ELECTROPHORETIC STUDIES ON ENZYMES OF THE SNAIL INTERMEDIATE HOSTS OF *SCHISTOSOMA MANSONI* FROM ETHIOPIA.

Tilahun Woldemichael*, MSc.

ABSTRACT: Enzyme patterns of three populations of *Biomphalaria pfeifferi* and two populations of *Biomphalaria sudanica* from Ethiopia were studied by starch gel electrophoresis. The preliminary results indicated that four of the six enzyme systems investigated appeared potentially useful in discriminating between these closely related species. Acid-phosphatase, esternases, 3-hydroxybutyrate dehydrogenase and xanthine oxidase showed distinguishing characteristics either in terms of mobilities, or number of bands. The migration of acid-phosphatase was consistently faster in the *B.sudanica* populations than in any of the *B.pfeifferi* populations. Unlike acid-phosphatase, intraspecific variation among the populations of a species was observed for the esterases, 3-hydroxybutyrate dehydrogenase and xanthine oxidase although clear differences were still apparent among these enzymes between *B.sudanica* and *B.pfeifferi*. The geographically closely located populations of *B.pfeifferi* from Wollo exhibited similar enzymatic activities but differed in most enzymes from the geographically separated population from Harar region. Further investigation is recommended to strengthen the present observation.

INTRODUCTION

In Ethiopia, both intestinal and urinary schistosomiasis are endemic. The former caused by *S.mansoni* is represented in nearly all of the administrative regions (1,2) while the urinary form is limited to some low and arid areas of the Awash Valley (3,4) in the Wabeshebelle valley (5) and a recently found focus in the western region bordering the Sudan (6).

The proven snail intermediate hosts are *Biomphalaria pfeifferi* and *Biomphalaria sudanica* for *S.mansoni* (7) and *Bulinus abyssinicus* (8) and *Bulinus africanus* (6) for *S.haematobium* in the Awash valley and in the western region respectively. The initial studies on the local fauna were conducted by Wright and Brown (9) followed by a thorough distribution study mainly on the intermediate hosts by Brown (7). Consequently, it is now known that *B.pfeifferi* is found in a wide range of habitats including small streams, rivers and lakes. *B.sudanica* on the other hand appears to be commonly reported from the southern Ethiopian Rift Valley lakes of Margherita, Awasa and Zeway (7,10,11).

A number of taxonomic features have been investigated for the identification of fresh water snails. Commonly, they are described on the basis of their shell morphology and internal anatomy.

* National Research Institute of Health

According to the initial guidelines of African snail identification by Mandahl-Barth (12) and a later review and compilation meant to suit local species by Meskal (13), the fully grown *B.sudanica* measures about 22 mm in diameter and *B.pfeifferi* about 15 mm. In practice, however, these measurements often overlap particularly when viewing young and young adults. With progressive age, many of the shell characters are also subject to change (12). The difficulties of the identification of snails on morphological and anatomical grounds have been adequately discussed elsewhere (14,15).

This project was designed to establish whether enzyme patterns could be utilized for species identification. To our knowledge, this diagnostic approach has not been employed on Ethiopian species for this purpose. Traditionally, identification has been based mainly on the nature of habitats following the initial studies on their distribution, because additional proven discriminating characters are lacking. Yet, the possibility of the snail species colonizing a variety of new habitats is not remote. For instance, experience in Tanzania (16) indicated that *B.sudanica* was spreading out into man-made dams, permanent and seasonal water courses and seepages around lakes. A similar spread of the Ethiopian *B.sudanica* to habitats other than the Rift Valley lakes is highly probable because of the extensive human migration, intensive and increased water related development schemes and resettlements that are presently being undertaken. Confirmation of the proper identification by enzyme characters could help towards the understanding of the ecological conditions that may favor the snail intermediate hosts and eventually may have a bearing on the choice of appropriate control approaches. As there is no documented experimental evidence on the comparative degree of susceptibility of the two species, the enzymatic pattern studies may also serve as a background information for a possible long term future work on the differential compatibility, if any, of the two species with Ethiopian strain(s) of *Schistosoma mansoni.*

MATERIALS AND METHODS

Three populations of *B.pfeifferi* were examined; two of these were from streams of Bati and Kemisie, Wollo region, and the third group was from the irrigation canals of Amibara along the Middle Awash Valley, Harar region.

The other species consisted of *B.sudanica* populations collected from Lake Zeway, Shoa region and from Tikurwoha, Sidamo region. The latter specimen was originally collected by David Brown in 1969 and has been maintained in the laboratory of the Department of Zoology, the Natural History Museum, London where this investigation was carried out. The other snail samples were collected at different times between 1986 and 1988 and colonies established at aquaria of the Zoology Department of the Museum. The snails were kept under identical laboratory conditions and fed on boiled and dried lettuce together with *Oscillatoria spp.* The aquaria temperature was maintained at 26 degree centigrade.

Bulinus truncatus from Dezful, Iran, which has been cultured at the Zoology Department of the Museum since 1977 and whose electrophoretic mobility has been determined, was used as an internal standard in the present work.

Each specimen used in the study was greater than 4 mm in diameter and all were free of schistosome infection. Water soluble extracts of snails were used for electrophoresis. Usually, five individuals of each population representing each species were processed at a time.

The enzyme systems analyzed were, esterases (Est), Acid-phosphatase (Acp), 3-hydroxybutyrate dehydrogenase (HBDH), Gulcose-phosphate isomerase (GPI), Diaphorase (DIA) and Xanthine Oxidose (XO).

The gel was made up with 12% hydrolysed starch and was prepared in accordance with the method of Smithies (17). The gel buffer systems varied according to the enzymes investigated. Jelnes B buffer system (18) was employed for DIA and XO. Buffers for the rest of the gels were prepared in accordance to the human buffer system (19). The tank buffers also differed according to each enzyme system. The range of voltages applied and the duration of electrophoresis were 200 to 500 volts for 3 to 3 1/2 hours depending on the buffer systems and enzyme types.

The activity or mobility of the enzyme on the electrophoretic plate was visualized by a colour reaction after the addition of specific substrates and staining chemicals and incubated at 37^0C. Detection methods of the enzyme activities under investigation were as described by Bergmeyer (20) and Harris and Hopkinson

(19). The schematic representation of the patterns are drawn to describe their position and numbers. The relative electrophoretic migration of the enzymes on the gels was .00.0determined by the distance covered by the enzyme bands from the application line. The relative mobility values (RM) for each snail species was thus calculated as the average electrophoretic mobility of the samples as measured in mms and this divided by the average electrophoretic mobility of the control also in mms.

RESULTS

The table shows the mobility values of the enzymes in both species expressed relative to the standard snail. Figures 1 to 11 also show representative enzyme patterns of one population of a species in relation to the standard.

Consistently, Acp migration was faster in the *B.sudanica* than in the *B.pfeifferi* populations. Each species population was represented by a single enzyme band pattern with a more marked anodic migration in the *B.sudanica* than in the *B.pfeifferi* group. A representative pattern and migration of this enzyme system is shown in figure 1. The migration of HBDH were also faster in *B.sudanica* (figure 2) except between the Tikurwoha population of *B.sudanica* and the Amibara *B.pfeifferi* population where their mobilities were reversed (figure 3).

There was generally poor resolution of the enzyme GPI. The few successful developments were with *B.pfeifferi* from Kemisie and *B.sudanica* from Tikurwoha which appeared to show similar and virtually inseparable band patterns and mobilities (figure 4). Similar mobilities and patterns were also observed in the populations of both species for the enzyme DIA showing differences only in the intensity of the bands (figure 5).

The *B.pfeifferi* populations from Wollo all tended to lack detectable XO actively. The *B.sudanica* populations from Tikurwoha and Zeway, on the other hand, showed consistently a one banded system. This pattern is represented in figure 6. The Amibara *B.pfeifferi* population, unlike the other *B.pfeifferi* populations,

Table. The average relative mobility values (Rm) of the enzymes after electrophoresis of whole snail representative populations of Ethiopian Biomphalaria pfeifferi and Biomphalaria sudanica with respect to the standard snail, Bulinus truncatus.

Snail species	Est	Acp	HBDH	GPI	DIA	XO
B.pfeifferi (Kemisie)	0.53	0.74	0.74	1.03	0.99	=
B.sudanica (Tikurwoha)	0.18	1.02	1.01	0.97	0.96	0.86
B.pfeifferi (Amibara)	1.91	0.61	1.14	+	0.97	0.86
B.sudanica (Tikurwoha)	0.18	1.04	1.07	+	1.00	0.85
B.pfeifferi (Amibara)	A-1.80* B 2.40*	0.69*	1.2*	+	0.96*	0.87
B.sudanica (Lake Zeway)	1.7*	0.8*	1.15*	+	0.98*	0.90*
B.pfeifferi (Bati & Kemisie)	0.54* 0	64*	0.72*	+	1.00*	=
B.sudanica (Tikurwoha)	0.18*	1.02	0.93*	+	1.00	0.89*

Note: * - not repeated, single value
= - no enzyme activity detected
+ - poor resolution
A - average value for pattern one
B - average value for pattern two

showed detectable XO activities (figure 7).

Figure 8 illustrates two band patterns in the Est of *B.pfeifferi* from Amibara and one pattern in *B.sudanica* from Zeway. In *B.pfeifferi* of this population, there were two faster bands in a member of the group (pattern A, fig.8-2) while three faster anodic bands were shown in the rest of the group (pattern B, fig.8-3). A one pattern of four strong anodic bands were observed in the *B.sudanica*. As shown in figure 9, the enzymes in the *B.pfeifferi* from Amibara revealed relatively more faster migrating bands and a moderate cathodal migration while the corresponding *B.sudanica* population from Tikurwoha showed fewer bands and a faster cathodal migration. The pattern for the same enzyme in a population of *B.pfeifferi* from Kemisie in comparison with a population of *B.sudanica* from Tikurwoha is shown in figure 10. One pattern type was observed in both species. Both exhibited cathodal migration but it was pronounced in the *B.sudanica* than in the other species population. The population from Bati and a specimen from Kemisie against *B.sudanica* from Tikurwoha is shown in figure 11. Est patterns with a cathodal migration were present in the *B.sudanica* but absent in the *B.pfeifferi*.

DISCUSSION

On the basis of the present electrophoretic study, four enzyme systems (Est, HBDH, Acp and XO) appear to show differences between the two species.

As shown in figures 8 to 11 major differences in the Est patterns exist between the two species and among members of the same species. Variations in patterns, number of bands and direction of migration were observed. For example, in *B.pfeifferi* from Amibara, two patterns were expressed (one with three and the other with two faster bands) while in *B.sudanica*, there was only one pattern. The marked patterns shown by the cathodal migration of the enzyme fraction (figure 11) also appeared distinctive only to the *B.sudanica* species. Pronounced cathodal migration was also observed in the *B.sudanica* than in the *B.pfeifferi* from Kemisie (figure 10). The polymorphism of Est patterns within members of a population such as in the *B.pfeifferi* from Amibara (figure 8) may be due to the existence of genetically distinct individuals. Conversely, the other populations of *B.pfeifferi* and the two populations of *B.sudanica* showed a uniform monomorphic

pattern for each population group which indicates similar genetic constitution in the population. Variation in Est patterns among populations of the same species was also observed by previous investigators. Wright and File (21) reported differences of the enzyme patterns in the populations of the genus *Bulinus*. Malek and File (22) studying *Biomphalaria spp* of the Western hemisphere and Wium-Anderson (23) working on African *B.alexanderina* all observed variations of the enzyme patterns within populations belonging to the same species. Clearly there is a need to examine many individuals from each population. Although variable within and between populations, Est may serve in discriminating between the two species. This impression is in line with the opinion of Wright et al (24) who emphasized the validity of electrophoresis of Est for taxonomic purposes of *Biomphalaria* and *Bulinus*. On the basis of Est from the hepatopancreas of African *Biomphalaria spp,* Wium-Anderson (25), using starch gel electrophoresis was able to separate four species among which were *B.pfeifferi* and *B.sudanica*. The proposal put forward by Henrickson and Jelnes (26) of the possibility of *Biomphalaria* species identification based on Est patterns is supported by the present observations.

As shown in figure 1, Acp patterns appear to offer a means of separating *Biomphalaria* species. The consistently faster mobility of the enzyme from *B.sudanica* populations, compared with that of the same enzyme in *B.pfeifferi* may prove to be a useful character to distinguish the two species with less ambiguity than the Est.

Discrimination of the two species involving the electrophoresis of XO appeared even simpler since the absence or undetectable activity of the enzyme in all *B.pfeifferi* populations (except that from the Amibara population) was a common feature. In contrast, the presence of detectable single pattern system of this enzyme in all of the *B.sudanica* populations studied under the same conditions indicates a parameter probably useful in separating at least the Wollo populations of *B.pfeifferi* from *B.sudanica* (figure 6). However, separation of the Amibara population, on the basis of XO from *B.sudanica* seems difficult because of the similarity of migration and patterns in both organisms (figure 7).

Failure to detect XO in the Wollo *B.pfeifferi* populations might reflect the scarcity of the enzyme in the entire tissues of the snail.

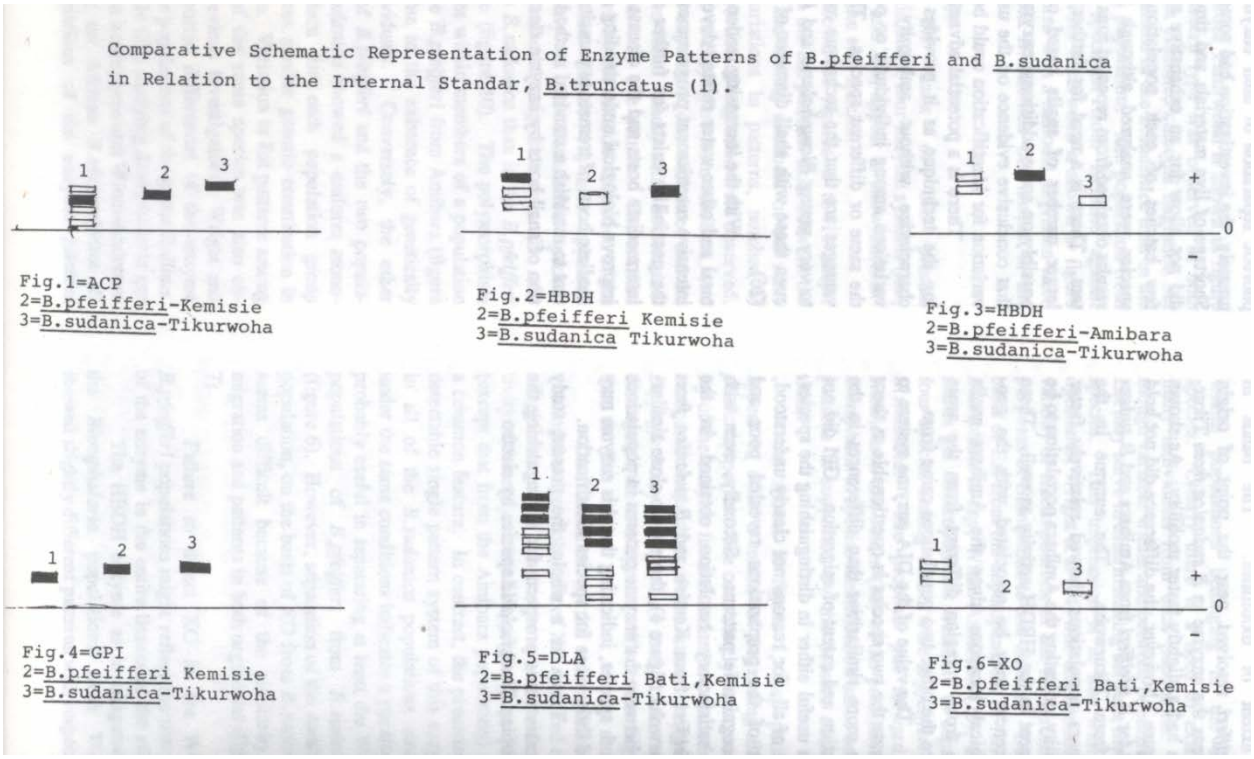
The HBDH enzyme as investigated in the *Biomphalaria* populations from Wollo showed slightly different patterns with respect to the extent of mobilities. The bands in *B.pfeifferi* resolved near the point of origin whereas the enzyme in *B.sudanica from* Tikurwoha had a slightly faster mobility. As shown in figure 3 however, the difference did not hold good for *B.pfeifferi* from Amibara and *B.sudanica* from Tikurwoha. The enzyme in the Amibara populations showed relatively faster mobility indicating the Amibara population to be different in the HBDH activity as well. These differences may be associated with the geographical locations since the Amibara snails were from a region different from the areas where the other two populations came from.

The value of the DIA enzyme system to separate the two species is questionable as there were more similarities than differences in the direction and extent of migration. GPI did not seem useful either in distinguishing the species. First of all, for reasons not clearly understood, most of the preparations revealed poor and unrecognizable patterns. Secondly, even with the satisfactory resolution obtained in the *B.pfeifferi* from Kemise and *B.sudanica from* Tikurwoha (figure 4), there were close similarities between the enzyme patterns in populations of both species, indicating that this enzyme may be of little value for species identification.

Taken as a whole, the present study indicates some prospects for distinguishing the Ethiopian *Biomphalaria* species by electro-phoretic separation of their enzymes. Unfortunately, the investigation had some limitations. Shortage of live materials and time constraints did not allow for an exhaustive study; only a few batches of each population from each species were analyzed although most of the results obtained from repeated runs were consistent. There is a need for further studies on a larger number of snails based on these and possibly on some additional enzyme systems so that conclusive evidence on the use of enzyme variation for identification could be provided.

There is a potential advantage in utilizing the technique as it provides a means to characterize without ambiguity the genetic variations among individuals or populations of the same or different species. The added advantages are that the technique can be applied to very young *Biomphalaria* and *Bulinus* snails even those with shell diameter of about 4 mm (26).

With the increasing tendency of agricultural and other water related developments and intensive resettlement programmes in Ethiopia, the possibility exists for further spread of the intermediate hosts and the disease. In order to improve biological understanding of snail intermediate hosts of trematode parasites it is important to establish a standard method of identification of snail hosts by enzyme electrophoresis.



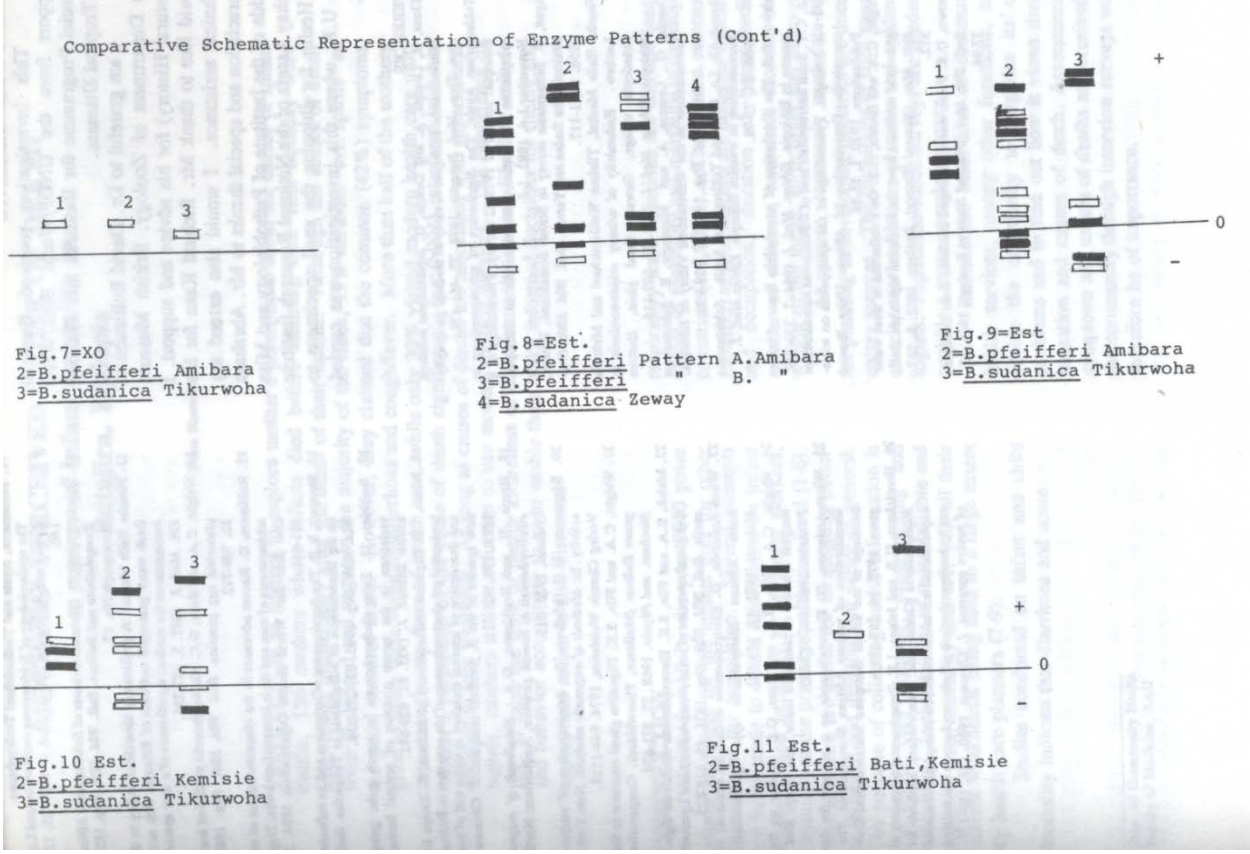


Fig.7=XO
2=B.pfeifferi Amibara
3=B.sudanica Tikurwoha

Fig.8=Est.
2=B.pfeifferi Pattern A.Amibara
3=B.pfeifferi " B. "
4=B.sudanica Zeway

Fig.9=Est
2=B.pfeifferi Amibara
3=B.sudanica Tikurwoha

Fig.10 Est.
2=B.pfeifferi Kemisie
3=B.sudanica Tikurwoha

Fig.11 Est.
2=B.pfeifferi Bati,Kemisie
3=B.sudanica Tikurwoha

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