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SURFACE ANTIGENS ON *SCHISTOSOMA MANSONI*

II. ADSORPTION OF A FORSSMAN-LIKE HOST ANTIGEN BY SCHISTOSOMULA

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SUMMARY

A study was made of the nature of mouse (host) antigens adsorbed by schistosomula of *Schistosoma mansoni.* Using the mixed antiglobulin test, extracts of a number of individual mouse tissues were tested for their ability to coat schistosomula. All were effective to some extent, with the greatest activity being found in extracts of the lung and spleen. Antibodies against the schistosomulum-coating antigen as well as surface host antigens of adult *Schistosoma mansoni* were removed by absorbing with erythrocytes from a number of Forssman-positive but not Forssman-negative animal species. These antibodies were also absorbed by Forssman-positive guinea-pig kidney extract and methanol soluble (Forssman-positive) but not insoluble fractions of sheep erythrocyte stromata and mouse lungs. Schistosomula could be coated *in vitro* with methanol soluble fractions of mouse lung and erythrocytes and sheep erythrocytes. Though both mouse and sheep coating antigens reacted with anti-mouse and anti-sheep antibodies, reactions were stronger with the homologous antiserum. It was concluded that schistosomula of *Schistosoma mansoni* adsorb from mice an antigen similar but not identical to the Forssman antigen of sheep erythrocytes, and that this antigen is also found on the surface of adult worms.

INTRODUCTION

In the first paper of this series (Sell & Dean, 1972), it was shown that schistosomula but not cercariae of *Schistosoma mansoni* adsorb mouse antigens onto their surfaces. The present

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The experiments reported herein were conducted according to the principles set forth in "Guide for Laboratory Animals Facilities and Care," prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences, National Research Council.

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study is a continuation of this work, designed to answer several questions about mouse antigens on the surface of *S. mansoni*: (1) What is the nature of these antigens? and (2) are the host antigens found on the surface of adult worms the same as those adsorbed by schistosomula?

There are numerous reports of erythrocyte surface antigens in adult *S. mansoni* (Oliver-Gonzalez & Torregrosa, 1944; Oliver-Gonzalez & Gonzalez, 1949; Damian, 1962; Smithers, Terry & Hockley, 1969; Clegg, Smithers & Terry, 1970). One such antigen, which has been demonstrated in other parasitic helminths as well (Damian, 1964), is the heterogenetic Forssman antigen. Evidence for the adsorption of a Forssman-like antigen by schistosomula is presented in this report. A preliminary report of this study has been presented by Dean (1971).

MATERIALS AND METHODS

Parasites

A Puerto Rican strain of *S. mansoni* was used throughout this study. Infected snails (*Biomphalaria glabrata*) were provided by the U.S., Japan Cooperative Medical Science Program (National Institute of Allergy and Infectious Diseases). Schistosomula were produced *in vitro* as described previously (Sell & Dean, 1972). Mice were infected by injection of cercariae intraperitoneally (Oliver-Gonzalez & Gonzalez, 1949) and subcutaneously (Peters & Warren, 1969). Adult worms were recovered by perfusion (Smithers & Terry, 1965).

Mice

Inbred C_3H mice (Flow Laboratories, Dublin, Virginia) were used throughout this study.

Protein determination

Protein concentrations of tissue extracts were determined by the method of Lowry *et al.* (1951), modified as described previously (Sell & Dean, 1972).

Antisera

Preparation of goat antiserum to rabbit whole serum, rabbit antiserum to intact sheep erythrocytes, and rabbit antiserum to whole newborn mouse extract has been described earlier (Sell & Dean, 1972). Rabbit antiserum to boiled sheep erythrocyte stromata was obtained from Dr T. Borsos of U.S. National Cancer Institute. It was prepared by repeatedly injecting rabbits intravenously with boiled stromata, exactly as described by Kabat & Mayer (1961).

Coating of schistosomula with host antigens

Schistosomula were coated with host antigens by incubating them in host tissue extracts for 20 hr at 37°C. The details of this procedure have been described previously (Sell & Dean, 1972).

Mixed antiglobulin test

Host antigens were demonstrated on the surfaces of schistosomula and adult worms by means of the mixed antiglobulin test. Sheep erythrocytes which had been coated first with

rabbit antibodies to sheep erythrocytes and then with goat antibodies to rabbit globulin were used as indicator cells. Attachment of indicator cells to the parasites indicated the presence of rabbit anti-mouse antiglobulin on the surface of the parasites and constituted a positive test, indicating that mouse antigens were present on the surface of the parasite. A detailed outline of the test procedure has been presented previously (Sell & Dean, 1972).

Mixed agglutination test

In order to show that the mouse antigens adsorbed by schistosomula are cross-reactive with antigens on the surfaces of mouse and sheep erythrocytes, they were linked directly by direct mixed agglutination using rabbit antiserum to whole mouse extract. Mouse erythrocytes were pretreated with 0·1% trypsin for 10 min at 37°C (Zmijewski, 1968) in order to enhance their reactivity. In this test parasites were incubated with antiserum, washed repeatedly, and then incubated with indicator cells. It should be emphasized that the mixed agglutination test reveals surface antigens common to the test organism and indicator cells. In contrast, the mixed antiglobulin test detects antibody globulin on the surface of the test organisms.

Haemolysis test

Haemolysis tests were carried out in disposable round ('U')-bottom Microtitre plates (Cooke Engineering Company, Alexandria, Virginia) with antisera titrated by Microtitre loop. The diluent was pH 7·2 barbitone-based complement fixation testing buffer (CFT) (Oxo Limited, London). Each well contained 0·05 ml of diluted serum to which was added 0·025 ml of a 1·0% sheep erythrocyte suspension and 0·025 ml of 1:10 guinea-pig serum as a source of complement. The guinea-pig serum complement was absorbed with an equal volume of packed sheep erythrocytes before dilution. Plates were incubated in a moisture chamber at 37°C for 3 hr. They were then centrifuged for 10 min at 1000 *g* and read. Titres were reported as the reciprocal of the highest dilution producing total haemolysis. Tests were carried out in duplicate. Each test was reproducible within one dilution.

Antigen preparation

Whole newborn mouse extracts were prepared with a freeze press, as described previously (Sell & Dean, 1972). Extracts of various mouse tissues were prepared by homogenizing the tissues isolated from ten adult male mice in two volumes of Hank's balanced salt solution (HBSS) for 1½ min with a high speed rotary blade (Microhomogenizer attachment of Sorvall OmniMixer, Ivan Sorvall, Inc., Norwalk, Connecticut). The homogenates were extracted overnight at 4°C, centrifuged for 1 hr at 4000 *g*, and the supernatants frozen for later use. Guinea-pig kidney extract was prepared in the same way. Liver and skin extracts were prepared by the freeze press method because of the large volume of the liver and the tendency of the skin to wrap around the blade of the homogenizer.

Methanol extraction of erythrocyte stromata and lungs

Mouse and sheep erythrocyte stromata were prepared by a modification of the method of Rapp & Borsos (1966). Erythrocytes from sheep and adult male mice were washed three times with pH 7·4 phosphate buffered saline (PBS), stirred into twenty volumes of ice cold distilled water acidified to pH 4·0 with glacial acetic acid, refrigerated overnight, and then

centrifuged at 10,000 *g* for 20 min. The stromata were washed three times with the acidified water.

The lungs of ninety-eight mice (2·93 g dry weight) were perfused with saline *in situ* and removed. The mouse lungs and stromata were dried by lyophilization while the sheep stromata were collected by filtration onto Whatman No. 1 filter paper, washed on the paper with acetone, and air dried. The dried stromata and lungs were ground to fine powders with a mortar and pestle, each suspended in 150 ml of absolute methanol, and extracted on a mechanical shaker for 3 days at room temperature. The methanol insoluble fractions were collected by centrifugation (1000 *g* for 10 min), washed three times with methanol, and air dried. The methanol soluble fractions were filtered once through paper and placed in a freezer at −15°C for 2 days. The resulting flocculent precipitates were collected by centrifugation (1000 *g* for 20 min) at −10°C. The precipitates were washed three times with acetone at room temperature and dried by heating in a boiling water bath for 15 min. The dried precipitates were resuspended in small volumes of HBSS and stored frozen. One millilitre of these suspensions contained the methanol soluble material from 80 ml of packed sheep erythrocytes, 5·0 ml of packed mouse erythrocytes, or 0·42 g dry weight of lung tissue (from approximately fourteen mice).

Absorption of antisera

Absorption of antisera with erythrocytes was carried out by slowly rotating the antiserum at room temperature three times for 15 min with equal volumes of washed packed erythrocytes. Erythrocytes used for absorption were first washed four times with PBS, with some cells being discarded after each wash to ensure removal of the buffy layer. Mouse and guinea-pig erythrocytes used to absorb antiserum were washed six times and at least half of the total volume discarded to ensure that white cells, which contain Forssman antigen, would be removed. Blood films of the washed cells were prepared and showed only occasional white cells. Absorption (inhibition) with soluble tissue extracts was carried out by mixing equal volumes of extract and antiserum and rotating for 30 min at room temperature. The mixture was then centrifuged at 1000 *g* for 5 min and all but the bottom few drops removed for testing. Absorption with the dry methanol insoluble fractions was carried out by mixing the dry material with antiserum, rotating for 30 min at room temperature and centrifuging at 1000 *g* for 10 min. Aliquots of antiserum (0·5 ml) were absorbed with amounts of dry material equivalent to the amounts of soluble material used (84·4 mg sheep stromata, 24·8 mg mouse stromata, and 155·7 mg mouse lungs).

RESULTS

Distribution of the coating antigen in various mouse tissues

In order to get an idea of the source of the mouse antigen which ‘coats’ schistosomula (Sell & Dean, 1972), saline extracts of various mouse tissues were prepared and tested for the ability to coat schistosomula *in vitro*. Schistosomula were incubated overnight with the various extracts (at 1·0 mg protein/ml final concentration) and tested for host surface antigens using the mixed antiglobulin test. The results (Table 1) indicated that the coating antigen is widely distributed in the mouse, with perhaps the greatest concentrations in the lungs and spleen. It is possible of course, that the tissues giving the strongest reactions contained

the antigen in a more reactive or a more readily extractable form rather than in greater concentrations.

Removal of antibody to the coating antigen by absorption with Forssman-positive cells

The presence of Forssman antigen in adult schistosomes reared in mice is well documented (Damian, 1964). The Forssman antigen is also found in most tissues of mice (Tanaka & Leduc, 1956). Experiments were therefore carried out to determine whether or not this antigen is adsorbed by schistosomula. A series of mixed antiglobulin tests was carried out with rabbit anti-whole mouse extract, which had been absorbed with erythrocytes from a number of Forssman-positive and Forssman-negative species (Buchbinder, 1935). To further assure that Forssman was being reviewed, antiserum was mixed with a saline extract of guinea-pig kidney, a traditional source of Forssman antigen, and then was tested for its ability to identify host antigen on schistosomula.

TABLE 1. Coating of schistosomula with extracts of various mouse tissues

Tissue	Results of mixed antiglobulin tests*		
	Exp. No. 1	Exp. No. 2	Exp. No. 3
Lungs	8+	5+	5+
Spleen	4+	5+	5+
Serum	5+	3+	3+
Skin	4+	3+	4+
Submax. gland	3+	4+	4+
Liver	2+	4+	4+
Erythrocytes	3+	4+	3+
Kidney	2+	4+	3+
Brain	+	3+	3+
Heart	3+	3+	+
Mesenteries	+	3+	3+
Testes	2+	3+	2+
Thigh muscle	+	3+	2+
Intestine	+	2+	2+
Stomach	+	+	3+

Antiserum: Rabbit antiserum to whole mouse extract.
Extract concentration: 1 mg protein/ml.
* Graded 1+ to 8+ depending on strength of reaction. See also Sell & Dean (1972), Materials and Methods.

The results (Table 2) indicated that the antibodies which were reactive with the coating (host) antigen on schistosomula were removed by absorption with erythrocytes from all the Forssman-positive species and were also blocked by the addition of guinea-pig kidney extract. In addition, in all of these absorbed sera the sheep haemolysin titres were reduced at least to the preimmunization level. In contrast, none of the erythrocytes from Forssman-negative species significantly reduced the reactivity of the rabbit anti-mouse serum with coated schistosomula. As expected, absorption with Forssman-negative red cells also failed to remove sheep haemolysins present in these sera.

TABLE 2. Absorption of antibodies to the coating antigen with Forssman-positive material

Absorbing material	Mixed antiglobulin results: Schistosomula Exp. No. 1	Schistosomula Exp. No. 2	Adults Exp. No. 1	Adults Exp. No. 2	Sheep haemolysin titre
Forssman-positive					
Chicken RBC	N*	N	N	N	96
Dog RBC	N	N	N	N	96
Sheep RBC	N	N	N	N	0
GP kidney extract	N	N	N	N	0
Forssman-negative					
Human A RBC	7+	6+	P†	P	4096
Human B RBC	6+	4+	P	P	1024
Human O RBC	6+	5+	P	P	1536
Baboon RBC	6+	7+	P	P	2048
Rat RBC	6+	4+	P	P	1024
Bovine RBC	6+	5+	P	P	2048
Unabsorbed	6+	7+	P	P	8192
Preimmunization	N	N	N	N	128

Schistosomulum-coating antigen: Whole mouse extract.
Antiserum: Rabbit antiserum to whole mouse extract.
RBC = Red blood cells.
GP = Guinea-pig.
O = Haemolysin titre indicates negative at 1:8 dilution, the lowest dilution tested.
* N = negative reaction.
† P = positive reaction (adult worm reactions were not graded further than positive or negative; see Sell & Dean, 1972).

TABLE 3. Absorption of antibodies to the coating antigen with mouse and guinea-pig erythrocytes

Absorbing material	Mixed antiglobulin results: Schistosomula Exp. No. 1	Schistosomula Exp. No. 2	Adults Exp. No. 1	Adults Exp. No. 2	Sheep haemolysin titre
Mouse RBC	N	N	N	N	128
Guinea-pig RBC	N	N	N	N	96
Unabsorbed	6+	7+	P	P	8192
Preimmunization	N	N	N	N	128

Schistosomulum-coating antigen: Whole mouse extract.
Antiserum: Rabbit antiserum to whole mouse extract.
RBC = Red blood cells.

Absorption of anti-mouse serum with Forssman-positive cells also removed all reactivity of these sera with adult worms. This was the first indication that the host material adsorbed by schistosomula may be the same as that found on the surface of adult worms.

Absorption of rabbit anti-mouse extract was carried out with rabbit erythrocytes in an identical manner to determine to what extent titre reductions could be explained by dilution and trapping of the antiserum during absorption. This antiserum showed a drop in sheep haemolysin titre of one dilution, indicating that only reductions greater than 50% are significant.

Absorption of anti-mouse serum with mouse and guinea-pig erythrocytes

Though most tissues of both the mouse and guinea-pig contain Forssman antigen, it has been reported that neither of their erythrocytes contain this substance (Davidson & Stern, 1950; Rapp, 1970 (personal communication); Forssman, 1911). Therefore, mouse and guinea-pig erythrocytes were tested for the ability to reduce the reactivity of rabbit anti-mouse serum against the coating antigen on schistosomula. It was found (Table 3) that antiserum absorbed well with either of these red cells was completely inactive in the mixed antiglobulin test with coated schistosomula. Furthermore, sheep haemolysin (anti-Forssman) titres of both absorbed antisera were reduced to the preimmunization level. These absorptions were repeated, with great care being taken to ensure removal of leucocytes during washing of the erythrocytes, and the same results were obtained.

These results indicated that the rabbit anti-mouse serum is unlike rabbit anti-sheep cell serum in reacting with a Forssman-like antigen which is unusual in that it is represented in the mouse and guinea-pig red cell. To test this finding, further studies were next carried out using the more conventional anti-Forssman serum (anti-sheep cell stomata) to assure ourselves that its reactivity would not be reduced by absorption with mouse or guinea-pig red cells.

Reactivity of anti-Forssman (anti-sheep red cell) serum with the coating (host) antigen on schistosomula

Attempts were made to detect the schistosomulum-coating antigen by reacting parasites with antiserum against sheep erythrocytes in place of antiserum against whole mouse extract which had been used in previous studies (Table 4). Two antisera were used—rabbit antiserum to intact sheep erythrocytes and rabbit antiserum to boiled sheep erythrocyte stromata. While both antisera have anti-Forssman activity, the latter is generally considered to be more specific for Forssman. Though the sheep haemolysin (anti-Forssman) titres of these sera were high (over 100,000 for antiserum to boiled stromata), the mixed antiglobulin reactions for detection of host antigen on schistosomula were weaker than those produced with rabbit antiserum to whole mouse extract. This suggested that the antigen in mouse tissue extract, which coats the schistosome, might not be identical to the Forssman antigen of sheep erythrocytes, although it probably was closely related.

Proof of the difference in the coating mouse antigen and sheep cell Forssman was provided by absorption of both of the anti-sheep cell antisera with guinea-pig or mouse erythrocytes. Such absorption did not remove their reactivity with the mouse-coating antigen of schistosomes nor did it significantly reduce their sheep haemolysin titres. These findings were in strong contrast to the removal of both activities from rabbit anti-whole mouse extract, after absorption with guinea-pig and mouse erythrocytes (Table 3). Further study of the nature of the coating antigen in mouse tissue is therefore necessary.

Extraction of the coating antigen with methanol

It has been reported that the Forssman antigen of sheep erythrocytes is a glycolipid which can be extracted from sheep erythrocyte stromata at room temperature with methanol (see Raffel, 1961). Since the schistosomulum-coating antigen seemed to be Forssman-like, experiments were carried out to determine whether it is also methanol soluble. Methanol extracts of mouse erythrocyte stromata and lungs were prepared and tested for the ability to coat schistosomula and to block detection of the coating antigen with the mixed antiglobulin reaction. The lung was chosen since it was the most effective tissue tested for the ability to coat schistosomula (Table 2). The presence of erythrocyte antigens has been reported repeatedly in schistosomes (Damian, 1967; Smithers, Terry & Hockley, 1969; Clegg, Smithers & Terry, 1970). A methanol soluble (Forssman-positive) extract of sheep erythrocyte stromata was also tested for the ability to coat schistosomula and to inhibit the mixed antiglobulin test. The method of preparing these extracts ensured that they were heat stable and acetone insoluble.

TABLE 4. Reactivity of antibodies to sheep erythrocytes with the mouse coating antigen on schistosomula

Antiserum	Sheep haemolysin titre	Mixed antiglobulin results	
		Exp. No. 1	Exp. No. 2
RAIS	4,096	3+	4+
RAIS ABS. Mouse RBC	1,024	3+	3+
RAIS ABS. GP RBC	1,024	2+	+
RASS	131,072	2+	2+
RASS ABS. Mouse RBC	65,536	2+	2+
RASS ABS. GP RBC	49,152	2+	3+
RAM (control)	4,096	6+	7+

Test organism: Live schistosomula.
Coating antigen: Mouse lung saline extract.
GP = Guinea-pig.
RBC = Red blood cells.
RAIS = Rabbit antiserum to intact sheep erythrocytes.
RASS = Rabbit antiserum to boiled sheep stromata.
RAM = Rabbit antiserum to whole mouse extract.

All three methanol extracts tested—mouse lungs, mouse stromata, and sheep stromata—were found to coat schistosomula (Table 5). In most cases homologous antisera gave stronger mixed antiglobulin reactions than heterologous antisera. This confirmed earlier indications that the coating antigen found in mouse tissues is not identical to sheep Forssman. Methanol extracts of mouse lungs and sheep stromata were both found to completely inhibit the reaction of rabbit anti-whole mouse extract in the mixed antiglobulin test with schistosomula coated with mouse antigen (Tables 6 and 7). Both of these extracts also reduced the sheep haemolysin (anti-Forssman) titre of the rabbit anti-whole mouse extract below the preimmunization level.

TABLE 5. Coating of schistosomula with methanol soluble mouse and sheep antigens

Coating antigen	Antiserum	Mixed antiglobulin results	
		Exp. No. 1	Exp. No. 2
Methanol soluble	RAM	2+	4+
Mouse lung	RASS	3+	5+
Methanol soluble	RAM	4+	4+
Mouse erythrocyte stromata	RASS	2+	3+
Methanol soluble	RAM	2+	+
Sheep erythrocyte stromata	RASS	7+	5+

Test organism: Live schistosomula.
RAM = Rabbit antiserum to whole mouse extract.
RASS = Rabbit antiserum to boiled sheep erythrocyte stromata.

TABLE 6. Blocking of antibodies against mouse coating antigen with methanol soluble mouse material

Coating antigen	Antigen used to inhibit antiserum	Sheep haemolysin titre	Mixed antiglobulin results	
			Exp. No. 1	Exp. No. 2
Mouse lung	—	4,096	6+	7+
Saline extract	Mouse RBC	128	N	N
	MSMS	4,096	4+	4+
	MIMS	4,096	3+	6+
	MSMS+MIMS	4,096	8+	7+
	MSML	64	N	N
	MIML	768	6+	5+
Methanol soluble	—		4+	4+
Mouse RBC stromata	MSMS		3+	3+
	MIMS		2+	2+
	MSMS+MIMS		2+	+
	MSML		N	N

Test organism: Live schistosomula.
Antiserum: Rabbit antiserum to whole mouse extract.
RBC = Red blood cells.
MSMS = Methanol soluble mouse erythrocyte stromata.
MIMS = Methanol insoluble mouse erythrocyte stromata.
MSML = Methanol soluble mouse lung.
MIML = Methanol insoluble mouse lung.

The methanol soluble fraction of mouse erythrocyte stromata did not inhibit the reactivity of rabbit anti-whole mouse extract in the mixed antiglobulin test (Table 6). This was true whether schistosomula were coated with mouse lung or mouse erythrocyte antigen. This extract also did not reduce the sheep haemolysin titre of the antiserum. These results are in contrast to removal of both anti-coating antigen and sheep haemolysin activities from antiserum with intact mouse erythrocytes (Table 3). It is possible that the amount of antigen present in the stromal extract was insufficient to completely inhibit the antiserum. Another possibility is that the attachment of the antigen to either the red cell or parasite surface is necessary before the molecular configuration is suitable for the reaction with antibodies.

No methanol *insoluble* fractions significantly reduced the effectiveness of rabbit antiserum to whole mouse extract in either the mixed antiglobulin or sheep haemolysis test.

TABLE 7. Blocking of antibodies to the mouse coating antigen with the methanol soluble fraction of sheep erythrocyte stromata

Test organism	Antigen used to inhibit antiserum	Sheep haemolysin titre	Mixed antiglobulin results	
			Exp. No. 1	Exp. No. 2
Schistosomula	MSSS	0	N	N
(live)	MISS	8,192	3+	3+
	—	8,192	3+	3+
Schistosomula	MSSS		N	N
(formalin-fixed)	MISS		+	2+
	—		+	+
Adults	MSSS		N	N
	MISS		P	P
	—		P	P

Schistosomulum-coating antigen: Whole mouse extract.
Antiserum: Rabbit antiserum to whole mouse extract.
MSSS = Methanol soluble sheep erythrocyte stromata.
MISS = Methanol insoluble sheep erythrocyte stromata.
0 = Haemolysin titre indicates negative at 1:8 dilution, the lowest dilution tested.

Cross-reactivity of the mouse coating antigen and surface antigens of mouse and sheep erythrocytes

Mixed agglutination (as contrasted with mixed antiglobulin) tests were carried out to demonstrate that the mouse coating antigen on schistosomula was cross reactive with antigens found on the surfaces of mouse and sheep erythrocytes. Both coated schistosomula and adult worms were directly linked to mouse or sheep red cells by rabbit anti-whole mouse extract. Normal mouse cells, however, gave very weak or negative results. In contrast, mouse cells treated with trypsin to expose deeper antigens gave strong reactions while still proving to be unreactive in negative control tests. The results (Table 8) confirmed the data shown in Table 7. The ± reactions observed in the mixed agglutination tests with adult worms reacted with normal mouse erythrocytes were characterized by only occasional firmly attached erythrocytes and very few agglutinated clumps of cells. Most attached cells could be dislodged

by tapping the coverslip or by the worms' own movements. In contrast, both sheep and trypsin-treated mouse erythrocytes gave very strong reactions with adult worms, which were similar to those described previously for mixed antiglobulin tests (Sell & Dean, 1972). Reactions with female worms were nearly always restricted to the anterior one-half of the body while those with male worms occurred over the whole surface. These results suggest that the antigen adsorbed on schistosomula is cross reactive with antigen on the surface of mouse and sheep red cells.

TABLE 8. Identity of the mouse coating antigen and surface antigens of mouse and sheep erythrocytes

Test organism	Indicator erythrocytes	Antiserum	Mixed agglutination results	
			Exp. No. 1	Exp. No. 2
Schistosomula	M	RAM	N	N
(live)	MT	RAM	2+	5+
		RAM ABS. MSSS	N	N
		—	N	N
	S	RAM	6+	8+
		—	N	N
Schistosomula	MT	RAM	3+	3+
(formalin-fixed)		RAM ABS. MSSS	N	N
		—	N	N
	S	RAM	5+	4+
		—	N	N
Adults	M	RAM	±	±
	MT	RAM	P	P
		RAM ABS. MSSS	N	N
		—	N	N
	S	RAM	P	P
		—	N	N

Schistosomulum-coating antigen: Mouse lung saline extract.
M = Normal mouse erythrocytes.
MT = Trypsin-treated mouse erythrocytes.
S = Normal sheep erythrocytes.
RAM = Rabbit antiserum to whole mouse extract.
MSSS = Methanol soluble sheep erythrocyte stromata.

Evidence that host antigens on the surface of adult worms are adsorbed from the host

Detection of mouse antigens on the surface of adult worms was completely inhibited by the methanol soluble fraction of sheep stromata (Tables 7 and 8). This provides strong evidence that the host antigens on the surface of adult worms are similar or even identical to those adsorbed by schistosomula, and that they might also be of host rather than parasite origin.

DISCUSSION

The results of mixed antiglobulin (Sell & Dean, 1972; Table 7) and mixed agglutination

(Table 8) tests with formalin-fixed schistosomula strongly suggest that surface host antigen is passively adsorbed from the host rather than synthesized by the schistosomula. Furthermore, since the only host material detectable by these techniques on the surface of adult worms, as well as live and fixed schistosomula, is that cross-reactive with the methanol soluble, acetone insoluble, heat stable fraction of sheep stromata, it would appear that there is no need to suggest that parasite synthesis plays any role. Though antigens of parasite origin, such as the 'eclipsed' antigens of Damian (1964) and the induced antigens of Capron *et al.* (1968), cannot be excluded in the case of schistosomes, and certainly not for other host-parasite systems, it seems unlikely that in the *S. mansoni*-mouse infection two completely different mechanisms would be involved in acquiring the same surface antigen.

It would appear that, in mice, schistosomula of *S. mansoni* adsorb onto their surfaces something similar but not identical to the Forssman antigen of sheep. Kagan (1958) found that rabbits infected with cercariae of *S. mansoni* did not develop Forssman haemolysins, while Damian (1967) reported that rabbits immunized with adult worms from mice did. These findings indicate that the Forssman may be only an occasional antigen of *S. mansoni* being present in worms reared in Forssman-positive hosts. The two tissues which were shown to be most active in coating schistosomula, lung and spleen (Table 2), also gave the strongest reactions for Forssman antigen in a fluorescent antibody study reported by Tanaka & Leduc (1956).

The presence of the schistosomulum-coating antigen in mouse erythrocytes and the absorption of anti-mouse and anti-sheep antibodies with guinea-pig and mouse erythrocytes, however, are not consistent with the usual definition of the Forssman antigen. Brown (1943) and Davidsohn & Stern (1950) could not produce sheep haemolysins by injection of mouse erythrocytes (including C_3H) into rabbits, and Rapp (1970) could not absorb homologous sheep haemolysins with erythrocytes from a number of mouse strains. However, it has been reported (Buchbinder, 1935) and confirmed many times that Forssman antigen extracted from tissues with lipid solvents is a glycolipid hapten, unable to elicit the production of antibodies unless it is mixed with some carrier substance. It is well known that the Forssman hapten loses the ability to inhibit sheep haemolysis during purification, but that the expected specific activity can be restored by providing an activator substance, such as the cerebroside phrenosine (Papirmeister & Mallette, 1955). It is also known that Forssman substance exists in tissues as a glycolipid–protein complex, which is antigenic (Raffel, 1961). It seems possible that the failure of mouse erythrocytes to elicit production of sheep haemolysins could be due to the absence of a suitable carrier rather than absence of a Forssman hapten altogether. An alternative explanation is the possibility that a heterogeneous population of related antigens is involved. The antigens found in mouse erythrocytes might not elicit production of sheep haemolysins, but might cross-react with such antibodies elicited by related antigens found in mouse tissue cells.

The finding that mouse and guinea-pig erythrocytes absorb sheep haemolysins from rabbit-anti-mouse but not rabbit-anti-sheep erythrocyte antiserum (Tables 3 and 4) suggests a basic difference in the two antibodies and therefore in the Forssman antigens of mice and sheep. Though it is generally accepted that the Forssman substances from guinea-pig kidney and sheep erythrocytes are very similar, it has recently been shown (Hager, 1967) that guinea-pigs will produce antibodies to sheep erythrocyte Forssman, and that these antibodies are auto-reactive and bind to other Forssman-containing tissues of the guinea-pig. Whereas sheep erythrocyte Forssman reacts with antibodies to both chicken and human A erythro-

cytes, anti-chicken antibodies do not react with human A antigen and anti-human A antibodies do not react with chicken antigen. Similarly, antibodies to some but not all sheep erythrocytes react with human A erythrocytes (Landsteiner, 1945; Raffel, 1961). The Forssman group of antigens thus seems to encompass a large number of cross-reacting but not identical antigens.

It is likely that Forssman substances from different species share certain chemical groups and in fact may share groups with glycolipids in species normally considered Forssman-negative. It is conceivable that some basic determinant, perhaps even more ubiquitous than that responsible for Forssman specificity, is readily bound to the schistosome tegument. Perhaps this determinant is found on the same molecule with the Forssman determinant in Forssman-positive species and with other determinants in Forssman-negative species. If host antigens do indeed provide a camouflage for schistosomes, then the ability to bind the more universal portions of molecules, regardless of the species or isogenic specificities, could be of great benefit to a parasite which infects multiple host species. Such a mechanism would not require the extreme versatility that is implied by the various hypothetical mechanisms involving active synthesis of host antigens by the parasite.

The idea that host surface antigens of schistosomes are shared with the host erythrocytes is supported by the *in vitro* coating of schistosomula with erythrocyte antigens (Tables 1 and 5) and the adsorption of antibodies to the coating antigen with intact erythrocytes (Tables 2 and 3). Damian (1962) reported mouse haemagglutinin production in rabbits immunized with adult worm antigen. Kusel (1970) reported that human erythrocytes will adhere to schistosomula in human serum for 4–12 hr, after which the red cells are released. It was suggested that such attachment might provide a means of acquiring host antigens. Since the activity was partially lost when the serum was inactivated at 56°C, suggesting that complement may play a role, it seems possible that this phenomenon may be related to immune adherence, brought about by the presence of 'natural' or acquired antibodies in the serum. So-called 'natural' antibodies to several parasitic and free-living nematodes have been reported in serum from humans thought to be free of helminth infections (Coombs, Pout, & Soulsby, 1965; Hogarth-Scott, 1968).

Previous reports of human blood group antigens on adult schistosomes from mice are contradicted by the failure of human erythrocytes to remove antibodies to the coating antigen (Table 2). It is possible that human blood group antigens are stored internally, but are not found on the surface of schistosomes. There is evidence for such internal stores of blood group substances in adult worms. Oliver-Gonzalez & Torregrosa (1944) reported that a polysaccharide preparation from adult *S. mansoni* inhibited isoagglutinins for human A and B erythrocytes. Oliver-Gonzalez & Gonzalez (1949) reported a gradual absorption of human anti-A_2 but not anti-A_1 or anti-B isohaemagglutinins during 24 hr incubation with live adult *S. mansoni*. They concluded that an 80–90% reduction in the worms' polysaccharide content during this period was related to the release of A_2 substance. Damian (1964) could not find human A antigen in *S. mansoni*, however, and felt that the cross-reactivity of Forssman and human A antigens might have been responsible for the previous reports. Internal stores of blood group and other host antigens have been reported in many studies with parasitic nematodes and cestodes (Damian, 1964; Williams & Soulsby, 1970).

Increases in anti-A and anti-B haemagglutinins have been reported in type B and A (respectively) schistosomiasis mansoni patients (Huntley, Lyerly & Patterson, 1969), suggesting that A and B antigens may be produced by *S. mansoni.* Nevertheless, data from patients

with hepatosplenic schistosomiasis indicate that humans of all ABO and Rh blood types are equally susceptible (Katz, Tavares & Abrantes, 1967).

The present study suggests that at least one and possibly more mouse antigens adhere to the surface of *S. mansoni*. Immunodiffusion and immunoelectrophoretic studies of Damian (1967) and Capron *et al.* (1965, 1968) indicate that at least four and five host antigens, respectively, are present in adult worms from mice and hamsters. Some of the bands observed could be internally stored. It would appear that a serious problem in studies with whole adult antigens is the inevitable contamination with antigens of ingested host blood. Capron *et al.* (1968) presented several arguments against the importance of such contamination. They pointed out that (1) at least some of the antigens are shared with several vertebrates in addition to the host from which the worms were collected; (2) the digestive enzymes could be expected to degrade the antigenic specificities of ingested blood; and (3) poor correlation was observed between shared antigens found and those expected from contamination with whole blood. The first point seems to verify the sharing of antigens by the vertebrate hosts but not to exclude the possibility that they are ingested by the parasite. The second point might explain the third, since digestive enzymes might degrade some ingested substances more rapidly and completely than others. Certainly, partially digested erythrocytes are present in the gut of *S. mansoni* adults and it must be assumed that this material influences the antigenic nature of whole worm homogenates. The possibility that host macromolecules are taken in through the tegument suggests another possible source of contaminating antigens.

The present findings agree for the most part with the reports of Smithers, Terry & Hockley (1969) and Clegg, Smithers & Terry (1970). By means of the mixed agglutination test and electron microscopy combined with ferritin-labelled antibody, Smithers *et al.* (1969) demonstrated antigens shared by the surfaces of mouse erythrocytes and adult *S. mansoni* from mice. They also favoured the idea that these antigens are acquired from the host. Experiments involving destruction of mouse worms transferred to monkeys immunized against various mouse antigens indicated that the erythrocyte membrane is apparently an important source of host antigens.

In the report by Clegg *et al.* (1970) it was reported that mouse worms were not killed upon transfer to monkeys immunized against sheep erythrocytes, suggesting that the Forssman antigen was not involved. This is a contradiction to the findings in the present study, in which the only host material demonstrated on the surface on schistosomula and adult worms was that cross-reacting with sheep erythrocytes. There are several possible explanations for this difference. The most obvious seems to be that the mouse antigen, not being identical to sheep Forssman, might not cross-react well with the monkey heterophile antibodies. In mixed antiglobulin tests (Tables 4 and 5) both rabbit-anti-mouse and rabbit-anti-sheep antisera gave stronger reactions with schistosomula coated with the homologous antigen. The degree of cross-reactivity of mouse antigen with the monkey anti-sheep antibodies might be even less. Apparently the monkey assay used in the study of Clegg *et al.* was not sensitive to low antibody titres since it was necessary to inject Freund's adjuvant with mouse antigens in order to produce the hypersensitivity sufficient to kill mouse worms, and even then not all of the worms were killed.

At this stage it appears that schistosomula of *S. mansoni* adsorb from the host environment a glycolipid substance. Further studies are being carried out to isolate and characterize the coating material found in tissue extracts of several hosts.

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