shorter time scale of a fluorescence experiment, each DPH molecule reports orientational order (Fig. 3 *B*) reflecting roughly a single site (6). (*b*) A recent study using electron-spin-resonance probes has stressed the ability of cytochrome oxidase to "immobilize" lipid structure beyond the primary or boundary layer (7). This apparent disagreement with our results can be resolved by recognizing that the shape of an electron-spin-resonance spectrum is sensitive to the rate of lipid acyl chain motion as well as to the average order in the bilayer. Thus, it may be that lipid molecules within the secondary layer experience slower acyl chain motions than in a bulk lipid bilayer. This would not preclude decreased average order relative to bulk lipid due to a greater extent of motion. (*c*) The spectra of electron spin probes attached to the $Ca^{2+}$-ATPase have been found to vary with lipid:protein ratio, despite the constant juxtaposition of the probe to the protein (8). This has been interpreted in terms of protein-rich domain formation, leading to increased protein-protein contacts (9). We agree that the properties of both the primary and secondary lipid layers should be sensitive to protein-protein separation. However, our calculations indicate that this complication is unnecessary to explain our data over the range of protein content considered here. In addition, we find in freeze-fracture electron micrographs of native SR membranes no evidence of particle patches which would indicate protein-rich domains, even at 4°C.[1]

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[1]Hoechli, B. R. Lentz, and G. Meissner. Unpublished results.

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# THE INTERACTION OF MEMBRANE-ACTIVE COMPOUNDS WITH THE SURFACES OF SCHISTOSOMULA AND ADULT *SCHISTOSOMA MANSONI*

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The adult *Schistosoma mansoni* is a multicellular parasite which lives in the portal blood vessels of man, and causes the disease called schistosomiasis (bilharziasis). The organism is covered by a complex pentalaminate surface membrane (Hockley and McClaren, 1973). The schistosomulum, the name given to the growth stage of the parasite formed after penetration through the mammalian skin by the infective larva (cercaria), also has a pentalaminate surface membrane, which changes in ultrastructure and antigenicity during the migration of the parasite through the tissues of the host (McClaren et al., 1978). These changes are accompanied by a decreased sensitivity of the membrane to the manifestations of the immune response of the host (McClaren, 1980). Changes in the lipid phase of artificial and natural membranes can affect the expression of antigenic determinants (Kinsky, 1978) and their ability to be damaged by immunological mechanisms (Schlager and Ohanian, 1980).



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TABLE I

EFFECTS OF SEVERAL COMPOUNDS ON SURFACE MEMBRANE OF *SCHISTOSOMA MANSONI*

| Effect on parasite surface membrane | Name of compound |
|---|---|
| 1. Releases both $^{51}Cr$ and $^{125}I$WGA from schistosomulum and adult | Linoleic acid (I serum) |
| | EM49 (squibb) |
| | Tween 20 |
| | Phospholipase C (*Clostridium perfringens*) |
| | Anionic Detergents (SDS, DOC) |
| 2. Releases $^{51}Cr$ from schistosomulum; both $^{51}Cr$ and $^{125}I$WGA from adult | Saponin (1–10 mg $ml^{-1}$) |
| | Melittin |
| | Phospholipase $A_2$ (bee or snake venom) |
| | Delta toxin (*Staphylococcus aureus*) |
| 3. Releases $^{51}Cr$ from schistosomulum; no release from adult | Retinol (vitamin A alcohol) |
| | Amphotericin B |
| | Hydrocarbon micelles |
| 4. Releases $^{125}I$WGA from schistosomulum; no release from adult | Tween 40 |
| | Tween 80 |

To detect possible changes in the lipid phase of the parasite membrane during development we have investigated the effects of a variety of lipophilic membrane-active compounds on both the schistosomulum and the adult parasite. The simultaneous release of $^{51}Cr$ and $^{125}I$ wheat-germ agglutinin (WGA) from labeled parasites was used as a measure of permeability change and of release of surface macromolecules from the surface membranes of the parasites during treatment with the reagents.

## MATERIALS AND METHODS

Adult worms were perfused from mice by the method of Smithers and Terry (1965). Schistosomula were mechanically transformed from cercariae shed from the infected intermediate host, *Biomphalaria glabrata* (Colley and Wikel, 1974).

Adult worms or schistosomula were labeled separately for 1 h with 100$\mu$Ci $^{51}Cr$ and 1$\mu$Ci WGA (5$\mu$g) in Eagle's medium (EM). After washing in EM the parasites were incubated in triplicate with 500$\mu$l of the membrane-active compound dissolved in EM. The release of isotope was expressed as a percentage of total isotope in the incubation mixture (Kusel et al., 1981).

All membrane-active compounds were obtained from Sigma (London, UK) Ltd., except the Tweens (Koch-Light Ltd.), EM49 (Squibb, Middlexex, U.K.), stock solutions of vitamin A alcohol (retinol), and amphotericin B, which were prepared in ethanol and dimethyl sulfoxide, respectively.

Spectrofluorimetric determination of retinol was done using the method of Kahan (1966).

## RESULTS

The membrane-active compounds examined for activity against the schistosomulum and adult worm can be classified into four groups as shown in Table I. In group 3 neither retinol (vitamin A alcohol) nor amphotericin B caused detectable damage to the adult but did damage the schistosomulum. This observation suggested that during the development of the parasite some change in organization or composition had occurred in the lipid phase of the membrane. Both reagents bind to the membrane of the adult (Table II and Torpier and Capron, 1980); indeed the uptake of retinol by both forms of the parasite can be enhanced under a variety of conditions (Table II). The adult membrane can accommodate retinol molecules apparently without being perturbed by them, as assessed by this isotope release assay. Thus Table II and Table III

TABLE II

UPTAKE OF RETINOL BY SCHISTOSOMULA AND ADULT *S. MANSONI* AND HUMAN ERYTHROCYTES

| Treatment | Adult worms | | Human Erythrocytes | | Schistosomula | |
|---|---|---|---|---|---|---|
| | 4° | 37° | 4° | 37° | 4° | 37° |
| Eagle's medium (EM) plus retinol | 0.04 | 1.08 | 0.058 | 0.064 | 1.0 | 1.0 |
| Tween 20 plus retinol | 1.66 | 6.82 | 0.444 | Lysis | 14.0 | 7.34 |
| Tween 40 plus retinol | 1.36 | 9.32 | 0.534 | 0.168* | 32.67 | 9.67 |
| Tween 80 plus retinol | 1.42 | 8.52 | 0.404 | 0.164* | 43.00 | 19.67 |

*Some lysis of erythrocytes.

Schistosomula and *S. mansoni* and human erythrocytes were incubated at 4°C or 37°C with retinol (200 $\mu$g $ml^{-1}$) dissolved in 10 mg $ml^{-1}$ Tween 20, 40, or 80. After being washed in EM the parasites or erythrocytes were incubated in ethanol, and the amount of retinol in the supernatant was determined by fluorimetry. The quantities extracted from the parasites are expressed as nM per 10 adults or 1,000 schistosomula; those from erythrocytes are expressed as nM per $10^8$ cells and are the means of triplicate determinations from which control values (incubations in EM alone) have been subtracted.

TABLE III

RELEASE OF $^{51}CR$ AND $^{125}I$ WGA FROM LABELED ADULT *S. MANSONI* BY RETINOL IN THE PRESENCE OF VARIOUS TWEENS

| Reagent | $^{51}Cr$ | $^{125}I$ |
|---|---|---|
| Control (EM) | 15.13 ± 3.97 | 10.90 ± 0.61 |
| Control (EM + 1% ethanol) | 15.82 ± 4.19 | 11.33 ± 2.79 |
| Retinol | 17.91 ± 4.39 | 14.22 ± 3.30 |
| Tween 20 | *26.65 ± 4.81 | *19.84 ± 3.61 |
| Tween 20 + retinol | *45.15 ± 4.85 | *60.25 ± 10.36 |
| Tween 40 | 16.41 ± 2.44 | 14.12 ± 2.24 |
| Tween 40 + retinol | 19.31 ± 4.13 | 16.41 ± 2.50 |
| Tween 80 | 17.56 ± 4.60 | 12.80 ± 2.68 |
| Tween 80 + retinol | 20.33 ± 4.20 | 14.79 ± 1.73 |

*Results significantly different from control ($P < 0.01$).

Retinol concentration was 1 mg $ml^{-1}$. Tween concentrations were 10 mg $ml^{-1}$ in Eagle's medium (EM). Radioactivity released into the supernatant is expressed as a percentage of the total radioactivity in each incubation. Means ± standard deviation for triplicate determinations are given.

show that retinol dissolved in Tween 40 or Tween 80 causes little isotope release from adult parasites although retinol is taken up into the surface and other membranes. If, however, the retinol is presented to the adult dissolved in Tween 20, considerable damage occurs to the membrane. This damage represents a synergism between Tween 20 and retinol (Table III). The Tweens are polyoxyethylene sorbitol esters of fatty acids. The fatty acid found in Tween 20 is lauric acid; that in Tween 80 is oleic acid (Schick, 1967). The synergism between Tween 20 and retinol is evident only when the two compounds are presented simultaneously. We therefore suggest that the mixed micelle (Tween 20/retinol) has the ability to penetrate areas of membrane unavailable to mixed micelles of other composition.

It may be that the inner bilayer of the complex pentalaminate membrane is perturbed by the Tween 20/retinol micelle. Certain properties (hydrophobicity, size) of this micelle may determine its effect, but the causes of the synergism are unknown. We suggest that the presentation of membrane-active drugs to this complex membrane may be facilitated by understanding why certain mixed micelles are particularly damaging and others are not so.

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# $^{31}$P NMR INVESTIGATION OF RHODOPSIN-PHOSPHOLIPID INTERACTIONS IN BOVINE ROD OUTER SEGMENT DISK MEMBRANES

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The interaction between the integral membrane protein, rhodopsin, and the surrounding phospholipids in bovine rod outer segment disk membranes was investigated using $^{31}$P NMR. Disks were prepared as described in (1). $^{31}$P NMR intensity measurements were obtained as described in reference 2 using spectra as shown in Fig. 1 and a standard curve as shown in Fig. 2. Intensity measurements indicated that ~ 20% of the phospholipid molecules in the disk membrane were immobilized. This is not to imply they are absolutely immobile or frozen, but it does imply that the immobilized phospholipids as reflected by the phosphate headgroup are in slow exchange ($\tau < 10^{-4}$ s) with the bulk phospholipids. These measurements are to be distinguished from those which detect motional properties of the hydrocarbon chain. It is reasonable to hypothesize a certain amount of independence with respect to the motional freedom experienced by the headgroup and by the hydrocarbon chain.



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