CRYOPRESERVATION OF SCHISTOSOME LARVAE. (U)

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UNCLASSIFIED
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by

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Seventeen genetically different strains of S. mansoni and sixteen stocks of B. glabrata showing genetic differences in susceptibility patterns are being composed. Isoenzyme studies on 13 S. mansoni strains and 8 B. glabrata stocks demonstrated differences in all. Histologic studies on B. glabrata stocks exposed to S. mansoni demonstrated three types of host reaction: active resistance, passive unsuitability, and susceptibility. El studies on one stock of B. glabrata showed amebocytes attacking and destroying muscle cells of the airium, a reaction against self. Molluscidial effect of cashew nut shell extract for B. glabrata was shown to be due primarily to the triene component of anacardic acid.
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.
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 one 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).
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, B·yluscide, and CuS0₄. 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, lactatedehydrogenase, 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 isozyme 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.

Accomplishments during the report period include:

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;

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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