CONTRIBUTIONS TO THE IMMUNOLOGY AND SEROLOGY OF SCHISTOSOMIASIS

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THE superfamily Schistosomatoidea (Stiles & Hassall, 1926: LaRue, 1957) comprises a large group of trematodes with species parasitizing many classes of the phylum Chordata. What were formerly thought to be three distinct human species, *Schistosoma haematobium, Schistosoma mansoni* and *Schistosoma japonicum,* are believed today to be complexes of closely related species or varieties which infect man and lower animals (Kuntz, 1955). With the incidence of malaria decreasing in the world, schistosomiasis is becoming one of the more important parasitic diseases of the human race. Millions of people are infected in the Middle East, the Orient, Africa, and South America. The economic toll and loss of productivity which schistosomiasis causes in these areas of the world makes it important that we study and understand the biology, physiology, epidemiology and host-parasite relationships of these parasites. Armed with this knowledge we may hope to establish effective control procedures to eradicate or alleviate manifestations of this disease.

In recent years advances made in the chemotherapy of schistosomiasis make it important the immunology of the disease be better understood. Increasingly larger groups of people are being treated in endemic areas and the effect of treatment on resistance and immunity toward this infection has not been thoroughly evaluated. To this day, the mechanisms of immunity against schistosomiasis in man and animals are still obscure. In this paper contributions made on the immunology and serology of schistosomiasis by the author and his co-workers will be reviewed. In an effort to discuss all aspects of the subject, papers by other workers will be cited.

It is not feasible to list all pertinent references relating to the immunology and serology of schistosomiasis. The number of published works is quite large. In general, recent references are cited and no attempt at completeness has been made. Two bibliographies list references up to 1948 (Khalil, 1931 and Bouillon, 1950). General reviews by Fairley (1951) and Newsome (1956) and books and monographs by Gelfand (1950), Skrjabin (1951), Girges (1934), and Martins (1949) plus many special reviews are available for reference.

In our laboratory we have been interested in the immunology and serology of *Schistosomatium douthitti* (Cort, 1914), a natural parasite of meadow voles (*Microtus pennsylvanicus*), and muskrats (*Ondatra zibethica*). This schistosome is endemic in the United States. We have also studied *S. mansoni*, a human species. Our *S. mansoni* strain was originally from Puerto Rico* and has been maintained by serial passage through laboratory reared *Australorbis glabratus* and hamsters. The infection with *S. douthitti* was obtained from naturally infected *Lymnaea palustris* collected in Ann Arbor, Michigan, and Chicago, Illinois, and maintained by serial passage through *L. palustris* and hamsters (Kagan, Short and Nez, 1954).

Our immunological studies were made with white mice, *Mus musculus*, and rhesus monkeys, *Macaca mulatta*. We have investigated various facets of the host-parasite relationship of *S. douthitti* because we believe there is fundamental value in studying the immunology of a near natural host-parasite relationship. We also believe that studies on animal schistosome species may bear directly on human species. In many respects *S. douthitti* appears to be related to *S. japonicum*. In our studies on chemotherapy with Miracil D (Kagan & Lee, 1952), we observed that *S. douthitti* was completely resistant to the action of this drug. Pesigan (1951) reported similar results with *S. japonicum*. These two species have eggs similar in size and shape which differ markedly from those of *S. mansoni* and *S. haematobium*. The nocturnal emergence of cercariae from the snail host and the behavior of the cercariae in clinging to the surface film of the water awaiting penetration of the mammalian host is identical for both species. The gross pathology in mice and rate of infection for mice for these two species are almost the same. These facts, plus the reported occurrence of *S. japonicum* in rodents (Hsü & Li, [in press], Magath & Mathieson, 1945), led us to believe that perhaps *S. japonicum* was a rodent schistosome species with broad host specificities (Kagan & Lee, 1952).

The presence of antibodies in infected or antigen-injected animals has been recognized in schistosomiasis for many years. The terms immunology and serology are used quite loosely and, in the minds of some, are synonymous. In this review serology will refer to antigen-antibody reactions whereas immunology will imply resistance to infection. Thus a host may manifest a serological response (the production of antibodies) in the absence of an immune response (resistance or protection). In many instances, but not in every case, the development of resistance is mediated through the action of an antigen-antibody reaction. The emphasis on the

* The original stock of infected snails was obtained in 1950 from the Laboratory of Tropical Diseases, National Institute of Allergy and Infectious Diseases, Public Health Service.

former circumstance (presence of antibodies) may account for the fact that the two terms are not always used correctly.

The mechanisms of helminth immunity for many related species involve both humoral and cellular factors (Taliaferro, 1950). To demonstrate a humoral antibody mechanism, three criteria have to be met: (1) antibodies must be present in the serum of the immune host; (2) the serum of the immune host on passive transfer to a normal animal must be protective; and (3) the protective antibody should be removed from the serum by absorption with the parasite (somatic antigens) or antigens produced by the living parasite (metabolic products antigen).

Cellular host factors in immunity may involve an enhanced allergic or phagocytic response or hyperplasia of the reticulo-endothelial system. These manifestations may, under certain circumstances, lead to the death, encapsulation, or isolation of the parasite, and/or the production of antibodies. In addition to the above humoral and cellular mechanisms, there may be non-specific defense mechanisms which do not require the mediation of antibodies or cells.

The following immune states are generally recognized and the discussion of immunity will follow this general outline (Boyd, 1956).

I. Innate Immunity (constitutional or racial)
II. Acquired Immunity
  1. Active —a. Infection (natural)
    b. Vaccination (artificial)
  2. Passive—c. Natural (congenital)
    d. Artificial (serum transfer)

The serology of schistosomiasis encompasses the detection of antibodies in the serum of an infected host by both *in vitro* and *in vivo* methods. Such standard serological tests as complement-fixation, precipitin, flocculation and hemagglutination, have been used in diagnosis. *In vitro* studies of serum antibodies on larval and adult schistosome stages have been made. These studies have characterized the CHR antibody, (Cercarienhüllen reaction of Vogel & Minning, 1949*a*, 1949*b*), miracidial immobilization test (Senterfit 1953), circumoval precipitin test (Oliver-Gonzalez, 1954) and cercarial agglutination test (Liu & Bang, 1950) (Fig. 1). *In vivo* serological studies include the Prausnitz-Küstner reaction and the skin test.

Innate Immunity

Native or innate resistance encompasses the hereditary defense mechanisms possessed by a species against infectious organisms. Susceptibility studies of host species for laboratory infections with schistosomes are in one sense investigations of innate resistance against infections (Moore,

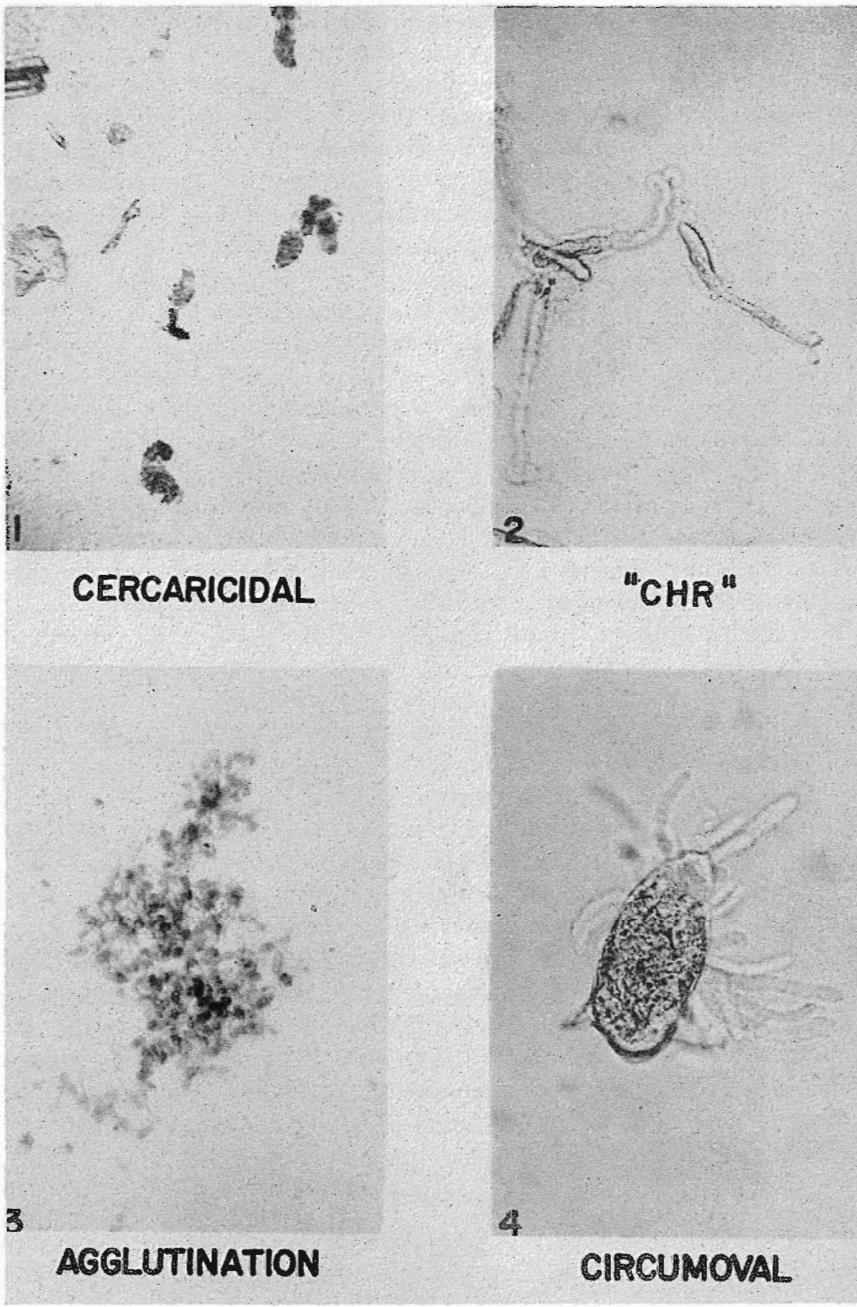

FIG. 1. *In vitro* reactions with eggs and cercariae of *Schistosoma mansoni*. Cercaricidal precipitins (Papirmeister & Bang, 1948), CHR (Vogel & Minning, 1949), agglutination (Liu & Bang, 1950) and circumoval precipitin reaction (Oliver-Gonzalez, 1954).

Yolles & Meleney, 1949; Stirewalt, Kuntz and Evans, 1951; Büttner, 1953; and Kuntz, Malakatis & Wells, 1955). As reviewed by Kuntz (1955) data from a number of workers indicate that the human schistosomes in Africa and South America are not as host specific as once supposed. S. *mansoni*, once thought to be restricted to primates, has been reported from wild rodents, insectivores and opossums (Martins, *et al.*, 1955; Schwetz, 1955).

Animal species can be divided into three categories with regard to infection with schistosomes: those that are totally resistant to infection, those that can be infected but are poor hosts, and those that are susceptible hosts.

We have infected mice, hamsters, rats, rabbits and monkeys in our laboratory with S. *douthitti*. The host-parasite relationship in these animals differs greatly. The monkey will tolerate an infection for 2-3 weeks, at which time the worms are suddenly killed and infection dramatically terminated. We found living worms in 9 monkeys 10-25 days after exposure, dead and moribund worms in 2 monkeys 22-25 days after exposure, and no worms in 5 monkeys necropsied 30-90 days after exposure (Kagan, 1953). A similar phenomenon was reported by Fairley, *et al.* (1927, 1930) for S. *spindalis* in *Macacus sinicus*. They found that monkeys remained infected for only 11-15 days. Spontaneous cure in monkeys infected with S. *mansoni* has also been reported (Cram & Files, 1947).

In the rat the termination of infection is not so dramatic. The rat is a poor host for S. *douthitti* (Price, 1931). It has been our experience that worms recovered from rats are smaller than worms from mice and hamsters. The cellular response in the rat is very extensive, with encapsulation and nodule formation occurring in the lungs of the animal early in the infection (Kagan and Meranze, 1957). The rat is also a resistant host for S. *mansoni*, and spontaneous cure after 6-8 weeks has been reported, (Stirewalt, Kuntz and Evans, 1951; Thompson, 1954). The rabbit is another poor host for S. *douthitti*. Massive doses of cercariae resulted in light infections.

The mouse and hamsters are very susceptible hosts for S. *douthitti* infections. Infections lasting 468-484 days in mice were reported by Short (1952), and a male infection of 828 days was reported in our work from an experimentally infected deer mouse, *Peromyscus maniculatus* (Kagan, Short and Nez, 1954). In the mouse the life span of the worms, once maturity is reached and when the infection is not too heavy, is often (possibly in most cases) limited by the death of the host. A similar situation may exist in man. Infections of over 20 years with S. *haematobium* have been reported (Berberian, *et al.*, 1953). In a survey of the incidence of S. *mansoni* in the Puerto Rican population, Ferguson *et al.* (in press) reported a relatively stable level of infection in the adult popu-

lation (Figure 2). The pleateau in the incidence curve after age 30 implies an infection of long duration. The possibility of reinfection in the female population cannot be ruled out since this is an endemic area. Rural women come in contact with infected waters in the washing of clothes and other household chores and may be continuously exposed. This is not true for the male population once maturity is reached. The incidence of infection in non-endemic parts of the island is very similar.

The schistosome cercariae are very invasive organisms. The skin is not

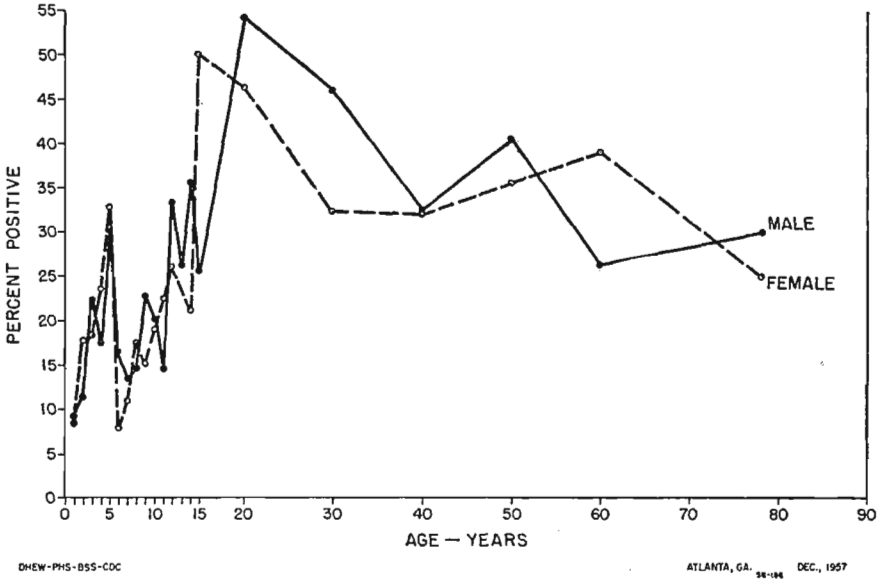

FIG. 2. Incidence of infection with *Schistosoma mansoni* in 5402 stool samples in an endemic area in Puerto Rico. (Ferguson, F. F., *et al.*, in press.)

as effective a barrier as was previously thought. It has been demonstrated that non-mammalian schistosome cercariae will penetrate mammalian hosts and migrate to the lungs before death (Olivier, 1953). The factor that causes the death of cercariae in unsuitable hosts is probably associated with the innate immunity of the host.

A number of workers have investigated the *in vitro* activity of cercariae in normal serum and have demonstrated a cercaricidal factor (Tubangui and Masilungan, 1936; Culbertson & Talbot, 1935; Culbertson, 1936; Standen, 1952; Stirewalt and Evans, 1955; Kagan and Levine, 1956). In our laboratory we were interested in determining if there was a correlation between the cercaricidal activity of the normal serum of resistant and

susceptible hosts. We bathed cercariae of S. *monsoni* in the serum of 18 animal species and noted that some sera were cercaricidal, others inhibited the locomotion of the cercariae, and two agglutinated cercariae, Table 1, (Kagan & Levine, 1956). We found that the sera of hosts with a high degree of resistance to infection with S. *mansoni* were more cercaricidal than the sera of hosts susceptible to infection. We also found that when the cercaricidal activity was eliminated by heat, the sera of additional species agglutinated cercariae (Standen, 1952; Kagan & Levine,

TABLE 1

In vitro ACTIVITY OF CERCARIAE OF *Schistosoma mansoni* IN SERA OF ANIMALS

Normal Sera			Normal Heated Sera	
Cercaricidal	Inhibition of Locomotion	Agglutination	Normal Swimming	Agglutination
Chicken	Cat	Cow	All species tested	Cow
Dog	Hamster	Horse		Dog (50%)
Goat	Man			Goat
Guinea pig	Mouse			Hamster (33%)
Pig	Monkey			Horse
Rat	Pigeon			Man (43%)
Sheep	Rabbit			Pig
Steer	Squirrel			Rabbit (11%)
				Sheep
				Steer

1956). The agglutinins we found in normal serum were non-specific antibodies unrelated to the agglutinins found in immune serum. Normal serum which agglutinated living cercariae would not agglutinate sensitized sheep cells in a tannic acid hemagglutination test (Kagan, 1955a).

Evans, Stirewalt and MacKenzie (1955) fractionated normal mouse serum electrophoretically and noted that the gamma and beta globulin fractions contained the cercaricidal factor. The alpha fraction inhibited motility, but the albumin fraction was without activity.

The cercaricidal factor of normal serum has been related to complement since it could be destroyed by heating for 30 minutes at 56°C (Tubangui and Masilungan, 1936; Culbertson, 1936; Standen, 1952). In our work we also thought initially that the cercaricidal activity was related to complement but later we decided it was not, because guinea pig serum frozen for 60 days and heated for 30 minutes at 56°C was still cercaricidal. Recent studies have indicated that the cercaricidal factor of normal serum is associated with the properdin level of serum (Iralu, unpublished). Properdin, a euglobulin containing lipid, carbohydrates

and phosphorus was discovered by Pillmer (1954) and is a minor constituent of normal serum (0.03%). With the cooperation of complement and magnesium ions it acts as a non-specific defense factor in the blood against all types of infectious agents.

There have been some experimental data with respect to host susceptibility by various schistosome species "strains" (Gönnert & Vogel, 1955; Büttner, 1953), and the effect of cortisone on innate immunity (Coker, 1957). There has been little work on the susceptibility of inbred strains of host for schistosome infection.

We did some preliminary work to determine if the incidence of infection to inbred strains of mice varied significantly when exposed to cercariae of *S. douthitti.* Although the number of animals was too small to be statistically significant, there appeared to be a tendency for the C_3H (57.79%) and the *dba* mouse (65.09%) to be slightly more susceptible for infection than the CFI mouse (48.84%) (Kagan, Short and Nez, 1954).

Cardoso (1953) after examining several hundred Negro and white Brazilians infected with *S. mansoni* came to the conclusion that the Negro race was more resistant to *S. mansoni* than the white race. These claims for man have to be evaluated very carefully because of the role that nutrition and general environmental conditions play in schistosome infection (Chandler, 1953; Le Grange, 1955; Smith, 1955).

Acquired Immunity

Although experimental evidence is meager, it is generally accepted that man acquires resistance for schistosomiasis (Fisher, 1934; Schwetz, 1956). We were able to demonstrate that the white mouse (*Mus musculus*) and the monkey (*Macaca mulatta*) developed an acquired immunity against *S. douthitti* (Kagan, 1952, 1953). In the mouse, resistance and immunity was manifested by a statistically significant decrease in the number of worms which developed from a challenging exposure of 50 cercariae in previously infected mice as compared to the number of worms which developed from 50 cercariae in initially uninfected mice. Immunization by repeated small exposure to cercariae of both sexes increased the resistance of mice to a challenging exposure. An initial infection with male and female worms evoked an immune response sooner than an initial infection with female worms. Infection with male worms over a 210-day period failed to protect mice from challenging infections (Fig. 3).

In addition to the fewer worms which developed in the immune animal, infection with female worms caused stunting of male worms in the challenging infection. Infection with male worms caused stunting of female worms, and initial infection with worms of both sexes caused

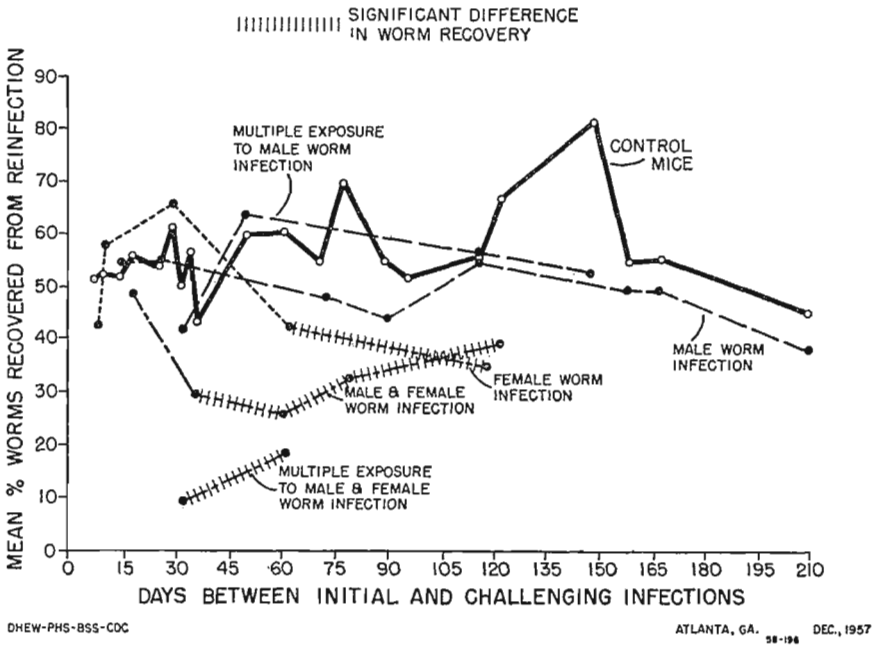

FIG. 3. Acquired immunity in mice infected with *Schistosomatium douthitti* and challenged at varying intervals of time after infection (Kagan, 1952).

stunting of all the worms in the challenging exposure (Kagan, 1952). In the immune monkey the cercariae (*S. douthitti*) of the challenging infection were eliminated before they had reached the lungs (Kagan, 1953).

Studies by Fairley (1926) for *S. spindalis,* Oliver and Schneidermann (1953), Stirewalt (1953), Pifano (1953), Thompson (1954), Laurie and de Meillon (1957), Meleney and Moore (1954) for *S. mansoni,* and Ozawa (1930), Lin, Ritchie and Hunter (1954), Vogel and Minning (1953), Hunter, *et al.,* (1956) for *S. japonicum,* all reported acquired immunity in experimental animals.

In mice infected with *S. douthitti,* the egg appears to be an important source of antigenic stimulus for the immune state. The evidence for this is primarily circumstantial. Resistance in mice infected with male and female worms or only with female worms coincided with egg deposition in the tissues by the sexually mature worm. The female *S. douthitti* can produce viable eggs hermaphroditically in the absence of male worms (Short, 1952). We found that the egg of *S. douthitti* was very resistant to chemotherapy and survived in the tissues 3-4 weeks after the death of the worms. This is also true for the longevity of the eggs of *S. mansoni*

(Gönnert, 1955) and S. *japonicum* (Vogel and Minning, 1947). Mice infected with S. *douthitti* and cured by chemotherapy lose their immunity approximately 3 weeks after the death of the worms (Kagan and Lee, 1953). This interval of time coincides with the longevity of the eggs in the tissues and suggests that perhaps the living embryo within the egg secretes or excretes substances that are necessary for the maintenance of acquired immunity in the mouse.

In addition to the eggs serving as an antigenic stimulus there is experimental evidence that in the monkey and perhaps in man, adult worms and/or their metabolic products may also be responsible for the antigenic stimulus of the immune state. Girges (1934) expressed his belief that a male infection in man was protective against superinfection. He based this statement on data obtained from the autopsy table since in chronic human infections he occasionally found only male worms. Vogel and Minning (1953) reported that monkeys infected with male S. *japonicum* worms were protected from lethal superinfections of cercariae 1-2 years after cure. Lin, Ritchie and Hunter (1954) also found that mice infected with male S. *japonicum* worms were resistant to superinfection. In our studies with rhesus monkeys, animals challenged after the initial infection had been eliminated by spontaneous cure were resistant to reinfection (Kagan, 1953).

Immunization Experiments

One of the most significant contributions in the immunology of parasitic disease has been the experimental demonstration that "metabolic antigens" play a significant role in parasitic resistance (Thorson, 1953, 1954; Thillet and Chandler, 1957). Workers with S. *japonicum* have reported some measure of success with immunization experiments with somatic or killed body antigens of adult worms. Ozawa (1930), Kawamura (1932) and Lin, Ritchie and Hunter (1954) reported that experimental animals were protected by previous injection of saline suspension of whole worms. Vogel and Minning (1953) were unsuccessful in thus immunizing monkeys against infection with S. *japonicum*. Watts (1949) reported that mice injected with a cercarial antigen of S. *mansoni* were more resistant to infection than non-injected mice. Thompson (1954) was unable to verify Watt's results; in his experiments, no protection was manifested in immunized mice. In our laboratory we conducted a series of experiments with somatic antigens of S. *douthitti* with negative results. In almost every instance antigen-injected mice were more susceptible to infection than the non-antigen injected controls. We believe this was due to their resistance being lowered by the injection of massive doses of foreign protein (Table 2).

TABLE 2

TABLE 2. IMMUNIZATION EXPERIMENTS WITH ANTIGENS FROM THE LIFE CYCLE OF *Schistosomatium douthitti*

Antigen	Immunizing Dose	Number of Mice		Cercariae Challenge	Number of Worms Recovered	
		Immunized	Controls		Immunized	Controls
1300 male and female adult worms	.1 ml×6	6	6	50	30.0	33.5
1200 male and female adult worms	.5 ml×3	4	4	50	20.5	20.0
100 male adult worms	.5 ml×7	8	9	50	33.0	29.0
Infected snail	.5 ml×6	8	4	50	15.1	20.0
Normal snail	.5 ml×6	8	4	50	16.8	20.0
Eggs in Liver	0.25–0.5×6	8	6	25	8.2	5.5
Eggs in Liver	0.5 ml×7	6	6	25	13.3	11.1
Eggs in Liver	0.5 ml×9	12	8	25	8.7	5.0

Recent work by my former graduate student with metabolic antigens of cercariae and adult worms of *S. mansoni* have indicated that the life of mice exposed to lethal doses of cercariae may be prolonged by immunization with these antigens (Levine, unpublished). The metabolic antigen was obtained by incubating approximately 400,000 cercariae in 5-10 ml of aquarium water at room temperature overnight. The cercariae were removed and the aquarium water containing the cercarial secretions, excretory and metabolic products was saved. This process was repeated four times using the same fluid, so that antigen from approximately 1.6 million cercariae was obtained. A similar antigen was made by substituting 300 male and female worms for each incubation period.

Five groups of mice were exposed to a lethal dose of 700 cercariae of *S. mansoni* after a period of immunization. Group A was unimmunized, group B received a sham immunization of aquarium water in Freund's adjuvant (Arlacel A plus Bayol F and BCG, Cohn, 1953), groups C, D, and E received cercarial and adult metabolic product antigen in Freund's adjuvant once each week for 5 weeks and were then exposed to 700 cercariae. Mice exposed to 700 cercariae begin to die in the 8th week due to passage of eggs through the wall of the intestine. Perhaps because of excess handling or the deleterious effect of the oil in the antigen, mice in the immunized and control group died during immunization and shortly after exposure to cercariae. In the analysis of the results, Levine took the 8th week after exposure as the start of the experiment and calculated the longevity of the immunized mice compared to the controls for all mice alive after 7 weeks of infection (Table 3). As can be seen from the

TABLE 3

IMMUNIZATION EXPERIMENTS WITH METABOLIC ANTIGENS OF *Schistosoma mansoni* (Levine, D. M., unpublished).

Group	A	B	C	D	E
No. of Mice	15	15	15	15	15
Protocol	No Vaccination	Aquarium Water	Cercarial metabolic antigen	Adult metabolic antigen	Cercarial and Adult metabolic antigen
No. of mice surviving immunization and exposure to Cercariae	10	9	12	13	11
No. of mice surviving 7 weeks of infection	10	4	8	11	8
Mean week of death for mice alive at 8 weeks of infection	10.1±1.8	10.0±1.4	12.8±3.1	11.9±2.8	12.5±2.4
Average mean week of death for control and experimentals	10.1±1.6		12.4±2.7		
Difference between means	2.3±0.67				
T value	3.4 (significant at 1% level)				

data the mean week of death for the immunized mice was significantly later than for the controls, indicating that immunization prolonged the life of these mice. If one applies the same analysis to longevity experiments reported by Olivier and Schneidermann (1953) the mean week of death for mice immunized by previous infection is also significant in some experiments.

With techniques for maintaining schistosomes *in vitro* (Robinson, 1956a; Newsome and Robinson, 1954), I believe sufficient concentrations of metabolic antigens can be obtained to evaluate the use of these antigens in the study of the immunology and serology of the disease.

PASSIVE TRANSFER

Natural (congenital): There has been to my knowledge no study on the transfer of immunity congenitally or in the colostrum. Infants and very young children are not infected with schistosomes because they do not

come in contact with infected cercariae. As soon as they are old enough to play in infected waters they probably are exposed (Ferguson, *et al.*, in press).

Artificial passive transfer: Antibodies are present in the serum of an infected animal. The nature and character of some of these antibodies will be described in detail in the serology portion of this paper. In order to demonstrate an antibody basis for schistosomal immunity passive transfer experiments should be successfully performed. Kawamura (1932) reported passive transfer in dogs and rabbits infected with *S. japonicum.* This report is difficult to evaluate since he does not describe the protocols of the experiment or the controls used. Vogel and Minning (1953) were unable to transfer passively resistance to monkeys with *S. japonicum* antiserum. Stirewalt and Evans (1953) were unsuccessful in passive transfer of protection to mice with hyperimmune *S. mansoni* rat serum.

In our laboratory we have been conducting passive transfer experiments for several years with serum from animals infected with *S. douthitti* with negative results. We have injected various doses of homologous and heterologous hyperimmune serum for varying lengths of time prior to, and following, infection. We injected serum at the site of cercarial penetration prior to and after exposure. The results of these experiments are tabulated in Table 4. In some instances, mice receiving heavy doses of immune and normal serum were rendered more susceptible to infection than the controls.

Another approach to this problem was made by Levine (unpublished). He bathed cercariae in diluted normal and immune serum for varying lengths of time prior to exposure. These studies are still in progress but may show that there are antibodies in immune serum which may impair the ability of the cercariae to develop to maturity in the host.

Serology

The diagnosis of infection has been the principal goal of serological tests. The detection of eggs in feces or urine may be very reliable in an acute infection, but for chronic or light infections even with improved methods for concentration of eggs the intermittent escape of eggs from the intestine makes diagnosis uncertain. For this reason a greater reliance on immunodiagnostic methods has been the trend in recent years. In addition to diagnosis, serological methods have been used to evaluate the efficacy of therapy, and for studies of the serological specificity of various life cycle stages of the schistosome parasite as potential sources of antigen.

Complement Fixation

The complement fixation test has been in use in the diagnosis of schistosomiasis for close to fifty years (Taliaferro, 1929). The principal

TABLE 4

PASSIVE TRANSFER EXPERIMENTS WITH ANTISERUM OF ANIMALS INFECTED WITH *Schistomatium douthitti*

Exp. No.	Number of Mice Autopsied			Serum Dose	No. Infecting Cercariae	Worms Recovered		
	Immune Serum	Normal Serum	No. Serum			Immune Serum	Normal Serum	No Serum
				Hyperimmune Mouse Serum				
1	3	2	4	.5–1.0 ml×1	50	20.0	20.5	38.5
2	6		6	.5– ml×5	25	6.7		7.8
3	8	8	6	.25–.5 ml×6	25	8.0	11.7	10.3
4	8		8	.5 ml×5	25	8.8		8.4
5	4	4	4	.25 ml×8	50	24.0	25.0	9.0
				Hyperimmune Rabbit Serum				
6	4	2	3	3.0×1	50	—	41.5	25.6
7	7	7	5	1.5×.5	25	11.0	10.7	11.3
8	5	5	5	0.5×7	10	4.4	5.6	4.4
9	8	6	6	0.5–1.0×17	25	10.5	—	10.0
				Hyperimmune Monkey Serum				
10	6	0	6	0.25×5	25	4.0		6.6
11	0	8	7	0.25×6	25		5.7	7.0
12	5	0	5	0.25×5	25	8.4		10.2
13	5	0	5	0.3–0.8×3	25	11.0		9.2
				Serum Injected at Site of Penetration at Time of Infection				
15	3	3	3	.5 ml×1	50–150	29	15	28
				Serum Injected at Site of Penetration before Exposure				
16	3	3	3	.5 ml×1	100	21.3	5.0	31.0

problem in the use of this test has been the development of a suitable antigen. The early Japanese workers utilized alcoholic and saline extracts of adult worms (Yoshimoto, 1910). A cercarial antigen of *S. spindalis* made from infected snail livers was introduced by Fairley (1919). As reviewed by Taliaferro (1929) the early workers were concerned with the nature of the antigen, whether it was protein (LeBas, 1922), or lipoidal in character (Fairley, 1925). The tendency for schistosome antigens to react with syphilitic serum led Chaffee, *et al.*, (1954) to prepare an improved antigen from desiccated adult schistosomes extracted with cold anhydrous ether prior to extraction with buffered salt solution, which would not cross-react with non-schistosome sera. This antigen proved to be very sensitive and specific in detecting schistosomiasis in a survey of Puerto Rican soldiers (Horstman, *et al.*, 1954). Eliahim & Davies (1954), and Davies and Eliahim (1954), evaluated antigen prepared from infected snails and adult *S. mansoni* worms by a variety of methods. They found

in contrast to Mayer and Pifano (1945*a*) and Williams (1947) that adult worm antigens of *S. mansoni* were superior to cercarial antigens. They prepared a lipo-protein antigen by extracting adult worms with Coca's solution (0.5g. NaCl, 0.05g $NaHCO_5$, 0.4g phenol, distilled water to 100 ml). Schneider, *et al.*, (1956, 1957) prepared a non-dialyzable aqueous extract of adult *S. mansoni* worms that is primarily carbohydrate and nucleoprotein, and which shows promise. Lurie, *et al.*, (1952) reported that the CF test was still the most sensitive test in the diagnosis of early schistosomiasis.

Precipitins

Precipitins play a unique role in helminth immunity. They are formed in the host in response to antigenic material secreted and excreted by the parasite and in response to the somatic antigens of the dead worm. They have been implicated in effecting immobilization of helminths in the tissue of the host, stunting of growth, prevention of food assimilation and inhibition of enzymatic activity (Taliaferro, 1940, 1943).

Antigens prepared from dried livers of *Australorbis glabratus* infected with *S. mansoni,* thoroughly extracted with ether-alcohol followed by a final extraction with Coca's solution, proved to be effective in a precipitin test developed by Taliaferro, Hoffman and Cook (1928). The precipitin test has not been readily accepted in diagnosis because of cross-reaction with syphilitic serum. In contrast to the complement-fixation test, alcoholic extracts of schistosome worms are not active in the precipitin test (Miyaji and Imai, 1928).

Oliver-Gonzalez, *et al.*, (1955) utilized the precipitin test in their studies of schistosome serology. Saline extracts of adults, cercariae and eggs of *S. mansoni* were tested by "ring tests" in microprecipitation tubes with sera of rabbits injected with adults, cercariae, and eggs of *S. mansoni.* In addition, sera from acute (early) and chronic (late) infections in humans were tested. They found that in the sera of individuals infected from 40-240 days (early infection) the titer of cercarial precipitins was higher than in the sera of chronic patients. Adult antigen titers were fairly constant for both groups. Rabbits immunized with the three stages of the life cycle produced stage-specific antibodies. Precipitin tests with adult worm antigens performed by Okabe, *et al.*, (1954) were reported to be more sensitive than skin tests in the diagnosis of infection.

Circumoval Precipitin Test

Precipitins will form about living eggs incubated in sera of infected humans and monkeys when placed at 37°C for periods up to 24 hours (Oliver-Gonzalez, 1954). The precipitates which appear are antigen-anti-

body complexes formed by secretions or excretions of the living miracidium in the egg and specific antibodies in the serum. This was shown to be so by tagging immune globulin with fluorescent isocyanate and demonstrating that the precipitates around eggs incubated in immune tagged serum were fluorescent (Lewert, Lee & Jackson, unpublished). The specificity of this reaction was shown by incubating eggs in immune serum plus tagged normal serum. The precipitates in this preparation did not fluoresce. Anti-egg antibodies in immune serum could not be absorbed with adult or cercarial antigens but only with living or lyophilized eggs (Oliver-Gonzalez, *et al.*, 1955b). The circumoval precipitin test was reported to be species specific (Oliver-Gonzales, *et al.*, 1955b). When mixtures of eggs of *S. mansoni, S. japonicum* and *S. haematobium* are incubated together in different antisera, a precipitate will form around the homologous egg. A slight cross-reaction between the eggs of *S. mansoni* and antiserum of *S. haematobium* and of *S. japonicum* was observed.

The circumoval precipitin test was found to be more reactive in chronic schistosomiasis. From 49.5-72.8 per cent of eggs were positive for precipitates in antisera from chronic schistosomiasis cases as compared to 29.4-53.2 per cent in recent acute infections. The serum of animals exposed to worms of one sex (and in the case of female *S. mansoni* producing no eggs) was negative for circumoval antibodies. The circumoval activity is greatly diminished in sera of treated individuals (Oliver-Gonzalez, Ramos and Coker, 1955). The test therefore has diagnostic and prognostic value in evaluating therapy. Approximately 120-180 days after successful therapy the circumoval precipitin test becomes negative.

Newsome and Robinson (1956), and Robinson (1956b), reported circumoval precipitin titers in sera of baboons infected with *S. mansoni.* Robinson (1956*b*), also observed precipitates around the oral sucker of 21-day-old schistosomulae in immune serum.

Cercarienhüllen Reaction *(CHR)* of Vogel and Minning (1949a, 1949b)

Papirmeister and Bang (1948) reported the formation of a precipitate around cercariae of *Schistosoma mansoni* incubated in sera of monkeys and humans infected with schistosomiasis. This reaction, which occurred in fresh serum, was destroyed by heating the serum at 56°C for 30 minutes, and could be restored by the addition of complement. Vogel (1948), and Vogel and Minning (1949a, 1949b) published their observations on the formation of a membrane (CHR reaction) around the tail and body of living cercariae placed in immune serum. This transparent envelope which surrounds the cercariae did not kill the organism. They studied the activity of this antibody in serum from experimentally infected

mice, rabbits, dogs, monkeys, and cats and in serum from naturally infected humans. They observed that precipitates form around cercariae in fresh serum and expressed the belief that this reaction was not related to the CHR and was similar to the reaction described by Papermeister and Bang (1948). They found that the CHR reaction was negative 5-7 months after treatment and cure in infected humans. The CHR antibody appeared in the serum 40-47 days after infection in experimental animals. The formation of a membrane around schistosome cercariae was very specific and did not develop around non-schistosome cercariae.

Mayer and Pifano (1951), and Pifano and Ron Pedrique (1957) tested the serum of patients passing eggs of *S. mansoni*. They were able to detect the CHR antibody in only 79.62% and 78% of their samples, respectively. This led them to conclude that the CHR test was not as reliable as other serological methods in the diagnosis of schistosomiasis. Mayer and Pifano (1951) also reported that the CHR antibody was detected in the sera of animals infected with worms of one sex after the worms became sexually mature. Standen (1952), Meleney and Moore (1954), Stirewalt and Evans (1955), Hendricks and Cort (1956), have also reported on the CHR phenomenon. Electrophoretically-separated immune serum revealed that the CHR membrane would only develop in the gamma and alpha fraction of the serum (Evans, Stirewalt and MacKenzie, 1955). Electron micrograph studies of cercariae in immune serum indicated that the membrane is formed from a thin film of secretory substance which is present on the cuticle when the cercariae emerge from the snail (Kruidenier and Stirewalt, 1955).

In our studies of the CHR antibody we injected rabbits with saline extracts of all stages of the life cycle of *S. douthitti* and were able to observe CHR reactions in all these antisera. Sera from rabbits immunized with another trematode species (*Haematoloechus medioplexus*) were negative for CHR antibody. The CHR antibody could be absorbed with massive concentration of living cercariae. We exposed hamsters, mice, guinea pigs, rats and rabbits to infection with *S. mansoni*. The CHR was detected in our studies as early as 10 days after exposure in the rabbit, 20 days in the hamster, 34 days in the guinea pig, 40 days in the rat and 52 days in the mouse. Sera of monkeys exposed to *S. douthitti* were positive for antibody 15 days after exposure. The CHR reaction was influenced by the severity of infection. Fewer cercariae showed membranes in sera from light than from heavy infections. In one instance the CHR was negative in a monkey shown to be immune to reinfection by challenge with *S. douthitti*. Positive reactions with cercariae of *S. mansoni* and *S. douthitti* were observed in infected human sera (Kagan 1955). We also studied the appearance of CHR in animals injected with a cercarial an-

tigen (Kagan & Levine, 1956). We injected 16 different species and each host developed a CHR antibody titer. CHR titers declined after the end of cercarial immunization. There was no correlation in these hosts between the appearance of the CHR after injection of antigen or after infection with cercariae. The appearance of the CHR in the serum after the start of antigen injection varied considerably from species to species.

The CHR reaction as a diagnostic tool has limitations. The test lacks sensitivity in the diagnosis of human infections (Mayer & Pifano, 1951), may cross-react with bird schistosome cercarial antibodies (Hendricks & Cort, 1956), and necessitates having living cercariae in the laboratory. This reaction must therefore be regarded as one of the less desirable diagnostic methods available to the parasitologist.

From a biological point of view the CHR reaction holds a great deal of interest. I have called it a "modified surface precipitin phenomenon" (Kagan, 1955b). Thought of as a precipitin phenomenon between a living organism and immune serum antibodies one finds parallels with *in vitro* reactions of other helminth species in immune serum (Taliaferro, 1940). This type of precipitin phenomenon is not limited to helminths. Similar *in vitro* manifestations such as immobilization of motile organisms, accumulations of semisolid products about the periphery of individual cells, have been reported for a variety of microorganisms (Harrison, 1955).

Flocculation Tests

Shortly following the publication of a flocculation slide test for trichinosis (Sussenguth and Kline, 1944), this technique was applied in the diagnosis of schistosomiasis (*S. mansoni*) by Brandt and Finch (1946). These workers used as antigen cholesterol particles coated with an alkaline phosphate buffer extract of dried adult worms plus lecithin. The test in their hands had a high degree of sensitivity. Wright, *et al.*, (1947) used a bentonite flocculation test in the diagnosis of S. *japonicum* in military personnel. They were able to report 77.3% of infected patients with their method. Bozicevich and Hoyem (1947) working with treated individuals found the flocculation test unreliable in diagnosis.

The ease and rapidity with which the flocculation test may be performed and read makes this test a very desirable serological procedure to develop.

Agglutination

Schistosome polysaccharide, and other helminth polysaccharide fractions, is able to inhibit the alpha and beta agglutinins of human serums and the Forssman hemolysin of rabbit serum (Oliver-Gonzalez and Torregrosa, 1944). Adult schistosome worms incubated in human serum reduce

the A_2 isoagglutinin titer to zero (Oliver-Gonzalez and Gonzalez, L. M., 1949). Sheep cell agglutinins present in 81.7% of 120 normal human sera are reduced to zero after incubation with living S. *mansoni* worms. The relationship between sheep red cell agglutinogens, the A_2 human blood substance and parasite antigens is not well understood at present. It is thought that perhaps the organism secretes or excretes an A_2 isoagglutinogen-like substance which is antigenic and which absorbs these agglutinins from the serum during active infection (Oliver-Gonzalez, 1952).

We attempted to determine whether Forssman antigen was present in schistosome cercariae. We exposed 3 rabbits which had developed high Forssman titers 6 months earlier as a result of immunization with sheep red cells. After exposure to massive doses of cercariae, complement fixation tests were made with the sera of these rabbits to determine if there was any rise in the Forssman titer (anamnistic effect). These rabbits showed no rise in titer and we concluded that under the conditions of the test no Forssman antigen could be detected.

Cercarial agglutinins were reported in the sera of individuals and animals infected with S. *mansoni* by Liu & Bang (1950). These workers found that living cercariae were agglutinated in the sera from acute human infections and the sera from 4 to 7 chronic infections showed reduced activity.

Standen (1952) reported that normal cattle sera agglutinated cercariae. Stirewalt and Evans (1955) found that agglutination of cercariae was related to the CHR reaction.

Oliver-Gonzalez, *et al.*, (1955a) reported cercarcial agglutinin titers in immunized rabbits injected with lyophylized cercariae and in sera of adult human infections. Following the pattern in the precipitin test, cercarial agglutinin titers of early schistosome cases were higher than in chronic late cases. In infections in mice with worms of one sex, where the circumoval precipitin titer was zero, cercarial agglutinin titers as high as 1:32 were observed.

In our laboratory we studied cercarial agglutination in the sera of normal and immunized animals (Kagan & Levine, 1956). The normal cercaricidal activity of serum masked the agglutinin antibody in all but cow and horse sera. When the cercaricidal factor was destroyed by heat, normal sera from 8 additional species agglutinated cercariae. The sera of 16 animal species agglutinated cercariae after being injected with a cercarial antigen. The number of cercariae that were agglutinated varied from 10-100% in some sera. Agglutinin titers of immune serum varied from 1:32 for the rabbit to 1:256 for the cow. The presence of CHR antibody inhibited the agglutination of cercariae. In sera in which the titer of the CHR antibody was exceeded, agglutination of the cercariae took place.

Hemagglutination

The presence of agglutinins in serum of antigen-injected animals led us to look for a more sensitive method of measuring these antibodies. We therefore investigated the use of tannic acid hemagglutination of sensitized sheep red cells.

The hemagglutination test used was the tannic acid modification introduced by Boyden (1951). Sheep red cells were sensitized with tannic acid and coated with a cercarial antigen (Kagan, 1955*a*). One drop of these coated cells was added to a series of dilutions in 0.5 ml. amounts of antiserum. The test was read from the pattern formed by the cells on the bottom of the tube. In a negative serum, the cells fall to the bottom and form a doughnut-shaped bullseye. In a positive serum, the cells agglutinate and form a thin layer of cells over the bottom of the tube.

One of the first findings in our hemagglutination studies was the differentiation of agglutinins found in normal serum and in serum of infected animals. Normal horse and cow serum (which actively agglutinates cercariae) is negative by hemagglutination. Agglutinins found after immunization will agglutinate sensitized sheep cells (Kagan, 1955*a*).

In our initial study we were also able to demonstrate that agglutinins were formed in rabbits in response to the injection of every stage of the schistosome life cycle. The test was specific for schistosomiasis, since antisera made against frog lung flukes (*Haematoloechus medioplexus*) were negative by hemagglutination.

The hemagglutination test was more sensitive than the CHR test, more sensitive than cercarial agglutination test but less sensitive than a miracidial immobilization test (Senterfit, 1953) in detection of antibody in immune serum.

During the summer of 1955 I was privileged to work in the laboratory of Dr. Oliver-Gonzalez. We studied the hemagglutination test with a variety of antigens. Cells were coated with antigens from cercariae, adults and eggs of *S. mansoni.* In addition, egg antigens of *S. japonicum, S. haematobium* and *Schistosomatium douthitti* were used and a cercarial and adult metabolic product antigen of *S. mansoni.* These antigens were tested against sera of rabbits injected with eggs, cercariae and adults of *S. mansoni,* sera from acute cases of infection in children with *S. mansoni,* and sera of patients before and after treatment. In contrast to the precipitin test, we were not able to demonstrate any marked stage specificity with this method. In 4 of the 5 children's sera tested, the egg antigen was the most sensitive antigen. With egg antigen and adult metabolic product antigen we were able to demonstrate differences in the titer of 4 of 10 sera from patients before and after treatment (Kagan and Oliver-Gonzalez, 1956).

Miracidial Immobilization Test

Senterfit (1953) published on the immobilization of miracidia in immune serum. He studied the reaction in serum of infected hamsters and monkeys. In the sera of infected monkeys, immobilization antibodies appeared 39-48 days after infection with S. *mansoni* and rose to a titer of 1:160 by the 75th day. In general the miracidial immobilization titer paralleled the cercarial agglutination titer for these animals. Monkeys infected with S. *japonicum* for 5½ years had a titer of 1:10.

We observed and studied the miracidial immobilization phenomenon independently of Senterfit's work (Kagan, 1955*b*), and found this test to be very sensitive in detecting antibodies in serum of antigen-injected and infected animals. All stages of the schistosome life cycle when injected into rabbits produced immobilization antibodies. Immobilization titers were observed in sera of monkeys and humans infected with S. *mansoni*. Immobilization titers were stronger with homologous miracidia. In certain immune sera, miracidia were agglutinated at the posterior end of the organism into "rosettes."

Miracidial agglutinins, circumoval precipitins, cercarial agglutinins, and complement fixation antibodies were studied by Lewert and Lee (unpublished). They fractionated a human serum from infection with S. *mansoni* on a starch block (Kunkel, 1954) and recovered 50 fractions. They observed the maximum immunological reaction in the following fractions: Circumoval precipitates, fraction 38 (range 32-42); miracidial agglutination, fractions 38-40 (range 32-50); cercarial agglutination, fractions 40-42 (range, 36-46); complement fixation adult antigen, fractions 42-44 (range 32-50), complement-fixing cercarial antigen, fractions 44-46 (range 32-50). Fractions 35-46 represent the gamma globulin portion of the immune serum. The miracidial agglutinins were by far the most sensitive antibody.

This ionographic approach to the serology of helminthiasis is a most useful tool. Perhaps with this method more sensitive and specific diagnostic antigens will be developed by fractionating complex helminth whole worm or tissue antigens.

Skin Tests

Because of its relative simplicity, ease of application and rapid result, the skin test as a diagnostic epidemiological tool has appealed to workers in endemic areas. A perusal of the literature reveals that the test has had to overcome lack of specificity and standardization of what constitutes a positive test. Pellegrino (unpublished) has reviewed "The Intradermal Test in the Diagnosis of Schistosomiasis" for the African Conference on Schistosomiasis, in Brazzalville, Belgian Congo (1956).

Practically every stage of the life cycle of the schistosome parasite has

been used for antigen: Adult worms (Mayer and Pifano, 1949, Coutinho, 1949, 1952), cercariae (Oliver-Gonzalez and Pratt, 1944, Pratt and Oliver-Gonzalez, 1947), infected snail livers (Fairley and Williams, 1927), miracidia (Sherif, 1956) and eggs (Oliver-Gonzalez, 1954). Various workers have claimed greater sensitivity with alcoholic extracts, saline extracts, polysaccharide fractions (Martins, 1949) or delipidized powders.

According to Pellegrino (unpublished) the following areas have to be developed: (1) Standardization of the cutaneous test on an objective basis, (2) availability of sufficient quantities of antigen for the intradermal test in epidemiological surveys, (3) use of purified antigens, (4) biological standardization of antigens, (5) evaluation of the skin test as a criterion for chemotherapeutic cure. I agree with him fully.

With regard to point (1) it is important for workers in any aspect of immunology and serology to quantitate their antigenic material in terms of nitrogen, protein or other chemical entities. Without chemical quantitation a 1:1000 dilution of an antigen in laboratory A could be equal to a 1:100 dilution in laboratory B. With regard to obtaining large amounts of antigen, there are published techniques for obtaining cercariae in quantity (Standen, 1950, Pellegrino and Macedo, 1955), miracidia (Sherif, 1956), adult worms (Yolles, *et al.*, 1949) and eggs (Coker and Lichtenberg, 1956).

Attempts have been and are being made to purify schistosome antigens. Research in this area must continue and with new techniques available to parasitologists such as serum-agar methods for assay of antigens (Kagan, 1957), ionographic methods for physical separation of antigens, chemical methods for isolation of specific fractions (Schneider, *et al.*, 1956), purified antigens can be made available to the clinician or public health worker in the near future.

Pellegrino, Memoria and Macedo (1957) recently demonstrated that a skin-testing antigen can be biologically assayed and objective criteria for a positive skin test established. They were able to show that when 0.01-0.05 ml of antigen was injected subcutaneously there was a linear relationship between the concentration of the antigen and the mean area of the wheal.

Several reports have appeared regarding the evaluation of chemotherapy and cure by means of the skin test. Oliver-Gonzalez and Pratt (1944) evaluated a cercarial antigen and were able to show that skin sensitization persisted years after cure. Oliver-Gonzalez, Bauman and Bennenson (1954, 1955*a*) reported that in 104 Puerto Rican individuals infected with *S. mansoni*, 35.6% were positive with an egg antigen of *S. mansoni*, whereas 95.2% were positive with a cercarial antigen. Skin tests on patients passing viable eggs were negative with an egg antigen,

whereas they were positive in those patients treated a year earlier. These results were elaborated in further studies in conjunction with the circumoval precipitin test. The negative skin tests in a treated patient became positive approximately 183 days after treatment. As a result of the treatment, the circumoval precipitin test becomes negative. A negative circumoval test and a positive skin test following treatment appears to be indicative of the termination of infection (Oliver-Gonzalez, Romas and Coker, 1955). Sherif (1956), using a miracidial antigen, reported that the skin test became negative in patients 3-6 months after cure.

The presence of metabolic antigens in the egg antigen probably accounts for the differences between the response of the miracidium and the egg antigen in the skin test. Sherif utilized the hatched and actively swimming miracidium as his source of antigen and did not observe a negative skin test during active infection. The shell of the egg in the egg antigen is probably non-specific and may mask or block the antigenic stimulation of the miracidium in the enclosed shell, which may explain why the miracidium in the egg antigen does not cause a positive test to develop. The fluid within the egg, however, can escape as evidenced by the formation of precipitates in the circumoval precipitin test, and acts as the antigenic stimulus in the skin test. The fluid bathing the miracidium is probably rich in secretory and excretory miracidial metabolic products. During active infection there may be an excess of antigen in the serum of the host which absorbs and binds the antibody available to the egg antigen. The skin test is therefore negative in the patient with an active infection. This phenomenon (negative test in the face of active infection) was also reported by Lurie, *et al.*, (1953) in skin tests with cercariae with children infected with S. *haematobium*. Approximately 6 months after cure the skin test with the egg antigen becomes positive. As a clinical criterion for cure this interval of time is too long. Perhaps if greater concentrations of metabolic antigens from adult and larval worms could be utilized in the skin test a shorter time interval between death of the worms and a positive skin test would result. The sero-evaluation of chemotherapeutic cure of helminth disease offers a challenging area in the serology of parasitic disease.

Prausnitz-Küstner Reaction

The P-K test (Prausnitz-Küstner) is based on the passive transfer of sensitivity to the skin of a normal individual by subcutaneous injection of serum containing antibody. Twenty-four hours later the site is injected with antigen and a wheal and erythema indicates a positive test. This is the standard test for atopic or reagin antibody of allergies. Taliaferro and Taliaferro (1931) were the first to demonstrate this reaction in schisto-

somiasis with sera of infected individuals. Pellegrino (unpublished) was able to demonstrate the P-K phenomenon in his patient's serum. Guerra, Mayer and Di Prisco (1945) demonstrated that the reaction was specific for schistosomiasis, since *Fasciola hepatica* antigen did not elicit a positive response on passive transfer.

Ritchken and Gelfand (1954) and Ritchken (1955) called attention to the allergic condition associated with infection 4 to 10 weeks after exposure to schistosomiasis. They believe that "River-Fever" or "Katayma Syndrome" associated with urticaria, fever and eosinophilia are the allergic manifestations of schistosomiasis.

Attempts to neutralize the antibody in the serum by absorption with antigen prior to injection into the skin of a donor were unsuccessful (Taliaferro and Taliaferro, 1931; Pellegrino, unpublished). These tests indicate that perhaps a true atopic or univalent antibody was being demonstrated in schistosomiasis. Coker and Oliver-Gonzalez (1956) were able to passively transfer immune serum to the skin of normal individuals and observe a positive skin test at the site of injection 24 hours later with egg antigen. In the skin of infected individuals the passive transfer was negative.

It is not clear from the preliminary report by Coker and Oliver-Gonzalez (1956) whether the serum was absorbed prior to passive transfer into the skin of the normal or infected individual. If a true atopic antibody is neutralized in the infected individual this reaction should be investigated further to determine how soon after cure the negative test becomes positive. This reaction may prove to be more sensitive than the immediate skin test in the evaluation of the curative effects of therapy.

Discussion

The host-parasite relationship of *Schistosomatium douthitti* (and other schistosome species) is complex and the possibility that more than one immunological mechanism may be operative in a single host or in several different hosts should not be ruled out. In the monkey infected with *S. douthitti,* the death of the worms in massive infection may indicate an antigen-antibody response or perhaps a metabolic or enzymatic impasse. In the rat the infection is eliminated by what appears to be an active cellular response. Perhaps there is an antigen-antibody basis for this resistance, or perhaps a non-specific response such as an increase in properdin levels in the serum of the host. In the mouse, a light infection will persist for the life time of the host, and whether the limited protection that an initial infection renders the host is dependent on an antigen-antibody basis or on an enhancement of non-antibody mechanisms remains to be experimentally demonstrated. Thompson (1954) believed that ac-

quired immunity against *Schistosoma mansoni* in experimental hosts was mediated through an enhancement of innate immunity. Stirewalt (1953) demonstrated the penetration of *S. mansoni* cercariae in mice within 24 hours after initial exposure was impeded by a non-specific mechanism in the host. Lindquist (1950) reported that the larvae of *Nippostrongylus muris,* the species with which Taliaferro and Sarles conclusively demonstrated an antigen-antibody basis for immunity in the rat (Taliaferro, 1943), is encapsulated and killed in the skin of cotton rats in the apparent absence of antibodies.

I personally believe that the immunology of schistosomiasis has an antigen-antibody basis. However, until this point is unequivocally proven by passive transfer experiments I believe that one should not rule out other kinds of immunological mechanisms that are not based on antibodies. The immunity to *Bacillus anthracis,* the causative agent of anthrax, is pertinent in this regard. In the mouse, acquired immunity to anthrax is dependent on the stimulation by capsular antigens and follows classical antigen-antibody priciples established for pneumococcus and other organisms. In the sheep, rabbit and guinea pig, in spite of the fact that acquired immunity can be so dramatically demonstrated with heat-attenuated organisms (which is fortunate since Pasteur used this organism in his dramatic demonstration of acquired immunity in sheep at Pouilly-le-Fort in 1881), the mechanism of this immunity is still not clearly elucidated but appears to be independent of capsular antigens and may be of a non-antibody type (Raffel, 1953).

The different response of hosts infected with schistosomes may have important bearing on our understanding of the immunology of schistosomiasis in man. From preliminary reports, monkeys infected with varying numbers of *S. mansoni* cercariae appear to respond differently after exposure (Naimark, *et al.,* 1957*a*). Heavily infected animals become refractive to reinfection whereas lightly infected animals do not. In areas such as Puerto Rico, where the incidence of schistosomiasis is about 10% (White, *et al.,* 1957) and from an epidemiological point of view is an area of light infection, the infection appears to be of long duration in man. This means that unless the infected individuals who come to the United States from Puerto Rico are treated, they may remain infected for life. From a public health point of view this is an important consideration and studies should be initiated in our larger cities where there are concentrations of Puerto Rican citizens to resolve this point.

From the information derived from animal experimentation, I believe we can make some hypotheses concerning the effect of treatment on immunity and resistance in man. Schwetz (1956) believed that for humans infected with schistosomes, a state of "premunition" exists. This type of

immunity necessitates the presence of an active infection to maintain and stimulate immunity. If this be true, then a clinical cure by chemotherapy would in time deprive the individual of his immunity. Schwetz therefore infers that treatment should be initiated to alleviate the patient's chronic symptoms but not to effect a complete cure. Our studies on the effect of chemotherapy on duration of immunity in mice (Kagan and Lee, 1952) supports the above hypothesis; however, studies in monkeys (Vogel and Minning, 1953, Kagan, 1953, Meleney and Moore, 1954) have shown that animals cured by chemotherapy or by "self-cure" are immune to reinfection years after the termination of the initial infection. I suspect that the immune response in man is similar to the mechanism of immunity in the monkey and differs from the immune response in the mouse. Therefore a clinical cure in man may not strip him of any acquired immunity against reinfection with schistosomes.

The immunodiagnosis of schistosomiasis is at a very formative stage. Many serological tests have been developed and the antigenicity of practically every stage in the life cycle of the parasite has been probed. Serological tests still fall short in detection of early infection (Naimark, *et al.*, 1957*b*) and early chemotherapeutic cure. Perhaps we will find the answers in refined techniques and extraction of more potent somatic antigens from the various life cycle stages of the parasite. This avenue of research must be pursued; but, I believe that much more emphasis must be directed toward collecting metabolic antigens and evaluating their efficacy in the immunology and serology of schistosomiasis.

As Newsome (1956) outlined in his recent review, there are many questions to be resolved in the immunology of schistosomiasis. With the application of modern research methods and ideas these problems will be solved in the near future.

Summary

Contributions made by the author and his co-workers on the immunology and serology of *Schistosomatium douthitti* and *Schistosoma mansoni,* have been discussed. In addition, the contributions of other workers have been reviewed. The discussion of immunology has been divided into innate immunity, natural acquired immunity, artificially acquired immunity, natural passive transfer of immunity and artificial transfer of immunity. The discussion on the serology has been subdivided into the following divisions: complement-fixation test, precipitins, circumoval precipitin test, CHR (cercarianhüllen reaction of Vogel and Minning), flocculation tests, agglutination, hemagglutination, miracidial immobilization test, skin tests and Prausnitz-Küstner reaction.

ACKNOWLEGEMENTS

The investigations of the author and his co-workers on schistosomiasis were made possible in part by a National Research Council Postdoctoral Fellowship sponsored by Dr. William H. Taliaferro, Chairman, Department of Microbiology, University of Chicago, during the years 1950-1952. I wish to thank Dr. Taliaferro for his encouragement and sustained interest in my research. From 1952-1957, when the author was at the University of Pennsylvania, research was supported by a grant from the National Institutes of Health, Division of Allergy and Infectious Diseases, Public Health Service.

REFERENCES

Berberian, D. A., Paquin, H. O., Jr., Fantauzzi, A. 1953. Longevity of *Schistosoma haematobium* and *Schistosoma mansoni:* Observations based on a case. *J. Parasitol.* **39,** 517-519.

Bouillon, A. 1950. Bibliographie des schistosomes et des schistosomiasis (Bilharzioses) humaines et animales de 1931 a 1949. *Mem. Inst. Royal Colonial Belge* **27,** 1-141.

Boyd, W. C. 1956. *Fundamentals of Immunology.* 3rd ed. 776 pp. Interscience Pub.

Boyden, S. V. 1951. The adsorption of proteins on erythrocytes treated with tannic acid and subsequent hemagglutination by antiprotein sera. *J. Exp. Med.* **93,** 107-120.

Bozicevich, J., & Hoyem, H. M. 1947. Intradermal and serological tests in patients with schistosomiasis japonica. *Nat. Inst. Health Bull.* **189,** 199-212.

Brandt, J. L., & Finch, E. P. 1946. A simple flocculation slide test for the diagnosis of schistosomiasis. *Am. J. Clin. Path.* **16,** 141-152.

Büttner, A. 1953. Sur l'aptitude de certains rongeurs sauvages a devenir reservoirs de virus de la bilharziose intestinale humaine (Note preliminaire). *Bull. Soc. Path. Exot.* **46,** 688-692.

Cardoso, W. 1953. A esquitosomiase mansonica no negro. *Medicina, Cirurgia Farmacia,* Rio de Janeiro, **202,** 89-95.

Chaffee, E. F., Bauman, P. M., and Shapila, J. J. 1954. Diagnosis of schistosomiasis by complement-fixation. *Am. J. Trop. Med. & Hyg.* **3,** 905-913.

Chandler, A. C. 1953. The relation of nutrition to parasitism. *J. Egyptian Med. Assoc.* **36,** 533-552.

Cohn, M. 1953. Production of antibodies in experimental animals. *Methods in Med. Res.* **5,** 271-283.

Coker, C. M. 1957. Effect of cortisone on natural immunity to *Schistosoma mansoni* in mice. *Proc. Soc. Exp. Biol. & Med.* **96,** 1-3.

Coker, C. M., & Lichtenberg, F. 1956. A revised method for isolation of *Schistosoma mansoni* eggs for biological experimentation. *Proc. Soc. Exp. Biol. & Med.* **92,** 780-782.

Coker, C. M., & Oliver-Gonzalez, J. 1956. Studies on immunity to schistosomiasis—passive transfer of anti-egg antibody in humans. *Am. J. Trop. Med. & Hyg.* **5,** 385.

Coutinho, J. O. 1949. Diagnostico da esquistossomose pela intradermoreacao com antigenos de esquistosomas adultos. *Rev. Clin. Sao Paulo* **25,** 7-15.

Coutinho, J. O. 1952. Nova tecnica de preparo de antigeno de vermes adultos para a intrademo—reacao na esquistossomose. *Folia Clinica et Biologica* **18,** 121-124.

Cram, E. B., and Files, V. S. 1947. Experimental mammalian infection with the schistosomes of man. 1. Laboratory animals as a source of supply of adult schistosomes and their ova. *Nat. Inst. Health Bull.* **189,** 101-105.

Culbertson, J. T. 1936. The cercaricidal action of normal serums. *J. Parasitol.* **22,** 111-125.

Culbertson, J. T., and Talbot, S. B. 1935. A new antagonistic property of normal serums: the cercaricidal action. *Science* **82**, 525-526.

Davies, A. M., and Eliakim, M. 1954. The value of different antigens in the diagnosis of chronic bilharziasis by the skin and complement fixation tests. *Am. J. Trop. Med. & Hyg.* **3**, 728-741.

Eliakim, M., & Davies, A. M. 1954. The complement-fixation test in bilharziasis. 1. The value of different extracts of *Schistosoma mansoni* and *Fasciola hepatica* worms as antigens. *Parasitology* **44**, 407-413.

Evans, A. S., Stirewalt, M. A., & MacKenzie, M. 1955. Serological reactions in *Schistosoma mansoni* infections II. Cercarial behavior in electrophoretically separated fractions of sera of infected and uninfected mice. *Exp. Parasitol.* **4**, 419-426.

Fairley, N. H. 1919. The discovery of a specific complement fixation test for bilharziasis and its practical application to clinical medicine. *J. Roy. Army Med. Corps* **32**, 449-460.

Fairley, N. H. 1925. Complement-fixation with bilharzia. Part I. The lipoidal nature of cercarial antigen as used in the complement-fixation test for mammalian bilharziasis. *J. Pathol. & Bacteriol.* **28**, 591-607.

Fairley, N. H. 1926. Studies in the chemotherapy and immunity reaction of schistosomiasis (*Schistosoma spindalis* and *S. haematobium*). *Trans. Roy. Soc. Trop. Med.* **20**, 236-267.

Fairley, N. H. 1927. The early spontaneous cure of bilharziosis (*S. spindalis*) in monkeys (*Macacus sinicus*) and its bearing on species immunity. *Indian J. Med. Res.* **14**, 685-700.

Fairley, N. H. 1951. Schistosomiasis and some of its problems. *Trans. Roy. Soc. Trop. med. & Hyg.* **45**, 279-306.

Fairley, N. H., & Williams, F. E. 1927. A preliminary report on an intradermal reaction on schistosomiasis. *Med. J. Australia* **2**, 811-818.

Fairley, N. H., Mackie, F. P., and Jasudasan, F. 1930. Further observations on spontaneous course in *Macacus sinicus*. *Ind. Med. Res. Mem.* **17**, 53-59.

Ferguson, F. E., Goodwin, M. H., Jr., Gerhardt, C. E., Klock, J. W., and Williams, E. R. Research on *Schistosomiasis mansoni* at the Puerto Rico field station of the United States Public Health Service. *Proc. Carib. San Eng. Conf.* (Nov. 1955), *Pan. Am. San. Bur.*, published by Puerto Rican Comm. Dept. Health, San Juan. In press.

Fisher, A. C. 1934. A study of the schistosomiasis of the Stanleyville district of the Belgian Congo. *Trans. Roy. Soc. Trop. Med. & Hyg.* **28**, 277-306.

Gelfand, M. 1950. *Schistosomiasis in South Central Africa—A clinical-pathological study.* 239 pp. Post-Graduate Press, Juta & Co. Ltd.

Girges, R. 1934. *Schistosomiasis (Bilharziasis).* John Bale, Sons and Danielsson, Ltd. London. 527 pp.

Gönnert, R. 1955. Schistosomiasis-Studien. II. Über die Eibildung bei *Schistosoma mansoni* und das Schicksal der Eier im Wirtsorganizmus. *Ztschr. Tropenmed. u. Parasitol.* **6**, 33-52.

Gönnert, R., and Vogel, H. 1955. Über die Abhängigkeit des Therapieerfolges von Wirts—und Parasitenstamm bei der experimentellen Schistosomiasis. *Ztschr. Tropenmed. u. Parasitol.* **6**, 193-198.

Guerra, P., Mayer, M., & Di Prisco, J. 1945. La especificidad de la intradermorreacciones con antigenos de *Schistosoma mansoni* y *Fasciola hepatica* por el metado de Prausnitz-Kuestner. *Rev. Sanid. y Asist. Soc.* **10**, 51-63.

Harrison, J. A. 1955. General aspects of immunological reactions with bacteria and protozoa. In *Biological Specificity and Growth*, Chap. 7, 141-156. Princeton Univ. Press.

Hendricks, J. R., & Cort, W. W. 1956. A study of the *in vitro* actions of antiserums on the cercariae of certain bird schistosomes. *J. Parasitol.* **42**, 557-564.

Horstman, H. A., Jr., Chaffee, E. F., and Bauman, P. M. 1954. Schistosomiasis mansoni in Puerto Rican soldiers. *Am. J. Trop. Med. & Hyg.* **3**, 914-917.

Hsü, H. F., and Li, S. Y. [in press]. Studies on *Schistosomiasis japonica* in Formosa IV. Wild rats and other small mammals as reservoir hosts of *Schistosoma japonicum* in Formosa. *Proc. 8th Pacific Sci. Congr.* Quezon City, Nov. 1953. In press.

Hunter, G. W., III, Ritchie, L. S., Lin, S., Pan, C., and Tanake, H. 1956. Immunological Studies. I. Experiments with bird and human schistosomes in small mammals. *Exp. Parasitol.* **5**, 551-559.

Iralu, V. Personal communication.

Kagan, I. G. 1952. Acquired immunity in mice infected with *Schistosomatium douthitti*. *J. Inf. Dis.* **91**, 147-158.

Kagan, I. G. 1953. Experimental infections of rhesus monkeys with *Schistosomatium douthitti* (Cort, 1914). *J. Inf. Dis.* **93**, 200-206.

Kagan, I. G. 1955*a*. Hemagglutination after immunization with schistosome antigens. *Science* **122**(3165), 376-377.

Kagan, I. G. 1955*b*. Studies on the serology of schistosomiasis. I. The *in vitro* activity of cercariae and miracidia in serum of experimental natural and immunized hosts. *Exp. Parasitol.* **4**, 361-376.

Kagan, I. G. 1957. Serum-agar double diffusion studies with ascaris antigens. *J. Inf. Dis.* **101**, 11-19.

Kagan, I. G., and Lee, C. L. 1952. Chemotherapy of experimental infections of mice with *Schistosomatium douthitti, Schistosoma mansoni* and *Schistosoma japonicum*. *J. Inf. Dis.* **91**, 224-230.

Kagan, I. G., and Lee, C. L. 1953. Duration of acquired immunity of *Schistosomatium douthitti* infections in mice following treatment. *J. Inf. Dis.* **92**, 52-57.

Kagan, I. G., & Levine, D. M. 1956. Studies on the serology of schistosomiasis. II. The *in vitro* activity of cercariae of *Schistosoma mansoni* in sera of normal and antigen-injected animals. *Exp. Parasitol.* **5**, 48-58.

Kagan, I. G., and Meranze, D. R. 1957. The histopathology of the liver in mice experimentally infected with *Schistosomatium douthitti*. *J. Inf. Dis.* **100**, 32-39.

Kagan, I. G., & Oliver-Gonzalez, J. 1955. Preliminary studies on hemagglutination in schistosomiasis. *J. Parasitol.* **41**(Suppl), 26.

Kagan, I. G., Short, R. B., & Nez, M. M. 1954. Maintenance of *Schistosomatium douthitti* (Cort, 1914) in the laboratory (Trematoda: Schistosomatidae). *J. Parasitol.* **40**, 1-16.

Kawamura, R. 1932. The recent researches on schistosomiasis in Japan. *Compt Rend. Cong. Internat. Med. Trop. & Hyg.* (Cairo, Dec. 1928). **4**, 311-319.

Khalil, M. B. 1931. The bibliography of schistosomiasis (bilharziasis), zoological, clinical and prophylactic. *Publ. Fac. Med.* Egyptian Univ. 506 pp.

Kruidenier, F. V., and Stirewalt, M. A. 1955. The structure and source of the pericercarial envelope (CHR) of *Schistosoma mansoni*. *J. Parasitol.* **41**(Suppl.), 22-23.

Kunkel, H. G. 1954. *Methods of biochemical analysis.* **1**, 141-170. Interscience Pub. Inc.

Kuntz, R. E. 1955. Biology of the schistosome complexes. *Am. J. Trop. Med. & Hyg.* **4**, 383-413.

Kuntz, R. E., & Malakatis, G. M., and Wells, W. H. 1955. Susceptibility studies in schistosomiasis. II. Susceptibility of wild mammals to infection by *Schistosoma mansoni* in Egypt with emphasis on rodents. *Am. J. Trop Med. & Hyg.* **4**, 75-89.

LaRue, G. R. 1957. The classification of digenetic Trematoda: A review and a new system, Exp. *Parasitol.* **6**, 306-344.

LeBas, G. Z. L. 1922. On the nature of the antigen in the complement fixation test for bilharziasis. *J. Trop. Med. & Hyg.* **25**, 49-55.

LeGrange, E. 1955. Considerations sur la pathologie de la bilharziose a *Schistosoma mansoni*. *Ann. Soc. Belge de Med. Trop.* **6**, 719-724.

Levine, D. M. Personal communication.

Lewert, R. M., and Lee, C. L. Distribution of various reactants in human anti-*Schistosoma mansoni* serum fractionated by starch electrophoresis. Manuscript in preparation.

Lewert, R. M., Lee, C. L., & Jackson, G. Unpublished.

Lin, S., Ritchie, L. S., & Hunter, G. W., III. 1954. Acquired immunologic resistance against *Schistosoma japonicum. J. Parasitol.* **40**(Suppl), 42.

Lindquist, W. D. 1950. Some abnormal host relationships of a rat nematode, *Nippostrongylus muris. Am. J. Hyg.* **52**, 22-41.

Liu, C., and Bang, F. B. 1950. Agglutination of cercariae of *Schistosoma mansoni* by immune sera. *Proc. Soc. Exp. Biol. & Med.* **74**, 68-72.

Lurie, H. I., deMeillon, B., Staffberg, N. 1952. Experimental bilharziasis in animals. I. Early diagnosis of bilharziasis. *S. Afr. Med. J.* **26**(56), 1005-1008.

Lurie, H. I., deMeillon, B., Staffberg, N., and Eiselen, H. H. 1953. Negative cercarial antigen skin tests in proven urinary bilharziasis in children. *S. Afr. Med. J.* **27**, 295-296.

Lurie, H. I., and deMeillon, B. 1957. Experimental bilharziasis in laboratory animals. V. Immunity in mice produced by repeated small infections. *S. Afr. Med. J.* **31**(4), 68-69.

Magath, T. B., and Mathieson, D. R. 1945. Infection of wild rats in Leyte with *Schistosoma japonicum. U. S. Naval Med. Bull.* **45**, 1195-1202.

Martins, A. V. 1949. Diagnostico de laboratorio da *Esquistosomose mansoni.* Thesis. Fac. Med. Univ. Minas. Geräis. 265 pp.

Martins, A. V., Martins, G., & de Brito, R. S. 1955. Reservatorios silvestre do *Schistosoma mansoni* no etado de Minas Geräis. *Rev. Brasileira Malariologia* **7**, 259-265.

Mayer, M., and Pifano, F. 1945. La reaccion de desviacion del complemento, segun Fairley, en la *Schistosomiasis mansoni;* estudio fundamentado en 1932 reacciones. *Rev. Sanid. y Asist. Soc.* **10**(1), 65-93 .

Mayer, M. y Pifano, F. 1949. Experiencias de ocho enos en la elaboracion y aplicacion de un antigeno de vermes adultos de *Schistosoma mansoni* para la intradermoreaccion diagnostica de la bilharziosis. *Arch. Venezolanos de Pathol. Trop. & Paras. Med.* **1**(2), 1-35.

Mayer, M., and Pifano, F. 1951. La cercaria-reaccion de Vogel-Minning en la *Schistosomiasis mansoni. Rev. Sanid. y Asist. Soc.* **16**, 461-474.

Meleney, H. E., und Moore, D. V. 1954. Observations on immunity to superinfection with *Schistosoma mansoni* and *S. haematobium* in monkeys. *Exp. Parasitol.* **3**, 128-139.

Miyaji, S., and Imai, B. 1928. Serologischen Studien bei *Schistosomiasis japonica. Centralbl. f. Bacteriol.* **106**, 237-246.

Moore, D. V., Yolles, T. K., and Meleney, H. E. 1949. A comparison of common laboratory animals as experimental hosts for *Schistosoma mansoni. J. Parasitol.* **35**, 156-170.

Naimark, D. H., Benenson, A. S., Oliver-Gonzalez, J., and Ritchie, L. 1957*a*. Studies on *Schistosomiasis mansoni* in primates. Observations on acquired resistance. Paper before Am. Soc. Trop. Med. & Hyg. Philadelphia, Oct. 1957.

Naimark, D. H., Oliver-Gonzalez, J., Chaffee, E. F., and Anderson, R. I. 1957*b*. Studies on *Schistosomiasis mansoni* in primates (1) Initial occurrence of serological antibodies correlated with egg recovery. *J. Parasitol.* 43(Suppl), 26.

Newsome, J. 1956. Problems of fluke immunity with special reference to schistosomiasis. *Trans. Roy. Soc. Trop. Med. & Hyg.* **50**, 258-274.

Newsome, J., and Robinson, D. L. H. 1954. Investigation of methods of maintaining *Schistosoma mansoni* in vitro. *Ann. Trop. Med. & Parasitol.* **48**, 194-200.

Newsome, J. and Robinson, D. L. H. 1956. Reactions of *Schistosoma mansoni* eggs and schistosomulae to immune serum. *Trans. Roy. Soc. Trop. Med. & Hyg.* (Demonstration) **50**, 310-11.

Okabe, K., Koga, Y., Shibue, H., and Matsuse, M. 1954. Immunological studies on schistosomiasis japonica. 3. Intradermal and precipitin tests for *Schistosoma japonica. Kurume Med. J.* **1**, 85-89.

Olivier, L. 1953. Observations on the migration of avian schistosomes in mammals previously unexposed to cercariae. *J. Parasit.* **39**, 237-243.

Olivier, L. and M. Schneidermann. 1953. Acquired resistance to *Schistosoma mansoni* infection in laboratory animals. *Am. J. Trop. Med. & Hyg.* **2**, 298-306.

Oliver-Gonzalez, J. 1952. Agglutinins for sheep cells in human serums and their relationship to the A_2 isoagglutinogen-like substance in infectious organism. *J. Inf. Dis.* **90**, 44-47.

Oliver-Gonzalez, J. 1954. Anti-egg percipitins in the serum of humans infected with *Schistosoma mansoni. J. Inf. Dis.* **95**, 86-91.

Oliver-Gonzalez, J., and Torregrosa, M. V. 1944. A substance in animal parasites related to the human isoagglutinogens. *J. Inf. Dis.* **74**, 173-177.

Oliver-Gonzalez, J., and Gonzalez, L. M. 1949. Release of the A_2 isoagglutinogen-like substance of infectious organisms into human blood serum. *J. Inf. Dis.* **85**, 66-71.

Oliver-Gonzalez, J., Bauman, P. M., and Benenson, A. S. 1954. Intradermal response to egg antigen in humans with active and treated (Fuadin) *Schistosoma mansoni* infections. *Proc. Soc. Exp. Biol. & Med.* **87**, 186-188.

Oliver-Gonzalez, J., Bauman, P. M., and Benenson, A. S. 1955*a*. Immunological aspects of infections with *Schistosoma mansoni. Am. J. Trop. Med. & Hyg.* **4**, 443-452.

Oliver-Gonzalez, J., Bauman, P. M., and Benenson, A. S. 1955*b*. Species specificity of the anti-egg precipitin in schistosome serums. *J. Inf. Dis.* **96**, 95-100.

Oliver-Gonzalez, J., Ramos, F. L., and Coker, C. M. 1955. Serological reactions against egg antigens as an aid in the evaluation of therapy in schistosomiasis. *Am. J. Trop. Med. & Hyg.* **4**, 908-912.

Oliver-Gonzalez, J., Pratt, C. K. 1944. Skin and precipitin reactions to antigens from cercariae and adults of *Schistosoma mansoni. Puerto Rico J. Publ. Health & Trop. Med.* **20**, 242-248.

Ozawa, M. 1930. Experimental studies on acquired immunity to schistosomiasis japonica. *Jap. J. Exp. Med.* **8**, 79-84.

Papirmeister, B., and Bang, F. B. 1948. The *in vitro* action of immune sera on cercariae of *Schistosoma mansoni. Am. J. Hyg.* **48**, 74-80.

Pellegrino, J. The intradermal test in the diagnosis of schistosomiasis. (Unpublished).

Pellegrino, J., and Macedo, D. G. 1955. A simplified method for the concentration of cercariae. *J. Parasitol.* **41**, 329-330.

Pellegrino, J., and Memoria, J. M. P. and Macedo, D. G. 1957. Quantitative aspects of the intradermal test with cercarial antigen in schistosomiasis. *J. Parasitol.* **43**, 304-307.

Pesigan, T. P., Pangilinan, M. V., Saniel, V. G., Garcia, E. G., Borzon, T. C., and Putong, P. B. 1951. Field studies on the treatment of schistosomiasis japonica with Nilodin. *J. Philip. Med. Assoc.* **27**, 242-247.

Pifano, F. 1953. La Resistencia del Huesped Vertebrado a les re-infecciones por el *Schistosoma mansoni. Rev. Sanid. y Assist. Soc.* **18**, 767-781.

Pifano, F., and Ron Pedrique, M. 1957. La cercaria–reaccion de Vogel y Minning en el diagnostico de la schistosomiasis mansoni. *Ztsch. Tropenmed. u. Parasitol.* **8**, 203-207.

Pillemer, L. 1954. Properdin system and immunity I. Demonstration and isolation of new serum protein, properdin, and its role in immune phenomena. *Science* **120**, 279-285.

Pratt, C. K., and Oliver-Gonzalez, J. 1947. Intradermal reactions to fresh and stored antigens prepared from cercariae. *Puerto Rico J. Publ. Health & Trop. Med.* **22**, 254-256.

Price, H. F. 1931. Life history of *Schistosomatium douthitti* (Cort). *Am. J. Hyg.* **13**, 685-727.

Raffel, S. 1953. *Immunity, Hypersensitivity, Serology.* Appleton-Century-Crofts, Inc. 531 pp.

Ritchen, J. 1955. The fevers of Africa. 1. Bilharzial fever. *Cent. Afr. J. Med.* **1**, 37-42.

Ritchen, J., and Gelfand, M. 1954. Katayama disease, early toxemic stage of bilharziasis with a report of a case showing pulmonary infiltration. *Brit. Med. J.* **1**(4876), 1419-1420.

Robinson, D. L. H. 1956*a*. A routine method for the maintenance of *Schistosoma mansoni in vitro*. *J. Helminthol.* **29**, 193-202.

Robinson, D. L. H. 1956*b*. The behavior of young and adult *Schistosoma mansoni* in immune serum. *Trans. Roy. Soc. Trop. Med. & Hyg.* (correspondence) **50**, 105-106.

Schwetz, J. 1955. Sur L'infection naturelle des rats domestiques (*Rattus rattus*) por *Schistosoma mansoni* en Afrique centrale. *Bull. Soc. Path. Exot.* **48**, 182-185.

Schwetz, J. 1956. Sur l'immunité clinique dans les bilharziases humaines. *Bull. Soc. Path. Exot.* **49**, 52-56.

Schneider, M. D., Radke, M. G., and Coleman, M. T. 1956. Immunologically reactive substance from *Schistosoma mansoni*. Exp. *Parasitol.* **4**, 391-397.

Schneider, M. D., Radke, M. G. 1957. Further observations on a complement fixing substance from *Schistosoma mansoni*. *Proc. Med. Soc. Wash.* **24**, 137-139.

Senterfit, L. B. 1953. Immobilization of *Schistosoma mansoni* miracidia by immune serum. *Soc. Exp. Biol. & Med.* **84**, 5-7.

Sherif, A. F. 1956. A new intradermal antigen for the diagnosis of schistosomiasis. *Ann. Trop. Med. & Parasitol.* **50**, 105-112.

Short, R. B. 1952. Sex studies on *Schistosomatium douthitti* (Cort, 1914) Price, 1931 (Trematoda: Schistosomatidae). *Am. Midland Nat.* **47**, 1-54.

Skrjabin, K. I. 1951. Trematodes of animals and man. *Principles of Trematodology*. Vol. 5, Moskow & Leningrad. Izdatelstov. Akademi Nauck SSSR, 624 pp. (In Russian).

Smith, D. A. 1955. Parasitic infections and nutrition. *Vitamins & Hormones*, **13**, 229.

Standen, O. D. 1950. The concentration of cercariae of *Schistosoma mansoni* for the preparation of cercarial antigen. *Trans. Roy. Soc. Trop. Med. & Hyg.* **43**, 527-530.

Standen, O. D. 1952. The *in vitro* effect of normal and immune serum upon the cercariae of *Schistosoma mansoni*. *J. Helminthol.* **26**, 25-42.

Stirewalt, M. A. 1953. The influence of previous infection of mice with *Schistosoma mansoni* on a challenging infection with the homologous parasite. *Am. J. Trop. Med. & Hyg.* **2**, 867-882.

Stirewalt, M. A., and Evans, A. S. 1953. An unsuccessful attempt to protect mice against *Schistosoma mansoni* by transfer of immune rat serum. *Proc. Helm. Soc. Wash.* **20**, 15-19.

Stirewalt, M. A., and Evans, A. S. 1955. Serologic reactions in *Schistosoma mansoni* infections. 1. Cercaricidal, precipitation, agglutination and CHR phenomena. *Exp. Parasitol.* **4**, 123-142.

Stirewalt, M. A., Kuntz, R. E., and Evans, A. S. 1951. The relative susceptibilities of the common-used laboratory mammals to infection by *Schistosoma mansoni*. *Am. J. Trop. Med.* **31**, 57-82.

Sussenguth, H., and Kline, B. S. 1944. A simple rapid flocculation slide test for trichinosis in man and in swine. *Am. J. Clin. Path.* **14**, 471-484.

Taliaferro, W. H. 1929. *The Immunology of Parasite Infections*. John Bale, Sons and Danielsson, Ltd., London.

Taliaferro, W. H. 1940. The mechanism of acquired immunity in infections with parasitic worms. *Physiolog. Rev.* **20**, 469-492.

Taliaferro, W. H. 1943. Antigen-antibody reactions in immunity to metazoan parasites. *Proc. Inst. Med. Chicago* **14**, 358-368.

Taliferro, W. H., Hoffman, W. A., and Cook, D. H. 1928. A precipitin test in intestinal schistosomiasis (*S. mansoni*). *J. Prev. Med.* **2**, 395-414.

Taliaferro, W. H., & Taliaferro, L. G. 1931. Skin reaction in persons infected with *Schistosoma mansoni*. *Puerto Rico J. Publ. Health & Trop. Med.* **7**, 23-35.

Thillet, C. D., and Chandler, A. C. 1957. Immunization against *Trypanosoma lewisi* in rats by injections of metabolic products. *Science* **125**, 346-347.

Thompson, J. H. 1954. Host-parasite relationships of *Schistosoma mansoni*. *Exp. Parasitol.* 3, 140-160.

Thorson, R. E. 1953. Studies on the mechanism of immunity in the rat to the nematode. *Nippostrongylus muris*. *Am. J. Hyg.* 58, 1-15.

Thorson, R. E. 1954. Absorption of protective antibodies from serum of rats immune to the nematode *Nippostrongylus muris*. *J. Parasitol.* 40, 1-4.

Tubangui, M., and Masilungen, A. 1936. Studies on the cercaricidal property of the sera of vertebrate animals. *Philip. J. Sci.* 60, 393-398.

Vogel, H. 1948. Fiat review of German science 1939-1946. *Trop. Med. & Parasitol.* Editor, E. G. Nauck.

Vogel, H., and Minning, W. 1947. Über die Einwirkung von Brechweinstein, Fuadin und Emetin auf *Bilharzia japonica* und deren Eier in Kaninchenversuch. *Acta Tropica* 4, 21-56, 97-116.

Vogel, H., and Minning, W. 1949*a*. Hüllenbildung bei Bilharzia-Cercarien im Serum Bilharzia-infizierter Tiere und Menschen. *Centralbl. f. Bakteriol.* 153, 91-105.

Vogel, H., and Minning, W. 1949*b*. Weitere Beobachtungen über die Cercarienhüllenreaktion, eine Seroprezipitation mit lebenden Bilharziacercarien. *Zeitsch. f. Tropenmed. u. Parasitol.* I, 378-386.

Vogel, H., and Minning, W. 1953. Über die erworbene resistenz von *Macacus rhesus* gegenuber *Schistosoma japonicum*. *Ztschr. Tropenmed. u. Parasitol.* 4, 418-505.

Watts, N. P. 1949. Prophylactic use of schistosomal antigen. *J. Immunol.* 62, 183-192.

White, P. C., Jr., Pimentel, O.. Garcia, F. C. 1957. Distribution and prevalence of human schistosomiasis in Puerto Rico in 1953. *Am. J. Trop. Med. & Hyg.* 6, 715-726.

Williams, F. E. 1947. The complement fixation reaction in Asiatic schistosomiasis employing cercarial antigen (*Schistosoma spindale*) *Trans. Roy. Soc. Trop. Med. & Hyg.* 40, 421-434.

Wright, W. H., Bozicevich, J., Brady, F. J., and Bauman, P. M. 1947. The diagnosis of *Schistosomiasis japonica* V. The intradermal and serological tests. *Am. J. Hyg.* 45, 150-163.

Yolles, T. K., Moore, D. V., De Giusti, D. L., Ripsom, C. A., and Meleney, H. E. 1947. A technique for the perfusion of laboratory animals for the recovery of schistosomes. *J. Parasitol.* 33, 419-426.

Yoshimoto, M. 1910. Ueber die Komplement Bindungsreaktion bie der Schistosomum Krankheit in Japan. *Zeitschr. Immunitäts. forsch. u. Exp. Therap.* 5, 438-445.