

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 8/31/88

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18
FROM: AID/SCI, Victoria Ose *VO*
SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 3, F-43

Attachment

- PR 19 Sept 84 - 31 Dec 84
- PR 1 Jan 85 - 1 July 85
- Visit Sept 18 - Oct 11, 1985
- PR 1 July - 31 Dec 85
- PR 1 Jan 86 - 31 Dec 86
- PR 31 Dec 86 - 30 Sept 87
- trip Jan - July 86
- PR 1 Oct 87 - 30 June 88

X

PN-ABA-921
3 F-43

TO: Office of the Scientific Advisor, U.S. Agency for International Development

FROM: Edward H. Michelson, Ph.D., Uniformed Services University of the Health Sciences

SUBJECT: Progress report for PSTC grant (#BST-5542-P-DZ-4259-00), "Characterization of Zambian Host-Snail Populations with Reference to Their Role in the Transmission of Schistosomiasis". For the period 1 October 1987 to 30 June 1988.

DATE: 15 July 1988

Since the last reporting period, Dr. Bailey's term as postdoctoral fellow expired and he has left the project. No replacement is anticipated at this time. In an effort to pursue the leads that resulted from contacts made at the University of Zambia during the trip to Lusaka last year, a second trip was undertaken from 5 May to 23 May 1988. This trip proved most successful and suggests that a fruitful collaboration might be possible. In the Department of Biology at the School of Natural Sciences, I have been offered both laboratory space and an area in the animal house to rear snails. More importantly, Miss Likezo Mungombe, who has been appointed as the new parasitologist on the faculty has expressed an interest in collaborating on problems of mutual interest. Ms. Mungombe has also expressed an interest in having me help her prepare a course in parasitology for undergraduate and graduate students. My major contact with the Department of Biology is through Dr. Denys Morgan, Professor of Microbiology and director of their graduate program. Dr. Howard, Professor of Ecology and Director of the Kafue Basin Project, has also extended an invitation to accompany me on field trips and use his jeep for transport. Likewise, the Department has transport that can be rented at a reasonable fee. This appears to be the most promising turn of events that has occurred during the past 18-24 months. Since the extension of the project runs through September, I have requested (June 30, 1988) a further extension with no increase in funding. As noted previously, there was no way to spend the allotted funds for the purpose they were granted and, consequently, I have about \$50,000 in my budget which should suffice for several more years of work in Zambia. If my extension is approved, I will spend 5-6 weeks in Zambia in the field from late September through October. This time was selected to avoid the beginning of the rainy season, during which field work is difficult or, in some places, impossible.

A manuscript reviewing the past and present status of schistosomiasis in Zambia has been completed and will shortly be submitted for publication. Dr. Bailey, likewise, is putting the finishing touches on another manuscript concerning our studies on snail lectins. It is planned that if the field studies are possible, susceptibility studies will be initiated in Zambia on various populations of Biomphalaria pfeifferi and Bulinus globosus. In these studies we plan to use both a bioassay system and look for corresponding electrophoretic markers.

3 F 43

TO: Scientific and Technical Committee, TDRC, Ndola, Zambia

FROM: Professor E.H. Michelson

SUBJECT: Report of trip to Zambia (June-July 1986) in conjunction with the project entitled "Identification and Characterization of Snail Intermediate Hosts of Schistosomiasis in Zambia"

DATE: 1 April 1987

General Comments: The investigator arrived in Lusaka on 17 June 1986 and spent until 19 June 1986 in Lusaka at the University Hospital and the Biology Department of the University of Zambia. Arrived in Ndola on the afternoon of 19 June. I should note that in many respects this was the least fruitful trip that I had taken to Zambia, both from the point of view of accomplishments and expectations of work to be done. Prior to my visit, I had corresponded and plans were made for collections and studies to be made in the south of the country. First to Kifue and Chilanga, and then to Mazubuka and Livingstone. Next via Lusaka to Siavonga, then back to collect and work in the Ndola area. Unfortunately, due to a variety of reasons and events, contacts were not notified, hotel reservations were not made, and the people at the Estate in Mazubuka did not know of our plans and had no room for us. Miss Mukange, who accompanied me, as well as the field collector (Boston) and the driver (Lester) were most helpful, but the situation was not of their making and there was little they could do. Consequently, we could not stay or work at the Sugar Estate since they were booked for a week. On returning from the field trip to Ndola, I could not stay at the hotel since this was fair time and arrangements were supposed to have been made for me to stay at the Guest House. This would have been fine if they had been notified in advance; they were not. In addition, there was no provision for meals, even though I made arrangements for such. It would have been prudent to have advised me before I came that housing at that time was difficult, and some other arrangements could have been made. I would also note that reservations were not made at Siavonga, although we did manage to find a place to stay. All in all, it would appear that, somehow or some way, things were not done, plans were not made and both peoples' time and money were spent with little to show for it.

Studies: In spite of the various obstacles, we did manage to make a few collections in the Livingstone area; however, these were limited since we had no local contact and many sites were undoubtedly missed. From Livingstone we went

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to Mazabuka, but since no accommodations were made, we continued on to Lusaka and the next day on to Siavonga. At Siavonga we had good collecting in the various inlets of the lake and collected a number of snails. We also made arrangements to examine about 18 students at the local hospital. Single urine specimens were taken, dipsticks for hematuria were run, and blood was collected from fingerpricks and placed on filter paper discs to be eluted later and used in an ELISA test. It should be noted that on examination of one urine slide per student, 50% were positive for eggs of S. haematobium and one student had S. mansoni eggs in the urine as well. The dipstick test for hematuria showed a high degree of correlation with positive egg samples, but also was positive in some individuals in which eggs were not detected. This is not surprising and an 85% correlation was noted. Our ELISA test, designed to detect antibodies, performed well and all those with eggs in the urine were positive; however, we got positive reactions with six others that had no eggs. This is to be expected as they may have S. mansoni and stools were not examined. According to the local physician, S. mansoni infection is extremely common in this area. Upon returning to Ndola via Lusaka, snails were mailed from the local Post Office in Lusaka. Unfortunately, these never arrived in the United States.

One high point of the trip was a visit arranged by Dr. Sukwa and Miss Mukanga to see Dr. Shehata at the National Council for Scientific Research Laboratories in Kitwe. Dr. Shehata was doing excellent work on the population dynamics of snails in the Mutenda River and in studying the plant molluscicide Phytolacca. I obtained some snails from him and did manage to get these back to Washington.

Comments: Although the last trip was not considered very successful, I am still optimistic enough to plan to return in the coming year. Our Department still has hopes of continued collaborative projects in Zambia, both in schistosomiasis and in other infectious diseases. I will probably contact Dr. Sukwa and try to see if July and August will be suitable times. I picked this time since we have a project with the University Hospital in Lusaka and I plan to accompany some of my colleagues at that time and would like to stay and do some additional work. I would note that I can visualize a joint project at Siavonga for the future and also in some other areas. I hope on my next trip to collect in the north at the Lake Bangweleulu region.

TO: Office of the Scientific Advisor, U.S. Agency for International Development

FROM: Edward H. Michelson, Ph.D., Uniformed Services University of the Health Sciences

SUBJECT: Progress report for PSTC grant (#BST-5542-P-DZ-4259-00), "Characterization of Zambian Host-Snail Populations with Reference to Their Role in the Transmission of Schistosomiasis". For the period 31 December 1986 to 30 September 1987.

DATE: 24 September 1987

This report covers the third and final official year of the grant. Unfortunately, progress has been sharply curtailed as a consequence of the worsening financial crisis facing Zambia and field work per se is practically impossible. My field collaborator, Miss Mukange, resigned from the TDRC in Ndola and it has not been possible to find another individual interested in going into the field. This is understandable, as local crime has increased and in many areas it is not safe to venture into the field, particularly in the Copperbelt Region. Transport for field work has also been difficult and the number of vehicles at the TDRC has been reduced to one or two. Since funding to the TDRC has been markedly reduced by the WHO/TDR Program, this situation is not likely to improve.

A short trip was made to Zambia from 27 July to 16 August in an effort to assess the situation and to see if alternative arrangements could be made. Dr. Sukwa, my collaborator, is leaving the TDRC and will be attending a graduate program at the Johns Hopkins University for three years. He has been unable to find a replacement for Miss Mukange and, at present, there is no one in Ndola or, for that matter, anywhere in Zambia doing epidemiological or field studies on schistosomiasis. The snail laboratory that I set up is idle and no work is being pursued. While in Zambia, I attempted to make contacts at both the Veterinary School and the School of Natural Sciences, University of Zambia, in Lusaka. This proved somewhat promising, as Dean Thomas of the Veterinary School and Professor Morgan at the School of Natural Sciences, were both interested in a possible collaboration.

Earlier in the year (8 May 1987), I had contacted Dr. William Oglesby at AID in Washington to inquire about extending my grant without additional funding. I have considerable funds still available, since it was not possible to spend the funds in support of either field work or helping my collaborators. I have just recently been informed of the extension and will now pursue the possibility of working in Lusaka.

In Bethesda, we are finishing up our studies on the lectins in Zambian snail hosts and, hopefully, will have a paper ready for publication. Our results suggest that the bulinids differ considerably from the biomphalarid snail hosts in having fewer lectins and these have affinities for binding sites on mammalian RBCs which are revealed only after enzyme modification and/or by the addition of suitable cations. Dr. Bailey also completed some studies on genetic inheritance of banding patterns in species of Bulinus. Dr. Bailey will be leaving after the first of the year, as his 3-year postdoctoral period will have been completed.

3 F43

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FROM: Edward H. Michelson, Ph.D., Uniformed Services University of the Health Sciences

SUBJECT: Progress report for PSTC grant (#BST-5542-P-DZ-4259-00), "Characterization of Zambian Host-Snail Populations with Reference to Their Role in the Transmission of Schistosomiasis". For the period 1 January 1986 to 31 December 1986.

DATE: 5 January 1987

This report, as noted above, cover the year 1986. No semi-annual report was submitted as I was in Zambia at the time (5/15/86-7/14/86) and I later neglected to submit it.

Although efforts to separate closely related South American snail hosts were successful by means of electrophoresing snail hemoglobin in agarose gels, difficulties were encountered with African species of Bulinus. Initial runs with snails from sites at Ndola and Kampumba were promising; however, when populations from additional sites were studied, considerable intraspecific variation was noted. One publication, supported only marginally by this grant, has been published. Dr. Bailey is continuing studies on lectins in the Zambian species of Bulinus and has demonstrated that, contrary to earlier reports in the literature, these substances do occur in Zambian species of Bulinus. In the micro-hemagglutinin system we employ, these substances have been detected when human and various species of mammalian blood cells have been used as receptors. Detection of the lectins, in most instances, requires enzyme treatment of the blood cells to reveal hidden receptor sites and often the presence of either calcium or magnesium ions. When present, the lectins are found in the hemolymph, albumin gland and eggs.

Several shipments of snails were received during the year; however, the continual depression in the Zambian economy has restricted transport and field work has been drastically curtailed. After a protracted correspondence, a field visit to Zambia was finally initiated and I arrived in the country on June 17, 1986. Unfortunately, this trip was riddled with minor disasters of one type or another and was far from satisfactory. Dr. Sukwa could not accompany us on the trips to the field as there had been a shift in personnel at the TDRC and he has been made Acting Director of Epidemiology. The original itinerary that I had planned would have included field work at the sugar estate at Mazabuka, visits to the Choma area, collecting at Livingstone, and then on to Lake Kariba and Siavonga. Although plans were made months in advance by correspondence, when I arrived nothing had been done with regard to our contacts or field housing. Consequently, it proved impossible to work either at Choma or at Mazabuka. Some field studies were done around the Livingstone area, but these were less than satisfactory. Prior to my visit, there had been some difficulties between South Africa and Zambia; consequently, working in the field was difficult and I had to obtain permission from the local police in each area in the South in which I worked. This was obtained, but did hinder our work. Our studies in the Siavonga area and on Lake Kariba were more successful. The region is hyperendemic for schistosomiasis and both S. mansoni and S. haematobium are

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present. We obtained permission to examine the urine from about 25 randomly selected students at the elementary school in Siavonga. Of 18 which submitted urine specimens, eggs were found in 9 (50%), and one individual had S. mansoni as well as S. haematobium eggs. In addition to a urine examination for eggs, the urines were compared for hemoglobin concentration by a dipstick method and blood obtained by fingerprick, placed on filter paper discs and later used to test an ELISA technique developed by Dr. Gene Hayunga. The dipstick test showed a high degree of correlation to egg-positive individuals as did the ELISA. Several hundred snails were collected, many which appeared to be infected, and sent by Express Mail to Bethesda from Lusaka. Again, disaster struck, for these snails never were received in our Bethesda laboratory.

After returning to Ndola, I had an opportunity to go to Kitwe for several days and observed the work of Dr. Shehata at the laboratories of the National Council for Scientific Research. Dr. Shehata is working on plant molluscicides and has a very impressive laboratory. I received several strains of local snails from him. Unfortunately, Dr. Shehata, who is Egyptian, is only on temporary assignment to Zambia and will leave before too long.

I should note that I have been informed by Miss Mukange that she may be leaving the TDRC. This is a direct result of the financial crisis in Zambia, as her reasons for leaving are poor housing provided by the TDRC and the need for a higher salary. If she should leave, this would be a serious setback to the project, as she is one of the few, if not the only individual in Zambia, working on the snail hosts of schistosomes. In a similar vein, I should note that transport from the TDRC is now severely limited and difficult to obtain for field use. Due to deteriorating conditions, it was not possible to arrange for another trip to Zambia later in the year.

Laboratory studies have continued with the material on hand, but we have not been able to obtain additional material from Zambia. Dr. Bailey is beginning some studies on the genetics and breeding behavior of B. tropicus.

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SUBJECT: Progress report for PSTC grant (#BST-5542-P-DZ-4259-00), "Characterization of Zambian Host-Snail Populations with Reference to Their Role in the Transmission of Schistosomiasis". For the period 1 July 1985 to 31 December 1985.

DATE: 3 January 1986

During this reporting period a second trip was made to Zambia (9/15-10/18/88) by Dr. Bailey and me. The goals of the trip were threefold: (1) to obtain snail-hosts from additional Zambian sites to establish additional colonies; (2) to obtain, if possible, infected snails and attempt to establish life cycles of both S. haematobium and S. mansoni; and (3) to test a micro-agrose electrophoretic technique to separate snail species. To some extent, all of the objectives were accomplished.

Trials with the agarose electrophoretic system demonstrated that rapid determinations could be made in differentiating between Bulinus tropicus and B. globosus. The technique promises to be of value with small specimens which are difficult to separate by morphological methods. This technique was demonstrated to various members of the TDR staff. Laboratory studies are continuing in Bethesda in an effort to see if the technique can differentiate between B. globosus and B. africanus. There are both important snail hosts which are difficult to separate from one another by standard taxonomic methods.

Through the efforts of Dr. Sukwa, we were able to visit the Zambian Sugar Company's Nakambala Estate at Muzubuka. We were briefed on the state of schistosomiasis on the estate by Dr. Sukthanker, a resident physician, and by the chief health officer, Mr. Simanga. Although data is skimpy and somewhat biased, in that it is based only on those reporting to the clinic with a defined illness, it would appear that since 1983 there has been an increase in the prevalence of schistosomiasis on the estate. During the period of January to August 1985, 72 cases of S. haematobium and 17 cases of S. mansoni have been confirmed. Admittedly, this may be an indication of a larger unrecognized problem. Storage ponds, irrigation canals, and fish ponds were sampled for the presence of snails. The source of the water for these sites is the Kafue River and water is continually drawn from this source and supplied to storage ponds by hydraulic pumping stations. Consequently, host snails are circulated throughout the open irrigation system. Host snails that were collected at various sites in canals and storage ponds were as follows: Biomphalaria pfeifferi, Biomphalaria rhodesiense, Bulinus tropicus, and Bulinus africanus/globosus. In addition to the host snails, numerous specimens of Lanistes, Lymnaea, Bellamyia and Cleopatra were obtained. Canals, fish ponds, and one storage pond were found to be the major sites for host snails; however, with an open system such as this, they may be expected to occur anywhere in the system. No infected snails were detected; however, the mortality was high among bulinids and some infected snails might have escaped detection. The potential for transmission on the estate is very high and water contact by the local population was evident.

A second field trip was taken to the Kampumba field station. Collections were made at the designated stations that are routinely collected at Kampumba field station. Collections were made at the designated stations that are routinely collected at by Miss Mukange and, also, outside the usual collecting area. Infected snails were found at several sites, both Bulinus globosus and Biomphalaria pfeifferi, and attempts are now being made to establish life cycles at USUHS.

Additional training was provided to the TDRC staff in the use of a HACH field laboratory kit to determine water quality in aquatic habits. The kit was left in the snail laboratory for use by the staff.

It should be noted that the field station at Kampumba has deteriorated, with respect to housing, laboratory facilities, and potable water. There is some question if this facility will be maintained or abandoned. Likewise, it is worth noting that the economy has taken a marked downward turn, with an increase in local crime, a lowering of morale in TDRC staff and restriction in obtaining transport.

Our studies in Bethesda have demonstrated that the agarose technique is of value in separating sibling species of Neotropical planorbids. A paper is being prepared for publication. Some preliminary studies have also been done on lectin patterns in Zambian bulinids.

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REPORT OF A VISIT TO THE TROPICAL DISEASE RESEARCH CENTRE
NDOLA, ZAMBIA, FROM SEPTEMBER 18 TO OCTOBER 11, 1985

TO: Dr. M. Mukunyandela, Director, Tropical Disease Research Center, Ndola, Zambia

PREPARED BY: Dr. E.H. Michelson, Professor of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, Bethesda, Maryland, U.S.A.

DATE: November 1, 1985

ACKNOWLEDGEMENTS: We wish to acknowledge the cooperation and hospitality extended to us during both the present trip and our previous visit to the TDRC by the Director, Dr. Mukunyandela, and by the entire staff. In particular, we wish to note the unstinting efforts of Dr. Thomas Y. Sukwa and Miss Harriet Mukange, whose help permitted us to accomplish the goals of the present trip. Likewise, we wish to note the efforts extended to us by Mr. John Morrison in obtaining laboratory equipment and field supplies.

BACKGROUND: The present trip was funded by a grant from the PSTC Program of USAID. This was the second trip by Dr. Michelson and the first by Dr. Bailey. The objectives of the grant proposal was to study and characterize the snail hosts of schistosomiasis in Zambia, with a view towards defining their role in disease transmission. The parameters to be studied include: (a) level of susceptibility of various host snail populations; (b) morphologic and electrophoretic discrimination of species; (c) feasibility of developing a probe to determine the species of cercariae shed from infected snails; and (d) to offer assistance and training opportunities in medical malacology to Zambian colleagues.

During the first trip to Zambia, Dr. Michelson, Dr. Sukwa, and Miss Mukange collected host snails in the environs of Ndola, at the Kampumba Field Station, and at Mutenda. Collections of snails were transported to USUHS and several laboratory colonies were established. Subsequently, we received snails from Miss Mukange to be used in experimental studies. Details of this trip have been reported in writing (September 9, 1985) to the Scientific and Technical Committee of TDRC.

ACTIVITIES FROM SEPTEMBER 18 THROUGH OCTOBER 11, 1985: During this trip to the TDRC, Dr. Michelson was accompanied by a post-doctoral fellow, Dr. Jim Bailey. Dr. Bailey is a trained malacologist with experience in Swaziland and Egypt and has particular expertise in the cultivation and biology of bulinid snails. The goals of the present trip were as follows: (1) To collect host snails from various localities in Zambia and obtain colonies to be established in the laboratory; (2) Obtain, if possible, infected snails and attempt to establish life cycles of S. mansoni and S. haematobium; and (3) Test a micro-agarose electrophoretic technique to separate snail species. To some extent, all of the objectives were accomplished.

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Trials with the agarose electrophoretic system demonstrated that rapid determinations could be made to separate Bulinus tropicus and B. globosus from one another. The technique promises to be of particular use in the separation of small specimens. Studies are being continued on collected specimens to determine if B. globosus can be differentiated from B. africanus. If this becomes possible, it will be a major advance in the identification of two important snail hosts from Central Africa.

Through the efforts of Dr. Sukwa, we were able to visit the Zambian Sugar Company's Nakambala Estate at Muzubuka. We were briefed on the state of schistosomiasis on the estate by Dr. Sukthanker, a resident physician, and by the chief health officer, Mr. Simanga. Although data is skimpy and somewhat biased in that it is based only on those reporting to the clinic with a defined illness, it would appear that since 1983 there has been an increase in the prevalence of schistosomiasis on the estate. During the period of January to August 1985, 72 cases of S. haematobium and 17 cases of S. mansoni have been confirmed. Admittedly this may be an indication of a larger unrecognized problem. Storage ponds, irrigation canals, and fish ponds were sampled for the presence of snails. The source of the water for these sites is the Kafue River and water is continually drawn from this source and supplied to storage ponds by hydraulic pumping stations. Consequently, host snails are circulated throughout the open irrigation system. Host snails that were collected at various sites in canals and storage ponds were as follows: Biomphalaria pfeifferi, Biomphalaria rhodesiense, Bulinus tropicus, and Bulinus africanus/globosus. In addition to the host snails, numerous specimens of Lanistes, Lymnaea, Bellamya and Cleopatra were obtained. Canals, fish ponds, and one storage pond were found to be the major sites for host snails; however, with an open system such as this, they may be expected to occur anywhere in the system. No infected snails were detected; however, the mortality was high among bulinids and some infected snails might have escaped detection. The potential for transmission on the estate is very high and water contact by the local population was evident.

A second field trip was taken to the Kampumba field station. Collections were made at the designated stations that are routinely collected at by Miss Mukange and, also, outside the usual collecting area. Infected snails were found at several sites, both Bulinus globosus and Biomphalaria pfeifferi, and attempts are now being made to establish life cycles at USUHS.

RECOMMENDATIONS AND OBSERVATIONS: It is recognized that it may be somewhat presumptuous for someone who has had such limited experience in Zambia to offer meaningful recommendations. However, I have been asked for such from both Dr. Munkunyandela and Dr. Wurapa and offer the following comments with considerable reservations.

First, I would like to offer my views on the role of Medical Malacology at the TDEC. The Centre should serve as a reference and resource facility for the country at large and possibly for South-Central Africa. Synoptic collections should be collected and both local and area-wide keys should be prepared. The interests of the Centre should extend beyond schistosomiasis and include all

molluscs of medical, veterinary, and wildlife importance. Initial research activities should deal with population dynamics, identification, susceptibility, and transmission potential of the various host snails. Snail colonies should be expanded and a ready source of infected snails should be maintained. To accomplish this, more space will be needed for the colonies and a dependable air source and water supply required. A small compressor would supply all the aeration that is required and simplify the task. Personnel will require additional training in various malacological techniques. I believe that the potential for this type of facility is great and will enhance the mission of the Centre. The malacology unit should be integrated with schistosomiasis projects and be assigned a specific adjunct function to aid in the understanding of transmission and to formulate means for control. Collecting snails without a definite objective is of little value and contributes almost nothing of a practical nature.

The Kampumba Field Station and its present status is well known to both the administration and staff and the benefit of more discussion is moot. It suffices to note that in its present state it is not conducive for housing personnel for long-term studies, particularly if laboratory support is required. While the station offers a definite advantage to those engaged in trypanosomiasis research, its continued use for schistosome studies is open to debate. Studies on the epidemiology of schistosomiasis, control studies, malacology and chemotherapy can surely be done at areas less than 900 km from Ndola.

The Sugar Estate at Muzubuka appears to be a promising site to study the epidemiology of schistosomiasis. It has the advantages of a circumscribed population that can be censused and surveyed, one in which chemotherapy can be evaluated and followup guaranteed, snail hosts which can be sampled and followed with little difficulty, excellent accommodations for staff, and the availability of laboratory facilities. A comprehensive program could be planned with little difficulty and the promise of significant results in the offing.

It suffices to note, in closing, that I shall be most happy to participate in any future activities and would be most willing to assist in both the development of malacology and in future schistosomiasis studies. Both Dr. Bailey and I enjoyed our brief stay and found it most profitable scientifically.

TO: Office of the Scientific Advisor, U.S. Agency for International Development

FROM: Edward H. Michelson, Ph.D., Uniformed Services University of the Health Sciences

SUBJECT: Progress report for PSTC grant (#BST-5542-P-DZ-4259-00), "Characterization of Zambian Host-Snail Populations with Reference to Their Role in the Transmission of Schistosomiasis". For the period 1 January 1985 to 1 July 1985.

DATE: 8 July 1985

Since submission of the previous report, Dr. James Bailey joined the project on the 15th of January, and is actively engaged in a series of studies with snails obtained from Zambia and in developing techniques for their maintenance in the laboratory.

My first trip to Zambia was made from March 3-29. The Tropical Diseases Research Centre was used as the base of operations. Dr. Sukwa introduced me to Miss Harriet Mukange, who has been assigned to work on schistosomiasis at the Centre and on snail hosts. At Dr. Sukwa's request, I have agreed to help train her and, during my stay, I helped set up a small snail laboratory with some plastic cages and pumps that I brought with me. During the period of my stay, Dr. Sukwa, Miss Mukange, and I went on a series of field trips in the vicinity of Ndola, to the swamps near Mutenda, and to the field station at Kampumba where we spent about a week in the field. These field trips resulted in the collection of numerous local snail species. To date the following species have been identified:

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|-------------------------------|---------------------------|
| <u>Biomphalaria pfeifferi</u> | <u>Lymnaea natalensis</u> |
| <u>Bulinus globosus</u> | <u>Gyraulus</u> sp. |
| <u>Bulinus tropicus</u> | <u>Lanistes ovum</u> |
| <u>Bulinus canescens</u> | Small bivalve |
| <u>Bulinus forskalii</u> | |

Only specimens of Bulinus globosus were found shedding schistosome-like cercariae. Several strains of B. pfeifferi, B. globosus, B. tropicus, and B. canescens have been brought back to Bethesda and are in culture.

Training was given to Miss Mukange in snail identification and maintenance and techniques for field collecting was demonstrating to local field workers attached to the TDRC.

Overall the trip to Zambia was successful and future field studies seem to be both possible and promising. Some restrictions on movement do occur in the form of military road blocks, and the extent of crime in the Ndola area makes movement after sunset unadvisable. Two other constraints occurred and, to some extent, these can be expected when long distance communication is involved. The first was some difficulty in getting equipment out of Customs. It took me nearly three hours to get supplies through Customs in Lusaka. The second difficulty was the transfer of funds from USUHS to TDRC. Hopefully, these problems will be rectified in the future.

Preliminary studies have been initiated using an agarose gel electrophoresis system for separating species of Zambian bulinids. Dr. Bailey and I have been experimenting with this system and have shown it to be of value in delimiting sibling species of planorbids from South America.

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TO: Office of the Scientific Advisor, U.S. Agency for International Development

FROM: Edward H. Michelson, Ph.D., Uniformed Services University of the Health Sciences

SUBJECT: Progress report for PSTC grant (#BST-5542-P-DZ-4259-00), "Characterization of Zambian Host-Snail Populations with Reference to Their Role in the Transmission of Schistosomiasis". For the period 19 September 1984 to 31 December 1984.

DATE: 5 January 1985

This first report is somewhat brief in that funding for the project was not initiated until September 15, 1984. During the three months that the project has been operating an aquarium room has been set up for handling African snail species, minor equipment has been purchased, correspondence has been established with my co-investigator Dr. Sukwa in preparing for a trip to Zambia in early 1985, and arrangements have been undertaken to hire a post-doctoral fellow to serve on the project.

Mr. James B. Bailey, currently a Ph.D. candidate at the University of Michigan and a student of Dr. Jack Burch, is finishing his doctoral requirements and will join the project during January 1985. He is working on African molluscs, has had experience in Africa, in both Swaziland and Egypt, and has experience in maintaining medically important molluscs.

Present plans call for me to go to Zambia on or around the first week of March 1985. I will spend some time in Lusaka to obtain necessary permission from the Ministry to conduct field studies and discuss aspects of the project with Dr. E.K. Njelesani, Director of Medical Services, Ministry of Health, and the majority of my time with Dr. Sukwa, my collaborator at the Tropical Diseases Research Centre, Ndola. It is hoped that at this time, I will be able to identify some Zambians interested in being trained in malacology, initiate a small laboratory and snail holding facility, and do some preliminary collecting.

**DIFFERENTIATION OF THE SIBLING SPECIES
BIOMPHALARIA OCCIDENTALIS AND *BIOMPHALARIA TENAGOPHILA*
BY THE ELECTROPHORETIC PATTERNS OF THEIR HEMOGLOBIN***

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*A simple and rapid method for differentiating the sibling species *Biomphalaria tenagophila* and *Biomphalaria occidentalis* by agarose gel electrophoresis (AGE) is described. Snail hemolymph is used as the test sample and the red coloration of the hemoglobin fraction permits visualization of the migration patterns without resorting to specific stains. Moreover, hemolymph samples may be obtained without killing the snail, thus permitting its use for other studies or for breeding.*

Key words: *Biomphalaria occidentalis* - *Biomphalaria tenagophila* - sibling species - hemoglobin electrophoresis - biochemical taxonomy

In 1981, Paraense described *Biomphalaria occidentalis*, a species which could not be differentiated from *Biomphalaria tenagophila* by shell characteristics or by the morphology of most of the genital organs. In the laboratory, the two species were found to be reproductively incompatible and consequently may be considered sibling species. Differentiation of the two species can be accomplished only by careful dissection; the male and female genitalia and the demonstration of the presence of a vaginal pouch in *B. tenagophila* and its absence in *B. occidentalis*. There are, in addition, morphometric differences in the prepuce/penile sheath complex of the two species. Differentiation, therefore, is a highly complex process requiring a high level of skill in dissecting these organisms and rigid control of relaxation and fixation of the snails.

B. tenagophila is a recognized host of *Schistosoma mansoni*, whereas *B. occidentalis* appears to be a non-susceptible species (Paraense & Corrêa, 1982). The need for a method, less tedious than manual dissection, to differentiate the species is obvious.

A technique employing snail hemolymph and agarose gel electrophoresis (AGE) for the differentiation of the two species is described. The technique has been found to be simple, rapid, repeatable, cost effective and suitable for rural laboratories.

MATERIALS AND METHODS

Six strains of *B. tenagophila* and four strains of *B. occidentalis*, all from Brazil, were used in the study (Tab. 1). Maintenance of the snail populations was as previously described (Michelson, 1966).

Two methods were used for collecting hemolymph. When large amounts of hemolymph (50-100 μ l) were required and snails were expendable, hemolymph was collected from the pericardial cavity as described by Michelson (1966). If snails were required for breeding or for other studies, hemolymph was drawn by puncturing the mantle collar with the tip of a sharp forceps and collecting the pooled fluid with a fine-tipped micropipet. Approximately 10-50 μ l of hemolymph could be obtained with this method and snail mortality was generally less than 10%.

Fresh hemolymph was mixed in a proportion of 5:2 with a sample solution comprised of 80% glucose containing 5% glycerol and sufficient 0.5% bromphenol blue to give a dark purple color. Ten microliters of the mixture were used as the sample for agarose gel electrophoresis (AGE). AGE was conducted in either Mini-vertical or Mini-subhorizontal cells (Bio-Rad Laboratories) and both methods gave comparable results; however, the vertical system appeared to give sharper bands. In both systems, the gel matrix consisted of 0.8% agarose in 0.15 M tris-borate buffer, pH 8.5. Although we used an ultrapure DNA grade agarose (Bio-Rad Laboratories), pre-

*The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of Defense or the Uniformed Services University of the Health Sciences. The investigation received support from an institutional grant awarded by the Uniformed Services University of the Health Sciences, and by a grant from AID.

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Received for publication January 27th and accepted March 27th, 1986.

TABLE I

Species and the geographic origin of strains from which hemoglobin was obtained*

| Species | Geographic Origin |
|----------------------------------|---|
| <i>Biomphalaria occidentalis</i> | Barão de Melgaço, Mato Grosso Guaira, Paraná Valparaíso, São Paulo Sena Madureira, Acre |
| <i>Biomphalaria tenagophila</i> | Joinville, Santa Catarina Rio de Janeiro, RJ Brasília, D F Taubaté, São Paulo São José dos Campos, São Paulo Vitória, Espírito Santo |

* All snail populations were derived from those maintained by Dr. W.L. Paraense, Instituto Oswaldo Cruz, Rio de Janeiro.

liminary studies demonstrated that less chemically pure agaroses would suffice. Horizontal gels, 2mm in thickness, were prepared by pouring 15ml of melted agarose directly onto a Gel Bond^(R) sheet (FMC BioProducts), 6.4cm x 11.0cm, containing a 10 comb well-spacer situated 1cm from a smaller edge. The gels were cured for 2hr at 50°C before use and AGE was run at 104V/70 min/25°C. Vertical gels, 1.5mm in thickness, were prepared in a casting cell and used approximately 9.3ml of agarose to make a gel 8cm wide x 7cm long. Gels were run at 104V/55 min/25°C. Routinely, ten sample wells were cast; however, the number may be varied to suit the needs of the investigator.

After electrophoresis was completed, gels were fixed in 12% acetic acid containing 5% glycerol for 1hr or until the bromphenol marker was no longer visible. Gels were then rinsed in deionized water and those run in a vertical cell were now mounted onto Gel Bond sheets. Permanent preparations were made by dehydrating the gels to a thin layer in accordance with the technique described by Saravia & Cook (1979). This consisted in covering the gels with a moist piece of filter paper, adding several layers of absorbent paper toweling, then placing an evenly distributed weight of 2kg on top of the gel-paper complex. After 30 min, the weight and the papers were carefully removed and the film further dried in a 37°C incubator for 4-6 hr or held overnight at room temperature. Stain was not required as the red-colored hemoglobin bands were clearly visible. The preparations may be photographed or photocopied and the gel films can be stored indefinitely.

Migration distances were determined by noting the distance of the bands from the point of origin; however, Rf values could be determined also in relation to the bromphenol dye front.

RESULTS

Our results indicated that the hemoglobin portion of *B. occidentalis* hemolymph migrated faster than did that of *B. tenagophila*. In most runs the bands were separated by a distance of 1-2mm. Although some variation in absolute distance was noted between runs, the distance between the bands of the two species did not show appreciable variation. No differences were noted among the geographical strains of the same species. Variation appeared to be less in gels run in the vertical system than those done in the horizontal system.

In the 62 analyses done in the horizontal system, the hemoglobin band of *B. occidentalis* migrated at least 1mm farther than that of *B. tenagophila* in 55 comparisons (Fig. 1A). In five sample runs, the hemoglobin of the two species migrated equally or so closely to one another that they could not be differentiated. Only two sample runs were observed in which the *B. tenagophila* band migrated faster than its sibling species.

In all of the 42 analyses done in the vertical system, *B. occidentalis* hemoglobin migrated at least 1mm farther than did the hemoglobin of *B. tenagophila* (Fig. 1B). When the results from both systems were combined, it was found that *B. occidentalis* hemoglobin migrated faster than *B. tenagophila* 93.3% of the time, migrated at an equal rate with the latter species 4.8% of the time, and had a slower migration 1.9% of the time.

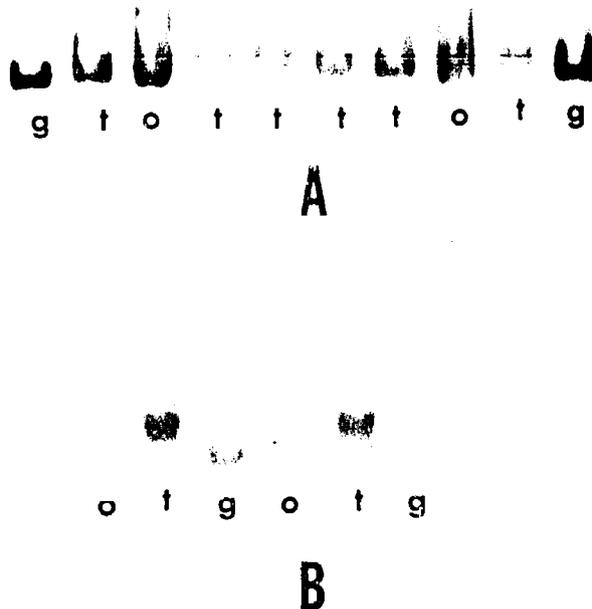


Fig. 1: Electrophoresis of snail hemolymph. A: Vertical gel; B: Horizontal gel; g: *Biomphalaria glabrata*; o: *B. occidentalis*; t: *B. tenagophila*. The vertical gel (A) was enhanced by staining with dianisidine and hydrogen peroxid due to faintness of the bands. The horizontal gel was unstained.

A limited number of experiments demonstrated that *B. glabrata* and *B. straminea*, both intermediate hosts of *S. mansoni* in Brazil, had hemoglobins which migrated faster than *B. occidentalis*; however, the two species could not be separated from one another in the present system.

DISCUSSION

Efforts to use hemolymph from species of planorbid snails as a taxonomic tool are not new and have been reviewed, in part, by Michelson (1973) and by Wright (1971, 1974). In past studies, electrophoretic analyses of hemolymph had been directed towards demonstrating differences in protein and isozyme patterns. Wright (1971) questioned the value of these techniques for taxonomic studies since he observed both quantitative and qualitative variations associated with age and/or size of individual snails. Subsequently, tissue extracts have replaced hemolymph as the sample of choice in applications of electrophoresis for taxonomic purposes.

The present study, however, demonstrates that the analyses of snail hemolymph hemoglobins by AGE may be a valuable adjunct to existing techniques for species identification. In *Biomphalaria* species, hemoglobin is a major constituent of the hemolymph and in some species may constitute as much as 40-65% of the total protein concentration (Michelson & Dubois, 1975). The hemoglobin fraction in these snails is characterized by being chromogenic, non-corpuseular, and of high molecular weight (1×10^6 or greater). Specific differences in hemoglobin mobility were not apparent in past studies, since the matrices employed for electrophoresis prevented or limited the migration of these molecules. AGE separates protein molecules by both charge densities and molecular sieving and, at concentrations of 0.6-0.8%, gels have pore sizes adequate for the migration of snail hemoglobins.

Our results indicate that differences in hemoglobin migration was a reliable criterion for separating the sibling species *B. occidentalis* and *B. tenagophila*, separation being demonstrated in 93.3% of the test samples. In addition, the mini-system has several advantages: (1) it permits the test snails to remain alive for use in other studies; (2) results are rapid; (3) the technique is simple and requires no special skills; (4) migration patterns can be demonstrated without the use of special stains or substrates; and (5) the method is relatively cheap, both in actual cost (estimated at \$0.03-\$0.08/sample) and materials required. Preliminary experiments suggest that the technique may be applicable to other groups of hemoglobin-bearing snails, and recent studies with African planorbids appear promising.

RESUMO

É descrito um método simples e rápido para distinguir as espécies crípticas *Biomphalaria tenagophila* e *B. occidentalis* por eletroforese em gel de agarose. A prova é feita com hemolinfa do molusco, permitindo a cor vermelha da fração hemoglobina visualizar os padrões de migração sem necessidade de recorrer a colorações específicas. Além disso, as amostras de hemolinfa podem ser obtidas sem sacrificar o molusco, que poderá ser usado para outros estudos ou para criação.

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