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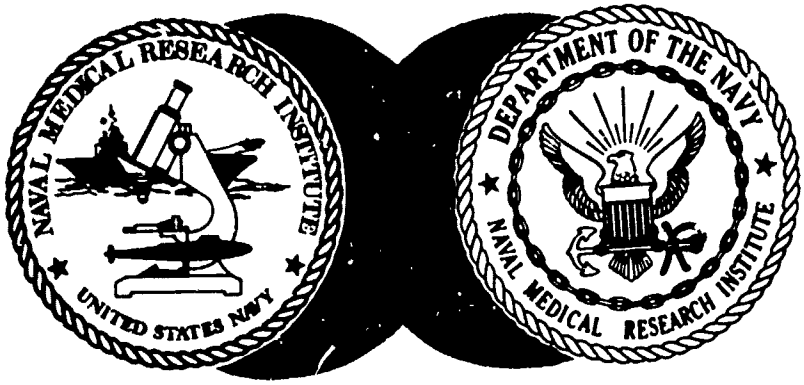

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EXPERIMENTAL VACCINES IN SCHISTOSOMIASIS

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A review of the nature of microbial and parasitic disease vaccines in use today is presented with a discussion of current work and progress towards the development of a schistosome vaccine.

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EXPERIMENTAL VACCINES IN SCHISTOSOMIASIS[1,2,3]

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Anti-schistosomal vaccines are, at present, little more than a hope and an idea, and work in this area is truly in the experimental stages. While considerable work and some progress have occurred, there does not exist at this time any vaccine that could qualify for trials in humans. To facilitate discussion of the possible approaches towards a schistosome vaccine, we think it would be helpful to review the nature of the vaccines in use today for a wide variety of other infectious diseases. Hopefully, we will benefit by considering the nature of such vaccines in terms of selecting approaches to a schistosome vaccine.

As seen in Table 1, vaccines for microbial diseases consist of three basic types : antigen extracts (especially in bacterial diseases), killed whole organisms, and attenuated-live organisms (especially in anti-viral vaccines). Not all of these vaccines are entirely satisfactory, and work continues in order to improve them. Of interest to schistosome immunologists is the fact that the immune mechanisms responsible for protection were, and in many cases are still, not well defined ; most of these vaccines were obtained by empirical methods.

When we review the work in vaccine development in parasitic diseases (Table 2), one is immediately struck by the fact that the majority are live organism-type vaccines. Some of these agents are already in commercial production, while others are only in varying stages of experimentation and development.

When we examine the work on anti-schistosomal vaccines, the use of live organisms is also prominent among the various experimental immunogens. Live parasite-type immunizations have taken two forms : avirulent, zoophilic or heterologous schistosome species or strains, and radiation-attenuated homologous species. The third approach, immunization with crude or purified antigens, represents an approach with perhaps the widest appeal among people in this field, but is one which, unfortunately, has provided the smallest degree of success.

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2) The opinions or assertions contained herein are the private ones of the authors and are not to be constued as office or reflecting the view of the Navy Department or the naval service at large.

3) The animals used in this study were handled in accordance with the provisions of Public Law 91-579, the «Animal Welfare Act of 1970» and the principles outlined in the «Guide for the Care and Use of Laboratory Animals», U.S. Department of Health, Education and Welfare Publication No. (NIH) 73.23.

TABLE 1. Nature of immunogens in vaccines for infectious disease (non-parasitic)

Disease	Immunogen
1. EXTRACTS	
Cholera	Crude Fraction of Vibrios
Plague	Crude Fraction of Bacilli
Diptheria	Purified Toxoid
Tetanus	Purified Toxoid
Meningitis	Purified Polysaccharide
Pneumonia	Purified Polysaccharide
2. KILLED ORGANISMS	
Rabies	Inactivated Virus
Influenza	Inactivated Virus
Typhus Fever	Killed Rickettsia
Thyphoid Fever	Killed Bacteria
Polio	Inactivated Virus
3. ATTENUATED ORGANISMS	
Yellow Fever	Infectious (Attenuated) Virus
Measles	Infectious (Attenuated) Virus
Mumps	Infectious (Attenuated) Virus
Rubella	Infectious (Attenuated) Virus
Polio	Infectious (Attenuated) Virus
Smallpox	Infectious (Attenuated) Virus
Tuberculosis	Infectious (Attenuated) Mycobacteria

TABLE 2. Nature of immunogens in vaccines for parasitic diseases

Disease	Immunogen
Coccidiosis	Attenuated Oocysts
Hookworm (Dog)	Attenuated Larvae
Lung Worm (Caule)	Attenuated Larvae
— "Very Experimental" —	
Malaria	Attenuated Sporozoite
Taeniasis (Sheep)	Normal Larvae in Abnormal Site
Filariasis (Various)	Attenuated Larvae
Trichinosis	Larval Extract

Before presenting some of our work on the use of heterologous cercariae as an immunizing agent, we would like to review quickly some of the work of others using this approach. As you can see (Table 3), not always consistent results have been obtained between different groups of investigators, particularly in experiments with monkeys. Taylor and his associates (1973) attempted a novel scheme whereby F_1 hybrid cercariae from the mating of *Schistosoma rodhaini* and *S. bovis* were used to immunize baboons against *S. mansoni* challenge. The results, however, were not encouraging. In the majority of these cases, the levels of immunity based on adult worm recoveries were moderate. In contrast, in rhesus monkeys immunized with the zoophilic strain of *S. japonicum*, egg excretion data indicated a very high level of induced resistance. This prompted our experiment (Murrell et al., 1973). Briefly, monkeys were exposed five times to about 1,700 cercariae of the zoophilic Formosan strain of *S. japonicum* and then challenged with the lethal Philippine strain. At autopsy 6 weeks later, 82% fewer challenge worms survived in the immunized monkeys (Table 4). Likewise, immunized monkeys shed far fewer eggs. When liver tissue egg counts were compared, however, no difference was noted. Since the egg is the primary agent of the disease, the level of immunity induced from a clinical standpoint was not satisfactory. The organ pathology was, as you would expect, considerable in both groups. We were further discouraged from pursuing this vaccination approach by the high degree of difficulty and impracticality in generating large numbers of immunizing cercariae where and when they were needed. These results do not, however, prove the unfeasibility of this particular method, since the zoophilic strain might be sufficiently immunogenic in humans to prevent the accumulation of unacceptable numbers of eggs. But it would be extremely difficult, based on these studies and those of others, to justify human experimentation, especially with *S. japonicum*.

In contrast, the work now going on in the laboratory of Taylor and Nelson at Winches Farm in London, using irradiated cercariae, appears to offer a more hopeful approach. These investigators have, with such an experimental vaccine, developed a truly effective procedure in large domestic animals. In Table 5, many of the earlier studies on the immunogenicity of irradiated cercariae are shown. Consistent, although moderate, levels of immunity were induced in rodents with 2-3 kilorads. Some discrepancy has appeared in the case of monkeys, however. Attenuating conditions in different laboratories, as well as variations in immunization protocols and numbers of cercariae used, may help to explain some of these contradictions. As already mentioned, the group at Winches Farm in London has had very good results in sheep. Dr. Taylor will be presenting their recent work at a later session which should be of great interest to all concerned with vaccines.

Our work along this line has produced some uncertainties regarding the case of standardization of protocols for immunizing with irradiated cercariae. In experiments in mice (Table 6), we were not able to induce significant levels of immunity with the irradiation doses utilized by others, usually 2-3 kilorads. When the dosage was increased to 8-16 kilorads, immunity was obtained. Importantly, no immunizing worms survived at these irradiation levels. Similar findings have been reported by Taylor in sheep. At this time we can only speculate that the discrepancy between our effec-

TABLE 3. Selected studies on heterologous immunity in Schistosominsis

Host	Immunizing species or strain	Challenge Species	Protection	Authors
Rhesus Monkey .	*Schistosomatium douthitti*	*Schistosoma mansoni*	(—)	Kagan (1953)
Rhesus Monkey .	*Schistosomatium douthitti*	*S. japonicum*	(+)	Hsü et al. (1964)
Rhesus Monkey .	*S. japonicum* (Formosan)	*S. japonicum*	(+)	Hsü and Hsü (1963)
Baboons	*S. rodhaini*	*S. mansoni*	(+)	Taylor et al. (1973)
Baboons	*S. bovis*	*S. mansoni*	(+)	Taylor et al. (1973)
Baboons	Hybrids	*S. mansoni*	(+)	Taylor et al. (1973)
Sheep	*S. mansoni*	*S. matiheei*	(—)	Taylor (1975)

TABLE 4. Immunization of Rhesus monkeys with the zoophilic Formosan strain of *Schistosoma japonicum* (Murrell *et al.*, 1973)

Measurement	Immunized	Controls	% Difference
Adult Worm (Recovery ($\bar{X} \pm$ SE)	81 ± 26	167 ± 15	52
Mean Eggs /g Stool ($\bar{X} \pm$ SE)	22 ± 6	95 ± 17	77
Mean Egg /g Stool/Female Worm	0.55 ± 0.16	1.40 ± 0.15	61
No. Eggs/10g Liver Tissue	75,375	71,700	0

TABLE 5. Studies on immunization against schistosomes with irradiated cercariae

Host	Schistosome Species	Radiation	Protection	Authors
Mice	*S. mansoni*	^{60}Co	(+)	Villella (1961)
Mice	*S. mansoni*	^{60}Co	(+)	Radke & Sadun (1963)
Mice	*S. mansoni*	X-ray	(+)	Perlowagora-Szumlewicz (1964)
Mice and Rats . .	*S. mansoni*	^{60}Co	(+)	Erickson & Caldwell (1965)
Rats	*S. mansoni*	X-ray	(+)	Smithers & Terry (1965)
Rhesus Monkey . .	*S. mansoni*	^{60}Co (2.5k)	(+)	Sadun et al. (1964)
Rhesus Monkey . .	*S. mansoni*	^{60}Co (4.0k)	(—)	Sadun et al. (1964)
Rhesus Monkey . .	*S. mansoni*	X-ray (2.0k)	(—)	Smithers & Terry (1967)
Rhesus Monkey . .	*S. japonicum*	X-ray (24k, 48k)	(+)	Hsü et al. (1969)
Sheep	*S. mattheei*	^{60}Co	(+)	Taylor (1975)

tive irradiation levels and those reported by others may lie in differences in strains of parasite and host. This experience should serve as a warning that different conditions may call for alterations in published protocols, in particularly with respect to minimum attenuating irradiation dose.

We have also investigated another possible source of irradiation for cercarial attentuation with the intent to make irradiation more adaptable to field-type conditions. As you know, gamma or x-ray irradiation is not easily performed in the field. As such, we have experimented with ultraviolet light, which has the advantages that it is relatively safe and can be generated with simple and easily available equipment.

Our experiments have as yet dealt only with mice, but as you can see in Table 7, we were able to achieve moderate to high levels of protection ; however, we have not yet induced immunity in the complete absence of residual immunization worms.

I wish to emphasize again that, although irradiated cercariae show great promise in relation to other vaccine approaches, their practicality is subject to debate. Because cercariae have such a short shelf life, on the order of hours, eventual wide application of the procedure, even as a domestic animal vaccine, will require the maintenance, under field conditions, of large snail-cercariae factories and sophisticated irradiation sources. This does not promise to be an inexpensive and simple task. However, a solution to this may be near. In an imaginative series of studies, James, Farrant, & Taylor (pers. comm., 1975), of the London School of Hygiene and Tropical Medicine, have shown that irradiated cercariae in large numbers can be easily transformed *in vitro* to the schistosomule stage, and injected by needle into the host, producing immune levels comparable to that achieved with cercariae. Going further, they have been successful in freezing schistosomules down to —20°C and recovering full infective organisms on thawing. If schistosomules can be cryopreserved, many of the practical drawbacks to the development of an irradiated cercarial vaccine can be corrected. Whatever the eventual outcome, the information to be gained by experimentation with this approach will be of great value to those interested in the immunology of schistosomiasis.

TABLE 6. Resistance induced in NIH/NMRI* mice with two exposures to 500 ^{60}Co-irradiated *Schistosoma mansoni* cercariae

Level of Irradiation (Kr)	Reduction in Challenge Worm Survival (%)
2.5	0
4.0	29
8.0	49
16.0	56

(*) National Institutes of Health/Naval Medical Research Institute inbred strain of white Swiss mice.

TABLE 7. Resistance induced in mice exposed to *Schistosoma mansoni* cercariae irradiated with ultra-violet light (2537 Å)

Number Immunizations	Number Immunizing Cercariae	Reduction in Challenge worm survival (%)
1	50	0
1	100	1
1	250	41
1	500	66
2	500	93
2	500	55

I would like to turn now to the third basic type of vaccine, that of antigen extracts. This approach has always had great appeal to immunoparasitologists for both its potential practicality and the assumed reduced hazards in its use. As reflected in Tables 8 and 9, the results have been less than promising inspite of considerable efforts toward this goal. Immunization with whole eggs, or cercariae, or with their extracts, has failed to induce protection in all but two studies of which we are aware. An exciting report was made 6 years ago by Dodin (1969). That author claimed to have reduced substantially the reinfection rate in Ambilhar-treated children by immunization with lyophilized cercariae previously treated with ascorbic acid and copper. To my knowledge, though, there has not been a published follow-up of this work.

The effectiveness of adult worm extracts has had more claims ; about two-thirds of published studies reported success in inducing protection, primarily in mice. It must be pointed out, however, that the levels of immunity induced were usually marginal.

TABLE 8. Immunization with inactivated egg and cercarial stages or their antigenic extracts

Immunogen	Protection	Authors
1. Egg Extracts	—	Kagan (1958)
	—	Smithers (1962)
	—	Ritchie et al. (1962)
2. Whole Cercariae	—	Thompson (1954)
Whole Cercariae treated with ascorbic acid and copper	+	Dodin (1969)
3. Cercarial Extracts	±	Ozawa (1930)
	—	Sadun & Lin (1959)
	—	Ritchie et al. (1962)

TABLE 9. Immunization with adult worm antigens

Immunogen	Resistance to Challenge	Authors
1. Adult Worm Extracts	+	Ozawa (1930)
Adult Worm Extracts	+	Kawamura (1932)
Adult Worm Extracts	+	Watts (1949)
Adult Worm Extracts	—	Vogel & Minning (1953)
Adult Worm Extracts	+	Sadun & Lin (1959)
Adult Worm Extracts	+	Sadun & Bruce (1964)
Adult Worm Extracts	—	Kagan (1958)
Adult Worm Extracts	—	Ritchie *et al.* (1962)
Adult Worm Extracts and BCG	+	Capron & Lesoin (1969)
2. Adult Culture Antigens	+	Sadun & Lin (1959)
Adult Culture Antigens	+(?)	Murrell & Clay (1972)
3. Adult and Cercarial Culture Antigens (Mixed)	(+)	Levine & Kagan (1960)

In our own study on adult culture antigen, we are compelled to say that, although a moderate level of protection was induced in the first trial, we were not able to confirm that result in two subsequent attempts. The highest levels of immunity induced by antigen extracts were those reported by Capron & Lesoin (1969) who used BCG (Bacille Calmette Guérin) as an adjuvant. However, these workers have not had consistent success in repeat experiments with this procedure which, unfortunately, seems to be true for most of these studies. To illustrate further the frustration experienced by ourselves, and perhaps by those others struggling with this problem, we have summarized our experimentation (Murrell et al., 1975) on antigen extracts made in a variety of ways in Table 10. As you can see, we consistently failed to produce immunity with crude adult worm extracts, regardless of the adjuvant employed, except in an initial experiment designed to recover surface-associated antigens using 3 molar potassium chloride (3M KCl). Frustratingly, we have not been able to confirm those initial results. Again, with cercarial autolytic antigen and, as mentioned, with adult culture antigen, initial success was followed with repeated failure.

TABLE 10. Immunization with *S. mansoni* antigen fractions (Murrell, Dean and Stafford, 1975)

Immunogen	Animal	Cytotoxic Antibody	% Reduction in Challenge worm Survival
1. Adult Worm Crude Extracts			
FCA	Mice	+	0
ICFA	Mice	+	0
Adjuvant*			
Alum & *Bordetella pertussis*	Mice	+	0
BCG	Mice	+	0
FCA	Guinea Pigs	+	0
2. Hypertonic Salt Extract (3M KCl)			
1	Mice	+	27
2	Mice	+	1
3	Guinea Pigs	+	0
3. Cercarial Enzyme Secretions	Mice	±	0
4. Cercarial Autolytic Antigen			
1	Mice	+	48
2	Mice	+	0
3	Mice	0	15
5. Adult Culture Antigen			
1	Mice	0	47
2	Mice	0	0
3	Rats	+	0

*FCA = Freund's complete adjuvant
IFCA = Incomplete Freund's adjuvant
BCG = Bacille Calmette Guérin

These results should not, however, deter anyone from undertaking the challenge of an antigen-extract vaccine. Rather, we hope they will serve to stimulate reflection on the problems associated with complex antigen extracts on which relatively little is known of their chemical and immunological nature. This is emphasized in Table 11 in which we have attempted to outline some major problems in this field that may account for the wide variations in results obtained by different groups of investigators.

Although it was stated in the beginning that most anti-microbial vaccines have come about by essentially empirical means, it is only fair to point out that, in contrast to human schistosomiasis, microbial diseases often induce very high levels of resistance, indicating that their antigens are immunogenically of a high order. In schistosomiasis, the levels of immunity, as a result of primary infection, are not always high and, in the case of humans, they may be quite moderate. A solution to the problem of low immunogenicity of schistosome antigens may lie in the manipulation and modification of antigens in such a manner as to elicit immune responses of a greater effectiveness than would result from normal infection. This undertaking, of course, will require detailed immunochemical knowledge of the antigens. At the same time, a precise understanding of the mechanism of immune resistance would be of immeasurable help in reaching that goal.

Towards that end, we are studying in our laboratory the role of humoral antibody in protection. I would like to present *one* current experiment which I think will illustrate the potential contributions that an understanding of the immune mechanisms can make towards vaccine development. In this experiment, we attempted to evaluate the role of reagins, or homocytotropic antibody, in immune protection. Ishizake et al. (1957) have suggested that reaginic antibody may have an important role in infectious diseases by promoting, through its induction of vascular permeability, the translocation of increased amounts of antibody and cells into the infected tissues. This has been adapted by us in a hypothesis illustrated in Fig. 1.

We tested the hypotheses by implanting intradermally (i.d.) in the abdominal skin of mice, mouse sera with a high titer of reagin. The mice were also injected intravenously (i.v.) with serum from immune mice having a chronic infection. Seventy-two hours after the implantation of reagin, the mice were challenged by allowing 100 cercariae to penetrate

TABLE 11. Difficulties encountered in reproducing immunization results obtained by different investigators

Some of these difficulties may be due to the:

1. Use of different schistosome and host strains;
2. Inadequate standardization of methods and protocols, particularly in preparing antigens and in assessing host resistance;
3. Faulty experimental designs resulting in inadequate numbers of test animals and insufficient controls; and
4. Scarce immunochemical information on schistosome antigens, particularly with regard to antigen susceptibility to enzymes and to changes in physical-chemical conditions.

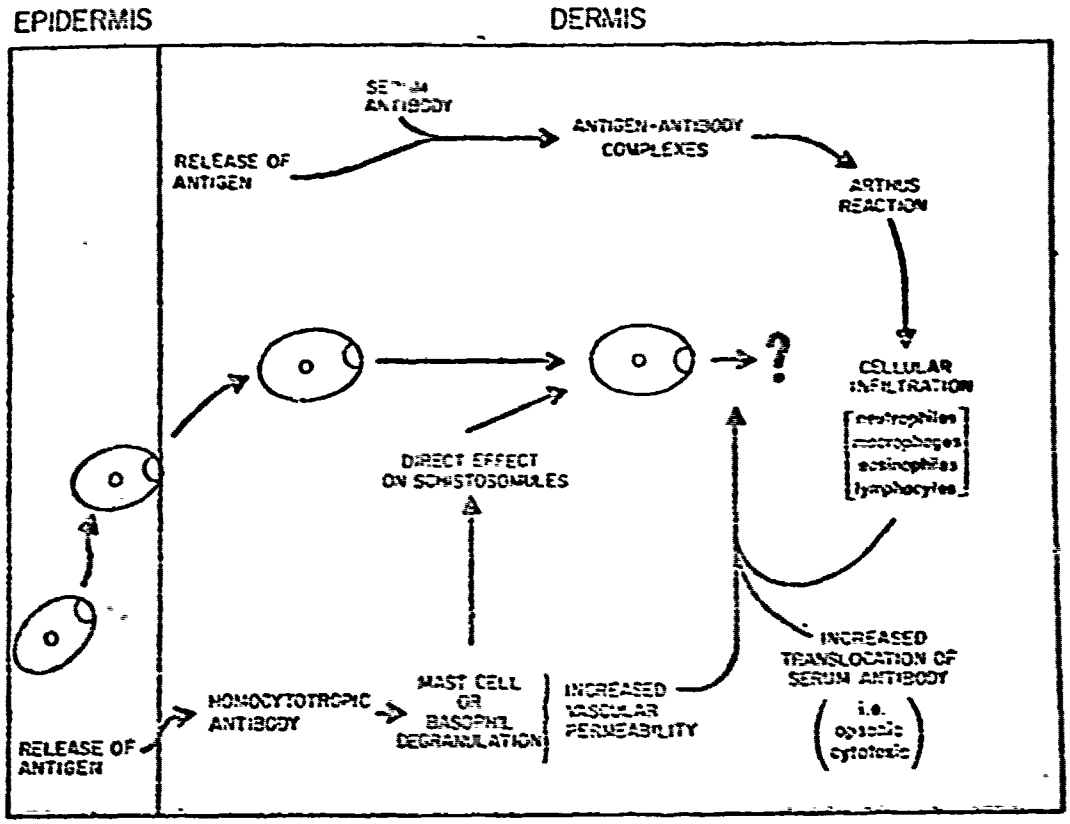

Fig. 1. The migration of the schistosomule through the dermis may, through the elaboration of antigens, trigger an immediate hypersensitivity reaction; this reaction may in turn promote the translocation of antibodies and killer cells into the tissue site. This sequence of interrelated immune reactions is the basis of of the "Gatekeeper Hypothesis"

through the serum-infiltrated skin site. Table 12 presents the results of that experiment. Mice receiving both immune serum i.v. and reagin i.d. had significantly fewer surviving challenge worms than the controls. However, these two antibodies, in combination with normal mouse serum, failed to protect mice.

These results in themselves do not prove the indispensable role of reagin and immune IgG and will require confirmation, but they do encourage us in the belief that it may be possible to induce such protective immune responses by selecting from the large array of schistosome antigens those capable of inducing effective reagin and effective IgG responses. Such knowledge would permit focusing on these antigens attempts to bolster their immunogenicity. Regardless of the eventual outcome of this particular work, the approach serves as an example of how defined immune mechanisms can further our search for a vaccine.

In summary, we feel that there do exist grounds for optimism that an immunological control of schistosomiasis can be developed ; the attenuated-live cercariae approach certainly warrants continued support and interest. At the same time, encouragement must be maintained for those attempting to unravel the complex immune response to schistosomiasis, for solutions to this problem will depend on a precise and complete understanding of this dangerous and successful parasite.

TABLE 12. Test of Gatekeeper Hypothesis: Mouse Protection Test

Group*	I	II	III	IV
Serum i.d	IgE	IgE	NS	NS
Serum i.v.	IgG	NS	IgG	NS
Adult Worms Recovered ($\bar{X} \pm SE$):	13.8±2.4	19.1±1.9	29.2±4.0	25.7±3.9

NS + NS *vs.*	IgE + IgG =	47%
	Ns + IgG =	0%
	IgE + NS =	26%

% worm reduction

* 10 mice/group; NIH/NMRI females
IgE = mouse reagin with PCA titer of 1 : 1200
IgG = serum from mice infected 7 months
NS = normal mouse serum

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