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BIOMEDICAL RESEARCH INST ROCKVILLE MD
SCHISTOSOME MATERIALS FOR VACCINE DEVELOPMENT.(U)
SEP 81 M STIREWALT, F LEWIS

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FINAL REPORT,

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Schistosome Materials for Vaccine Development .

by

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Rockville, Maryland 20852

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September 1981

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SUMMARY

This contract has been funded through the Office of Naval Research by the Naval Medical Research Institute (NMRI). It has had ^{two} purposes: (1) to supply investigators in schistosome immunology at NMRI with parasite and infected-host materials for their research; and (2), researchwise, to define the snail-schistosome-mouse interactions involved in maintenance of a constant, dependable, large-volume source of parasite and infected host materials.

From the beginning, *Biomphalaria glabrata* of mixed Puerto Rican-Brasilian origin (Nmri strain) have been grown and infected each week in sufficient numbers to supply 4 million or more cercariae of Puerto Rican *Schistosoma mansoni* (Nmri strain) weekly. Albino mice have been infected, held for 7 or 8 weeks, sacrificed and perfused, and their adult schistosomes recovered and cleaned for use in antigen preparations. In addition, schistosome eggs, schistosomules, miracidia, infected snails, infected mice and mouse tissues, and cercarial penetration enzymes have been available upon request. (Annual reports No. 1 through 5).

Research has been focussed on three areas. The first was on defining the means of schistosome maintenance at a level which insured a constant dependable supply of large quantities of research material. To this end, maintenance techniques and the harvests of parasite materials have been monitored constantly while one procedure at a time was changed in an effort to understand its effect on parasite production. These techniques included: number of miracidia to which snails were exposed — 1, 5, 6 to 8, and 8 to 10 (ms prepared for submission for publication); temperature and light changes; size of snails at exposure — 4 to 6 mm diameter optimal; and suitability of mouse strains for provision of miracidia (in progress). (Annual reports No. 1 to 5; Stirewalt, M. in press. *Schistosoma mansoni*: Conditions contributing to maximal cercarial harvests. Journal of Parasitology).

The second area of research interest was in development of procedures for collecting penetration enzymes of cercariae and analyzing its total protein content (Lowry) and its activity against an azocoll substrate. Total protein content varied widely from collection to collection but the proteolytic activity remained fairly constant. (Annual Report No. 1; Campbell, D. L., Frappaolo, P. J. E., Stirewalt, M. A. and Dresden, M. H. 1976. *Schistosoma mansoni*: partial characterization of enzyme(s) secreted from the preacetabular glands of cercariae. Experimental Parasitology 39, 33-40). Immunogenicity of the enzymes was demonstrated by induction in mice of precipitating and reaginic antibodies, but no protection was afforded mice against a challenge infection. (Annual report No. 1; Minard, P., Murrell, K. D., and Stirewalt, M. A. 1977. Proteolytic, antigenic and immunogenic properties of *Schistosoma mansoni* cercarial secretion material. American Journal of Tropical Medicine and Hygiene 26, 491-499). This work was supported also by N00014-76-0053.

^{in area indicated}
Research of the third type resulted in the finding that certain rotifers which colonized the shells of infected snails strongly inhibited cercarial emergence. (Annual report No. 2; Stirewalt, M. and Lewis, F. A. 1981. *Schistosoma mansoni*: effect of rotifers on cercarial output, motility and infectivity. International Journal for Parasitology 11, 301-308). Methods of controlling rotifer colonization on snails were developed. They consist of treating each snail with 10% ethanol applied to the shell by swab; or washing the rotifers from snails individually by a jet stream of water. (Annual report No. 4).

Drastic reduction in support for this contract has eliminated funding for research since FY78.

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INDEX OF ANNUAL REPORTS

1. Annual report, 15 July, 1976
2. Annual report, 30 September, 1977
3. Annual report, 21 September, 1978
4. Annual report, 24 September, 1979
5. Annual report, 24 September, 1980

INDEX OF PUBLICATIONS SUPPORTED COMPLETELY OR
IN PART BY THIS CONTRACT

1. Attallah, A. M., Lewis, F. A., Urrutia-Shaw, A., Folks, T., and Yeatman, T. J. 1980. Natural killer cells and antibody-dependent cell-mediated cytotoxicity components of *Schistosoma mansoni* infection. International Archives of Allergy and Applied Immunology. 63, 351-354.
2. Attallah, A. M., Smith, A. H., Murrell, K. D., Fleischer, T., Woody, J., Vannier, W. E., Scher, I., Ahmed, A. and Sell, K. W. 1979. Characterization of immunosuppressive state during *Schistosoma mansoni* infection. Journal of Immunology 122, 1413-1420.
3. Beaudoin, R. L., Armstrong, J. C., and Vannier, W. E. 1980. Production of radiation-attenuated vaccines against malaria and schistosomiasis. International Journal of Nuclear Medicine and Biology. 7, 113-124.
4. Campbell, D. L., Frappaolo, P. J. E., Stirewalt, M. A. and Dresden, M. H., 1976. *Schistosoma mansoni*: partial characterization of enzyme(s) secreted from the preacetabular glands of cercariae. Experimental Parasitology 39, 33-40.
5. Catto, B. A., Lewis, F. A., and Ottesen, E. A. 1980. Cercarial induced histamine release: a factor in the pathogenesis of schistosomal dermatitis? American Journal of Tropical Medicine and Hygiene. 29, 886-889.
6. Chen, P. and Dean, D. A. 1977. Immune response of guinea pigs to *Schistosoma mansoni*. II. *In vitro* blastogenesis and macrophage migration inhibition factor production in response to cercarial, adult worm and egg antigens. American Journal of Tropical Medicine and Hygiene 26, 963-969.
7. Cousin, D., Stirewalt, M. and Dorsey, C. H. 1981. *Schistosoma mansoni*: Comparative early transformation of skin- and shear pressure-derived schistosomules. Experimental Parasitology. 51, 341-365.
8. Dean, D. A. 1977. Decreased binding of cytotoxic antibody by developing *Schistosoma mansoni*. Evidence for a surface change independent of host antigen absorption and membrane turnover. Journal of Parasitology 63, 418-426.

9. Dean, D. A. 1978. Studies on the mechanism of acquired immunity to *Schistosoma mansoni*. Proceedings of the International Conference on Schistosomiasis, Cairo, Egypt. Vol. II pp. 674.
10. Dean, D. A. and Strong, D. M. 1977. Improved assay for monocyte chemotaxis using frozen stored responder cells. *Journal of Immunological Methods* 14, 65-72.
11. Dean, D., Bukowski, M. A., and Cheever, A. W. 1981. Relationships between acquired resistance, portal hypertension and lung granulomas in 10 strains of mice infected with *Schistosoma mansoni*. *American Journal of Tropical Medicine and Hygiene* 30, 806-814.
12. Dean, D., Bukowski, M. A., Clark, S. S. 1981. Attempts to transfer the resistance of *Schistosoma mansoni*-infected and irradiated-cercaria-immunized mice by means of parabiosis. *American Journal of Tropical Medicine and Hygiene* 30, 113-120.
13. Dean, D., Cioli, D., and Bukowski, M. A. 1981. Resistance induced by normal and irradiated *Schistosoma mansoni*. Ability of various worm stages to serve as inducers and targets in mice. *American Journal of Tropical Medicine and Hygiene* 30, 1026-1032.
14. Dean, D. A., Minard, P., Murrell, K. D. and Vannier, W. E. 1978. Resistance of mice to secondary infection with *Schistosoma mansoni*. II. Evidence for a correlation between egg deposition and worm elimination. *American Journal of Tropical Medicine & Hygiene* 27, 957-965.
15. Dean, D. A., Minard, P., Stirewalt, M. A., Vannier, W. E. and Murrell, K. D. 1978. Resistance of mice to secondary infection with *Schistosoma mansoni*. I. Comparison of bisexual and unisexual infections. *American Journal of Tropical Medicine & Hygiene* 27, 951-956.
16. Dorsey, C. H. and Stirewalt, M. A. 1977. *Schistosoma mansoni*: localization of calcium-detecting reagents in electron-lucid areas of specific preacetabular gland granules. *Zeitschrift fur Parasitenkunde* 54, 165-173.
17. Hussain, R., Vannier, W. E. and Murrell, K. D. Submitted. *Schistosoma mansoni*: hypersensitivity to antigens. II. Gel filtration studies of allergens from adult worms. *Experimental Parasitology*.

18. Landsperger, W. J., Stirewalt, M. A. and Dresden, M. H. submitted. Purification and properties of a proteolytic enzyme from *Schistosoma mansoni* cercariae. *Biochemical Journal* (Br.).
19. Lewis, F. A. and Wilson, E. M. 1981. Strain differences in lymphocyte responses and *in vitro* suppressor cell induction between *Schistosoma mansoni*-infected C57Bl/6 and CBA mice. *Infection and Immunity* 32, 260-267.
20. Lewis, F. A., and Wilson, E. M. in press, *Schistosoma mansoni*: splenic lymphocyte responses of mice following an initial exposure to highly irradiated cercariae. *Experimental Parasitology*.
21. Minard P., Murrell, K. D. and Stirewalt, M. A. 1977. Proteolytic, antigenic and immunogenic properties of *Schistosoma mansoni* cercarial secretion material. *American Journal of Tropical Medicine and Hygiene* 26, 491-499.
22. Minard, P., Murrell, K. D. and Stirewalt, M. A. 1978. Penetration enzymes of *Schistosoma mansoni* cercariae. *Proceedings of the International Conference on Schistosomiasis, Cairo, Egypt, Vol. II, p. 599-602.*
23. Minard, P., Dean, D. A., Vannier, W. E., and Murrell, K. D. 1978. Effect of immunization on migration of *Schistosoma mansoni* through lungs. *American Journal of Tropical Medicine Hygiene* 27, 87-93.
24. Minard, P., Dean, D. A., Jacobson, R. H., Vannier, W. E., and Murrell, K. D. 1978. Immunization of mice with Cobalt-60 irradiated *Schistosoma mansoni* cercariae. *American Journal of Tropical Medicine & Hygiene* 27, 76-86.
25. Murrell, K. D., Stirewalt, M. A., and Lewis, F. A. 1979. *Schistosoma mansoni*: vaccination of mice with cryopreserved irradiated schistosomules. *Experimental Parasitology* 48, 265-271.
26. Murrell, K. D., Clark, S. S., Dean, D. A., and Vannier, W. E. 1979a. *Schistosoma mansoni*: immunization of cynomolgus monkeys by injection of irradiated schistosomula. *Experimental Parasitology*. 48, 415-420.

27. Murrell, K. D., Clark, S. S., Dean, D. A., and Vannier, W. E. 1979b. Influence of mouse strain on induction of resistance with *Schistosoma mansoni* cercariae. *Journal of Parasitology* 65, 829-831.
28. Murrell, K. D., Clark, S. S., Dean, D. A., and Vannier, W. E. 1979c. *Schistosoma mansoni*: immunization of cynomolgus monkeys by injection of irradiated schistosomula. *Experimental Parasitology* 48, 415-420.
29. Murrell, K. D., Minard, P., Vannier, W. E. and Dean, D. A. 1978. Immunization against schistosomes. *Proceedings of the International Conference on Schistosomiasis, Cairo, Egypt, Vol. II, p. 545-555.*
30. Murrell, K. D., Taylor, D. W., Vannier, W. E. and Dean, D. A. 1978. *Schistosoma mansoni*: analysis of surface membrane carbohydrates using lectins. *Experimental Parasitology* 46, 247-255.
31. Murrell, K. D., Vannier, W. E., Minard P. and Schinski, V. D. 1977. *Schistosoma mansoni*: extraction and partial characterization of membrane antigens using an assay based on competitive inhibition of human antibodies binding to schistosomes. *Experimental Parasitology* 41, 446-463.
32. Schinski, V. D., Clutter, W. C. and Murrell, K. D. 1976. Enzyme and ¹²⁵I- labelled anti-immunoglobulin assays for the study of schistosome antigens and human immunological responses. *American Journal of Tropical Medicine and Hygiene* 25, 824-831.
33. Schinski, V. D., Clutter, W. G. and Murrell, K. D. 1978. Labelled antiglobulin assays for the study of schistosome antigens and human immune responses. *Proceedings of the International Conference on Schistosomiasis, Cairo, Egypt, Vol. II, p. 583-585.*
34. Stek, M. J. and Kassim, O. O. in press. Specific circulating schistosomal antigens and antibodies in Nigerian children. *Tropical Geographic Medicine.*
35. Stek, M., Dean, D. and Clark, S. S. in press. Attrition of schistosomes in an irradiation-attenuated-cercaria immunization model of *Schistosoma mansoni*. *American Journal of Tropical Medicine and Hygiene.*

36. Stek, M., Minard, P., Dean, D. and Hall, J. E. 1981. Immunization of baboons (*Papio anubis*) with *Schistosoma mansoni* cercariae attenuated by gamma-irradiation. *Science* 212, 1518-1520.
37. Stirewalt, M. A., 1975. Hemolymph Components in Invertebrate Immunity, In "Invertebrate Immunity" (K. Marmarosch and R. E. Shope, eds.), Academic Press Inc., New York, New York, pp. 289.
38. Stirewalt, M. A. 1978. Quantitative collection and proteolytic activity of preacetabular gland enzyme(s) of cercariae of *Schistosoma mansoni*. *American Journal of Tropical Medicine & Hygiene* 27, 548-553.
39. Stirewalt, M. in press. *Schistosoma mansoni*: conditions contributing to maximal cercarial harvests. *Journal of Parasitology*.
40. Stirewalt, M. and Lewis, F. A. 1981. *Schistosoma mansoni*: effect of rotifers on cercarial output, motility and infectivity. *International Journal for Parasitology* 11, 301-308.
41. Stirewalt, M. A., Lewis, F. A., and Murrell, K. D. 1979. *Schistosoma mansoni*: cryopreservation of schistosomules. *Experimental Parasitology* 48, 272-281.
42. Taylor, D. W., Hayunga, E. G. and Vannier, W. E. 1981. Surface antigens of *Schistosoma mansoni*. *Molecular and Biochemical Parasitology* 3, 157.
43. Vannier, W. E., Hussain, R. and Murrell, K. D. 1978. Schistosome allergens. *Proceedings of the International Conference on Schistosomiasis, Cairo, Egypt, Vol. II*, p. 587-596.
44. Wistar, R., Murrell, K. D., Lewert, R. M., Yogore, M. G., Cole, C. and Clutter, W. G. 1976. The development of anti-immunoglobulin antibodies in rhesus monkeys repeatedly exposed to *Schistosoma japonicum*. *American Journal of Tropical Medicine & Hygiene* 24, 632-637.

INDEX OF ORAL PRESENTATIONS OF WORK SUPPORTED
COMPLETELY OR IN PART BY THIS CONTRACT

1. Cousin, C. Cunningham, K. and Solano, L. *Schistosoma mansoni*: tegumental changes in cercariae as they develop into adult worms. 8th Annual Minority Biomedical Support Symposium, Georgetown University, Washington, D. C. April, 1980.
2. Cousin, C., Solano, L. and Cunningham, K. *Schistosoma mansoni*: early morphological changes in *in vivo* schistosomules following penetration. 8th Annual Minority Biomedical Support Symposium, Georgetown University, Washington, D. C. April, 1980.
3. Cousin, C. Stirewalt, M. and Dorsey, C. H. Comparative transformation of *in vivo* and *in vitro* schistosomules, Program and Abstracts of the 52nd Annual Meeting of the American Society of Parasitologists, 6-11, Nov. 1978, Chicago, Ill.
4. Dean, D. A. 1977. Role of *Schistosoma mansoni* eggs in resistance of mice to reinfection. 61st Annual Meeting, Federation of American Societies for Experimental Biology, Chicago, Ill. 3-8 April, 1977.
5. Dean, D. A. and Bukowski, M. A. Ability of various developmental stages of *Schistosoma mansoni* to serve as inducers and targets of immunity. American Society of Tropical Medicine and Hygiene Meeting, Atlanta, Georgia, 5-7 November 1980.
6. Dean, D. A., Murrell, K. D., Minard, P. and Vannier, W. E. 1976. Evidence for mast cell requirement in immunity of mice to schistosome infection. Fed. Proc. 35: 228.
7. Dean, D. A., Stirewalt, M., Murrell, K. D. and Vannier, W. E. Role of *Schistosoma mansoni* eggs in resistance of mice to reinfection. FASEB Program, 5 April, 1977, Chicago.
8. Dean, D. A., Stirewalt, M. A., Murrell, K. D. and Vannier, W. E. 1977. Mechanisms involved in immune destruction of schistosomes. 52nd Annual Meeting, American Society of Parasitologists, Las Vegas, Nevada, 14-19 August, 1977.
9. Dean, D. A., Minard, P. Stirewalt, M., Murrell, K. D. and Vannier, W. E. Role of eggs in mouse resistance to reinfection with *Schistosoma mansoni*. Program and Abstracts of the 52nd Annual Meeting of the American Society of Parasitologists p. 44, 14 August, 1977.

10. Hayunga, E. G. and Vannier, W. E. Evidence of heterologous antigens in *Schistosoma haematobium* and *Schistosoma mansoni*. American Society of Tropical Medicine and Hygiene Meeting, Atlanta, Georgia, 5-7 November 1980.
11. Lewis, F. A. and Wilson, E. M. Murine spleen cell suppressor activity induced by *Schistosoma mansoni* cercarial membrane antigens. Federation Proceedings 39 (3), 1980.
12. Lewis, F. A., Campbell, D. L. and Stirewalt, M. Murine cellular reactivity to preacetabular gland contents of *Schistosoma mansoni*. American Society of Tropical Medicine & Hygiene, 7-11 Nov. 1978, Chicago, Ill.
13. Lichtenberg, F. von, Byram, J. E., Lewis, F. A. Stirewalt, M. Pathology of a live anti-schistosomal vaccine. Sixteenth Joint Conference on Parasitic Diseases, The U. S.-Japan Cooperative Medical Science Program, 13 July 1981, Bethesda, Maryland.
14. Minard, P., Murrell, K. D. and Stirewalt, M. A. Penetration enzymes of *Schistosoma mansoni* cercariae. International Conference on Schistosomiasis, Cairo, Egypt. October 18-25, 1975.
15. Murrell, K. D., Vannier, W. E. and Minard, P. 1977. Extraction and partial purification of surface-associated antigen of *Schistosoma mansoni*. Federation Proceedings. 36 (3): 1058.
16. Murrell, K. D., Clark, S. S., Weaver, N., Stirewalt, M., Vannier, W. E. and Dean, D. A. Immunization with attenuated *Schistosoma mansoni* schistosomules, Fourth International Congress of Parasitologists, Warsaw, Poland, 19-26 Aug. 1978.
17. Smith, A. H. and Vannier, W. E. Production of a hybrid cell line secreting reaginic antibody to adult *Schistosoma mansoni* freeze-thaw antigen by cell fusion. American Society of Tropical Medicine and Hygiene Meeting, Atlanta, Georgia, 5-7 November 1980.
18. Stek, M., Jr. Circulating antigens, antibodies, and immune complexes in experimental and clinical schistosomiasis. 4th International Congress of Immunology, Abstract 19. 6. 31, 1980.

19. Stek, M., Minard, P. and Dean, D. Successful immunization of baboons (*Papio anubis*) with gamma-irradiation-attenuated *Schistosoma mansoni* cercariae. Twenty-ninth Annual Meeting of the American Society of Tropical Medicine and Hygiene, Atlanta, Georgia, 5-7 November, 1980.
20. Stek, M., Stirewalt, M., Lewis, F. and Schinski, V. Immunization of cynomolgus monkeys with cryopreserved irradiation-attenuated *Schistosoma mansoni* schistosomula. Proceedings of the International Symposium on the Use of Nuclear Techniques in the Study of Parasitic Diseases of Man and Animals 1981.
21. Stirewalt, M. A. Biochemical aspects of secreted enzyme(s) of *Schistosoma mansoni*. Annual Meeting of the American Society of Parasitologists, 10-14, November, 1975, New Orleans, La. (Abstract).
22. Stirewalt, M. A. 1976. Activity of enzymes secreted by cercariae of *Schistosoma mansoni*. Program and Abstracts of the 51st Meeting of the American Society of Parasitologists (Abstract).
23. Stirewalt, M. and Cousin, C. When is a Schistosomule? Annual Meeting of the American Society for Tropical Medicine and Hygiene, 17 Nov. 1981.
24. Stirewalt, M. and Lewis, F. A. Effect of rotifers on schistosome cercariae. Program and Abstracts of the 52nd Annual Meeting of the American Society of Parasitologists, 6-11 Nov. 1978, Chicago, Ill.
25. Stirewalt, M., Leef, J. L. and Lewis, F. A. Evaluation of cryopreservation techniques for *Schistosoma mansoni* schistosomules. Program and Abstracts of the 56th Annual Meeting of the American Society of Parasitologists, p. 37, 11 August, 1981.
26. Stirewalt, M., Murrell, K. D., and Lewis, F. A. Cryopreservation of schistosomules of *Schistosoma mansoni*. American Society of Tropical Medicine & Hygiene, 7-11 Nov. 1978, Chicago, Ill.
27. Vannier, W. E., Hussain, R., Murrell, K. D., Dean, D. A. and Attallah, A. M. Immediate hypersensitivity and protective immunity in schistosomiasis. United States-Japan Cooperative Medical Science Program, New Orleans, Louisiana, August 1979.

CONCLUSIONS

1. It is possible to produce in quantity parasite materials for research on *Schistosoma mansoni*. Our production has been as follows: 1 to 2 million cercariae per day; 30,000 adult worms per week; 200,000 schistosomules per day; other materials as needed.
2. Requirements for such production are: money, time, space, personnel and constant monitoring.
3. Intraspecific strains of the *Biomphalaria glabrata* and *Schistosoma mansoni* in the model association vary in productivity level. A suitable model must be set up.
4. Base line parameters and standards were: 2500 or more cercariae/snail/day; 100 or more adult worms/mouse; snail infection rates of 90% or more; death rates of infected snails 10% or less biweekly.
5. Exposure level of snails need not exceed 6 to 8 miracidia/snail, since only 2 or 3 primary sporocysts appear to develop concurrently in a snail.
6. Temperature and light changes stimulate cercarial emergence. Shedding snails should be maintained in the dark at about 27 C and placed in strong light at about 32 C for cercarial collection.
7. Shedding snails must be kept free of colonizing rotifers.
8. Proteolytic penetration enzymes can be collected from cercariae by holding them at 37 C over a substrate of skin surface lipid or its active ingredients, linolenic or linoleic acids.
9. These secreted enzymes are antigenic, but the antibodies which are stimulated are not protective.

MAJOR ACCOMPLISHMENTS

1. Maintenance of an intraspecific *Schistosoma mansoni-Biomphalaria glabrata* strain model which produces a constant high level of research material.
2. Recorded cercarial output, miracidial infectivity and snail death rates from 1976 to present as base line information for our snail-schistosome model.
3. Development of the optimal cercarial production system which relies on (a) 2 groups of infected snails from each of which cercariae are harvested twice weekly; (b) design of the 27 C dark maintenance and 32 C lighted cercarial harvest conditions.
4. Report of differences in cercarial productivity by two intraspecific strains of the *Schistosoma mansoni-Biomphalaria glabrata* model.
5. Discovery that colonization of infected snail shells with rotifers decreases cercarial output and inhibits cercarial motility.
6. Design of jet spray washing of rotifers from snails.

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