CHANGES IN THE PROPERTIES OF THE SURFACE MEMBRANE OF *SCHISTOSOMA MANSONI* DURING GROWTH AS MEASURED BY FLUORESCENCE RECOVERY AFTER PHOTOBLEACHING

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1. Introduction

Schistosomiasis is a widespread tropical disease of human beings. Infection occurs when a free-living, water-borne cercaria penetrates the skin, becomes transformed into a schistosomulum which then migrates through the blood stream of the host to develop into an adult parasite in the portal blood system [1]. The surface membrane of both the schistosomulum and adult worm is unusual; it is a double bilayer, and forms a continuous surface covering the peripheral syncytium [2]. The properties of this membrane change during growth and development in the host so that it becomes progressively less susceptible to damage by a variety of agents [3–8]. An understanding of the nature of these changes is sought in the hope that immunological control or chemotherapy of the disease may be made more effective. The technique of fluorescence recovery after photobleaching (FRAP) can measure the lateral diffusion of protein and lipid in the cell surface [9–13]. We describe here its use in comparing the outer surfaces of the schistosomulum and adult *Schistosoma mansoni*.

2. Materials and methods

2.1. *The parasite*

The life cycle of a Puerto Rican strain of *Schistosoma mansoni* was maintained in a variety of mouse strains [14]. Schistosomula were transformed from cercariae mechanically and cultured for 24 h in Eagle's Medium (EM) plus 10% calf serum [25]. Adult worms were recovered by perfusion [14] and cultured for 24 h in Eagle's medium plus calf serum [15].

2.2. *Fluorescent labels for the surface of the parasite*

The following fluorescent probes were used: rhodamine isothiocyanate labelled succinylated concanavalin A (RITC–con A) or rhodamine isothiocyanate-labelled, succinylated wheat germ agglutinin (RITC–WGA); 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl indocarbocyanine iodide (DiI–C_{18}-(3)). For FRAP measurements, these probes were excited with the 514.5 nm argon ion laser line. For fluorescence photomicrography fluorescein isothiocyanate-labelled wheat germ agglutinin was used.

2.3. *Labelling the parasites*

Schistosomula and adult parasites were incubated at 37°C for 30 min in the following solutions: fluorescent probes were dissolved in Eagle's medium (EM) at: (a) RITC–con A 20 μg/ml; (b) RITC–WGA 20 μg/ml; (c) DiI–C_{18}-(3) 3 μg/ml. Parasites were then washed 4 times in EM at 37°C.

2.4. *Microscopy of the parasites*

Labelled schistosomula and adult parasites were mounted on glass microscope slides in the centre of a raised square of silicone vacuum grease in 50 μl EM containing 100 μg carbachol (carbamyl choline) to stop movement of the parasite during the FRAP experiments.

2.5. *Fluorescence recovery measurements*

In the FRAP technique the molecules of interest are fluorescently labelled. The laser beam is focused to a small spot on the membrane, and a brief but intense pulse of laser light irreversibly bleaches the fluorescence of the spot. Subsequent recovery of fluorescence in the bleached spot occurs by lateral diffu-

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sion of unbleached molecules from the surrounding membrane, and is measured by excitation with the same laser beam but attenuated by a factor of 10^3–10^5. The lateral diffusion coefficient (D_L) and percentage of immobile molecules was calculated according to [9]. The apparatus was as described [16] with further details in [17]. All FRAP measurements were made with a 20X objective (numerical aperture, 0.65) at 37°C. Measurements were made on adult male worms and schistosomula in the dorsal mid-region.

3. Results

The surface of the adult male worm is known to be highly folded and pitted [2]. This is illustrated in the fluorescence photomicrograph taken after staining with FITC–WGA (fig.1). A similar pattern is seen with RITC–WGA and RITC–con A. The regions of enhanced fluorescence in fig.1 are likely to be due to the geometry of the surface foldings. However, it may be that there is an increased concentration of lectin receptors within the pits or folds. Measurements of D_L with the FRAP method assume a planar membrane normal to the laser beam [9]. A folded membrane lowers the apparent value of D_L, but this effect is probably $\leqslant$2–3-fold [18].

Some results of the FRAP experiments are shown in fig.2. They illustrate the method and show some striking differences between the behaviour of a lipid probe (DiI–C_{18}-(3)) in the adult and schistosomulum. In the case of the schistosomulum recovery of fluorescence after photobleaching was only half-complete (i.e., 50% recovery). The $t_{1/2}$ for the recovery phase was 9 s, yielding $D_L = 2.2 \times 10^{-10}$ cm^2/s. The incomplete recovery indicates that a fraction (in this case 50%) of the fluorescent probe was effectively immo-

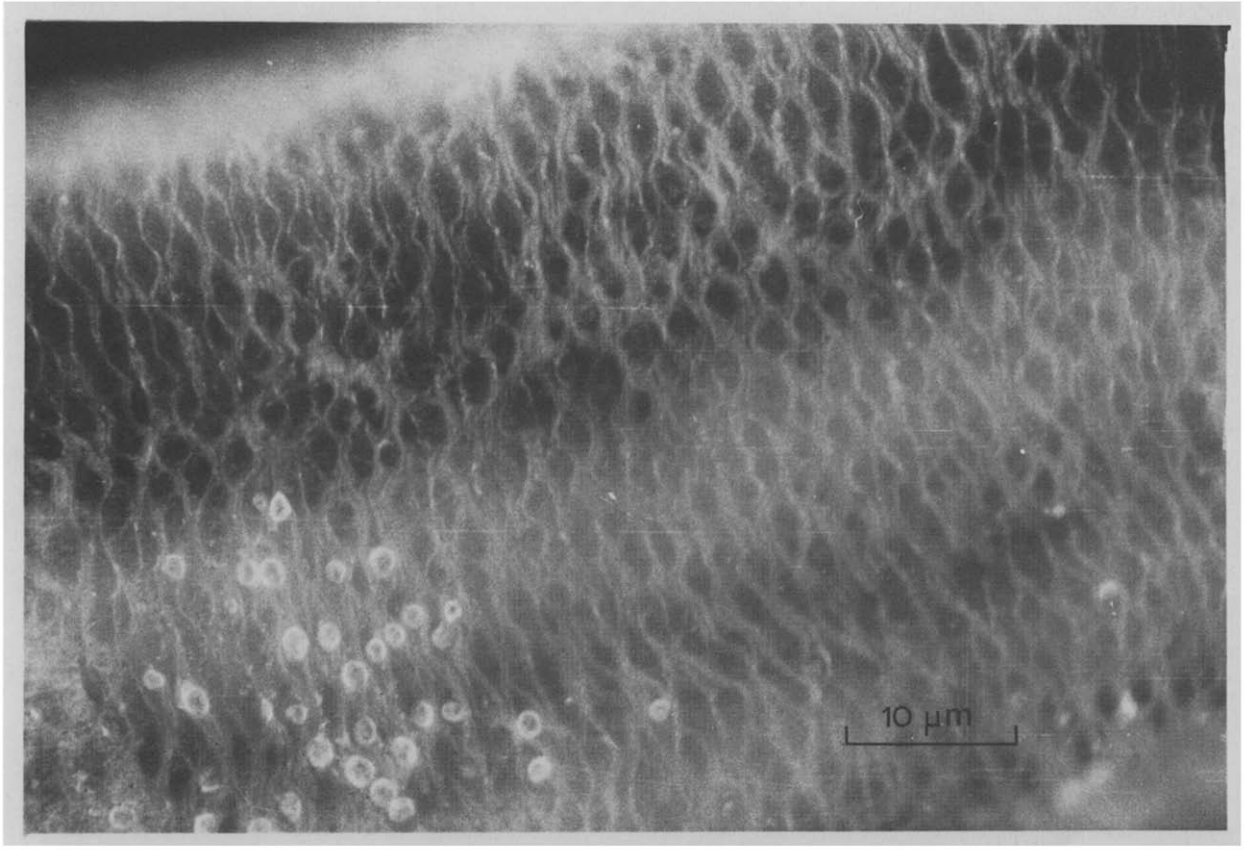

Fig.1. Photomicrograph of the surface of adult male worms of *Schistosoma mansoni* after staining with FITC–WGA. Worms were viewed with a Leitz Ortholux II fluorescence microscope with Ploëmpak Epi-illuminator, and photographed on Kodak TRI-X ASA 400 film.

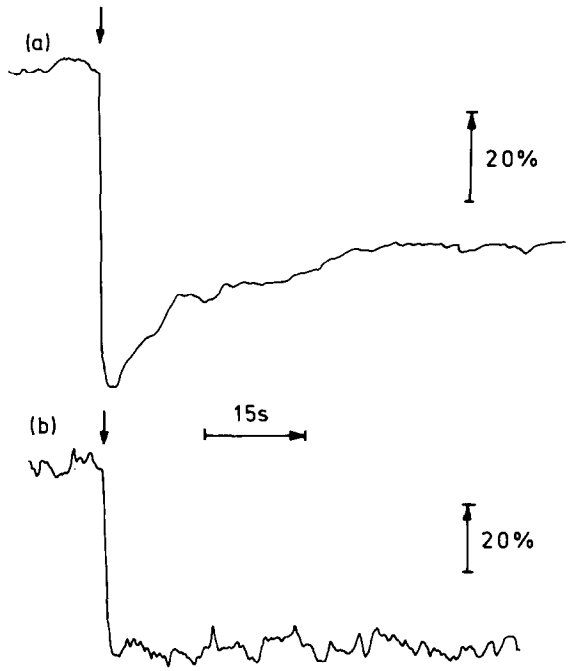

Fig.2. Fluorescence recovery curves of (a) schistosomulum and (b) adult worm treated with DiI–C_{18}-(3). The laser line wavelength was 514.5 nm, and the $1/e^2$ spot radius was 1 μm. The laser intensity was increased to the photobleaching level for a few hundred ms at the point marked with an arrow. The fluorescence intensities before bleaching are given as 100% and the vertical bars designate changes in fluorescence from the original level. In each case the laser spot was situated in the dorsal region of the parasite.

bile over the time period of the measurements ($D_L < 10^{-12}$ cm^2/s). We did not observe any fast recovery (i.e., $t_{\frac{1}{2}} < 0.5$ s) following the photobleaching, although this would have been readily observable with our equipment. Thus we can exclude the presence of faster D_L values of $\sim 10^{-8}$ cm^2/s for this lipid probe. Table 1 summarises results of FRAP experiments on 24 h schistosomula and adult male worms, using either fluorescent lectins or the lipid probe, DiI–C_{18}-(3). The lectins bind to glycoproteins and glycolipids of appropriate structure [19], whereas the lipid probe is considered to enter the membrane and behave analogously to a phospholipid [20]. The main features of our observations are:

(i) RITC–WGA was practically immobile in the schistosomulum, but not in the adult;
(ii) Recoveries of the fluorescence were markedly incomplete, both with lectins and the lipid probe;
(iii) The D_L-values of the lipid probe were $\geq 10^2$-fold lower than those expected for a fluid bilayer. This effect was most marked in the adult worm, where the probe was effectively immobile.

4. Discussion

Immobility of surface proteins in eukaryotic cells is frequently observed, and is usually attributed to constraints on lateral diffusion imposed by the underlying cytoskeleton [21]. It is not known at present whether the lectin receptors on the schistosome surface are glycoprotein or glycolipids [19] or a mixture, so we cannot conclude that their immobility was due to cytoskeletal interactions. Lectins do not diffuse across membranes, and the sites labelled in our experiments are presumably on the outer bilayer of the parasite's surface [19], separated from the cytoskeleton by the inner bilayer. Thus it remains only an intriguing possibility that cytoskeletal effects can be transmitted through 2 bilayers as distinct from 1. The D_L values and % immobile molecules in table 1

Table 1
Lateral diffusion coefficients (D_L) of the mobile fraction and the percentage of immobile fluorescent probe molecules on the surface of schistosomula and adult worms

Fluorescent probe	Schistosomulum		Adult worm	
	D_L (cm^2/s) × 10^{10}	% Immobile	D_L (cm^2/s) × 10^{10}	% Immobile
RITC–con A	1.0–7.6	74 ± 4 (4)	1.9–3.7	56 ± 11 (5)
RITC–WGA	n.c.	90 (3)	0.5–3.7	52 ± 16 (4)
DiI–C_{18}-(3)	1.7–2.2	33 ± 19 (4)	n.c.	90 (4)

The immobile fraction has $D_L < 10^{-12}$ cm^2/s; D_L was not calculated (n.c.) when the immobile fraction was ≥90%. Ranges are given for D_L, and means, with standard deviations for the percentage of immobile molecules. The number of molecules is in parentheses

showed some variability between individual parasites. Studies with clones of parasites [22] should diminish this variability. Certain regions behind the oral sucker of the adult gave fast and almost complete recovery after photobleaching (unpublished). The surface properties of the parasite may thus vary with the anatomical position.

Immobility of lipid molecules is unusual, and when it is observed, it is attributed to the presence of gel-like domains of bilayer [23]. These may be of functional significance. The lipid composition of tumour cell membranes can influence their ability to be damaged by specific antibody and complement [24]. A more rigid membrane is less readily damaged. Hence gel-like domains in the schistosome surface may prevent some forms of immune damage.

In conclusion, the FRAP technique can be used on eukaryotic parasites with a complex surface morphology, to reveal some important differences in surface properties at different stages of development.

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References

[1] Smithers, S. R. and Terry, R. J. (1976) Adv. Parasitol. 7, 41–93.
[2] Hockley, D. J. and McLaren, D. J. (1973) Int. J. Parasitol. 3, 13–25.
[3] Clegg, J. A. and Smithers, S. R. (1972) Int. J. Parasitol. 2, 79–88.
[4] Ramalho-Pinto, F. J., McLaren, D. J. and Smithers, S. R. (1978) Parasitology 77, 313–324.
[5] Butterworth, A. E., Sturrock, R. F., Houba, V. and Rees, P. A. (1974) Nature 252, 503–505.
[6] Anwar, A. R. E., Smithers, S. R. and Kay, A. B. (1974) J. Immunol. 122, 628–637.
[7] Ellner, J. J. and Mahmoud, A. A. F. (1979) J. Immunol. 123, 949–951.
[8] Kusel, J. R., Stones, L. and Harnett, W. (1981) Biosci. Rep. 1, 253–261.
[9] Axelrod, D., Koppel, D. E., Schlessinger, J., Elson, E. L. and Webb, W. W. (1976) Biophys. J. 16, 1055–1069.
[10] Dragsten, P. R., Blumenthal, R. and Handler, J. S. (1981) Nature 294, 718–722.
[11] Axelrod, D., Koppel, D. E., Schlessinger, J., Elson, E. and Webb, W. W. (1976) Proc. Natl. Acad. Sci. USA 76, 5650–5654.
[12] Cherry, R. J. (1979) Biochim. Biophys. Acta 559, 289–327.
[13] Wolf, D. E., Kinsey, W., Lennarz, W. and Edidin, M. (1981) Dev. Biol. 81, 133–138.
[14] Smithers, S. R. and Terry, R. J. (1965) Parasitology 55, 695–700.
[15] Kusel, J. R. and MacKenzie, P. E. (1975) Parasitology 71, 261–273.
[16] Garland, P. B. (1981) Biophys. J. 33, 481–482.
[17] Johnson, P. and Garland, P. B. (1982) Biochem. J. 201, in press.
[18] Aizenbud, B. and Gershon, N. (1981) Proc. Intl. Workshop on Photobleaching Techniques, North Carolina, pp. 89–91.
[19] Simpson, A. J. G. and Smithers, S. R. (1980) Parasitology 81, 1–15.
[20] Wolf, E. S., Schlessinger, J., Elson, E. L., Webb, W. W., Blumenthal, R. and Henkart, P. (1977) Biochemistry 16, 3476–3483.
[21] Schlessinger, J., Elson, E. L., Webb, W. W., Yahara, I., Ruthishavser, U. and Edelman, G. M. (1977) Proc. Natl. Acad. Sci. USA 74, 1110–1114.
[22] Smith, M. A. and Clegg, J. A. (1979) Parasitology 78, 311–321.
[23] Klausner, R. D. and Wolf, D. E. (1980) Biochemistry 19, 6199–6203.
[24] Schlager, S. I. and Ohanian, S. H. (1980) J. Immunol. 124, 626–634.
[25] Colley, D. and Wikel, S. K. (1974) Exp. Parasitol. 35, 44–51.