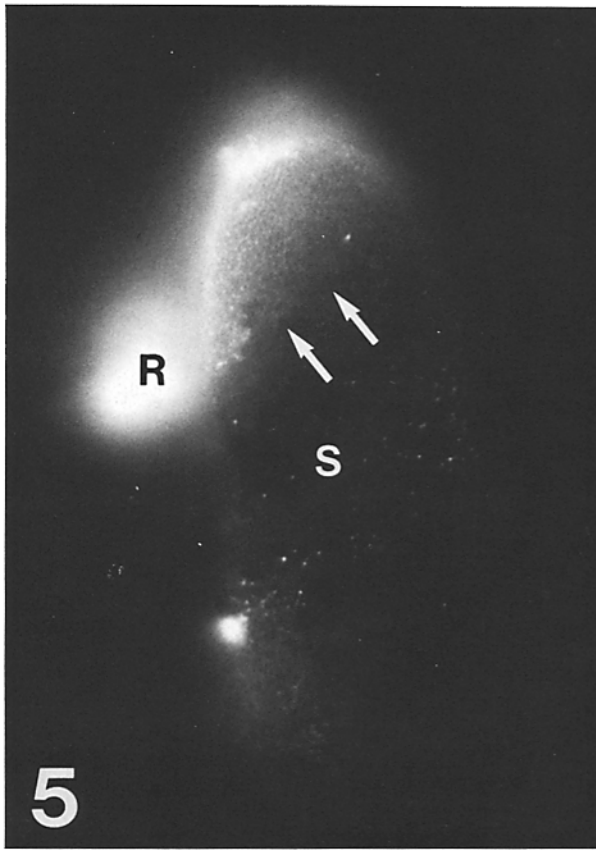
By transmission microscopy, RBCs adherent to either WGA-coated or uncoated parasites were distorted and often had elongated extensions reaching to the parasite surface (Fig. 9). High power examination of the areas where the cells were attached showed that the RBC plasma membrane and the parasite membrane were separated by a 200-Å gap containing electron dense material (Fig. 9, inset). In addition to the distorted intact RBCs, RBC ghosts were seen adherent to the parasite (Fig. 10). The ghosts were attached to the parasite in the same manner as the intact cells (Fig. 10, inset).

The distribution of RBC membrane components was demonstrated immunocytochemically with R $\alpha$ HRBC followed by Au-G $\alpha$ RlgG. The gold particles bound specifically to intact RBCs (Fig. 11), RBC ghosts (Fig. 12), and fragments of RBC membrane (Fig. 13) but not to the parasite tegumental membrane (Figs. 11–13). When the first antibody was omitted there was no labeling of the intact RBCs or ghosts by the Au-G $\alpha$ RlgG (Fig. 14).

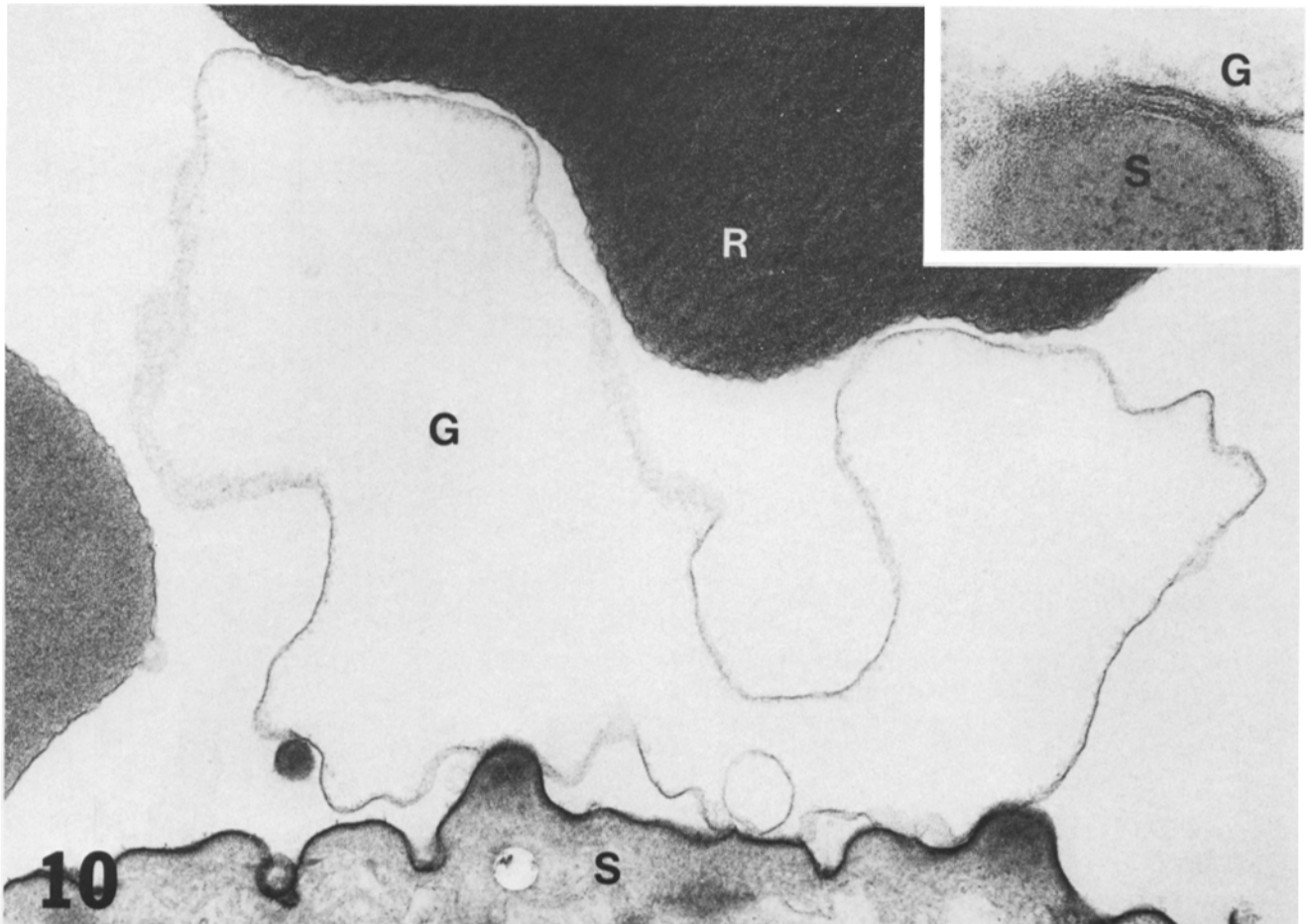
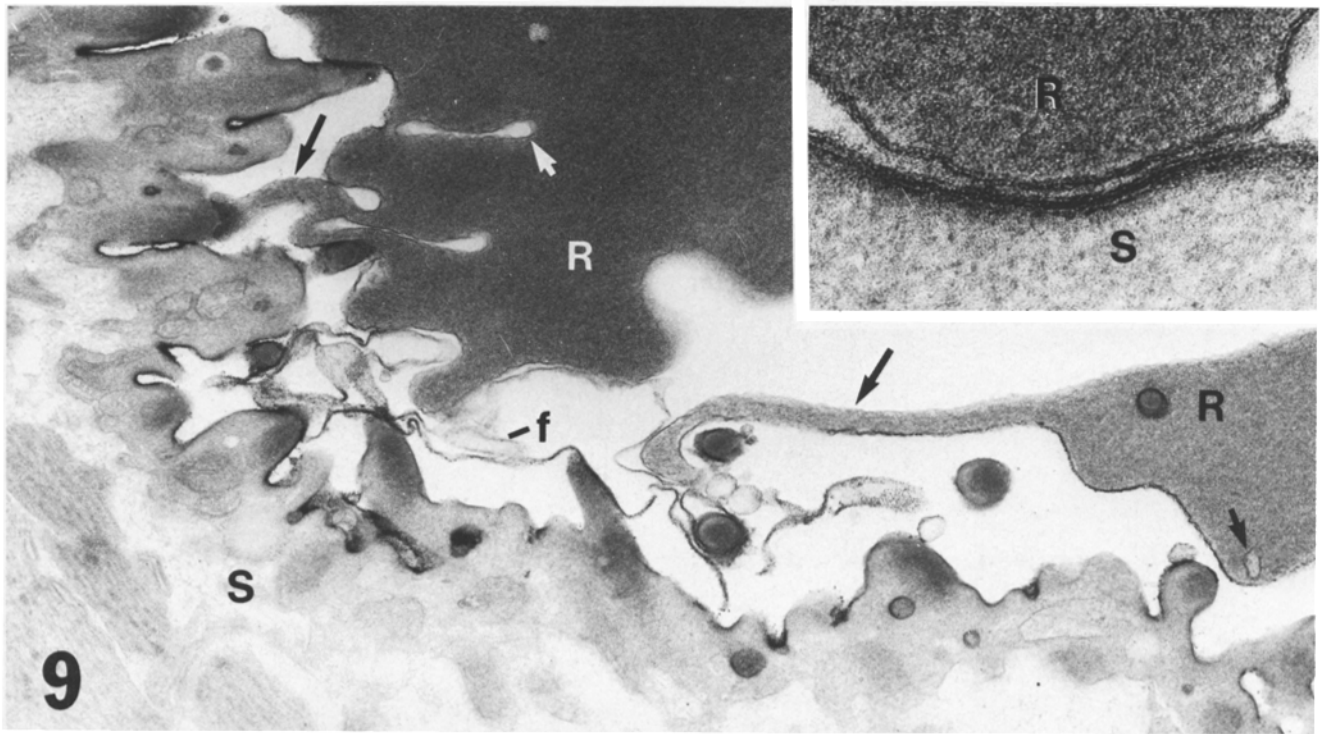
In freeze-fracture preparations of RBCs incubated with WGA-coated schistosomula, 45 worm profiles showed no specialization in the parasite outer membrane (Fig. 15). In particular, there were no membrane fusions such as occur between human neutrophils and schistosomula (2, 4). With neutrophils fusions are seen on 20% of the parasite profiles.

FIGURES 1–4 Figs. 1 and 2: Paired interference contrast and fluorescence micrographs of RBCs labeled with FI-TSC and incubated with WGA-coated schistosomula for 3 h. In Fig. 1, the intact cells (R) are deformed and attached to the schistosomulum (S) by “tethers” (arrows). In Fig. 2, lysed cell membranes (G) as well as intact cells are seen, and the parasite membrane is not fluorescent (compare with Fig. 4). Figs. 1 and 2,  $\times$  800. Figs. 3 and 4: Paired interference contrast and fluorescence micrographs of RBCs labeled with DilC<sub>14</sub> and incubated with WGA-coated schistosomula for 3 h. In Fig. 3, a few intact RBCs (R) are adherent to the schistosomulum (S). In Fig. 4, fragments of RBC membrane are seen also and the parasite tegumental membrane is fluorescent (compare with Fig. 2). Figs. 3 and 4,  $\times$  800.

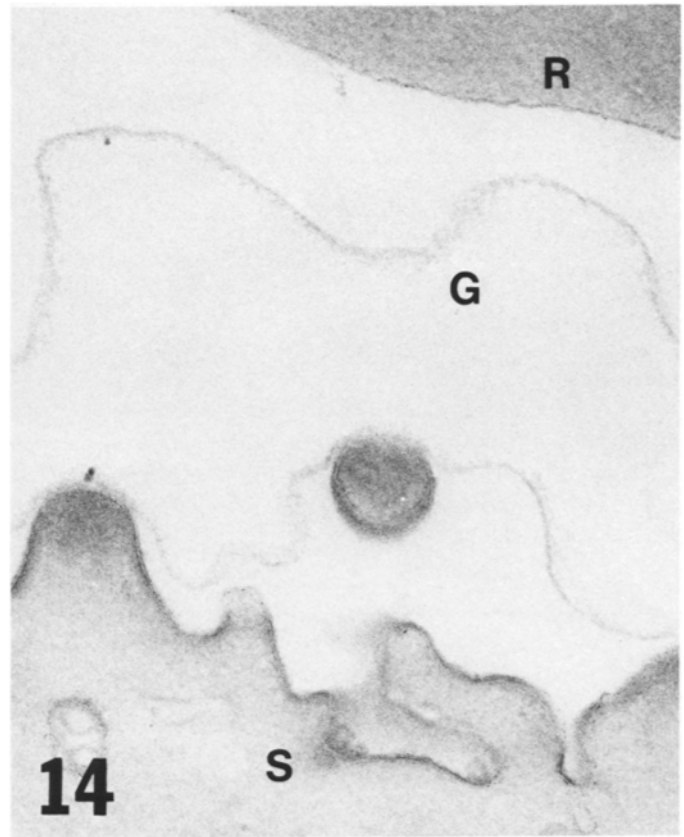
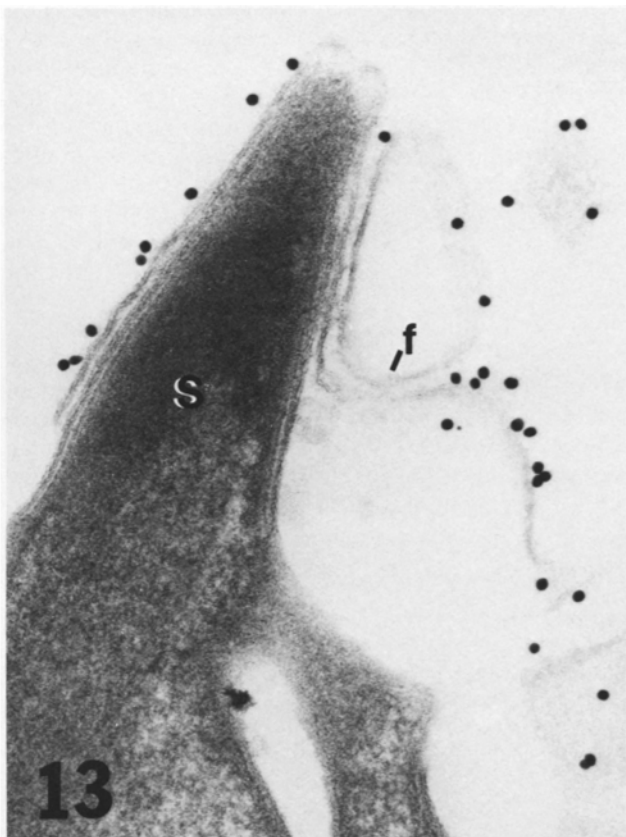
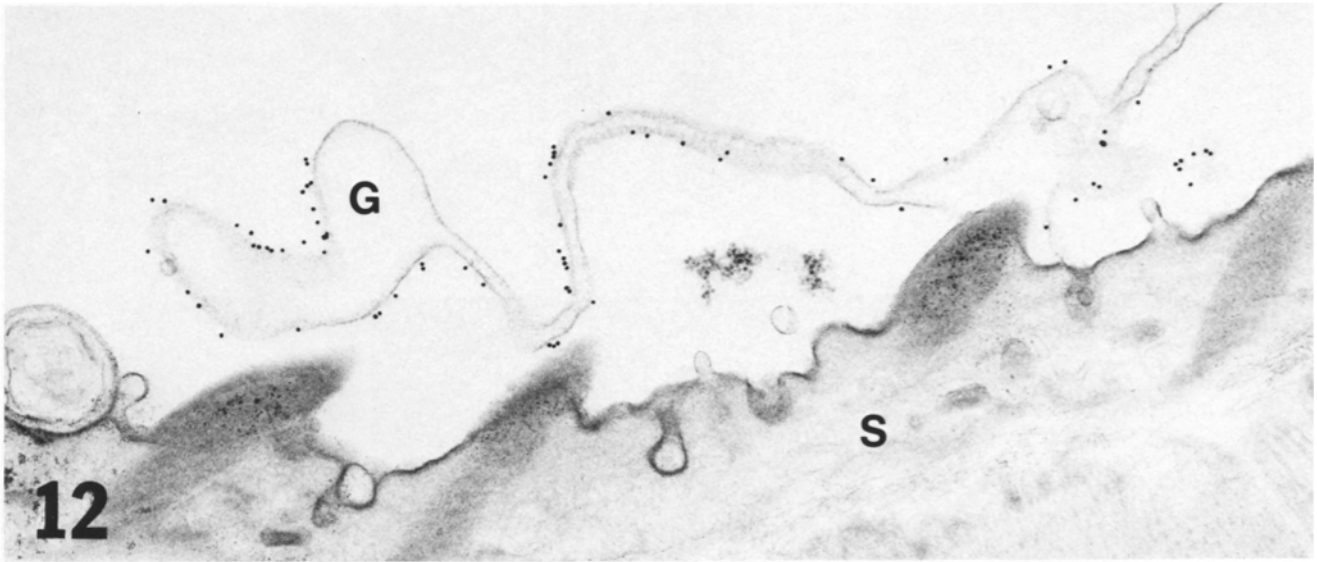
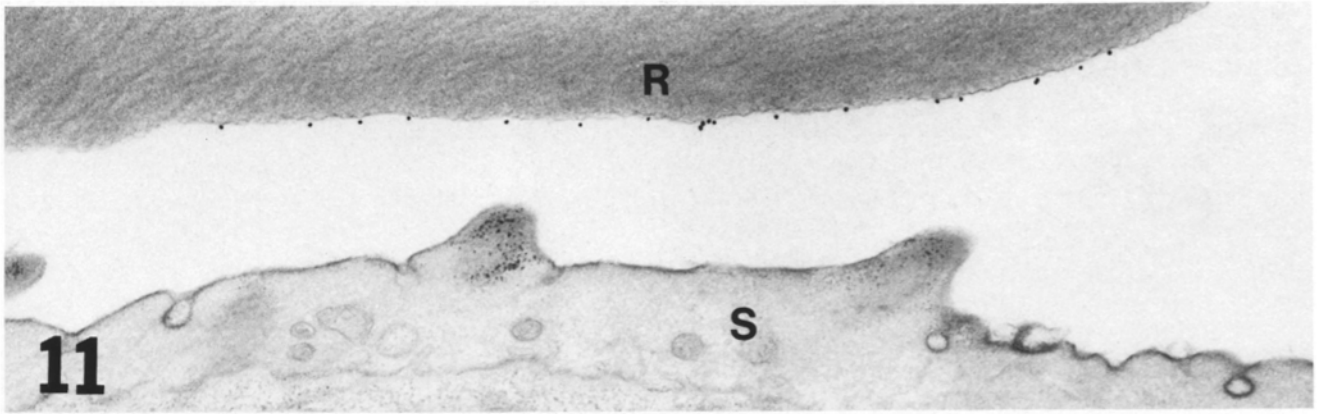




FIGURES 5-8 Fig. 5: Fluorescence micrograph of RBCs labeled with DiIC<sub>14</sub> and incubated with schistosomula in the absence of WGA. A clump of adherent cells (R) is attached to the parasite (S) out of the focal plane. The tegumental membrane is fluorescent in the area where the cells are attached (arrows).  $\times 800$ . Fig. 6: Fluorescence micrograph of RBCs incubated with WGA-coated parasites for 24 h and then reacted with Rh-R $\alpha$ HRBC. A few intact cells (R) and fluorescent patches (arrows) are present on the parasite surface, which is not generally fluorescent (compare with Fig. 4).  $\times 800$ . Fig. 7: Fluorescence micrograph of type B RBCs incubated with WGA-coated schistosomula for 26 h and then reacted with monoclonal  $\alpha$ B and then with Rh-G $\alpha$ MlgG. Cells (R) and patches (arrows) are fluorescent but the tegumental membrane is not.  $\times 800$ . Fig. 8: Fluorescence micrograph of RBCs incubated for 2 h with parasites coated with Rh-WGA. Note that the fluorochrome is not confined to the parasite surface but is also staining the membranes of the RBCs.  $\times 800$ .



FIGURES 9 and 10 Fig. 9: Transmission micrograph of RBC (R) incubated with WGA-coated schistosomula (S) for 22 h. The RBC membrane is distorted into long projections (long arrows). Membrane also appears internalized into the RBC (short arrows), and membrane fragments (f) are present on the parasite surface. The inset shows the adherence of an RBC to a WGA-coated schistosomulum after 3 h of incubation. The RBC plasma membrane and the double tegumental membrane of the parasite are separated by an  $\sim 200$  Å gap containing electron dense material.  $\times 29,000$ ; inset,  $\times 130,000$ . Fig. 10: Transmission micrograph of an RBC ghost (G) adherent to a WGA-coated schistosomulum (S) after 22 h of incubation. The inset shows a high power view of the attachment of a ghost to the parasite. Compare with Fig. 9, inset. R, RBC.  $\times 33,000$ ; inset,  $\times 130,000$ .



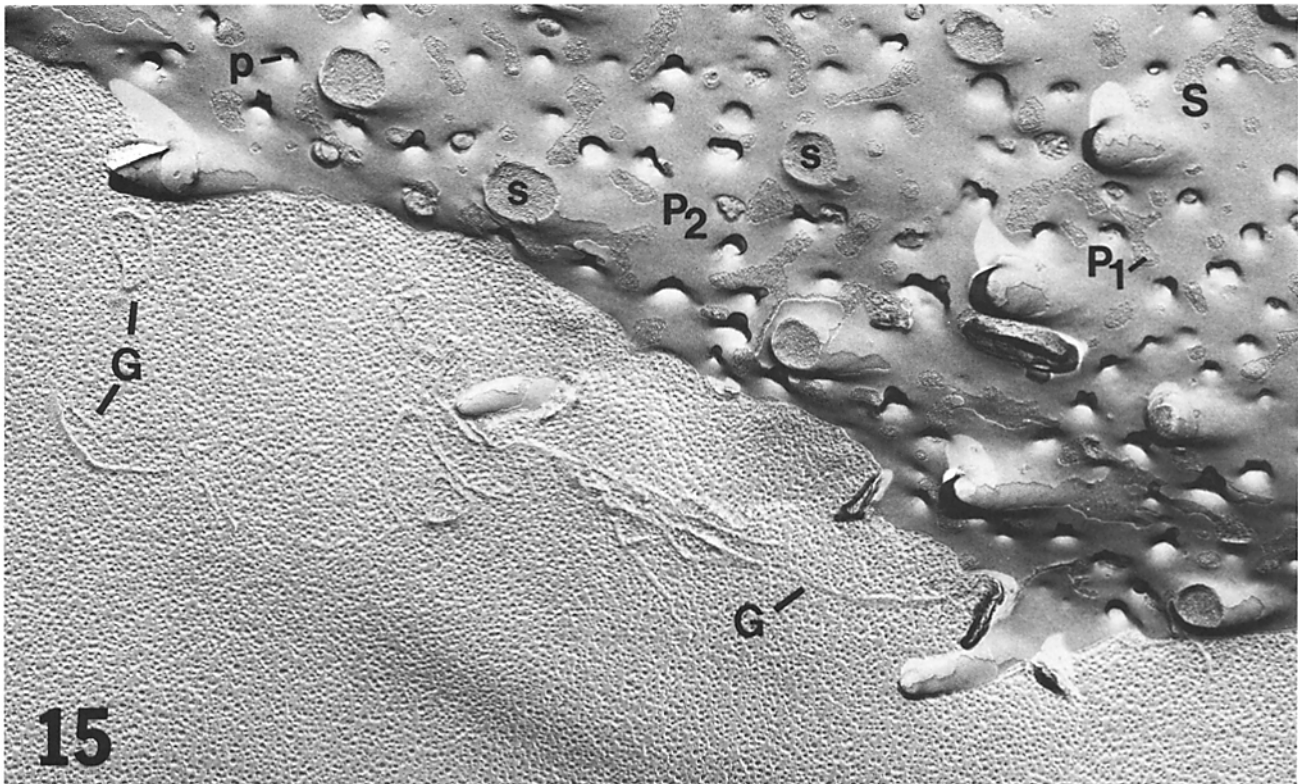


FIGURE 15 Freeze-fracture micrograph showing an RBC ghost membrane (G) adherent to a WGA-coated schistosomulum (S) after 3 h of incubation. The protoplasmic faces of the tegumental membranes are seen with the fracture plane passing generally through the intramembrane particle-poor outer membrane ( $P_2$ ) and occasionally exposing the intramembrane particle-rich inner membrane ( $P_1$ ). No alterations are seen in the area where the ghost is attached. The fracture was performed under mild etching conditions. *s*, spines; *p*, pits.  $\times 22,000$ .

If fusions were occurring between RBCs and the parasite at the same rate, then 9 of the 45 profiles should have had fusions.

## DISCUSSION

Human erythrocytes do not fuse to the outer tegumental membrane of schistosomula. Adherent cells also do not transfer any of the membrane proteins or glycolipids tested to the parasite membrane within 48 h, even when WGA is used to promote adherence. However, carbocyanine dyes transfer from the cells to the worm within minutes. Finally, adherent cells lyse on the parasite surface.

The simplest mechanism to explain DiI transfer is exchange through the medium. The dye diffuses from the RBC membrane into the medium and from the medium into the parasite membrane. Exchange must be occurring because worms that are not treated with lectin to promote RBC adherence acquire the dye from cells, albeit more slowly than lectin-treated organisms. Such exchange is known to occur with other lipid moieties such as cholesterol (1, 17) and phosphatidyl choline (17), although fluorescent derivatives appear to exchange

more rapidly than native membrane lipids (8, 18). The increased appearance of dye in the membrane of lectin-coated parasites is most probably not due to a simple reduction in the distance between the cell membrane and the parasite membrane because the dye diffuses so rapidly through the water from nonadherent cells, i.e.,  $\sim 10 \mu\text{m}$  in 0.25 s. Instead, it is far more likely that the dye is more easily removed from the adherent cell membrane or that direct interaction of the two membranes promotes transfer. Exchange may be a mechanism for the acquisition of host glycolipids or other membrane lipids since the parasite picks up ABH blood group (6, 12) or Forssman antigens (7) from serum (6, 12), saliva (6), and cell extracts (6, 7).

The question must be raised, however, whether ABH blood group glycolipids are inserted into the parasite membrane or are present only in RBC membrane fragments on the parasite surface. Our study supports the latter case since we failed to observe transfer of B and H antigens to the parasite membrane within 2 d of culture. This is significant because the half-time of the shedding of tegumental membrane glycoproteins and glycolipids is  $\sim 13$  h (20, 22). Thus, an antigen has to be

FIGURES 11–14 Transmission micrographs of the distribution of  $\text{R}\alpha\text{HRBC}$ . RBCs (R) were incubated with WGA-coated schistosomula (S) for 3 h (Figs. 11 and 12), or 22 h (Fig. 13), and reacted with  $\text{R}\alpha\text{HRBC}$  and  $\text{Au-G}\alpha\text{RIgG}$ . The colloidal gold is seen on the membrane of the intact RBC in Fig. 11, on a ghost (G) in Fig. 12, and on membrane fragments (f) in Fig. 13 but not on the parasite membrane. The distribution of gold on the fragment in Fig. 13 does not lead to any conclusions about the sidedness of the antigens in membrane because of the polyclonal nature of the  $\text{R}\alpha\text{HRBC}$ . Fig. 14 is a control showing that the  $\text{Au-G}\alpha\text{RIgG}$  does not bind to the RBC or ghost membranes in the absence of  $\text{R}\alpha\text{HRBC}$ . Fig. 11,  $\times 32,000$ ; Fig. 12,  $\times 50,000$ ; Fig. 13,  $\times 100,000$ ; Fig. 14,  $\times 70,000$ .



acquired in less than this time to reach an appreciable concentration in the membrane.

The results in the literature are more difficult to interpret. In particular, ABH antigens were screened by the mixed agglutination reaction, which employs RBCs of the same sera type as indicators of antigenicity (6, 12). Fragments of RBCs adherent to the worm may have caused adherence of the indicator cells and created the impression that antigens were inserted into the parasite membrane. In previous studies localization of ABH antigens by fluorescence or ultrastructure is not described. In the one study where immunofluorescence was done the result is described as "positive" and not illustrated (12). Thus, at present, there is no unequivocal demonstration of ABH blood group antigens inserted into the membrane of the parasite and, more important, no demonstration of such antigens on parasites removed from infected humans. On the other hand, this study does not prove that blood group antigens are not in the membrane of other stages of the life cycle. Other studies have used longer culture times, 15 d, and adult worms and, thus, tested conditions different from those used here. In particular, adult parasites eat and digest RBCs and regurgitate their fragments, so glycolipids could be released into the medium and then inserted into the parasite membrane. Another possibility is that the parasite could degrade the RBC membrane and biosynthetically incorporate the glycolipids into its own membrane.

The presence of RBC membrane fragments adherent to the parasite membrane represents a novel form of "host antigen acquisition." These fragments are easily recognized by either fluorescent or ultrastructural immunochemical techniques but they may present problems in other assay systems. In particular, immunological assays employing large particles such as RBCs, as described above, bacteria, or beads, can give an erroneous impression of the distribution of the antigen in the parasite membrane. Further, the biochemical analysis of parasite extracts could be influenced by the presence of adherent membrane fragments. However, these fragments may serve to mask host antigens since they clearly overlie fairly large areas of parasite membrane.

The failure to demonstrate transfer of labeled glycophorin or other RBC membrane proteins agrees with the observations of others who showed that the worm does not acquire MN blood group antigens (6, 12). In contrast, other integral membrane proteins, the murine histocompatibility antigens (9, 10, 23), are clearly acquired *in vivo* as shown by immunofluorescence (23). These molecules still have their membrane-spanning amino acids (25) and are apparently derived from both the circulating blood cells and tissue cells such as the endothelium (24). These antigens may be transferred to the parasite by membrane fusions similar to those shown to occur between human neutrophils and the parasite (2-4). Thus, the failure to observe transfer of RBC membrane proteins correlates with the failure to observe membrane fusion in this system.

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