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Enzyme analysis of *Schistosoma haematobium**

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Results are reported of enzyme analyses, by isoelectric focusing in polyacrylamide gels, of adult Schistosoma haematobium *worms derived from 22 isolates originating from 13 countries. Polymorphisms have been identified in the glucose-6-phosphate dehydrogenase (G6PD) and phosphoglucomutase (PGM) systems. Certain forms appear to be restricted in their geographical distribution and their occurrence outside their usual areas suggests human population movements resulting in mixing of parasite strains. The possible implications of minor variations in some PGM patterns and the apparent absence of heteropolymer fractions in presumed G6PD heterozygotes are discussed. The use of the technique to detect natural multiple miracidial infections in snails is reported and discussed.*

The distribution of *Schistosoma haematobium* is confined to Africa and some adjacent regions, extending eastward through Arabia to the Khuzistan province of Iran and to the Indian Ocean islands of Madagascar and Mauritius. This distribution is restricted by the range of the snail intermediate host genus *Bulinus*, a characteristic element of the tropical freshwater fauna in the area. Four species groups are recognized within *Bulinus* and they have various roles as hosts for *Schistosoma* species. In general, *S. haematobium* in Africa south of the Sahara is transmitted by snails of the *B. africanus* group, in the Mediterranean area and south-west Asia by tetraploid members of the *B. tropicus/truncatus* complex, and in Arabia and Mauritius by members of the *B. forskali* group. In West Africa all three of these snail groups are known to act as hosts for *S. haematobium*, and in Arabia the *B. reticulatus* group is also implicated. Of particular significance is the major division between the northern *B. truncatus*-borne *S. haematobium* and the sub-Saharan *B. africanus* group-borne parasites for, with few exceptions, neither of these forms can develop in the snail host for the other. Within this broad division there are further, more localized, forms whose intermediate host range is restricted to certain species or even populations within the snail groups. Thus *S. haematobium* consists of a complex of populations which differ in their ability to utilize various potential intermediate hosts. Conflicting reports concerning the pathological consequences of *S. haematobium* infection in man suggest that there may also be differences in the parasites' relationship with their definitive hosts. Laboratory studies in hamsters (*17-19*) have shown differences between geographically distinct isolates of the parasite in infectivity of cercariae, adult worm growth rates and maturation times, egg productivity, and host-organ distribution of eggs. The present study of enzymes was undertaken with a view to more precise characterization of variation in *S. haematobium*.

Most earlier electrophoretic studies on the proteins and enzymes of schistosomes employed techniques that required the pooling of large numbers of worms in order to obtain extracts of sufficient concentration for the resolution of clear patterns (*1-5, 8, 10, 12, 22, 25, 26*). While these methods were able to detect gross differences between species and even some local strains, individual variation tended to be masked. This deficiency was overcome, to a limited extent, by the use of snails infected by a single miracidium so that all the resulting adult worms were of the same genotype (*3*). The development of the high resolution technique of isoelectric focusing in polyacrylamide gels permitted the analysis of enzymes from small numbers and, eventually, from individual adult worms (*11, 13, 14, 20, 21, 23, 24*). Recently Fletcher et al. (*6, 7*) have successfully used a starch gel method for single worms in studies on *S. mansoni* and *S. japonicum*.

The present paper reports the results of a study carried out over the last 5 years, using isoelectric focusing in polyacrylamide gels to examine a number of isolates from sources throughout most of the geographical range of *S. haematobium*. Because the data provided by the glucose-6-phosphate dehydrogenase (G6PD) and phosphoglucomutase (PGM) systems proved to be particularly valuable in two previous studies (*20, 23*), this preliminary survey of *S. haematobium* has concentrated mainly on these two enzymes.

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MATERIALS AND METHODS

The geographical origins and sources of the *S. haematobium* isolates are given in Table 1. With the exception of the material from Madagascar, which was maintained in the laboratory for several generations, all the samples were examined after a single passage through hamsters. Where isolates were derived from naturally infected, wild-caught snails the number of snails is given in brackets. The isolates derived from urine samples were collected in endemic areas, prepared according to the method described by Wright & Bennet (*17*), and brought to London where the eggs were hatched and laboratory-bred snails were infected.

Table 1. Origin of *S. haematobium* isolates examined and their G6PD and PGM types

Country	Locality	Source[a]	Natural snail host	G6PD type	PGM type
Iraq	Baghdad	Snails (3)	*B. truncatus*	2	
Egypt	Cairo	Urine, pooled sample	*B. truncatus*	2	1
Sudan	Khartoum	Urine, pooled sample	*B. truncatus*	2	1, 1 × 2
Sudan	Gezira	Urine, pooled sample	*B. truncatus*	1, 2, 3, 1 × 2, 1 × 3	1, 3, 1 × 2, 1 × 3
Kenya	Tiengre, Nyanza Province	Urine	*B. africanus* or *B. nasutus*	1	1
Kenya	Benes and Bamburi dams, near Mombasa, Coast Province	Snails (3)	*B. globosus*	1, 2	1
United Republic of Tanzania	Kibaranga, Tanga	Urine, pooled sample	*B. nasutus* or *B. globosus*	1	1, 2, 1 × 2
United Republic of Tanzania	Mwanza, Sukumaland	Urine, pooled sample	*B. nasutus*	1	1
Malawi	Sucoma Estate N'chala	Snails (3)	*B. globosus*	1	1
Zambia	Ndola, Copperbelt	Urine, pooled sample	*B. globosus*	1	1
Zambia	Lusaka	Urine, pooled sample	*B. globosus*	1	1
Madagascar	(Ciba-Geigy, Basle laboratory passaged)	Snails laboratory infected	*B. obtusispira*	1	1, 2, 1 × 2
South Africa	Durban	Urine, pooled sample	*B. africanus*	1	1, 1 × 2
United Republic of Cameroon	Barombi Mbo, Kumba	Urine, pooled sample	*B. rohlfsi*	1	1
Nigeria	Ngala, South Chad Irrigation Project	Snails (3)	*B. globosus*	1, 1 × 2	
Ghana	Tokuse, Weija Lake, near Accra	Snails (3)	*B. rohlfsi*	1, 3	1, 3
Gambia	Prufu bolon, Upper River Division (1978)	Snails (2)	*B. jousseaumei*	2	1 × 3
Gambia	Prufu bolon, Upper River Division (1979)	Snails (2)	*B. jousseaumei*	3	3
Gambia	Njoben, MacCarthy Island Division	Snails (2)	*B. senegalensis*	1, 2	
Gambia	Kunchude, MacCarthy Island Division	Snails (6)	*B. senegalensis*	1, 2	
Gambia	Bansang, MacCarthy Island Division	Urine, single sample	*B. senegalensis*	1	1, 1 × 3
Gambia	Allahein bolon, Western Division	Urine, single sample	*B. jousseaumei*	3, 2 × 3	3, 1 × 3

[a] Figure in brackets gives number of snails.

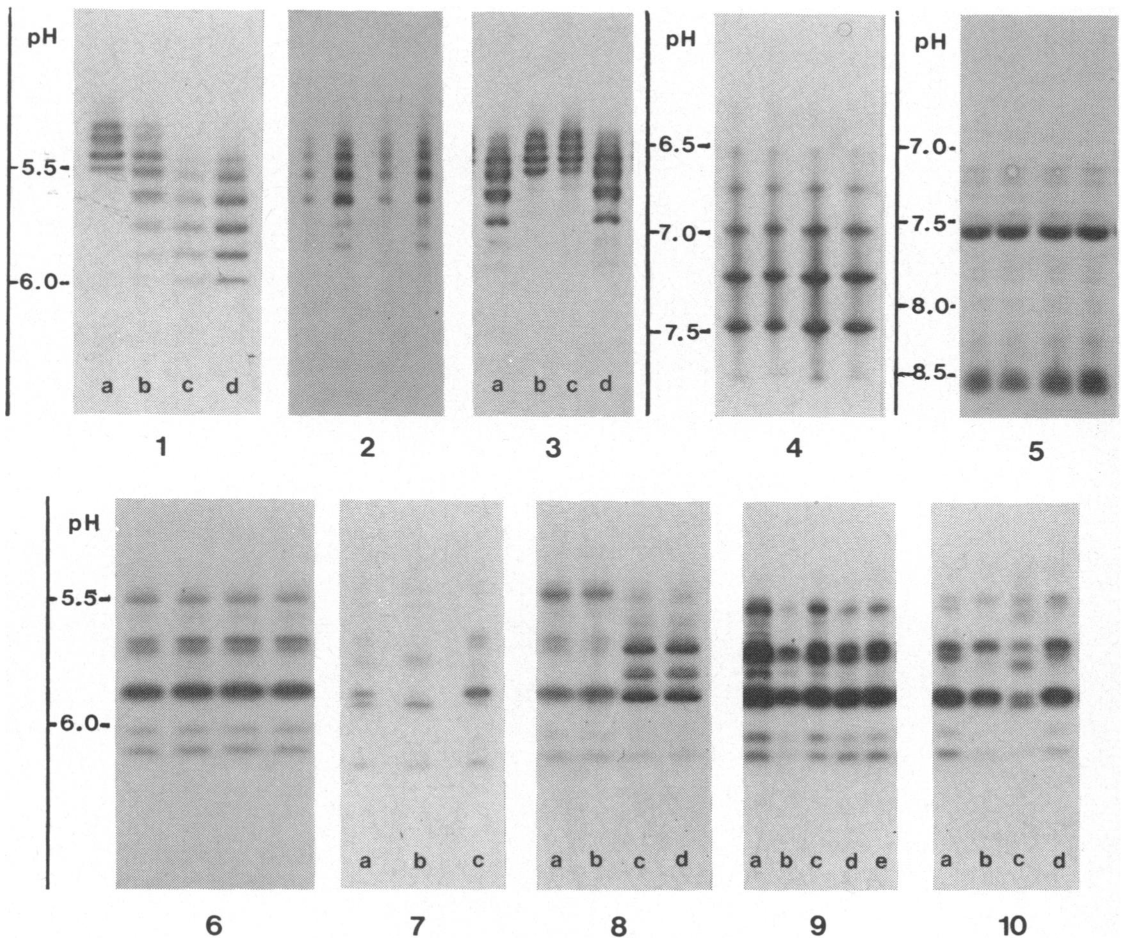

Fig. 1. Enzyme patterns obtained from adult worms of *S. haematobium*.

1.1 G6PD patterns in unpaired male *S. haematobium* from Gezira, Sudan;
a: G6PD 1; b: G6PD 1 × G6PD 3; c, d: G6PD 3.

1.2 G6PD 2 patterns in paired female *S. haematobium* from Benes dam, Kenya.

1.3 G6PD patterns in paired male *S. haematobium* from Ngala, Nigeria;
a, d: G6PD 1 × G6PD 2; b, c: G6PD 1.

1.4 LDH patterns in paired male *S. haematobium* from Benes dam, Kenya.

1.5 MDH patterns in paired male *S. haematobium* from Benes dam, Kenya.

1.6 PGM 1 patterns in paired male *S. haematobium* from Benes dam, Kenya.

1.7 PGM patterns in unpaired male *S. haematobium* from Kibaranga, United Republic of Tanzania;
a: PGM 1 × PGM 2; b: PGM 2; c: PGM 1.

1.8 PGM patterns in paired male *S. haematobium* from Tokuse, Ghana;
a, b: PGM 1; c, d: PGM 3.

1.9 PGM patterns in paired male *S. haematobium* from the Allahein Bolon, Gambia;
a, c: PGM 1 × PGM 3; b: PGM 3; d, e: PGM 1.

1.10 PGM patterns in unpaired male *S. haematobium* from Khartoum, Sudan;
a, d: PGM 1; b: PGM 1 variant lacking less acid fraction of each acidic pair of bands; c: PGM 1 × PGM 2.

The number of worms examined varied widely according to the success of the infection in the snails, the consequent number of cercariae produced, and the subsequent success of the infection in hamsters. The results for the isolates from the Gezira irrigation scheme in Sudan and from Durban are based on the examination of several hundred adult worms, while the isolates from Baghdad, Cairo, and the two Gambian samples derived from wild-caught infected *B. senegalensis*, yielded only a few dozen worms each. The number of worms examined in the other samples lay between these extremes. Only one enzyme system could be examined in each worm and, although data from both male and female worms were obtained, most of the observations reported are based upon males which, because of their larger size, usually gave more distinctive results. Preparation of adult worm extracts and procedures for isoelectric focusing with subsequent visualization of separated enzymes have been described by Wright et al. (*23*).

RESULTS

Examples of the enzyme patterns obtained from adult worms of *S. haematobium* are illustrated in Fig. 1. The results for the G6PD and PGM systems for each isolate are summarized in Table 1.

Glucose-6-phosphate dehydrogenase (EC 1.1.1.49)

Three patterns have so far been distinguished in this enzyme on the basis of the pI values of the most active fractions. Within each pattern there is some variation as a result of differences in the quantitative expression of individual fractions. G6PD 1 shows four major bands of activity, close together, between pH 5.25 and pH 5.5 (Fig. 1.1 and 1.3). In some populations, additional fractions are present on the less acid side of the pattern up to pH 5.65. G6PD 2 is characterized by 6 or 7 more widely spaced bands between pH 5.35 and pH 5.85 (Fig. 1.2). G6PD 3 resembles G6PD 2 but with up to 9 fairly evenly spaced fractions shifted towards the less acid region and lying between pH 5.42 and pH 6.2 (Fig. 1.1). Presumed heterozygotes between G6PD 1 and 2 (Fig. 1.3) and G6PD 1 and 3 (Fig. 1.1) are common in some populations and appear to be characterized by a slight reduction in the intensity of the extreme acidic and alkaline fractions of the parental patterns with pairing of closely adjacent bands in the zone of overlap.

Phosphoglucomutase (EC 2.7.5.1)

Three basic patterns have also been distinguished in the PGM system. In all three, the major active fractions lie within the pH range of about 5.5–6.2, although in some individuals, on strongly developed plates, an additional band appears at about pH 6.5. PGM 1 (Fig. 1.6) is characterized by a major fraction with a pI value of about 5.82. On the alkaline side of this band are two fractions of moderate strength with pI values of about 6.05 and 6.15. On the acidic side of the main fraction there is a pair of bands close together at about pH 5.75 with another closely adjacent pair at about pH 5.5. In strongly stained plates an additional weak fraction can sometimes be detected between these two pairs.

PGM 2 (Fig. 1.7) has the major fraction of the PGM 1 pattern shifted slightly in the alkaline direction to about pH 5.85, and the first pair of bands on the acidic side of this fraction is similarly shifted slightly. Homozygotes of PGM 2 have rarely been seen in this study (a few each in the Kibarangan (United Republic of Tanzania) and Madagascan isolates), but the presumed heterozygote PGM 1 × PGM 2 (Fig. 1.7 and 1.10) has occurred in both of these samples, in a single individual among 30 examined from Durban, and in 5 of 80 paired males and 3 of 9 unpaired males in the sample from Gezira.

PGM 3 is distinguished from the other two patterns by having the major fraction at pH 5.82 very sharply defined, the less acid band of the pair lying at pH 5.75 strongly emphasized, and an additional strong fraction lying between these two features at about pH 5.78 (Fig. 1.8 and 1.9). In PGM 3, the most acidic pair of bands and the two bands on the alkaline side of the major fraction are usually less well defined than they are in PGM 1. The presumed heterozygote PGM 1 × PGM 3 appears as a combination of the two parental patterns with stronger development of some minor fractions (which are, at best, only poorly resolved in both the homozygous patterns) lying between the two pairs of acidic bands (Fig. 1.9).

Throughout the wide geographical distribution of PGM 1 the basic pattern of bands is fairly consistent. However, variations occur in the relative quantitative expression of the four fractions that make up the two pairs of acidic bands in the pattern and these variations do not appear to be attributable to technical differences in the development of the plates. An extreme example of variation in this area is shown in Fig. 1.10, where only the more acidic band of each of these pairs is present. This was seen in 6 out of 30 unpaired males from the Khartoum isolate. The possible significance of these variations is discussed below.

Lactate dehydrogenase (LDH, EC 1.1.1.27)

Little attention was paid to the LDH system or to those described below because, in the early stages of the study, it was found that the patterns showed no

apparent differences between isolates. The usual LDH pattern consists of four evenly-spaced, well-developed bands between about pH 6.7 and pH 7.5 with minor fractions extending the pattern at both ends to about pH 6.5 and pH 7.65 (Fig. 1.4). A variant has recently been found in the Ghanaian isolate which, although poorly resolved, shows additional fractions.

Acid phosphatase (AcP, EC 3.1.3.2)

The AcP system has proved difficult to resolve in *S. haematobium*. There is a major area of activity between about pH 6.9 and pH 7.8, another less strong region between about pH 6.0 and pH 6.7, and a weak "streak" on the acid side of pH 5.7.

Malate dehydrogenase (MDH, EC 1.1.1.37)

MDH yields a complex pattern between pH 7.0 and pH 8.6 with major fractions at about pH 7.6 and at the extreme alkaline end. Between these two well-marked features there are at least three pairs of minor fractions and others on the acidic side of the pH 7.6 band (Fig. 1.5). This pattern suggests that there may be two MDH loci involved. No variants have yet been seen in the material studied from six isolates.

Glucosephosphate isomerase (GPI, EC 5.3.1.9)

The basic GPI pattern shows four bands between pH 6.9 and pH 7.5, with lesser fractions on the alkaline side extending to about pH 8.25. No variation in this pattern has been seen but, in a more strongly developed plate of male worms from the Ghanaian isolate, a further group of minor fractions, showing intrapopulation variation, has been found in the region between pH 5.5 and pH 6.0. Resolution of these fractions, which almost certainly represent another locus, has so far proved difficult.

Hexokinase (HK, EC 2.7.1.1)

Because of the relatively weak HK activity seen in the early part of the study, little work was done on this system. However, more recent technical improvements have succeeded in resolving a fairly complicated pattern of bands in the region between pH 5.8 and pH 6.75. In the isolates from both Gezira and Ghana there is evidence of intrapopulation variation in this system but analysis of the limited material available would be premature.

DISCUSSION

The validity of gene-frequency analysis from enzyme data of the kind described in this paper is highly dependent upon the nature of the original isolates. There are problems not only in defining the limits of a digenean population but also in estimating the adequacy of the sample size relative to the population as a whole (*15*, *16*). Many hundreds of miracidia can be hatched from the eggs in a single urine sample from one infected person. Whether these larvae are the progeny of all the paired female worms present or of only some of them is unknown. Of the miracidia that are successfully hatched, only a small proportion will establish infections in snails in the laboratory. Using five miracidia of *S. haematobium* per snail, an infection rate of 30% in exposed snails is considered high. Differences in cercarial output by individual snails mean that not all of the miracidia contribute equally to the group of adult worms eventually subjected to enzyme analysis. Thus, from the initial sample (the adequacy of which, with respect to the whole population, is distinctly questionable) there is a progressive diminution in the representative status of the material throughout the process leading to analysis. Even in fresh isolates, such as those used in this survey, it is questionable whether detailed analysis of gene-frequencies would contribute significantly to interpretation of the data.

Despite this criticism there is much to be learned from this preliminary study. Not only has it confirmed the heterogeneous nature of *S. haematobium* but it has also provided the first evidence of geographical trends in the pattern of variation. The isolates from southern, southeastern and south-central Africa were all homogeneous for G6PD 1 while those from Egypt and Iraq yielded G6PD 2. At one stage in the investigation, it seemed that there might be a clear distinction between the northern *S. haematobium*, which develops in *Bulinus truncatus*, and the sub-Saharan form, which uses snails of the *B. africanus* group. This impression was dispelled when G6PD 1 was found in the isolate from Barombi Mbo in the United Republic of Cameroon, where the snail host is *B. rohlfsi*, a member of the *B. truncatus* complex. Both G6PD 1 and G6PD 2 have now been found in mixed populations in the coastal region of Kenya where the snail host is *B. globosus* (*B. africanus* group) and in the Sudan where the host is *B. truncatus*. Isolates from Ghana and the Gambia in West Africa have revealed the existence of G6PD 3 in the *B. rohlfsi*-transmitted population in Ghana, and in two Gambian populations that use *B. jousseaumei* (*B. africanus* group). In the Ghanaian isolate, G6PD 3 occurred together with G6PD 1, and in the Gambian populations it was associated with G6PD 2. The Gambian isolates derived from *B. senegalensis* transmission sites have shown G6PD 2 associated with G6PD 1. The recent sample from the Gezira irrigation scheme in Sudan has yielded all three forms of G6PD.

In the PGM system, PGM 1 has been found throughout Africa. PGM 2 has been seen in the homozygous condition only in the isolate from the coastal region of the United Republic of Tanzania and in the passaged Madagascan strain. The heterozygote PGM 1 × PGM 2 has also been found in these two isolates, in a single worm from Durban, and in 8 out of 89 male worms from Gezira. PGM 3 was present in several of the Gambian samples, in the Ghanaian isolate, and in that from Gezira.

The wide enzymic variation in the material from the Gezira, one of the most extensive irrigation systems in Africa, is perhaps not surprising. The presence of all of the G6PD and PGM types that have been identified so far probably reflects the fact that an agricultural enterprise of this magnitude attracts people from many areas, some of whom would be already infected. This mixing of different strains may have implications in the pathology of the disease caused by the parasites and their response to chemotherapy. A significant finding in this laboratory has been that, whereas in the Gezira region of the Sudan *S. haematobium* is transmitted only by *B. truncatus* snails, this isolate can also be passaged through snails of the *B. africanus* group. Since snails of the latter group are not known to occur in the immediate area, it appears that parasites normally transmitted by these snails have been introduced and have interbred with the local endemic strain, yielding hybrids capable of development in either host group. This dual host infectivity is a characteristic of hybrid schistosomes (*21*).

Brief reference has already been made to the occurrence of some anomalous PGM patterns. Fletcher et al. (*6*) have reported the presence of two loci for PGM in *S. mansoni*, one monomorphic in all populations and the other polymorphic with three detected alleles. Our data, based on isoelectric focusing, have shown the existence of three distinct patterns for PGM in *S. haematobium*, each consisting of at least six fractions. Each of these patterns appears to behave as a single unit, as though under the control of a single locus, and presumed heterozygotes between PGM 1 and either PGM 2 or PGM 3 show additive patterns combining the characteristics of both parental types. It is possible that variations in each of these basic patterns can be attributed to occasional post-transcriptional changes. However, the genetic background of the patterns produced by isoelectric focusing techniques is not yet fully understood. The multiple fractions revealed by this method may be merely technical artefacts or there may be a genetic relationship with each fraction. If such a polygenic relationship exists, then it is probable that the loci concerned are very closely linked and thus normally behave as a single unit. An explanation along these lines might account for the aberrant PGM 1 pattern illustrated in Fig. 1.10 where a null allele could be present at the locus controlling the missing fraction. This hypothesis is highly speculative at this stage but, should further investigation provide support for the idea, it is possible that such an explanation may also help to account for variations in the expression of some fractions in other systems.

Another so far unexplained result in this study is the apparent absence of heteropolymer fractions in presumed G6PD heterozygotes. In the hybrid *S. haematobium* × *S. mattheei* the extensive development of such bands lying between the two parental patterns is a characteristic feature (*20*). However, in presumed *S. haematobium* heterozygotes between G6PD 1 and either G6PD 2 or G6PD 3, no additional bands have been seen. It is possible that, with the close juxtaposition of the bands in these patterns, heteropolymer fractions are hidden by overlap with the parental bands. If this is the case, one would expect the whole area of overlap to be obscured into a single region of strong activity with no clear distinction of any fractions. Instead, it appears that fractions from the two parental patterns with slightly different pI values merely occur as pairs in the heterozygote without any additional activity, as though the G6PD of *S. haematobium* is monomeric. Here again, speculation upon the reasons for this phenomenon is premature but it is appropriate to identify it as a subject in need of further investigation.

Analysis of enzymes of adult schistosomes derived from naturally infected, wild-caught snails has provided an additional insight into the biology of schistosome transmission. Until now the only way in which multiple miracidial infections of individual snails could be detected was by the recovery of both male and female worms from animals exposed to cercariae shed from a single snail. In areas where the parasites exhibit sufficiently high levels of enzyme polymorphism, it is now possible to identify different genotypes of the same sex and thus extend the possibilities for detection of multiple miracidial infections. In several cases, we found that an all-male infection actually yielded adult worms with two genotypes. Our material from Tokuse in Ghana provided particularly impressive evidence of multiple infection. About 70 *B. rohlfsi* were collected from *Ceratophyllum* along a short stretch (about 10 m) of the shore of Weija Lake in front of a small settlement. Of these snails, 3 were shedding cercariae. Some hamsters were exposed to pooled larvae, while others were exposed separately to cercariae from each snail. One snail produced only female worms showing G6PD 3; the second snail gave rise to male worms showing G6PD 1 and G6PD 3, and PGM 3; the third yielded both male and female worms, the females monomorphic for G6PD and PGM but the males showing G6PD 1, G6PD 3, PGM 1, and PGM 3. Thus this third snail must have

been infected by at least three miracidia and the second by at least two. That miracidial densities could have been so high as to achieve multiple infections in two snails while the majority remained uninfected is surprising. It is unlikely that differences in potential susceptibility among the snail population alone could account for the result. It is more probable that the infected snails had been close to an intense input of miracidia and that miracidial dispersal in this kind of habitat resulted in rapid diffusion of the larvae so that only the hosts immediately adjacent to the site of contamination were at high risk of infection. A similar, and possibly even more complex case of natural multiple miracidial infection in a single snail has been recorded by Southgate et al. (*14*) for *S. bovis* in *B. africanus* from a stream in the United Republic of Tanzania.

Some minor problems still exist in the precise correlation of the data obtained by isoelectric focusing of enzymes with current genetic theory. The necessary breeding experiments required to elucidate these problems are at present hampered by the logistics of maintaining *S. haematobium* in the laboratory. Further progress will be possible when the techniques for cloning individual parasites by direct intermolluscan transplantation, as has been achieved for *S. mansoni* by Jourdane & Theron (*9*), have been modified for use with *S. haematobium* and bulinid snails. However, these temporary deficiencies in interpretation do not detract significantly from the value of the technique in population studies on *Schistosoma* because the high resolution of the results permits the detection of finer differences than are possible with conventional methods of enzyme electrophoresis. As more enzyme systems are analysed in greater detail in more species of schistosome, it becomes apparent that superficially similar enzyme patterns occur in more than one species. At the same time the existence of polymorphisms has eroded the original concept of "diagnostic" types. Nevertheless, provided that adequate background knowledge of local variation is available, it is now possible to make definite identification of unpaired worms derived from cercariae shed by individual naturally-infected snails.

Finally, in the study of strains of *S. intercalatum*, Wright et al. (*22*) concluded that interpretation of the enzyme data in terms of taxonomic status must await similar studies in other species. The results reported here for *S. haematobium* suggest very strongly that the enzyme differences found between isolates of *S. intercalatum* from Lower Guinea and Zaire probably do not, on their own, justify their recognition as distinct species.

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RÉSUMÉ

ANALYSE DES ENZYMES DE *SCHISTOSOMA HAEMATOBIUM*

Des études de laboratoire sur hamsters ont montré que des isolements de *Schistosoma haematobium* correspondant á des zones géographiques bien définies se distinguent par un certain nombre de caractères biologiques tels que l'infectiosité des cercaires, le taux de croissance et la durée de maturation du vers adulte, la production d'œufs et leur distribution dans les organes de l'hôte. Les différents isolements du parasite présentent également une compatibilité variable avec les diverses espèces de gastéropodes-hôtes du genre *Bulinus*. La présente étude enzymatique portant sur 22 isolements en provenance de 13 pays a été conduite par électrofocalisation en gel de polyacrylamide; elle a été entreprise en vue de caractériser avec plus de précision les variations présentées par ce parasite. Ce sont surtout les systèmes de la glucose-6-phosphate-déshydrogénase (G6PD) et de la phosphoglucomutase (PGM) qui ont été étudiés, mais dans certains cas également, la glucose-phosphate-isomérase, la malate-déshydrogénase, la lactico-déshydrogénase, la phosphatase acide et l'hexokinase. On a pu observer ainsi un polymorphisme au niveau de la G6PD et de la PGM et une certaine limitation géographique dans la distribution de certaines des formes de ces deux enzymes. La présence de ces formes géographiquement limitées au-delà de leur zone habituelle est probablement attribuable à la migration de personnes infectées vers de nouvelles régions. C'est ainsi que dans un isolement provenant du réseau d'irrigation de Gézireh au Soudan, on a relevé la présence des trois formes de G6PD et de PGM; le parasite était d'ailleurs compatible avec des espèces de *Bulinus* absents de la zone de transmission.

Le déterminisme génétique exact des fractions enzymatiques séparées par électro-focalisation n'est pas encore élucidé. L'apparition d'hétérozygotes présumés pour diverses formes de PGM et de certaines structures anor-

males du même système a conduit à former l'hypothèse que ces enzymes pourraient se trouver sous la dépendance de plusieurs gènes correspondant à des loci étroitement liés. Les auteurs soulignent également l'absence inexpliquée de fractions hétéropolymériques chez les hétérozygotes présumés pour la G6PD.

L'analyse des enzymes des vers adultes a permis d'identifier de multiples infections miracidiennes naturelles chez les gastéropodes capturés. Sur un échantillon de 70 *Bulinus rohlfsi* ramassés en un point d'une rive du lac Weija au Ghana, on en comptait trois qui excrétaient des cercaires de *S. haematobium*. En étudiant les enzymes des vers adultes obtenus à partir de chacun de ces gastéropodes, on a constaté que l'un avait été infecté par un miracidium, un autre par au moins deux et un troisième par au moins trois larves. Selon les auteurs, seuls les gastéropodes situés à proximité immédiate du point exact de contamination courent un risque élevé d'infection du fait de la dispersion rapide des miracidiums dans ce type d'habitat.

Enfin, à la lumière des résultats relatifs à *S. haematobium*, les auteurs estiment que les différences enzymatiques relevées antérieurement chez *S. intercalatum* entre les isolements du Zaïre et de Basse-Guinée ne permettent pas à elles seules de considérer que ces deux formes constituent des espèces distinctes.

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