Partial and Complete Detachment of Neutrophils and Eosinophils from Schistosomula: Evidence for the Establishment of Continuity between a Fused and Normal Parasite Membrane

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ABSTRACT   Neutrophils and eosinophils adhering to the surface of schistosomula of *Schistosoma mansoni* have been partially or completely detached with hypertonic sucrose or by pipetting. The sucrose-treated neutrophils are attached only in areas where there are pentalaminar fusions between the neutrophil and tegumental membranes, suggesting that these fusions attach the cells to the parasites. Pipetting breaks many of the attached cells. In thin section, the tegumental membrane underlying these cells is seen to be pentalaminar. By freeze-fracture techniques, modified attachment areas are found. The edge zone often appears as a single strand of intramembrane particles (IMPs) on the P_2 face and as a groove on the E_2 face. The edge zone may also have large discontinuities, in which case it no longer separates membrane faces of unequal IMP density from one another. In addition, the IMPs on the IMP-rich areas become aggregated and surrounded by craters in the membrane. These experiments suggest that the fusions may be the mechanism by which the parasite acquires some host membrane components on its surface.

On the other hand, eosinophil plasma membranes are seen adhering to a layer of electron-dense material on the parasite after the cells have been disrupted by pipetting. This suggests that eosinophils adhere to the parasite surface through their discharged granule material and not by membrane fusions.

Human neutrophils adhering to antibody- and complement-coated schistosomula form a fused membrane derived from the plasma membrane of the neutrophil and the outer tegumental membrane of the schistosomulum (2). Eosinophils do not generally fuse their plasma membrane with the parasite but, instead, exocytose onto the schistosomulum (8, 11) and appear to adhere to this discharged material (2). In the present paper we wish to test whether, indeed, the two cell types do adhere to the parasite by the fused membrane and by the discharged material, respectively. Cells adherent to the parasite surface were stressed by two mechanisms. First, hypertonic sucrose was used to partially detach the cells, leaving the cell and parasite in contact only at areas where they adhere. Second, pipetting was used as a mechanical stress to damage the cells and remove them. It was expected that only adherent portions of the cell plasma membrane would remain with the parasite. This second technique offers the additional advantage that the fate of the fused membrane between the neutrophil and parasite can be followed after the cell is disrupted. This is particularly important because the fusions may be a mechanism by which the schistosomula acquire host membrane components on their surface (4, 9, 16).

MATERIALS AND METHODS

Sucrose Stress Experiments

Cells and schistosomula, after incubation as previously described (2) to allow adherence, were centrifuged and the supernate was removed. The pellet was

resuspended in 1 ml of Eagle's minimal essential medium (MEM; Grand Island Biological Co., Grand Island, N. Y.), containing 0.25, 0.37, or 0.5 M sucrose. Resuspension took 10 s, and the mixture was allowed to stand for 1 min at room temperature, after which it was fixed as previously described (2), and processed for transmission electron microscopy (Table I).

TABLE I

Number of Experiments

Conditions of incubation	Hyper-tonic stress*	Mechanical stress‡	
	Thin-section	Thin-section	Freeze-Fracture
Schistosomula + NP + Ab + C	4	4	2
Schistosomula + Eo + Ab	3	—	—
Schistosomula + N + Ab + C	—	—	4

NP, nonpurified buffy coat cells; Ab, antischistosomal antibody preincubation; C, complement preincubation; Eo, purified eosinophils; N, purified neutrophils.
* In each experiment the incubation mixture was mixed with 0.25, 0.37, and 0.5 M sucrose 1 min before fixation.
‡ Each experiment consisted of a zero time sample and three other time points (see Table II for the times sampled in the six freeze-fracture experiments).

TABLE II

Leukocyte Adherence to Schistosomula after Washing

Experiment	Cells*	Initial incubation	Time after washing	Schistosomula bearing 5+	10+	20+
				adherent cells‡		
		min	min	%		
1	Mixed	60	Pre	71	25	7
			60	49	22	5
			120	23	1	0
			180	17	2	0
2	Mixed	90	Pre	72	26	9
			60	8	0	0
			120	6	0	0
			18 h	1	0	0
3	Neutrophils	60	Pre	85	37	9
			30	39	14	5
			90	26	8	2
4	Neutrophils	60	Pre	64	18	6
			30	44	7	0
			180	10	1	1
			18 h	1	0	0
5	Neutrophils	60	Pre	85	48	15
			8	71	10	2
			15	53	5	0
			45	23	5	0
6	Neutrophils	60	Pre	62	18	6
			8	29	2	0
			15	15	1	0

‡ Schistosomula were incubated for 30–60 min with a 1:8 dilution of antischistosomular antiserum at ambient temperature, then for 30–60 min at 37°C with a 1:20 or 1:30 dilution of fresh guinea pig serum as complement, and, finally, for 60 or 90 min at 37°C with leukocytes at a concentration of 2 or 4 × 10^6 cells per tube. At the end of the incubation, the schistosomula were washed free of excess cells by repeated sedimentation at 1 g in three changes of fresh medium. Duplicate or triplicate aliquots were taken for microscopic scoring either before (Pre) or at various times after washing. Adherence was scored as the percent of organisms in each sample that bore 5 or more (5+), 10 or more (10+), or 20 or more (20+) adherent cells.
* Experiment 1: mixed leukocytres (7% eosinophils, 67% neutrophils, 26% mononuclears) at 2 × 10^6 cells per tube. Experiment 2: mixed leukocytes (5% eosinophils, 61% neutrophils, 32% mononuclears) at 4 × 10^6 cells per tube. Experiment 3: neutrophils (99% neutrophils, 1% eosinophils) at 4 × 10^6 cells per tube. Experiment 4: neutrophils (96% neutrophils, 4% mononuclears) at 4 × 10^6 cells per tube. Experiment 5: neutrophils (98% neutrophils, 1% eosinophils, 1% mononuclears) at 2 × 10^6 cells per tubes. Experiment 6: neutrophils (97% neutrophils, 3% mononuclears) at 2 × 10^6 cells per tube.

Mechanical Stress Experiments

Cells, either purified neutrophils or unpurified buffy coat cells, were allowed to adhere to schistosomula preincubated with antibody and complement for 60–90 min at 37°C (2). Cells were removed by washing. Cells and schistosomula were resuspended by two aspirations into a Pasteur pipette and allowed to sediment at 1 g. This procedure was repeated three times in fresh changes of medium. The beginning of washing was arbitrarily chosen to be halfway through the washing procedure, which lasted ~15 min. This procedure led to a reduction in the number of cells that were free in the medium and that were attached to schistosomula (Table II). Adherence was scored by light microscopy on at least 100 organisms in each of two or three aliquots by transferring the aliquots to slides that had been coated with 0.1% toluidine blue in methyl alcohol. The preparations were covered and examined at × 100. The adherence of cells to living schistosomula, which were defined by their ability both to move and to exclude toluidine blue, was scored by recording the percent of organisms in each preparation that bore 5 or more, 10 or more, or 20 or more adherent cells (Table II). The remainder of the sample was processed for microscopy.

Microscopy

Schistosomula were prepared for freeze-fracture transmission and scanning electron microscopy as previously described (2).

RESULTS

Mechanical Stress Experiments — Light Microscope Observations

Light microscope estimates of adherence, both before and after washing, were carried out in six experiments (Table II). There was a marked reduction in the numbers of cells bound to schistosomula, as reflected by a progressive reduction in the percent of schistosomula bearing a given number of adherent leukocytes. Comparable decreases in the numbers of bound cells were observed with mixed leukocyte preparations and with purified neutrophils. In the former case, no attempt was made to distinguish between eosinophils and neutrophils. The reaction was extremely heterogeneous in that, although a marked reduction in the number of adhering cells could be demonstrated as early as 8 min after the beginning of the washing procedure, many organisms still bore some adherent cells 180 min later.
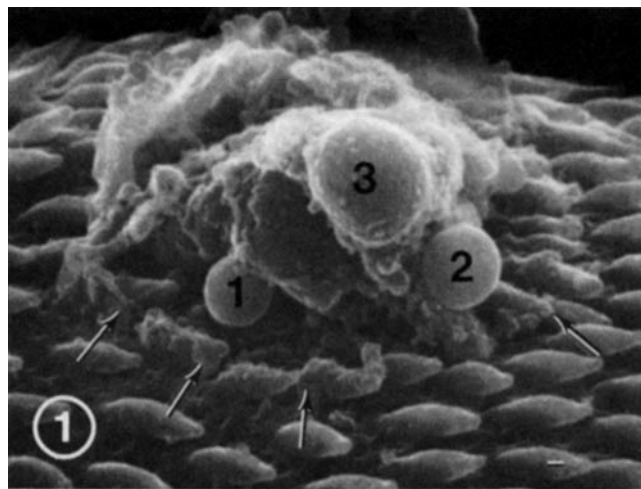

FIGURE 1 Scanning micrograph of a buffy coat cell adhering to the surface of a schistosomulum 30 min after mechanical stress. Note that the plasma membrane is broken at the edges of the area where the cell is attached (arrows). Two of the granules (1 and 2) are not covered by the plasma membrane, whereas a third (3) is. Bar, 1 μm. × 1,500.
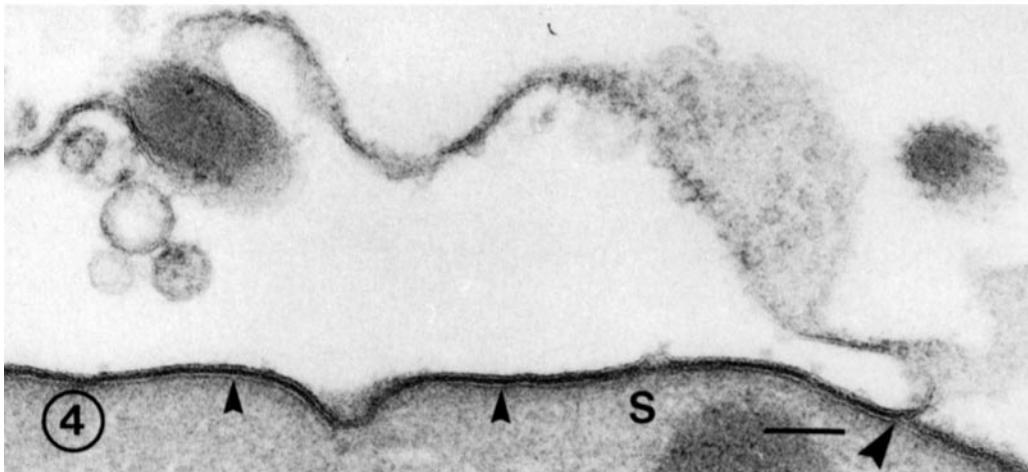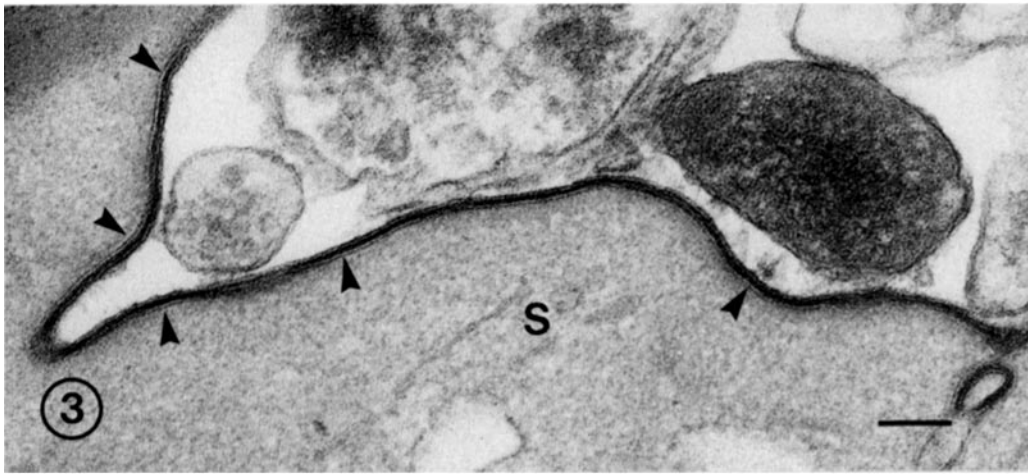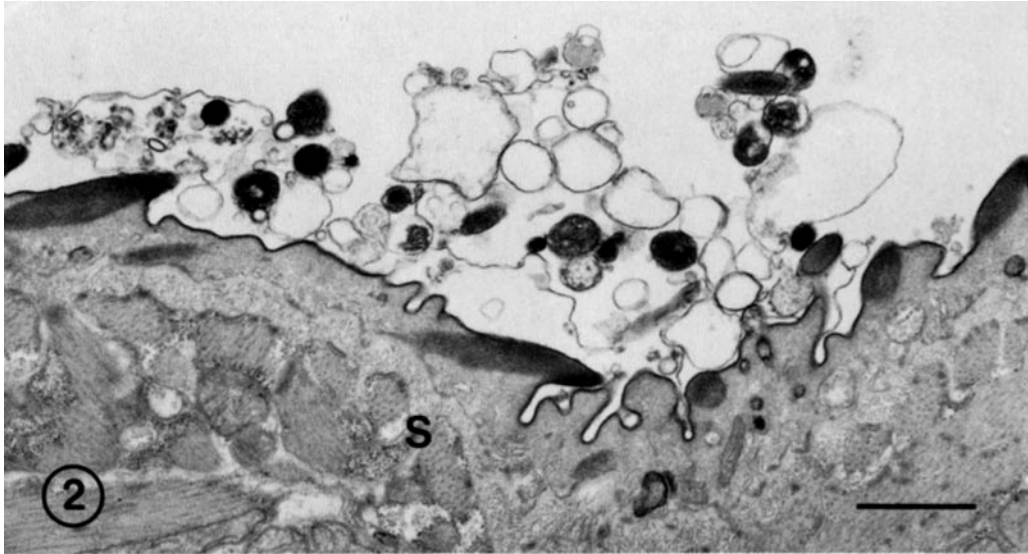
FIGURE 2   Low-power transmission micrograph of a neutrophil adhering to the surface of a schistosomulum (S) 10 min after mechanical stress. Note that the cell is disrupted and extracted. Bar, 1 μm. × 16,000.

FIGURE 3   High-power transmission micrograph on a neutrophil adhering to the surface of a schistosomulum (S) 10 min after mechanical stress. Note that the tegumental membrane is pentalaminar (arrowheads). Bar, 0.1 μm. × 97,000.

FIGURE 4   Remnant of a cell membrane adhering to the surface of a schistosomulum (S) 30 min after mechanical stress. Note that the cell membrane appears to insert into the tegumental membrane (large arrowhead) and that the tegumental membrane is pentalaminar (small arrowheads). Bar, 0.1 μm. × 105,000.
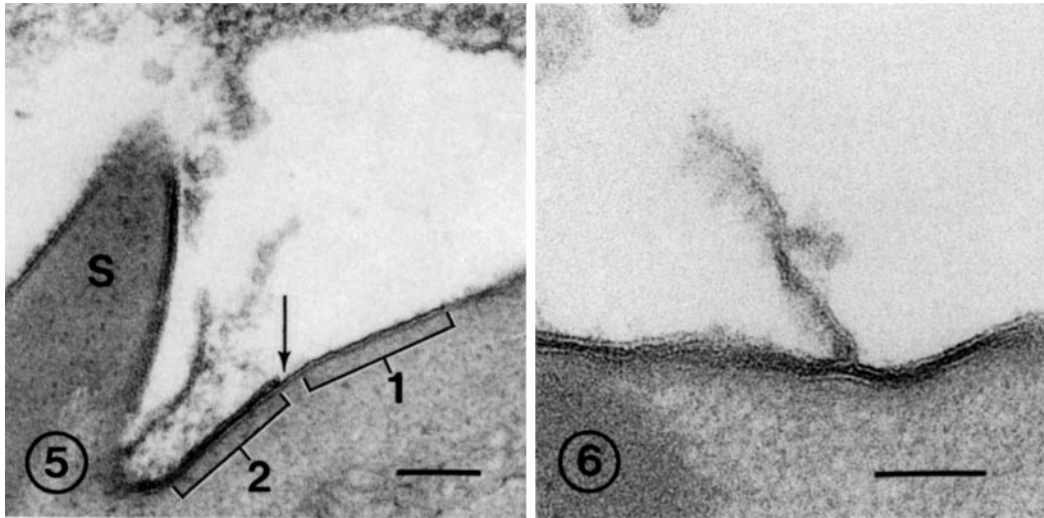
FIGURE 5   Transmission micrograph of neutrophil and schistosomulum (5) 10 min after mechanical stress. Note that the parasite is covered by both a trilaminar (1) and a pentalaminar (2) membrane. Electron-dense material is attached to the pentalaminar membrane. The outer membrane appears to be broken at the point where the tegumental membrane becomes trilaminar (arrow). Bar, 0.1 μm. × 107,000.

FIGURE 6   High-power micrograph of a membrane fragment attached to the surface of a schistosomulum taken immediately after mechanical stress. The trilaminar fragment inserts into the outer membrane like an inverted T. Note the continuity of the outermost leaflet of the outer membrane with the outer leaflets of the fragment and the continuity of the lucent layers of the two membranes. Note also that the tegumental membrane adjacent to the fragment, in areas where it is cut in normal section, is pentalaminar. Bar, 0.1 μm. × 147,000.
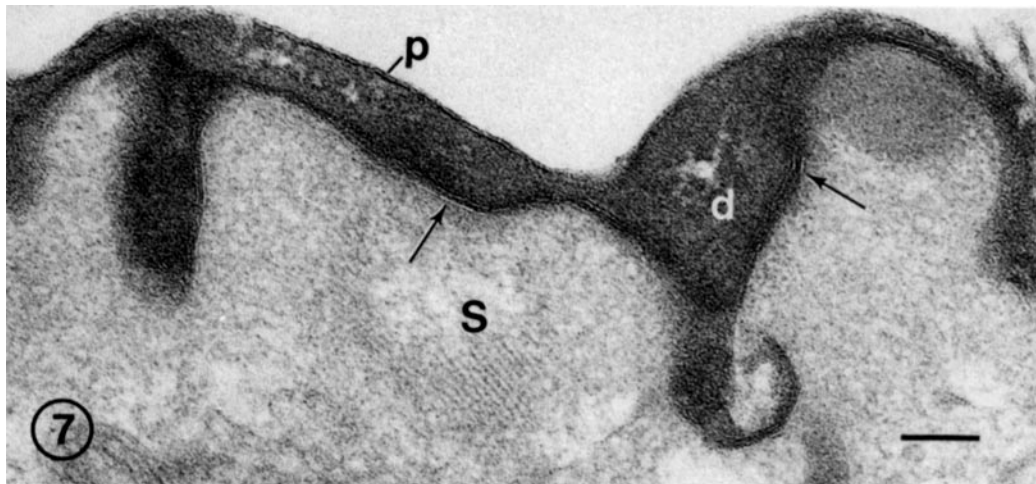

FIGURE 7   Remnants of an eosinophil adhering to a schistosomulum immediately after mechanical stress. Note that the plasma membrane (p) is adhering to the electron-dense material (d), which is in turn attached to the pentalaminar tegumental membrane (arrows) of the schistosomulum (5). Bar, 0.1 μm. × 98,000.

Mechanical Stress Experiments — Ultrastructural Observations

In general, cells attached to the parasite throughout the entire time-course of the experiment (3 h) can either appear as they do in the normal unstressed experiments (2), or they may be broken. Most broken cells that are seen are observed immediately after the washing. By scanning electron microscopy, the plasma membrane is disrupted, usually near the point where it attaches to the parasite surface, and granules are seen without any covering of plasma membrane (Fig. 1). By thin-section microscopy at low power, fragmented neutrophils composed of a few cytoplasmic organelles and pieces of membrane are seen adhering to the surface of the parasite (Fig. 2). At high power, the tegumental membrane underlying the broken neu-

trophils is most often seen to be pentalaminar (Figs. 3 and 4). However, occasionally the outer membrane appears disrupted, and only the inner membrane covers the tegument (Fig. 5). At the edge of the broken cell, trilaminar membrane fragments 0.25–1 μm in length are seen inserting into the outer membrane in an inverted T shape (Fig. 6). The outer leaflets of the trilaminar membrane fragment are continuous with the outermost leaflet of the outer membrane of the schistosomulum, and the lucent layers of the fragment and the outer membrane are also continuous (Fig. 6). Eosinophils are also disrupted by this procedure, but their plasma membranes are attached to the tegument through a layer of electron-dense material (Fig. 7).

By freeze-fracture, normal attachment areas, such as have been described previously (2), and modified attachment areas, as described below, were found at all times after washing that
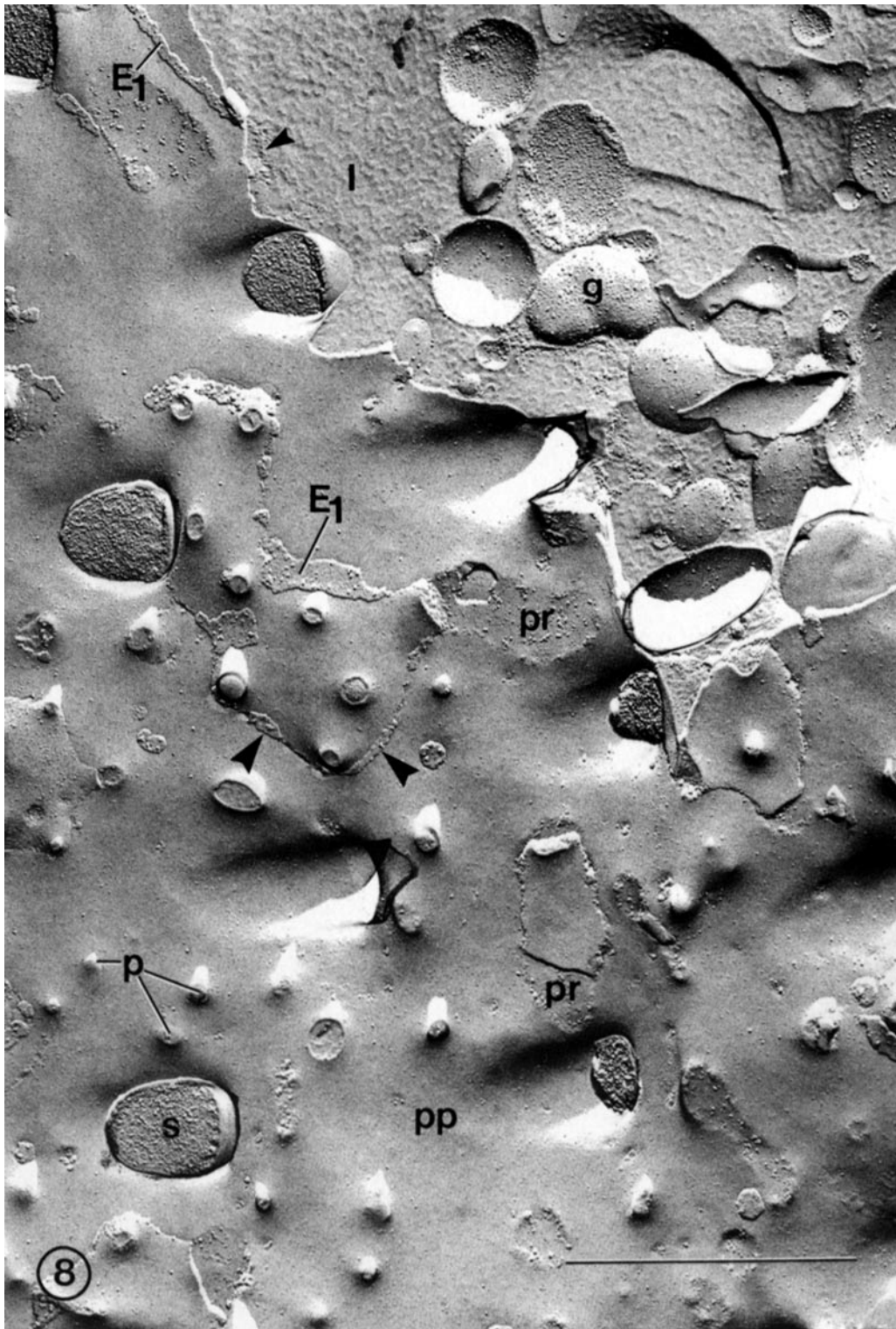
FIGURE 8 Schistosomular surface 2 h after mechanical stress. At the upper right, membrane faces typical of neutrophil granules (g) can be seen. Note that no cross-fracture typical of a plasma membrane is seen around the granules or extending from the edge of the schistosomulum where the E_1 ridge meets the ice (/). However, a few filaments (small arrowhead) are seen in the ice on the granule side of the E_1 ridge, whereas the other side of the ridge has no such material. At the bottom of the picture is an attachment area with IMP-rich (pr) and IMP-poor (pp) areas. The attachment area is modified in that there are linear step fractures into the E_1 membrane around what appears to be an IMP-rich area (large arrowhead). s, spine. p, pits. Bar, 0.1 μm. × 44,000.

were examined (8 min to 3 h). However, the modified areas are more often seen at early time points (8–30 min). At low power, fractures which pass from the modified attachment areas across the edge of the schistosomulum and onto the surrounding ice do not usually pass into a cell, unlike such fractures associated with normal attachment areas (2). Occasionally, neutrophil granules can be seen in the ice next to the attachment area, but these granules do not hve a cross-fractured plasma membrane around them (Fig. 8). The attachment areas under such cells resemble those seen where intact cells are attached except that some of the intramembrane particle (IMP)-rich areas are surrounded by a zone of skip fracture into the inner membrane (Fig. 8).

The major alterations seen in the modified attachment areas occur in the edge zones, which are either true edge zones at the border of the cell or are possibly derived from edge zones formed around IMP-rich areas (Figs. 9–12). There are three major changes. First, the edge zones are most often composed of strings of IMPs in the P-face view and a linear depression in the E-face view, although areas of step-fracture into the inner membrane are still seen (Figs. 9–12). Second, in many areas, the edge zones are longer than those seen in the normal attachment areas in that they no longer simply surround a large area with a 5–8-μm diameter but wind back and forth to form a network (Figs. 10–12). Third, these edge zones may be continuous, in which case they separate areas of differing IMP concentration from one another (Fig. 10), or they may be discontinuous. If the discontinuities in the edge zone are small (<50 nm) and infrequent, the concentration of IMPs on either side of the edge zone is different (Figs. 9 and 11). On the other hand, if the discontinuities are large and numerous, the IMP concentration is the same on both sides of the edge zone (Figs. 11 and 12).

A second set of alterations is seen in the IMP-rich areas (Figs. 13 and 14). The fracture plane no longer passes smoothly through these areas. The membrane faces are rough, and small dimples or craters appear (Fig. 13). In other images, the IMPs are aggregated with a ring of craters around them (Fig. 14). As a consequence of this aggregation, the IMP-rich areas appear smaller (Fig. 14). Finally, plaques of densely packed IMPs can be seen in slightly depressed areas of the outer membrane (Fig. 14, inset). These plaques often have linear arrays of IMPs at their edges (Fig. 14).

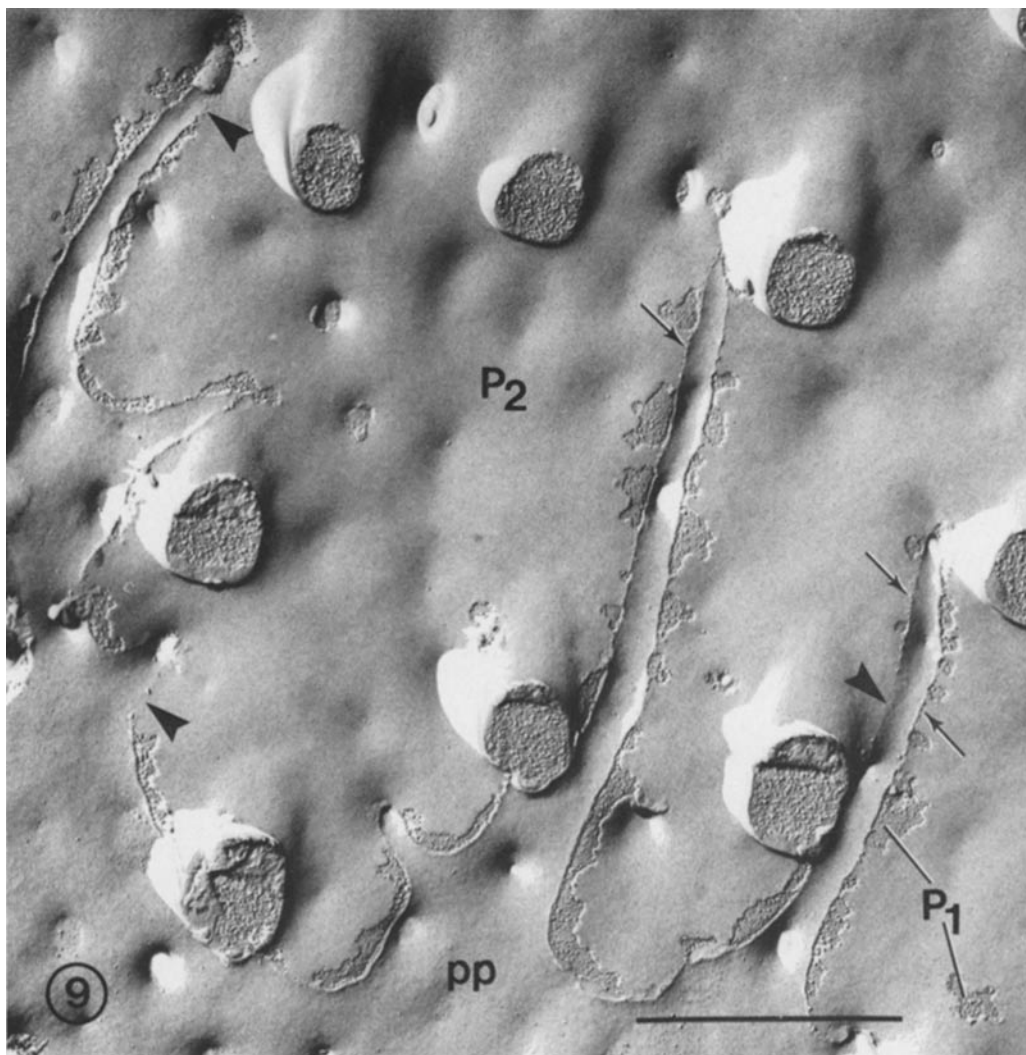Finally, there are occasional broad areas, 3–4 μm in diameter,

FIGURE 9 Schistosomular surface 2 h after mechanical stress, E-face view. An attachment area (pp) is seen at the bottom of the picture and projects along the left side and in the center into a normal P₂ face. The edge zone is composed of step-fractures into P₁ and, in a few places, of a string of IMPs (arrows). In other areas the edge zone is discontinuous (arrowheads), and the membrane faces of the fusion area and the outer membrane are continuous. Bar, 1 μm. X 35,000.
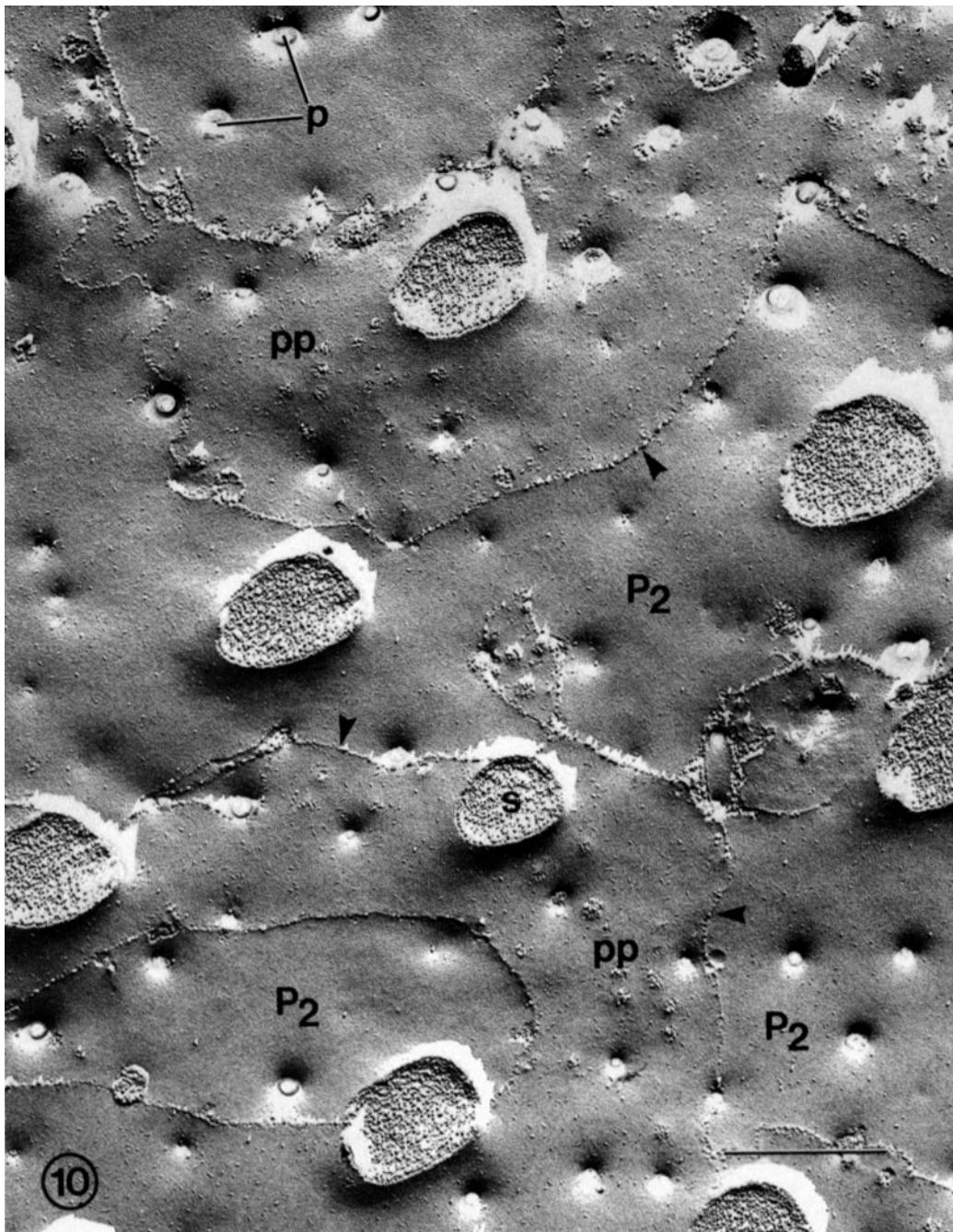
FIGURE 10 Schistosomular surface 3 h after mechanical stress, P-face view. Note that the edge zones (arrowheads) are composed of a single string of IMPs. They separate fused membrane (pp) from the outer membrane (P_2). p, pits. s, spine. Bar, 1 μm. × 23,000.

of fractures into the inner membrane (Fig. 15). They often contain circular step-fractures into the outer membrane (Fig. 15).

Sucrose Stress Experiments

At 0.25, 0.37, and 0.50 M sucrose, neutrophils are shrunken and pulled away from the parasite surface, except in areas where they are tightly attached (Fig. 16). High-power examination of these areas shows that a pentalaminar membrane is present between the cell and the parasite (Figs. 17 and 18). Heptalaminar fusions are not seen between cells and the parasite in preparations treated with these concentrations of sucrose. In the area where the cell is lifted off, the tegument is covered by a pentalaminar membrane in preparations incu-

bated in 0.25 M sucrose (Fig. 17), and by either a pentalaminar or a trilaminar membrane in preparations incubated in 0.37 or 0.50 M sucrose (Fig. 18). At the edge of the cell, the plasma membrane of the neutrophil inserts into the outer tegumental membrane in an inverted T (Fig. 16, inset), as do the membrane fragments seen in the mechanically stressed preparations (Fig. 6). Eosinophils are also shrunken by the sucrose and adhere to the tegument through a layer of electron-dense material similar to that seen in the mechanically stressed preparations (Fig. 7).

DISCUSSION

These experiments show that the membrane formed by the fusion of the neutrophil plasma membrane and the schistoso-

mular outer membrane serves to attach the cell to the parasite because the fused pentalaminar membrane is the only structure that connects the cell to the parasite in the presence of hypertonic sucrose. Further evidence for the strength of the fused membrane is provided by experiments in which the cell that contributes to the membrane is broken or pulled away by pipetting. Under such mechanical stress, the fused membrane most often remains with the parasite and is seen as a pentalaminar membrane in thin section or as a modified attachment complex in freeze-fracture. In some cases, however, the fused
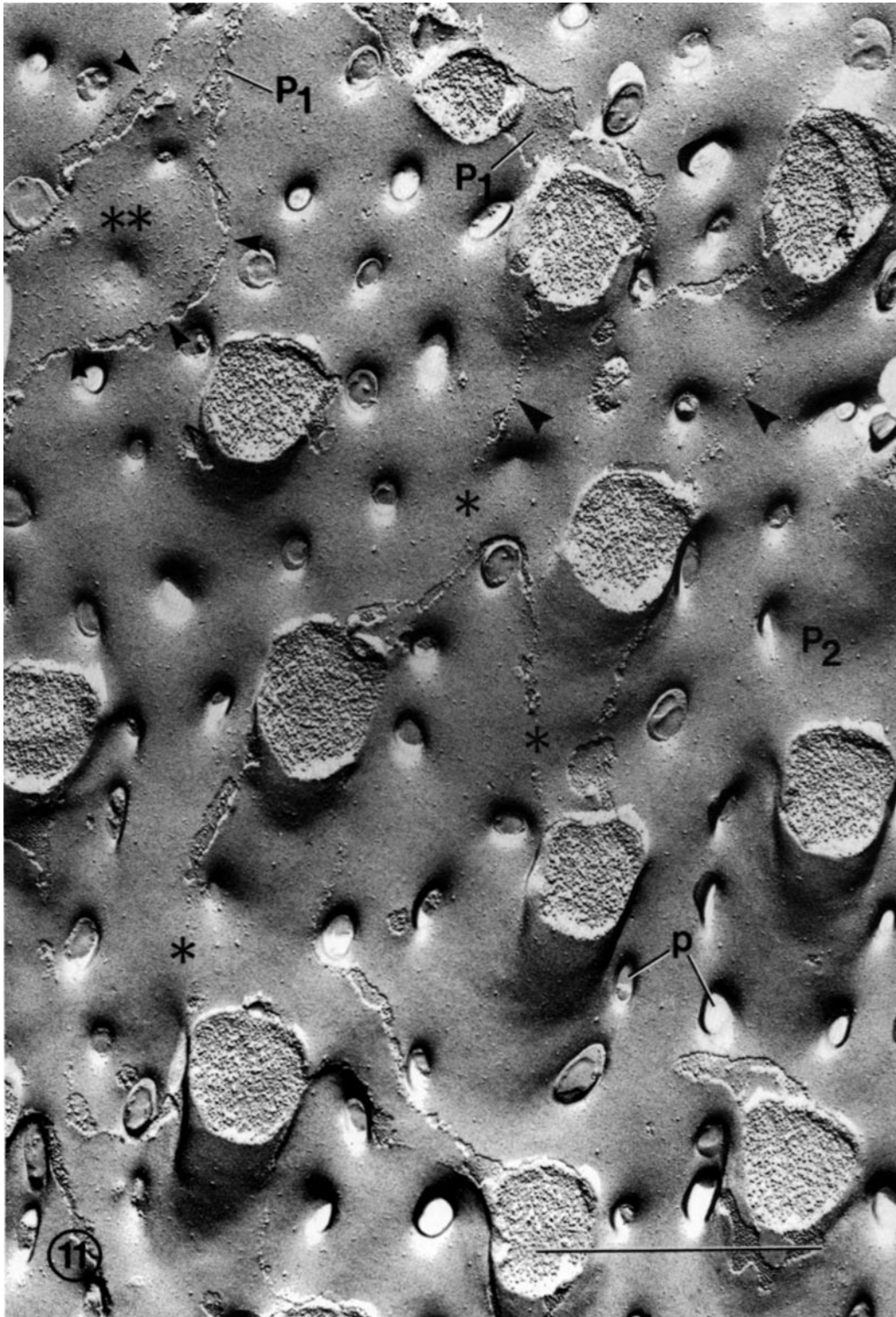

FIGURE 11   Schistosomular surface 45 min after mechanical stress, P-face view. There is an extensive edge zone formed both by step-fractures into P_1 and by linear arrays of IMPs (large arrowheads). Note that the edge zone is broken in several places (*) and that the membrane faces on either side of the zone are continuous. In the upper left hand corner, there is a small membrane face (**) with a higher IMP density than the P_2 face. This face is surrounded by a trench of P_1 face, which is bridged by membrane in several places (small arrowheads). Bar, 1 μm. × 45,000.
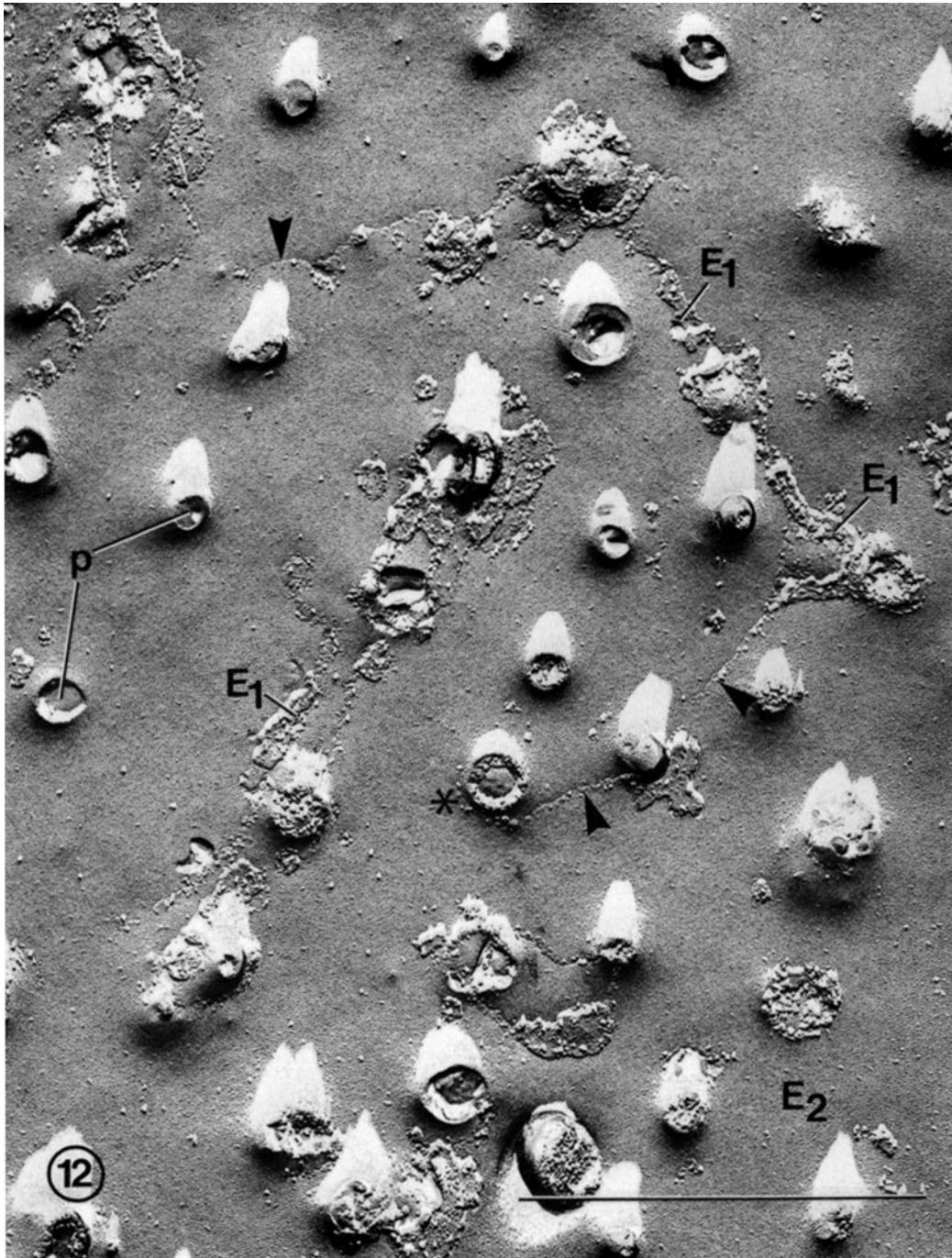
FIGURE 12   Schistosomular surface 2 h after mechanical stress, E-face view. Note the edge zone composed of fragments of E_1 and linear depressions in the E_2 face (arrowheads). These depressions have IMPs associated with them in some areas. Note that the zone is broken in the middle of the picture (*) and that the membrane faces on either side of the zone are continuous and appear similar. p, pits. Bar, 1 μm. $\times$ 58,000.
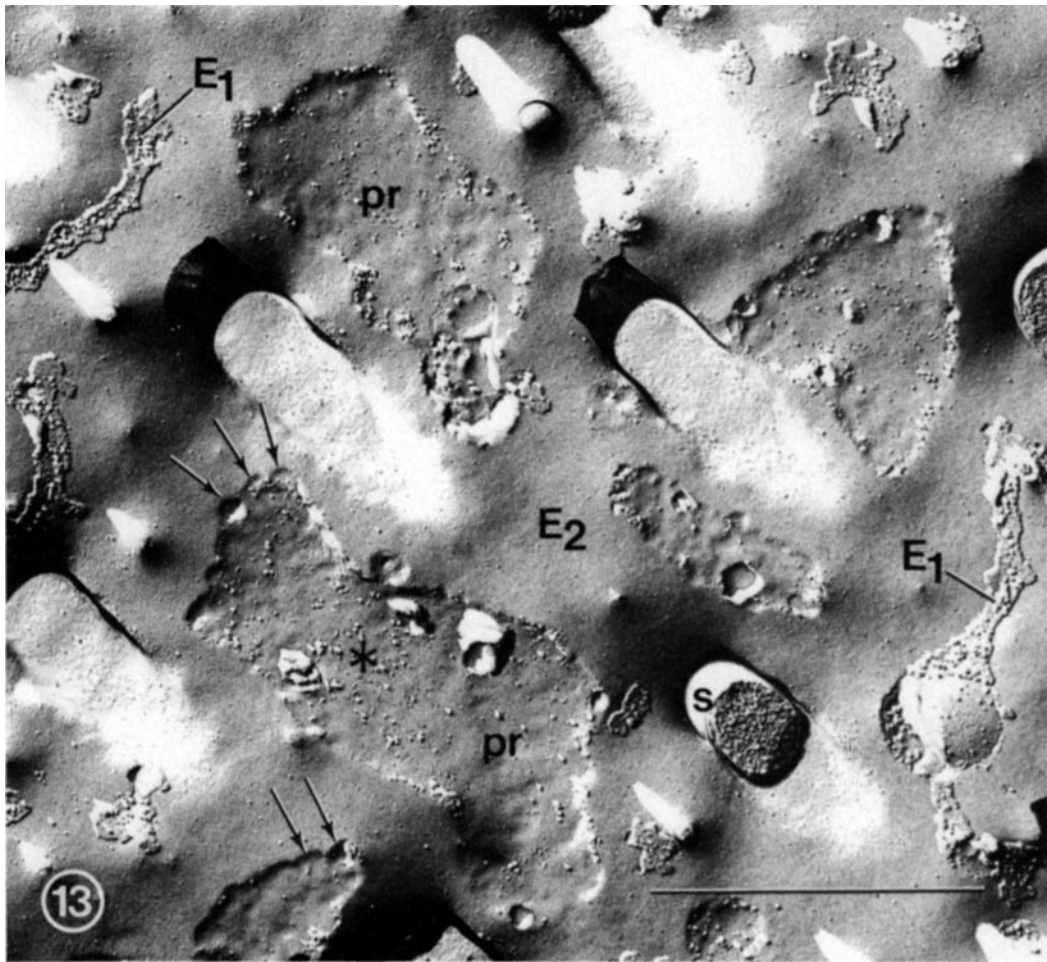
FIGURE 13 Schistosomular surface 3 h after mechanical stress, E-face view. Note that the IMP-rich (pr) areas appear depressed and that the fracture face is rough, unlike the surrounding E_2 membrane. Note also the scalloping of the edges of these areas formed by small craters or dimples (arrows). Several linear fragments of E_1 face, which resemble edge zones, are also seen. s, spine. Bar, 1 μm. $\times$ 45,000.

membrane may be pulled off with the cell, leaving the tegument covered only by a trilaminar membrane, as seen in thin section. In freeze-fracture, these areas are seen as extensive fractures into the inner membrane. Such a separation of the fused membrane from the inner parasite membrane can also be seen in the specimens incubated in 0.37 and 0.50 M sucrose but not in those incubated in 0.25 M sucrose. In all of these experiments, the fused membrane appears stable and strong enough to maintain its identity against forces which deform or destroy the cell from which it is derived.

These experiments also show that the edge zone separating the fused membrane from the normal outer membrane is analogous to an occluding junction in an epithelium. Structurally, the edge zone may be seen in freeze-fracture as a step-fracture into the inner membrane or as a string of IMPs in the P_2 face and as a linear depression or groove on the E_2 face. The linear array of IMPs is similar to that seen in low-resistance occluding junctions found in the proximal tubule and other epithelia (3, 5, 6, 17). On the other hand, the thin-section appearances of the edge zone and occluding junctions are dissimilar in that the edge zone appears as an inverted T in which the cell membrane inserts into the parasite outer membrane, whereas an occluding junction is composed of two membranes running side by side that are fused over a variable distance (5, 6, 17). However, there is structural similarity in

that the central lucent layers are in contact in both types of junction (6, 17). In addition to structural similarity, there is functional similarity between the edge zone and occluding junctions in that both separate membranes that are different from one another. The edge zone separates the fused membrane from both the unfused neutrophil membrane and the normal outer membrane (2). Occluding junctions separate apical from basolateral plasma membranes in epithelia (3, 5, 6, 12, 13, 17). Furthermore, when the occluding junctions are disrupted, the membranes on either side of the junctions become continuous and mixing can occur (12, 13, 17). Similarly, when the edge zone is disrupted, the membrane faces on either side are continuous, and the IMP concentration is similar. This observation strongly suggests that the edge zone, as does the occluding junction, serves as a barrier to diffusion in the plane of the membrane.

The equilibration of the membrane faces on either side of the broken edge zone also implies that the outer membrane is fluid. This interpretation is in agreement with measurements made on the clearance from the surface of schistosomula of a variety of exogenous labels, namely, concanavalin A, C3, which had been activated by the alternative pathway, and antischistosomal antibody (14, 15). All three labels are cleared with a half-time of 4.5-5 h and follow the kinetics of an exponential decay curve (14, 15), which suggests that the membrane is
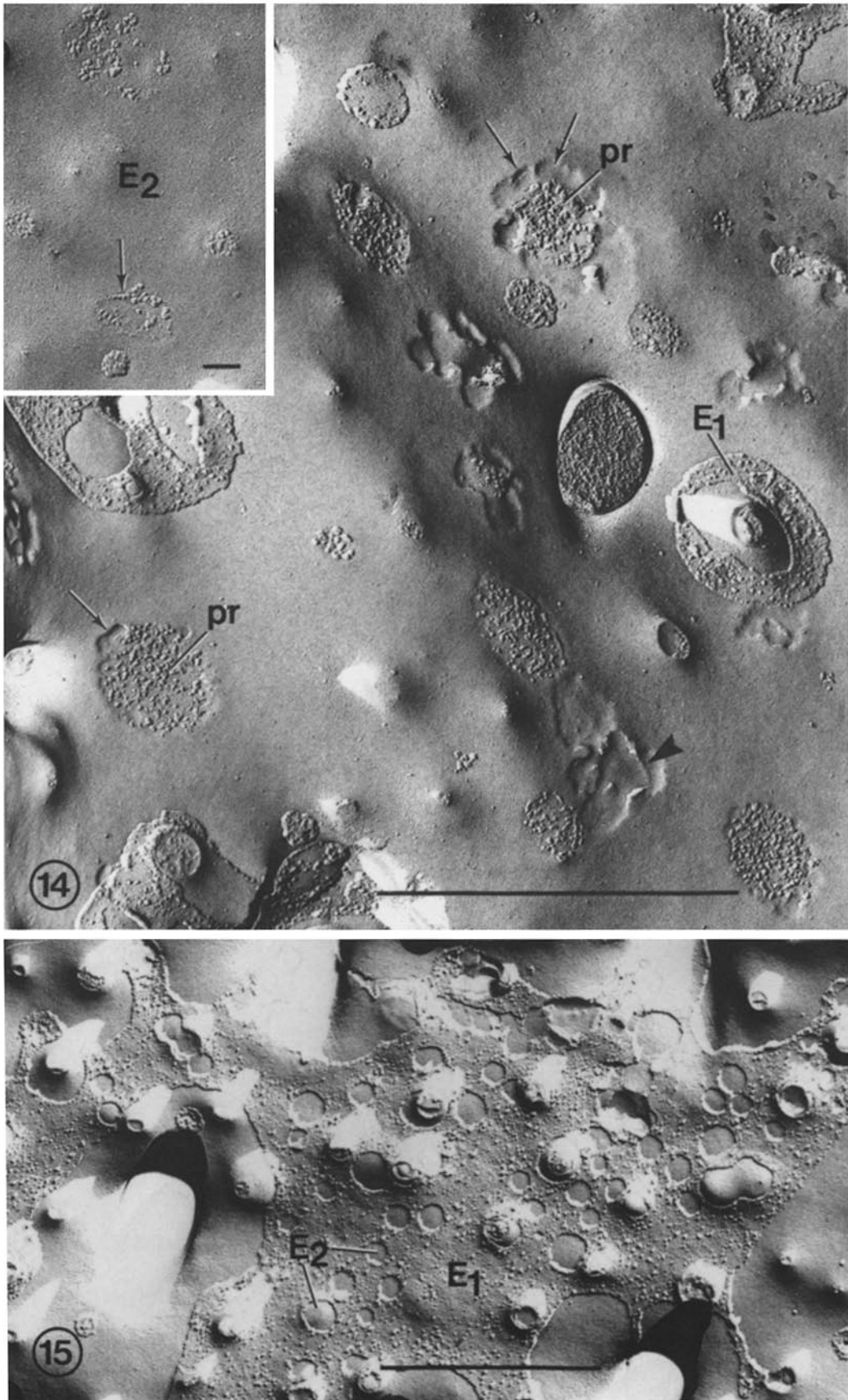
FIGURE 14   Schistosomular surfaces 3 h after mechanical stress, E-face view. Note the dense aggregations of (*pr*) of IMPs in the E_2 face, which in some cases are surrounded by depressions or craters (arrows). These aggregated IMPs appear slightly depressed from the plane of the E_2 face. In some places, the craters appear to be unrelated to the IMPs (arrowhead). *Inset* shows aggregates of IMPs in the E_2 membrane face. Note the linear array of some of the IMPs (arrow). Bar, 1 µm. × 58,000. (*inset*) Bar, 0.1 µm. × 65,000.

FIGURE 15   Schistosomular surface 90 min after mechanical stress, E-face view. Note the extensive area of fracture revealing the E_1 face. The E_2 face is seen as small circular step-fractures beneath the E_1 face. Bar, 1 µm. × 36,000.
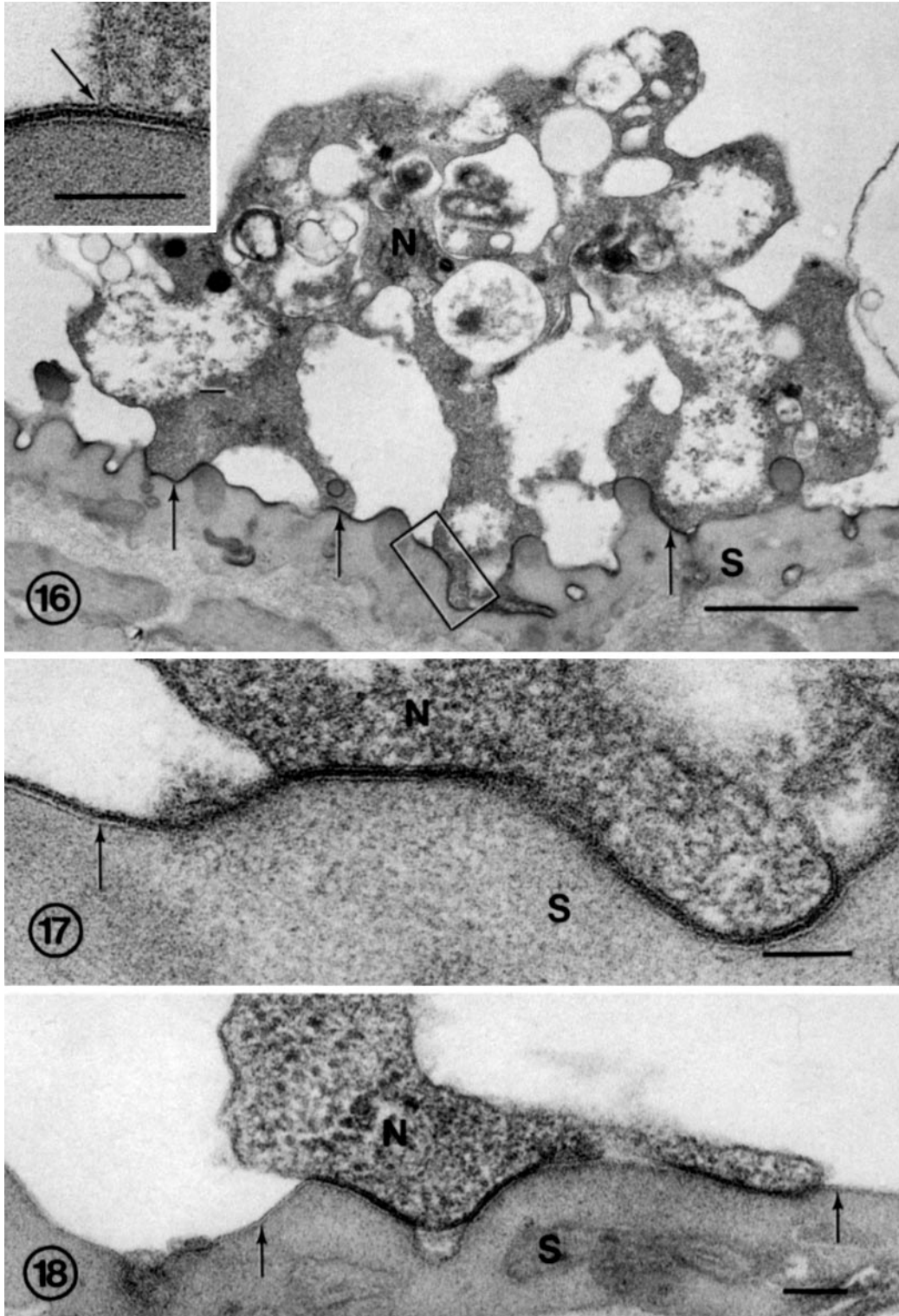
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FIGURE 16 Neutrophil (N) adhering to the surface of a schistosomulum (S) 1 min after incubation in 0.25 M sucrose. Note that the cell is shrunken and pulled away from the parasite surface, except in areas where it is tightly attached (arrows). *Inset* shows a neutrophil plasma membrane inserting into the outer tegumental membrane (arrow). Bar, 1 μm. × 23,000. (*inset*) Bar, 0.1 μm. × 200,000.

FIGURE 17 High-power view of the area in the rectangle in Fig. 16. Note that a pentalaminar membrane covers the tegument both where the cell (N) is attached and where it is pulled away (arrow). Bar, 0.1 μm. × 136,000.

FIGURE 18 High-power view of a neutrophil attached to a schistosomulum 1 min after incubation in 0.37 M sucrose. Note that the membrane between the cell (N) and the parasite (S) is pentalaminar, whereas the surrounding membrane is trilaminar (arrows). Bar, 0.1 μm. × 93,000.

continuously mixing with itself.

A much more tentative conclusion of these experiments is that the schistosomular outer membrane may acquire molecules from the neutrophil plasma membrane via the fused membrane. It has been known for some time that schistosomula can acquire host membrane components on their surface. In particular, when cultured with human erythrocytes, schistosomula can be stained indirectly with antisera against the ABH group glycolipid of the cells with which they were cultured but not with antisera against the MN group (9), which is found on glycophorin (7). When grown in vivo in mice, they can also be stained by indirect immunofluorescence for H-2 antigens, which are integral membrane proteins (16). If such parasites are transplanted into mice of a different haplotype, then they will lose the original haplotype and acquire the new one (16). Additional evidence for the acquisition of host membrane components comes from experiments in which the IMP concentrations of the outer membrane of schistosomula grown in vitro and in vivo were compared (10). It has been found that the IMP concentration of the E_2 face increased from 314 to 1,104 IMPs/μm^2 in parasites grown in vivo but decreased to 38 IMPs/μm^2 in parasites grown in vitro (10). These observations taken together with the present ones, which show that the neutrophil plasma membrane can fuse with the schistosomular outer membrane and that this fused membrane appears to mix with the outer membrane when the cell is disrupted, suggest that the parasite may acquire host membrane molecules through these fusions. This conclusion is not proven by these experiments. We have not shown the insertion of a properly oriented, intact neutrophil membrane component into the parasite membrane as a result of the fusion. However, the experiments do provide a hypothesis for further experimentation.

These experiments also suggest that eosinophils adhere to the surface of the schistosomulum through the electron-dense material that has been discharged from the granules (1, 2). The plasma membrane can be seen adhering to this layer after the rest of the cell has been torn off, suggesting that the cell has created a sequestered space between itself and the larva. In such a space, the cell may be able to maintain conditions, particularly low pH and high protein concentration, that would allow the lysosomal constituents to damage the parasite.

REFERENCES

1. Butterworth, A. E., M. A. Vadas, D. L. Wassom, A. Dessein, M. Hogan, B. Sherry, G. J. Gleich, and J. R. David. 1979. Interactions between human eosinophils and schistosomula of *Schistosoma mansoni*. II. The mechanism of irreversible eosinophil adherence. *J. Exp. Med.* 150:1456–1471.
2. Caulfield, J. P., G. Korman, A. E. Butterworth, M. Hogan, and J. R. David. 1980. The adherence of human neutrophils and eosinophils to schistosomula: evidence for membrane fusion between cells and parasites. *J. Cell Biol.* 86:46–63.
3. Claude, P., and D. A. Goodenough. 1973. Fracture faces of zonulae occludentes from "tight" and "leaky" epithelia. *J. Cell Biol.* 58:390–400.
4. Dean, D. R., and K. W. Sell. 1972. Surface antigens on *Schistosoma mansoni*. II. Adsorption of a Forssman-like host antigen by schistosomula. *Clin. Exp. Immunol.* 12:525–540.
5. Farquhar, M. G., and G. E. Palade. 1963. Junctional complexes in various epithelia. *J. Cell Biol.* 17:375–412.
6. Friend, D., and N. B. Gilula. 1972. Variations in tight and gap junctions in mammalian tissues. *J. Cell Biol.* 53:758–776.
7. Furthmayr, H. 1978. Structural comparison of glycophorins and immunochemical analysis of genetic variants. *Nature (Lond.).* 217:519–524.
8. Glauert, A. M., A. E. Butterworth, R. F. Sturrock, and V. Houba. 1978. The mechanism of antibody-dependent, eosinophil-mediated damage to schistosomula of *S. mansoni* in vitro: a study by phase-contrast and electron microscopy. *J. Cell. Sci.* 34:173–192.
9. Goldring, O. L., J. A. Clegg, S. R. Smithers, and R. J. Terry. 1976. Acquisition of human blood group antigens by *Schistosoma mansoni*. *Clin. Exp. Immunol.* 26:181–187.
10. McLaren, D. J., D. J. Hockley, O. L. Goldring, and B. J. Hammond. 1978. A freeze fracture study of the developing tegumental outer membrane of *Schistosoma mansoni*. *Parasitology.* 76:327–348.
11. McLaren, D. J., C. D. Mackenzie, and F. J. Ramalho-Pinto. 1977. Ultrastructural observations on the in vitro interaction between rat eosinophils and some parasitic helminths (*Schistosoma mansoni*, *Trichinella spiralis* and *Nippostrongylus brasiliensis*). *Clin. Exp. Immunol.* 30:105–118.
12. Meldolesi, J., G. Castiglioni, R. Parma, N. Nassivera, and P. De Camilli. 1978. Ca^{++}-dependent disassembly and reassembly of occluding junctions in guinea pig pancreatic acinar cells. Effects of drugs. *J. Cell Biol.* 79:156–172.
13. Pisam, M., and R. Ripoche. 1976. Redistribution of surface macromolecules in dissociated epithelial cells. *J. Cell Biol.* 71:907–920.
14. Samuelson, J. C., J. P. Caulfield, and J. R. David. 1979. Loss of antischistosomal antibody, complement and concanavalin A binding from *S. mansoni*. *J. Cell Biol.* 83(2, Pt. 2):125a (Abstr.).
15. Samuelson, J. C., A. Sher, and J. P. Caulfield. 1980. Newly transformed schistosomula spontaneously lose surface antigens and C3 acceptor sites during culture. *J. Immunol.* 124:2055–2057.
16. Sher, A., B. F. Hall, and M. A. Vadas. 1978. Acquisition of murine major histocompatibility complex gene products by schistosomula of *Schistosoma mansoni*. *J. Exp. Med.* 148:46–57.
17. Staehelin, L. A. 1974. Structure and function of intercellular junctions. *Int. Rev. Cytol.* 39:191–283.