The Taxonomy of Schistosomes

BY

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One of the duties of the Bilharzia Research Unit of the South African Institute for Medical Research is to screen snails sent in from the field. The examinations can take several forms. If "mammalian" type cercariae are obtained they can be examined live with phase contrast illumination or vital staining, or after fixation and staining. The papillary pattern or subtleties in morphology can then give a clue to the species. These methods are intricate; I have seen them demonstrated by Dr. John Goodman, but feel that the variability in results and the time, patience and skill required to interpret them put the methods outside those useful in routine work.

The usual method and the method used in our work is to expose laboratory rodents to the cercariae and hope that the resultant infection is bisexual when the most diagnostic feature of the various species of schistosome—the shape of the eggs—can be examined. Even this method is not infallible, as eggs intermediate between the typical forms can be obtained, but it is the method which is least open to objection.

Occasionally the resultant infection is unisexual and of course no eggs will be present in the host tissue. It is then necessary to fall back on the morphological characteristics of the adult flukes, which also can be notoriously variable. The overall size and the ratio and pattern of the gut elements may be of some use. In the male the number of testes and in the female the position of the ovary are also pointers, but once again careful and lengthy histological techniques are required. Unfortunately the results are as often as not equivocal, as the individuals in a single sexed infection tend to show an atypical morphology. The importance of identifying these single sexed individuals has led us to consider other techniques.

During the last few years the separation of proteins (amongst many other complexes) by electrophoretic methods has become a popular and often invaluable technique, particularly as a diagnostic tool in clinical medicine. Homologous proteins of different animal species or even strains often show different mobilities. As an example, Dr. Wright in London has detected differences in the protein patterns between species, sub-species and strains of the snails *Lymnaea*, *Bulinus* and *Biomphalaria* (Wright and Ross, 1965).

I thought that protein patterns of schistosomes might be useful in our work, so I subjected supernatants of flukes ground up with either water or saline to thin-layer starch-gel electrophoresis. The pattern of bands I obtained was very complex and variable. There were between 15 and 20 bands of differing intensities, and it was most difficult to obtain a standard separation. I then tried immersing the gel in an α-naphthyl acetate solution after an electrophoretic run, and was able to show three or four bands of non-specific esterase activity. However, again the starch-gel results were too variable to be of much use, although the number of bands were large enough to show differences in patterns, but not too large as to submerge slight variations. I then constructed a vertical flat-bed acrylamide apparatus (Reid and Bieleski, 1968), which gave a gel 2-3 mm. thick, which enabled six samples to be compared in one run of less than two hours and which needed less sample than the corresponding starch-gel method—an important consideration in view of the limited amount of material available.

There were other advantages, but they were technical and therefore need not concern us now.

The effect of pH on the length of the pattern of schistosome extracts produced the expected results. The higher the pH the faster the run, but the greater the distortion. We finally decided that pH 8.3 was the best compromise.

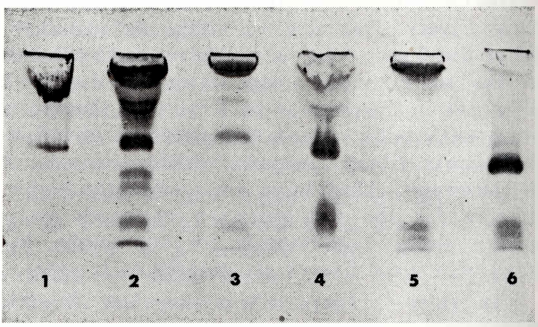

Fig. 1—Male flukes, α-naphthyl acetate esterases. 1, *S. mansoni* (Egyptian strain); 2, *S. mansoni* (South African strain); 3, *S. rodhaini* (Kampala strain); 4, *S. mattheei* (S.A.); 5, *S. haematobium* (S.A.); 6, *S. bovis* (Iranian strain).

We then compared the non-specific esterase patterns of extracts of male and female *S. mansoni* adult flukes and several features emerged (Fripp and McSheehy, 1969). First, that the male and female flukes showed similar patterns, and secondly that the intensities of the corresponding bands differed. These runs also demonstrated that eserine, an inhibitor of the cholinesterases (Pearse, 1960), inhibited the slowest band of both male and female extracts. This band was acetylcholinesterase, since it was identical with a band which hydrolyses acetylthiocholine iodide, but not the pseudocholinesterase substrate butyrylthiocholine iodide. [This work, together with the methodology, is dealt with at greater length in Fripp and McSheehy, 1969.]

We then applied samples from six different strains of schistosomes and after electrophoresis stained the gels for acetylcholinesterase. Extracts of male *S. mansoni* (South Africa), *S. mansoni* (Egypt) and *S. haematobium* (South Africa) seemed to have one strong and one weak band of activity, whilst *S. mattheei* had one strongly and several weakly reacting acetylcholinesterase isoenzymes. *S. bovis* (Iran) and *S. rodhaini* (Kampala, Uganda) produced only one band.

Amongst females, *S. mattheei* had two distinct bands, one weak and one strong. The extracts of the other schistosomes produced only one strong band.

Returning to the α-naphthyl acetate series, we found that the patterns were also distinctive (Figs. 1 and 2).

Extracts of male *S. mansoni* (E.), *S. mansoni* (S.A.) and *S. rodhaini*, all of which have *Biomphalaria* as the intermediate host, were clearly different from *S. mattheei, S. haematobium* and *S. bovis* which parasitise snails of the genus *Bulinus* (*Physopsis*). The patterns of the two cattle schistosomes are quite similar and enable them to be separated from *S. haematobium* by the presence of a strong intermediate band.

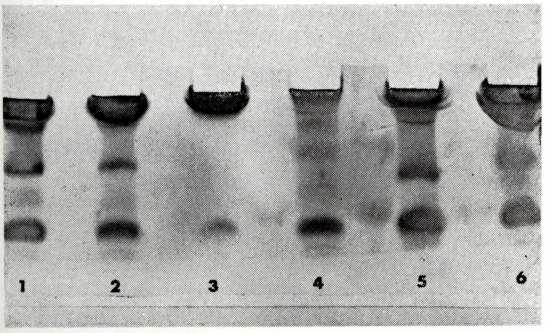

Fig. 2—Female flukes, α-naphthyl acetate esterases. 1, *S. mansoni* (S.A.); 2, *S. mansoni* (E.); 3, *S. haematobium* (S.A.); 4, *S. rodhaini* (K.); 5, *S. bovis* (I.); 6, *S. mattheei* (S.A.).

The ability to distinguish between *S. mattheei* and *S. haematobium* is probably the most useful diagnostic feature of the technique.

The differences between the female flukes are less distinct than those of the males. Once again *S. haematobium* and *S. mattheei* are easily distinguishable, and *S. mansoni* can be separated from *S. rodhaini,* although not quite so easily.

In summary, we found that the pattern of the isoenzymes which hydrolyse α-naphthyl acetate after separation on a polyacrylamide gel differed not only between species and strains, but between the sexes.

It is tempting to link the patterns with phylogenetic relationships. If we do this we can see close similarities between *S. mattheei* and *S. bovis* (as one would expect) and, although not so close, between *S. mansoni* and *S. rodhaini.* The patterns of *S. mansoni* (S.A.) and *S. mansoni* (E.) are slightly different, but no more so than those between *S. mattheei* and *S. bovis,* which might underline the close relationship of the two cattle schistosomes.

In addition to its diagnostic value, this polyacrylamide gel technique is valuable in the screening of drugs. Although we have only just started this facet, we can show differences between homologous enzyme systems in their response to inhibitors. Thus, the schistosome esterase isoenzymes seem to react towards eserine in a manner similar to the corresponding human serum enzymes (Pearse, 1960), but the inhibitor which is specific against mammalian acetylcholinesterase 62.C.47 (1:5-bis-(4-trimethylammoniumphenyl) pentane -3-one, diiodide) (Burroughs Wellcome Ltd.) is without effect. The method thus affords a quick and easy way to screen potential drugs against enzyme systems of schistosomes and to compare the effects of these compounds against the corresponding systems of the host.

Discussion

Dr. Lawrence: How often do you meet single sexed infections?

Dr. Fripp: Unfortunately we do get them fairly frequently. Each miracidium produces sporocysts of one sex, and unless you get more than one miracidium going into the snail, all the cercariae from one snail will be of the same sex. Since the incidence of infected snails in our collections is often low, the chances of cercariae being of the same sex is high.

Dr. Pitchford: Is it possible to use the electrophoretic technique with cercariae?

Dr. Fripp: Yes, it works, but it is difficult to get sufficient cercariae to carry out the process.

Dr. Shiff: What is the ratio of *mattheei* to *haematobium* in wild infections? Do you find a predominance of *mattheei?*

Dr. Fripp: Yes. Many more.

Dr. Reinecke: Do you get hybridisation of schistosomes in experimental infections?

Dr. Fripp: Dr. Pitchford claims you can, but I would think that this technique could resolve the question.

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