Biochem. J. (1981) **198**, 467–473
Printed in Great Britain

Properties of a series of tegumental membrane-bound phosphohydrolase activities of *Schistosoma mansoni*

Italo M. CESARI,* Andrew J. G. SIMPSON and W. Howard EVANS
Divisions of Parasitology and Biochemistry, National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.

(*Received 23 January 1981/Accepted 13 May 1981*)

1. Incubation of *Schistosoma mansoni* for 5 min in a phosphate-buffered medium, pH 7.4, released tegumental material containing the following phosphohydrolase activities: alkaline phosphatase, 5'-nucleotidase, glycerol-2-phosphatase, glucose 6-phosphatase, phosphodiesterase and ATPase. 2. Maximum activity of these enzymes was measured at pH 9.5; however, the phosphodiesterase and ATPase activities were also appreciable at pH 7.0. 3. Solubilization of the released tegumental material in 1% Triton X-100 followed by gel filtration distinguished three peaks of enzyme activity: an ATPase (mol.wt. >1 000 000), a phosphodiesterase (mol.wt. 1 000 000) and an alkaline phosphomonoesterase with broad specificity (mol.wt. 232 000). 4. The ATPase activity was highly activated by 10 mM-Mg^{2+} or 1 mM-Ca^{2+} and was inhibited by chelating agents. Ouabain, Na^+ and K^+ had little effect on enzyme activity, whereas activity was increased by 50% in the presence of calmodulin. The phosphodiesterase activity was highest in the presence of 100 mM-Na^+ or -K^+, and 10 mM-Mg^{2+} or -Ca^{2+}. Alkaline phosphatase activity was also stimulated by 100 mM-Na^+ or -K^+, and 10 mM-Mg^{2+}; however Ca^{2+} inhibited at >1 mM. 5. Surface iodination of parasites followed by detergent solubilization and gel filtration of the released tegumental membranes indicated that these enzymes were not accessible. A major surface component, apparent mol.wt. 80 000, was iodinated. 6. Rabbit anti-(mouse liver 5'-nucleotidase) antibodies did not inhibit the phosphohydrolase activities. However, an immunoglobulin G fraction from sera of mice chronically infected with *S. mansoni* partially inhibited alkaline phosphatase activity, but was without effect on the phosphodiesterase and ATPase activities. 7. The location of the enzymes in the double membrane of the tegument and their significance in host–parasite interactions is discussed.

The tegument of the adult *S. mansoni* is delimited by a double membrane that completely surrounds the parasite (Hockley & McLaren, 1973). The outer of the two membranes is an interface between the parasite and its host and is thus positioned to play an important role in both immunological and metabolic interactions. Various methods have been described for isolation of the tegument (Kusel, 1972; Cain & Oaks, 1978) and, more recently, a technique that released tegumental material characterized by double-membrane fragments has been developed (Simpson *et al.*, 1981).

A striking feature of the *S. mansoni* tegument fraction is that it contains phosphohydrolase activity directed against a variety of substrates (Cesari & Santos, 1974; Wheater & Wilson, 1976; Ernst, 1977; Simpson *et al.*, 1981) and, for example, alkaline phosphatase, 5'-nucleotidase and ATPase activities have featured as marker enzymes in the isolation of surface fragments (Cain & Oaks, 1978; Simpson *et al.*, 1981). In the present study, the topographical location, molecular size, pH optima, ionic requirements and sensitivity to antibodies of the phosphohydrolase activities are investigated.

* Permanent address: Instituto Venezolano de Investigaciones Cientificas, Apartado 1827, Caracas 1010A, Venezuela.

Experimental

Materials

Enzyme substrates were obtained from Sigma (London) Chemical Co., Poole, Dorset, U.K.; [^{3}H]AMP was obtained from The Radiochemical Centre, Amersham, Bucks., U.K.; Earle's salts plus

0306-3283/81/090467-07$01.50/1

lactalbumin hydrolysate medium (termed 'modified Earle's medium') was obtained from GIBCO Biocult (Paisley, Scotland, U.K.), bovine serum albumin fraction V was obtained from Armour Pharmaceutical Co., Eastbourne, Sussex, U.K., and Ultrogel AcA 22 was from LKB Produckter A.B., Stockholm, Sweden. Calmodulin was prepared from bovine brain by the method of Grand *et al.* (1979) and provided by Dr. C. Mitchison of this Institute. Rabbit anti-(mouse liver plasma-membrane 5'-nucleotidase) antibodies were prepared as described by Gurd & Evans (1974). Mouse immunoglobulin G was purified on protein A–Sepharose CL-4B columns (Pharmacia) from sera of animals chronically infected with *S. mansoni* and provided by Mrs. R. de Rossi of this Institute.

Parasites

Adult *S. mansoni* of a Puerto Rican strain were collected from Syrian hamsters infected 6 weeks previously with 1000 cercariae, as described by Smithers & Terry (1965). The worms were washed thoroughly in modified Earle's medium and stored briefly in the same medium at 4°C until used.

Preparation of schistosome tegument fractions

Live parasites were incubated for 5 min at 37°C in phosphate-buffered saline, pH 7.4. After the parasites had settled under unit gravity, the tegumental material released was sedimented from the supernatant by centrifugation at 55000 $g_{av.}$ for 1 h. The pellet was dispersed in 8% (w/v) sucrose/5 mM-Tris/HCl, pH 7.4 and either used directly, or centrifuged for 2 h at 100000 $g_{av.}$ in a Beckman SW 50.1 rotor containing a discontinuous gradient constructed of 1.5 ml of 54%, 50% and 37% (w/v) sucrose. Interfacial material was collected and centrifuged at 100000 $g_{av.}$ for 1 h and the resultant pellets resuspended in 8% (w/v) sucrose. Parasites from which tegumental material was removed were dispersed in 8% (w/v) sucrose/5 mM-Tris/HCl (Simpson *et al.*, 1981).

Solubilization and gel filtration

Tegumental material was solubilized by addition of 1% Triton X-100 in 5 mM-Tris/HCl, pH 7.4, and centrifuged at 100000 $g_{av.}$ for 1 h; the supernatant (accounting for >90% of the protein) was applied to Ultrogel AcA 22 columns (50 cm × 1 cm) equilibrated with 0.1% Triton X-100 in 5 mM-Tris/HCl, pH 7.4. Fractions were collected and analysed for enzymic activity and radioactivity.

Surface iodination of parasites

Parasites were incubated for 2 min at 4°C in 0.3 M-borate buffer, pH 8.5, containing 200 μCi of ^{125}I-labelled Bolton and Hunter reagent (Bolton & Hunter, 1973). After dilution and washing several times with cold modified Earle's medium, they were mixed with unlabelled parasites and a tegumental fraction was prepared as described above. Isolated teguments were solubilized in 1% Triton X-100 as described for enzyme solubilization, and the detergent extract was applied to an Ultrogel AcA 22 column. Radioactivity in fractions was determined by using a well-type γ-counter.

Enzyme assays

For measurement of activity at various pH values, the following buffers (50 mM) were used: pH 4.0–6.0, sodium acetate/acetic acid; pH 6.9–8.0, Tris/maleic acid; pH 8.0/10.0, 2-amino-2-methylpropane-1,3-diol/HCl. Chelators, ions and antibody were included in various experiments as stated in the text. Alkaline phosphatase (*p*-nitrophenyl phosphatase, EC 3.1.3.1) was measured spectrophotometrically at 410 nm by measuring the release of *p*-nitrophenol for 10 min at 37°C. Phosphodiesterase (EC 3.1.4.1) was measured spectrophotometrically at 410 nm by measuring the release of *p*-nitrophenol from thymidine 5'-monophosphate *p*-nitrophenyl ester (Razzel, 1963). 5'-Nucleotidase (5'-ribonucleotide phosphohydrolase, EC 3.1.3.5) was measured by using a modification of the radioactive method of Avruch & Wallach (1971). In some instances, activity was determined by measuring release of P_i. ATPase (EC 3.6.1.3), glycerol-2-phosphatase (EC 3.1.3.19) and glucose 6-phosphatase (EC 3.1.3.9) activities were measured by measuring release of P_i by the method of Carson (1976).

Results

Brief incubation of *S. mansoni* in phosphate-buffered saline resulted in the release of material derived predominantly from the worm's tegument (Simpson *et al.*, 1981). The phosphohydrolase activities present in this fraction are now investigated in detail.

Effect of pH on the enzyme activities

Measurement of the enzyme activities from pH 4 to 10 showed that all the enzymes were most active at pH > 9 (Fig. 1). However, the pH profiles of the ATPase and phosphodiesterase activities showed that significant activity also occurred at pH 7.0. This result suggested that the ATPase and phosphodiesterase activities were due to different enzyme(s) than the other activities examined.

Solubilization and separation of the enzyme activities

Solubilization of tegumental material in 1% Triton X-100 followed by gel filtration resolved the enzymic activities into three distinct peaks (Fig. 2). Excluded at the void volume of the column was ATPase

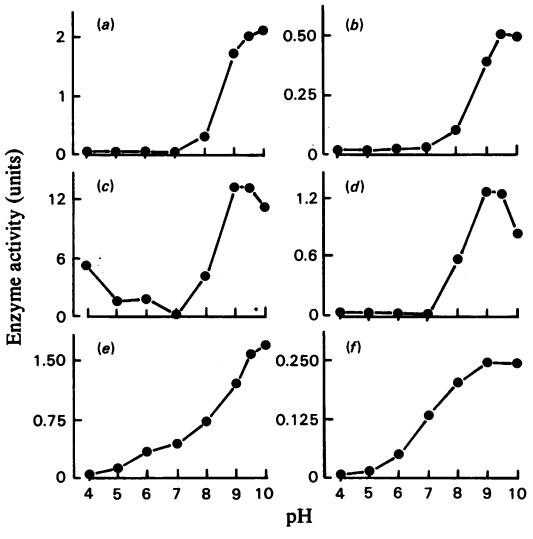

Fig. 1. *The dependence of the schistosome tegumental phosphohydrolase activities on pH*

Samples of tegumental membrane (5–7 μg) were incubated in various media at 37°C (see the Experimental section). (*a*) Alkaline phosphatase (μmol of *p*-nitrophenol liberated/min); (*b*) glycerol-2-phosphatase (μmol of phosphate liberated/min); (*c*) 5′-nucleotidase (10^{-4} × c.p.m. in adenosine liberated/15 min); (*d*) glucose 6-phosphate (μmol of phosphate liberated/min); (*e*) ATPase (μmol of phosphate liberated/min); (*f*) phosphodiesterase (μmol of *p*-nitrophenol liberated/min).

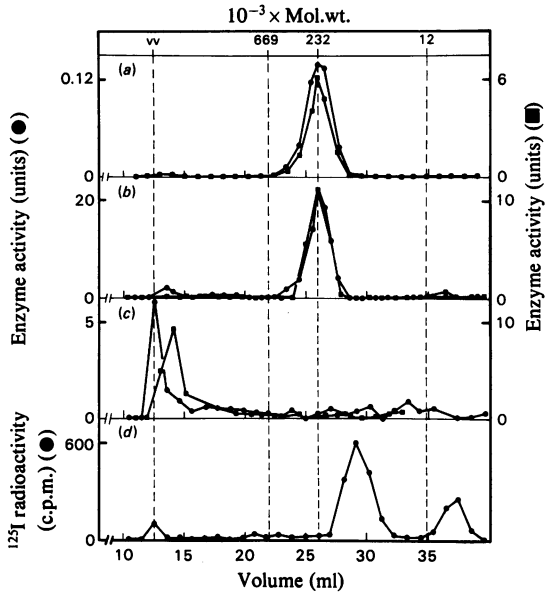

Fig. 2. *Separation of schistosome tegumental phosphohydrolase activities by gel filtration*

The tegumental material, solubilized in Triton X-100, was applied to an Ultrogel column. Samples (10–15 μl) of each fraction collected from the column were incubated with each substrate (1 mM) in 50 mM-2-amino-2-methylpropane-1,3-diol/HCl. (*a*) Alkaline phosphate (●) (μmol of *p*-nitrophenol liberated/min) and 5′-nucleotidase (■) (10^4 × c.p.m. from adenosine liberated/30 min); (*b*) glycerol-2-phosphatase (●) (nmol of phosphate liberated/min) and glucose 6-phosphatase (■) (nmol of phosphate liberated/min); (*c*) ATPase (●) (nmol of phosphate liberated/min), and phosphodiesterase (■) (nmol of *p*-nitrophenol liberated/min); (*d*) ^{125}I radioactivity of tegumental fraction from worms labelled with Bolton and Hunter reagent. Abbreviation used: vv, void volume.

Table 1. *Effect of various substrates on alkaline phosphatase activity of a schistosome tegumental fraction*

Samples of 10 μl of the alkaline phosphatase peak prepared by gel filtration (see Fig. 2) were incubated for 15 min at 37°C in 50 mM-2-amino-2-methylpropane-1,3-diol/HCl, pH 9.5, containing 1 mM-$MgCl_2$, 1 mM-*p*-nitrophenyl phosphate and an alternative substrate at 10 mM.

Substrate additions	Activity (%)
	100
Adenosine 2′-monophosphate	9.2
Adenosine 3′-monophosphate	8.5
Adenosine 5′-monophosphate	4.0
Glycero-2-phosphate	11.4
Adenosine 5′-triphosphate	13.6
Glucose 6-phosphate	30.0
Thymidine 5′-monophosphate *p*-nitrophenyl ester	104.2
150 mM-Phosphate	35.0
4 mM-Ammonium molybdate	6.2

activity (mol.wt. approx. 1 × 10^6) closely followed by a peak of phosphodiesterase activity. Coincident peaks of *p*-nitrophenyl phosphatase, 5′-nucleotidase, glycerol-2-phosphatase and glucose 6-phosphatase activities were obtained at a molecular weight of 230000, suggesting that the activities resulted from a single enzyme of broad specificity. This was further investigated by competition experiments using the Triton X-100-solubilized *p*-nitrophenyl phosphatase peak collected from the Ultrogel AcA 22 column. A 10-fold excess of AMP, glycerol-2-phosphate and ATP caused a >85% inhibition (Table 1) of *p*-nitrophenyl phosphate hydrolysis. Thus ATP is bound by the active site of the alkaline phosphatase but is not hydrolysed. The inhibition of the activity by P_i and ammonium molybdate showed that the enzyme was probably similar to that described by Nimmo-Smith & Standen (1963), who demonstrated a similar inhibition of alkaline phosphatase activity in schistosome homogenates.

Surface-labelled schistosome components

Surface iodination of worms using the Bolton and Hunter reagent followed by preparation of a tegumental fraction, detergent solubilization and gel filtration showed a major peak of iodinated protein of apparent mol.wt. 80000 (Fig. 2*d*). Since the ^{125}I-labelled peak did not coincide with any of the three peaks of enzyme activity it was concluded that the enzymes were unlikely to be directly exposed at the parasite surface. Surface labelling of adult worms is known to be extremely difficult (Hayunga *et al.*, 1979), but the labelled peak has a similar molecular weight to the major component labelled with ^{125}I in adult schistosomes by the lactoperoxidase method (Snary *et al.*, 1980).

Effect of ion concentration on the tegumental enzymes

To demonstrate further that the ATPase, phosphodiesterase and alkaline phosphatase were distinct enzymes and to characterize further ionic requirements of the enzymes, the effect of increasing Na^+, K^+, Ca^{2+} and Mg^{2+} concentrations was investigated (Figs. 3–5). The different patterns of stimulation provided further evidence that these three enzymes were indeed distinct.

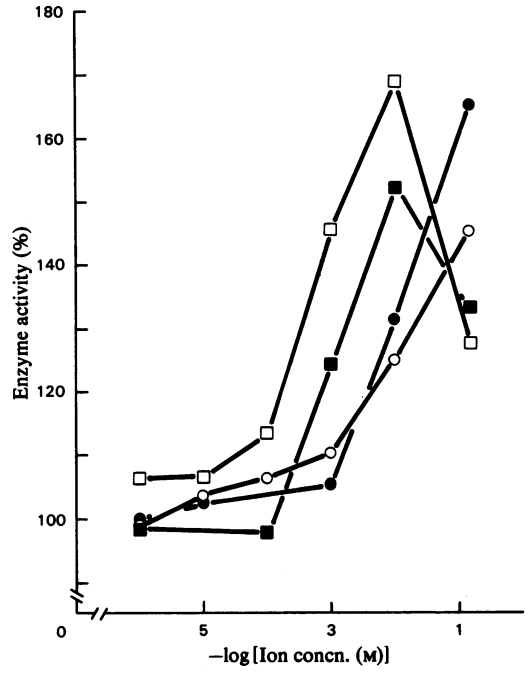

Fig. 4. *The dependence of phosphodiesterase activity on ion concentration*

Symbols: ○, Na^+; ●, K^+; ■, Ca^{2+}; □, Mg^{2+}. Enzyme activity is expressed relative to that measured in 50mM-2-amino-2-methylpropane-1,3-diol/HCl.

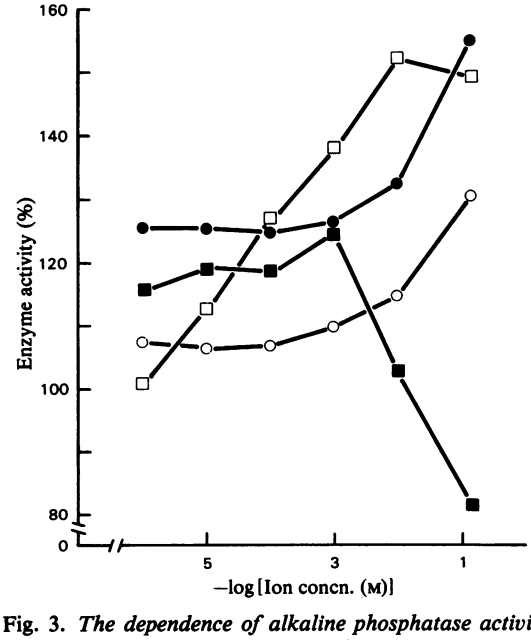

Fig. 3. *The dependence of alkaline phosphatase activity on ion concentration*

Symbols: ○, Na^+; ●, K^+; ■, Ca^{2+}; □, Mg^{2+}. Enzyme activity is expressed relative to that measured in 50mM-2-amino-2-methylpropane-1,3-diol/HCl.

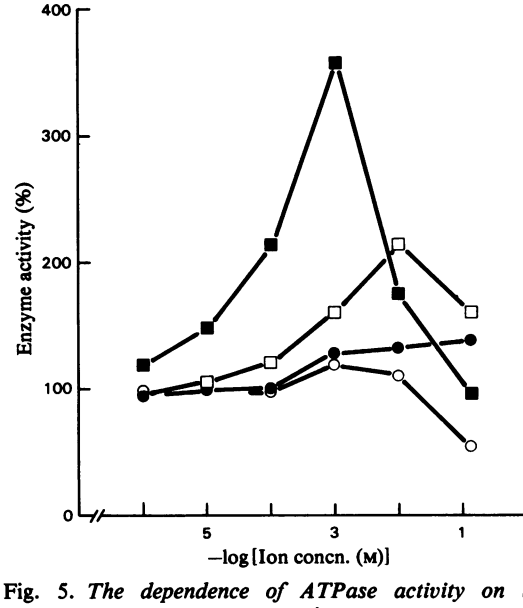

Fig. 5. *The dependence of ATPase activity on ion concentration*

Symbols: ○, Na^+; ●, K^+; ■, Ca^{2+}; □, Mg^{2+}. Enzyme activity is expressed relative to that measured in 50mM-2-amino-2-methylpropane-1,3-diol/HCl.

The low stimulation of the ATPase activity by Na^+ and K^+, together with the marked stimulatory effect of Ca^{2+}, indicated that this tegumental enzyme was a Ca^{2+}-stimulated ATPase rather than an $(Na^+ + K^+)$-stimulated ATPase. Further studies on the ion requirement and the effects of incubation in the presence of ouabain and calmodulin (Table 2) were also consistent with this conclusion and suggest that the enzyme can be further defined as a Ca^{2+}-stimulated Mg^{2+}-dependent ATPase.

Subcellular distribution of the phosphohydrolase enzymes

The distribution of ATPase and phosphodiesterase was compared with alkaline phosphatase in subfractions of parasite tegument. Alkaline phosphatase has been demonstrated to be associated with tegumental outer membrane of the schistosome by cytochemical techniques, and used as a marker enzyme in subtegumental fractionation studies (Simpson *et al.*, 1981). Both the ATPase and phosphodiesterase activities had a similar distribution to alkaline phosphatase when the tegumental fraction was centrifuged on sucrose gradients (Table 3). The highest specific activities of the three enzymes were found in subfraction 1, shown to contain vesicles of parasite outer membrane, many of which contained a double-lipid bilayer (Simpson

Table 2. *Effect of ions, chelators, ouabain and calmodulin on schistosome tegumental ATPase*

Samples of tegument (5 μg) were incubated for 30 min at 37°C in 50 mM-2-amino-2-methylpropane-1,3-diol/HCl, pH 7.5, containing substrate at 1 mM and ATPase activity was determined.

Added compounds	ATPase activity (%)
(*a*) Salts	
No addition	100
1 mM-NaCl	120.0
10 mM-KCl	122.8
1 mM-NaCl + 10 mM-KCl	122.8
1 mM-$CaCl_2$	324.5
10 mM-$MgCl_2$	189.7
1 mM-$CaCl_2$ + 10 mM-$MgCl_2$	189.1
1 mM-NaCl + 1 mM-$CaCl_2$ + 10 mM-KCl + 10 mM-$MgCl_2$	191.0
(*b*) Chelators	
0.1 mM-EDTA	0
0.01 mM-EDTA	43.8
0.001 mM-EDTA	101.0
10 mM-EGTA	33.7
1 mM-EGTA	30.9
0.1 mM-EGTA	62.0
0.001 mM-EGTA	121.1
(*c*) Other modulators	
0.2 mM-ouabain + 100 mM-NaCl + 20 mM-KCl	89.3*
Calmodulin (50 μg/ml)	151.2

* Relative to the same salts without ouabain.

Table 3. *Distribution of ATPase, p-nitrophenyl phosphatase and phosphodiesterase activities in schistosome tegumental subfractions*

Teguments were shed from schistosomes by incubation for 5 min in phosphate-buffered saline, pH 7.4. The tegumental membranes were fractionated on sucrose gradients and the distribution of protein and enzymes was determined as described in the Experimental section. ATPase activity was determined in the presence of 1 mM-$MgCl_2$ and 1 mM-$CaCl_2$.

Fractions	Protein content (mg)	Activity (nmol of substrate hydrolysed/min per mg of protein)		
		ATPase	*p*-Nitrophenyl phosphatase	Phosphodiesterase
Denuded worms	69.400	13.9	147.5	12.4
Tegumental membranes	1.170	207.7	865.6	92.9
Supernatant	6.060	0.0	0.0	0.8
Subfraction 1	0.052	646.9	1937.4	360.2
Subfraction 2	0.059	480.2	1460.4	277.7
Subfraction 3	0.020	79.7	164.4	62.2
Subfraction 4	0.072	28.1	74.2	15.7

et al., 1981). Thus it is likely that these phosphohydrolase enzymes are located in the tegumental outer-membrane region.

Effect of antibodies on the tegumental enzymes

An immunoglobulin G fraction purified from mice infected with highly irradiated (20krd) cercariae (*S. mansoni* larvae) had no inhibitory effect on the enzymes. Immunoglobulin G from mice chronically infected with cercariae inhibited alkaline phosphatase by up to 25% but did not affect the other activities or alkaline phosphatase activity isolated from host (hamster) liver. An immunoglobulin G fraction prepared against mouse liver 5′-nucleotidase also had no effect on parasite alkaline phosphatase activity, although these antibodies almost completely inhibited mammalian membrane-bound 5′-nucleotidase enzyme (Gurd & Evans, 1974).

Discussion

The present studies identify and separate in a tegumental membrane fraction stripped from the surface of adult *S. mansoni* three classes of enzyme activities. The phosphomonoesterase activity hydrolysed AMP, glycerol-2-phosphate, glucose 6-phosphate and *p*-nitrophenyl phosphate. This activity probably corresponds to an alkaline phosphatase demonstrated by cytochemical techniques to be located predominantly at the parasite's tegument (Morris & Threadgold, 1968; Bogitsch & Krupa, 1971; Ernst, 1977) and also shown to be present at high specific activity in fragments of the tegument released by parasites after treatment with saponin and $CaCl_2$ (Cesari, 1974) and by the phosphate-buffered-saline release method (Simpson *et al.*, 1981) used in the present work. The schistosome phosphomonoesterase activity we now designate as an alkaline phosphatase, for many of the properties described in the present paper are similar to alkaline phosphatases studied in a wide range of mammalian epithelial cells (Narayanan & Appleton, 1972; Seargeant & Stinson, 1979; Skillen & Rahbani-Nobar, 1979). The emergence of all phosphomonoesterase activities as a single peak of apparent mol.wt. 232000 from the gel-filtration column (Fig. 2) is unlikely to reflect the presence of a number of enzymes aggregated in micelles, for the product after further purification by lectin-affinity chromatography again shows the broad substrate specificity typical of alkaline phosphatase enzymes (G. Payares & W. H. Evans, unpublished work). The results also show that the tegumental membranes contain both Ca^{2+}-stimulated ATPase and alkaline phosphodiesterase activities. Little ouabain-sensitive ATPase activity was detected, although Fetterer *et al.* (1981) have suggested that an electrogenic Na^+- and K^+-transport system is present in the schistosome tegument. Nechay *et al.* (1980) have also described a $(Ca^{2+} + Mg^{2+})$-activated ATPase in the schistosome tegument.

The three classes of enzyme activities showed a similar distribution when teguments released from schistosomes were fractionated into sucrose gradients, suggesting that all three enzymes are located in the same, or at least similar, membranes. Furthermore, the enzyme activities were present in membranes that were perturbed to an equal extent to higher densities in sucrose gradients after exposure to digitonin, indicating that the enzymes were present in membranes containing sterols, e.g. cholesterol (A. J. G. Simpson, F. D. Rumjanek, G. Payares & W. H. Evans, unpublished work).

The properties of the enzymes now described suggest that in some respects the surface-membrane network of schistosomes, which is convoluted to give a high surface/volume ratio, performs similar functions to brush-border surface regions of epithelial cells, which contain similar enzymes. It has been suggested that the schistosome surface is actively involved in solute and nutrient transport (Asch & Read, 1975; Rogers & Bueding, 1975). Thus, the Ca^{2+}-stimulated ATPase and alkaline phosphatase may be involved in transporting Ca^{2+} (Lane & Lawson, 1978; Ghijsen & Van Os, 1979) and phosphate ions (Moog & Glazier, 1972; Shirazi *et al.*, 1978) respectively.

The surface membrane of adult *S. mansoni* consists of a double-lipid bilayer (Hockley & McLaren, 1973) and in evaluating the functional roles of the enzymes present, their assignment to one or other of these membranes is desirable, but at this stage one can only discuss their possible topographical position. Since the schistosome maintains a lower Ca^{2+} concentration within the tegument than the surrounding host medium (Fetterer *et al.*, 1980), the Ca^{2+}-stimulated ATPase is probably positioned for pumping Ca^{2+} out of the parasite by having its active site at the cytoplasmic side of the inner membrane of the surface-membrane double-lipid bilayer complex. The enzymes appear to have their polypeptide chains well shielded from the external environment, for they were not iodinated in intact worms by the Bolton and Hunter and the Iodogen (G. Payares & W. H. Evans, unpublished work) reagents. However, the parasite can hydrolyse externally added phosphomonoesterase substrates (Levy & Read, 1975) and in the present work the alkaline phosphatase activity was inhibited partially by sera prepared from animals chronically infected with *S. mansoni*. These observations are difficult to reconcile with the alkaline phosphatase being positioned entirely at the inner of the two lipid bilayers. The present results indicate that there may well be important biochemical and structural differences

between the inner and outer lipid bilayers of the schistosome surface membrane.

A. J. G. S. thanks the M.R.C. for a research studentship.

References

Asch, H. L. & Read, C. P. (1975) *J. Parasitol.* **61**, 378–379

Avruch, J. & Wallach, D. F. H. (1971) *Biochim. Biophys. Acta* **233**, 334–347

Bogitsch, B. J. & Krupa, P. L. (1971) *Exp. Parasitol.* **30**, 418–425

Bolton, A. E. & Hunter, W. J. (1973) *Biochem. J.* **133**, 529–539

Cain, G. D. & Oaks, J. A. (1978) *Abstr. Am. Soc. Parasitol. Annu. Meet. 53rd*, 70–71

Carson, S. D. (1976) *Anal. Biochem.* **75**, 472–477

Cesari, I. M. (1974) *Exp. Parasitol.* **36**, 405–414

Cesari, I. M. & Santos, I. (1974) *Proc. 3rd Int. Cong. Parasitol.* **3**, 1481–1482

Ernst, S. C. (1977) *Rice Univ. Stud.* **62**, 81–95

Fetterer, R. H., Pax, R. A. & Bennett, J. L. (1980) *Exp. Parasitol.* **49**, 353–365

Fetterer, R. H., Pax, R. A. & Bennett, J. L. (1981) *Parasitology* **82**, 97–109

Ghijsen, W. E. J. M. & Van Os, C. H. (1979) *Nature (London)* **279**, 802–803

Grand, R. J. A., Perry, S. V. & Weeks, R. A. (1979) *Biochem. J.* **177**, 521–529

Gurd, J. W. & Evans, W. H. (1974) *Arch. Biochem. Biophys.* **164**, 305–311

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

Hockley, D. J. & McLaren, D. J. (1973) *Int. J. Parasitol.* **3**, 13–25

Kusel, J. R. (1972) *Parasitology* **65**, 55–59

Lane, S. M. & Lawson, D. E. M. (1978) *Biochem. J.* **174**, 1067–1070

Levy, M. G. & Read, C. P. (1975) *J. Parasitol.* **61**, 648–656

Moog, F. & Glazier, H. (1972) *Comp. Biochem. Physiol.* **42A**, 321–336

Morris, G. P. & Threadgold, L. T. (1968) *J. Parasitol.* **54**, 15–27

Narayanan, S. & Appleton, H. D. (1972) *Biochim. Biophys. Acta* **284**, 175–182

Nechay, B. R., Hillman, G. R. & Dotson, M. J. (1980) *J. Parasitol.* **66**, 596–600

Nimmo-Smith, R. H. & Standen, O. D. (1963) *Exp. Parasitol.* **13**, 305–322

Razzel, W. E. (1963) *Methods Enzymol.* **6**, 236–238

Rogers, S. H. & Bueding, E. (1975) *Int. J. Parasitol.* **5**, 369–371

Seargeant, L. E. & Stinson, R. A. (1979) *Can. J. Biochem.* **57**, 1000–1007

Shirazi, S. P., Colston, K. W. & Butterworth, P. J. (1978) *Biochem. Soc. Trans.* **6**, 933–935

Simpson, A. J. G., Schryer, M. D., Cesari, I. M., Evans, W. H. & Smithers, S. R. (1981) *Parasitology* **83**, 163–177.

Skillen, A. W. & Rahbani-Nobar, M. (1979) *Biochim. Biophys. Acta* **571**, 86–93

Smithers, S. R. & Terry, R. J. (1965) *Parasitology* **55**, 701–710

Snary, D., Smith, M. A. & Clegg, J. A. (1980) *Eur. J. Immunol.* **10**, 573–575

Wheater, P. R. & Wilson, R. A. (1976) *Parasitology* **72**, 99–109