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OFFICE OF NAVAL RESEARCH

Contract No. N00014-78-C-0081

Task No. NR 204-072

Annual Report No. 3

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6 Cryopreservation of Schistosome Larvae

by

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7. 1980

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2 October 1980

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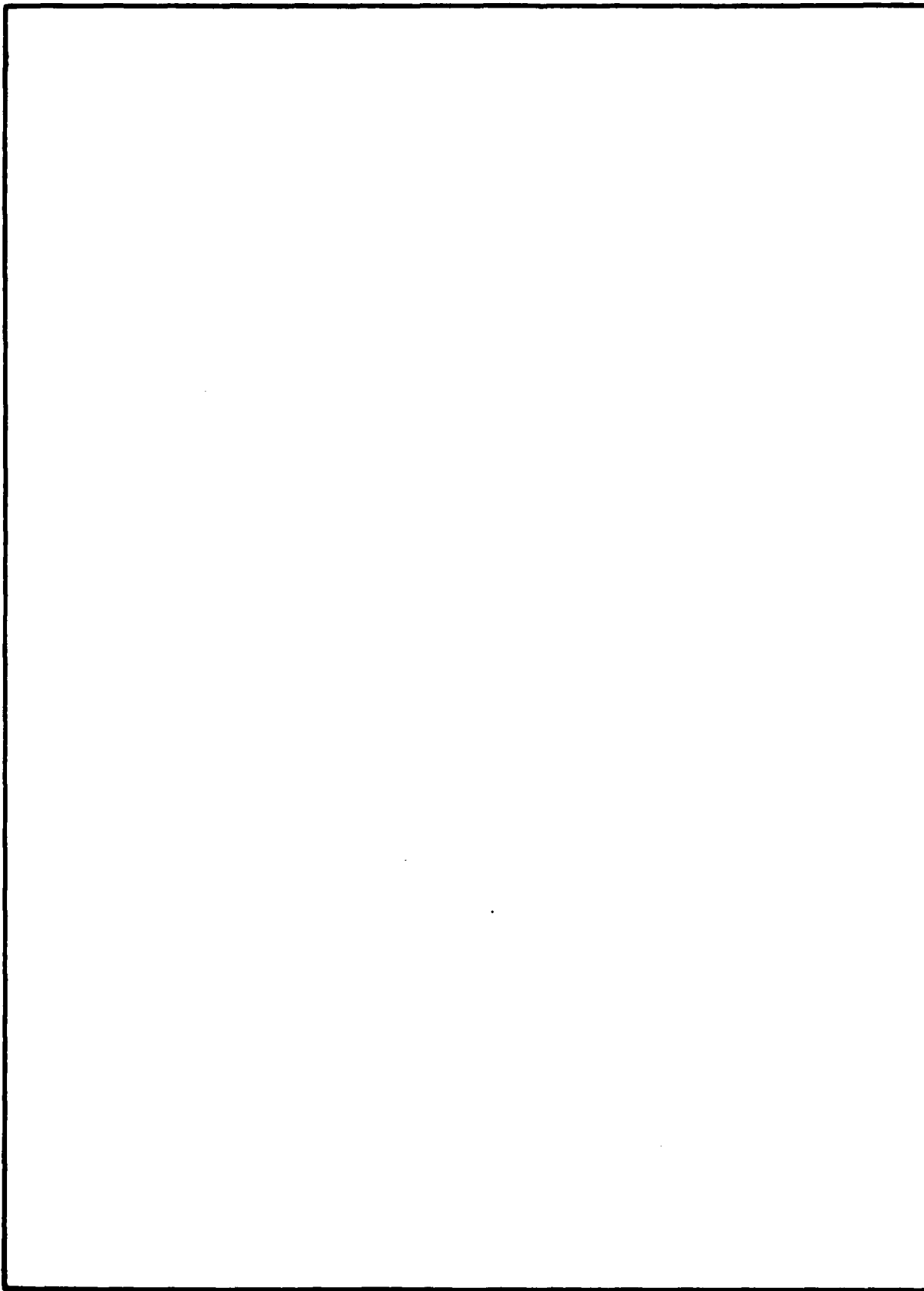
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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER 3	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Cryopreservation of Schistosoma Larvae		5. TYPE OF REPORT & PERIOD COVERED ANNUAL REPORT 30 Sept. 1979- 1 Oct 1980
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) M. A. Stirewalt, C. S. Richards & J. T. Sullivan		8. CONTRACT OR GRANT NUMBER(s) ✓ N00014-78-C-0081
9. PERFORMING ORGANIZATION NAME AND ADDRESS American Foundation for Biological Research 12111 Parklawn Drive, Rockville MD 20852		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS NR 204-072
11. CONTROLLING OFFICE NAME AND ADDRESS Procurement Contract Officer Office of Naval Research (433) Dept. of the Navy Washington, D. C. 20360		12. REPORT DATE 2 October 1980
		13. NUMBER OF PAGES
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) Standard Distribution List		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) <u>Schistosoma mansoni</u> ; trematode; cryopreservation; vaccine; irradiated cercariae; protective immunity; genetic variations of <u>S. mansoni</u> ; isozymes of <u>S. mansoni</u> ; <u>Biomphalaria glabrata</u> ; susceptibility of <u>B. glabrata</u> ; genetics of <u>B. glabrata</u> ; amebocytes, molluscan; encapsulation; molluscicides		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Seventeen genetically different strains of <u>S. mansoni</u> and sixteen stocks of <u>B. glabrata</u> showing genetic differences in susceptibility patterns are being composed. Isozyme studies on 13 <u>S. mansoni</u> strains and 5 <u>B. glabrata</u> stocks demonstrated differences in all. Histologic studies on <u>B. glabrata</u> stocks exposed to <u>S. mansoni</u> demonstrated three types of host reaction: active resistance, passive unsuitability, and susceptibility. EM studies on one stock of <u>B. glabrata</u> showed amebocytes attacking and destroying muscle cells of the atrium, a reaction against self. Molluscicidal effect of cashew nut shell extract for <u>B. glabrata</u> was shown to be due primarily to the triene component of anacardic acid.		

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### Background

Significant protective immunity to Schistosoma mansoni develops in several mouse strains (a) during a patent chronic infection, and (b) following immunization with highly irradiated viable cercariae. Since injection of highly irradiated cercariae does not lead to a patent schistosomal infection this type of immunization shows potential for human vaccine use.

Antigenic polymorphism within a single isolate of Schistosoma mansoni appears to affect the resistance of mice to second infection with various clones developed from the same isolate (Smith and Clegg, Parasitology 78: 311-321, 1979). Such antigenic polymorphism has important implications for experimental studies of immunity to schistosomiasis and also for the development of a vaccine.

Strains of S. mansoni, including isolates from different geographic areas, multiple isolates from some areas, and multiple substrains selected from individual isolates have been differentiated on the basis of infectivity for intermediate host snails. This series of genetically different S. mansoni strains provides parasites for studies on variations in immunologic and pathologic aspects of different S. mansoni strains, and differences in cryopreservation qualities as means of storing them for vaccine production.

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## Methodology and Results

B. glabrata snails have been exposed, both as juveniles and adults, to a variety of strains of S. mansoni. By selection on the basis of susceptibility and isolation of snails as juveniles with reproduction by self-fertilization only, a series of inbred snail lines has been established showing different patterns of susceptibility. Exposures of snails from these characterized lines to miracidia of S. mansoni serves to differentiate genetic infectivity patterns in different parasite strains.

(1) Intraspecific genetic variations in susceptibility for S. mansoni strains were compared in 16 stocks of B. glabrata. About 10 of these snail stocks are routinely used in testing variations in S. mansoni infectivity. Isozyme studies on 5 of the B. glabrata stocks demonstrated differences. Snail crosses, involving 16 stocks of B. glabrata, have been made in studies on inheritance of genetic factors influencing susceptibility for S. mansoni infection. Some of these crosses, and analyses of the results, are still in progress.

(2) Studies on intraspecific genetic variations in S. mansoni have been continued. Seventeen strains have been maintained and compared, including: 13 of Puerto Rican origin, 3 of St. Lucian origin, and on Egyptian. Differences in isozymes (Fletcher et al., submitted) and in snail infectivity patterns were demonstrated in these S. mansoni strains. Isozyme variations for one enzyme system, lactate dehydrogenase (LDH), appear to be associated with variations in infectivity for B. glabrata. Development of inbred lines of the S. mansoni strains, by brother-sister matings, are in progress. Crosses have so far been performed involving six of the S. mansoni strains, to study inheritance of snail infectivity factors.

(3) Cercariae of several different strains of S. mansoni have been provided to NMRI and to LPD, NIAID, NIH for immunologic studies.

(4) Histologic studies on several different stocks of B. glabrata exposed to one strain (NIH-Sm-PR2) of S. mansoni helped clarify variations in host-parasite interactions (Sullivan and Richards, submitted). Snail host reactions are of three types (with some intergradations): (1) resistance (active encapsulation and destruction of parasites by amebocytes); (2) unsuitability (failure of parasite development with minimal or no host reaction); and (3) susceptibility (normal parasite development with no host reaction). Snail crosses indicated that resistance is dominant over unsuitability and susceptibility, and unsuitability, dominant over susceptibility.

(5) EM studies demonstrated that genetically regulated amebocytic accumulations in the atrium in one stock of B. glabrata produced increased amounts of lysosomal enzymes and attacked and destroyed muscle cells of the atrium, a phenomenon resembling vertebrate autoimmunity (LoVerde et al., submitted).

(6) Among 7 genetic stocks of B. glabrata nonsusceptible to the NIH-Sm-PR2 strain of S. mansoni, 5 stocks reverted to susceptibility, one to partial susceptibility, and one remained nonsusceptible, when first infected with Echinostoma paraensei (Sullivan et al., submitted).

(7) B. glabrata sensitized by infection with x-irradiated miracidia of Ribieroa marini acquire a strong resistance to a challenge infection with normal R. marini miracidia (Sullivan et al., submitted).

(8) Tolerance to four molluscicides was compared in a laboratory stock and a field stock of B. glabrata (Sullivan and Richards, submitted). The laboratory snail stock demonstrated more resistance for PCP, Bayluscide, and  $\text{CuSO}_4$ . The field stock was slightly more resistant to Frescon.

(9) The saturated monene, diene, and triene components of anacardic acid, obtained by fractionation of an extract from the cashew nut shell, were tested for toxicity to B. glabrata. The triene form was most toxic, the di- and monene forms less toxic, and the saturated form relatively nontoxic. Results suggested that definitive laboratory testing of this naturally-occurring molluscicide is justified (Sullivan et al., submitted).

#### Discussion

Six isolates of S. mansoni from three geographic areas, have yielded 17 parasite strains by selection on the basis of infectivity for B. glabrata snails. These parasite strains showed genetic variations in infectivity patterns. Isozyme studies on 13 of the strains demonstrated enzyme differences. The results indicated the need for further genetic characterization of parasite strains used in biomedical research, and comparative studies on the influence of strain differences on immunology, pathology, epidemiology, etc. Strains from additional geographic areas should be tested, characterized, and compared.

Collaborative studies at Purdue University (Fletcher and LoVerde) suggested that isozyme variations for at least one enzyme system, lactate dehydrogenase, in our S. mansoni strains were associated with variations in snail infectivity. Such studies should be continued.

Studies on the histology of the snail-parasite interaction, comparing a series of stocks of B. glabrata exposed to S. mansoni strain NIH-Sm-PR2, demonstrated that the host relation was not simply nonsusceptible or susceptible. Nonsusceptible stocks could be actively resistant, passively unsuitable, or combinations of these phenomena. These studies should be extended to compare results with different parasite strains, and the genetics of these variations in both host and parasite explored further.

Snail exposures and selection resulted in establishing 13 strains of S. mansoni from 5 isolates of Puerto Rican origin. These strains differed genetically in patterns of infectivity for B. glabrata. Electrophoretic studies on 11 of the strains demonstrated izozyme differences. Four samples of B. glabrata from different localities in Puerto Rico are demonstrating differences in susceptibility for the Puerto Rican S. mansoni strains.

Crosses are in progress between snails of different stocks and parasites of different strains. The studies provide some information on the epidemiology of schistosomiasis, but more snail samples and parasite isolates should be obtained and compared.

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Accomplishments during this report period include:

Significant Accomplishments

- (1) A wide range of intraspecific genetic variations has been demonstrated in both S. mansoni and B. glabrata: 17 strains of S. mansoni showing different patterns of snail infectivity, and 16 stocks of B. glabrata showing different patterns of parasite susceptibility are under study;
- (2) Isozyme differences were demonstrated in 13 strains of S. mansoni and 5 stocks of B. glabrata studied;
- (3) Histologic studies on a series of stocks of B. glabrata exposed to one strain of S. mansoni showed the host reaction could be active resistance, passive unsuitability, or susceptibility. Resistance is genetically dominant over unsuitability and susceptibility; unsuitability, dominant over susceptibility;
- (4) EM studies showed that in one stock of B. glabrata with genetic accumulations of amebocytes in the atrium, the amebocytes attacked and destroyed muscle cells of the atrium, a reaction against self; and
- (5) Molluscicidal effect of cashew nut shell extract for B. glabrata was shown to be due primarily to the triene component of anacardic acid.



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