BIOCHEMICAL ASPECTS OF SCHISTOSOME BEHAVIOUR

P.J. FRIPP

Bilharzia Research Unit, South African Institute for Medical Research, Johannesburg.

ABSTRACT

The haematophagous blood trematodes that comprise the genus *Schistosoma* live in a stable environment with an adequate food supply and an efficient waste disposal system.

The main food is glucose obtained from the host blood and assimilated through the tegument. Other dietary factors are assimilated through the tegument or the gut epithelium. The male fluke supplements and augments the diet of the female who is thus able to concentrate on egg production.

Live flukes resist attack by the host by secreting an immunologically inert envelope and by incorporating unchanged host serum protein into their structures. They can also incorporate host structural fatty acids unchanged.

A schistosome is a "good" parasite. It is "good" in the sense that the host can tolerate its presence, and is little harmed by it. Most of the damage to the mammalian host arises as a result of the passage of the eggs from the blood vessels to the bladder or gut lumena. The scope of this paper is restricted to a consideration of the adult fluke and its association with its host so that the major failing of the schistosome will not concern us. Adult schistosomes are haematophagous, dioecious, dimorphic, invertebrate metazoa that parasitise mammals and birds, and their zoological position can be represented as follows:

Phylum: Platyhelminthes (flat worms);
Class: Trematoda (the flukes);
Order: Digenea ("2 host");
Family: Schistosomatidae
Genus: *Schistosoma* ("split bodied", referring to the ventral groove or gynaecophoric canal formed by the ventrally flexed lateral extensions of the body of the male in which the female lies).

The schistosomes that parasitise mammals live in the mesenteric veins of the hepatic portal system or in those around the bladder. They thus lie bathed in nutrient – the blood – which is constantly changing so that there is no lack of food. Neither do the schistosomes have any difficulty in getting rid of waste products; the parasites simply void them so that they are carried away in the blood stream of the host. Schistosomes do not have to contend with the problems of storage, concentration or detoxication of waste products that exist with other endo-parasites.

The schistosome's habitat could not be bettered from the point of view of an endo-parasite for the blood of the host supplies all the requirements necessary for the existence of the parasite in a soluble form. This fact was underlined when it was found that glucose (particularly plentiful in the hepatic portal system) is the main food of the schistosome. Schistosomes are surprisingly active animals, but even more remarkable is the fact that *Schistosoma mansoni*, which is responsible for intestinal schistosomiasis, utilizes glucose at a rate such that a weight of glucose equivalent to one fifth its own dry weight is metabolised each hour (Bueding 1950), which is a very high turnover.

Some of the glucose is converted to glycogen which is stored throughout the body, the rest (80-90%) supplies energy by being fermented to lactic acid, which is excreted through the flame cells of the nephridial system, and removed by the host blood. This anaerobic glycolysis takes place along the usual Embden-Meyerhof pathway with glucose entering the system via the hexokinase reaction.

The oxygen consumption increases in the presence of glucose, but the metabolism of glucose is independent of the presence of oxygen, which is rather strange considering that the schistosomes live in an environment of relatively high oxygen tension. It may be that this reflects an earlier stage in their evolution in which they occupied a more anaerobic site within the host.

A weak cytochrome system has been demonstrated, and it seems that such a terminal oxidase system (Bueding & Charms 1951) is unimportant. Until recently it was thought that an aerobic conversion of pyruvate to carbon dioxide and water was also superfluous and the Krebs tricarboxylic acid cycle was either absent or restricted, but we have recently demonstrated several of the enzymes involved in aerobic glycolysis using polyacrylamide gel electrophoresis (Fripp 1970). So far we have separated a succinate dehydrogenase, two malate dehydrogenases and an iso-citrate dehydrogenase from schistosome homogenates. However, this system seems to be as unimportant to the survival of the individual schistosome as the terminal oxidase system.

The parasite's disinterest in the aerobic cytochrome oxidase and Kreb's cycle systems which are of vital importance to free living animals, can be understood if their main function in the host is considered. This function is to remove the products formed in the metabolism not only of carbohydrates but proteins and fatty acids as well. The schistosome converts pyruvate to lactate and possibly to fat; there is simply little need for the aerobic systems. On the other hand since the metabolism of glucose and glycogen is so important to the parasite, the energy producing Embden-Meyerhof system is well represented.

Although the enzymes found in the schistosomes have similar functions to those in the host, they have characteristics of their own, and it is this facet of the biochemistry of schistosomes that is occupying a lot of our attention at the moment. The differences in the behaviour of homologous enzymes could possibly be made use of by the pharmacologist in the synthesis of drugs that would inhibit a vital enzyme of the parasite – and thus kill it – but would be less toxic to the host enzyme. This, by hind sight, for the drugs were in use before the fact was known, is the rationale for the use of trivalent antimonial drugs in the treatment of bilharziasis. Antimony inhibits a key enzyme in the Embden-Meyerhof chain, phosphofructokinase, which catalyses the reaction

The schistosome enzyme is inhibited by low concentrations of antimony, whereas the phosphofructokinase of the host is much less sensitive to the trivalent metal ions (Mansour and Bueding 1954).

We have found other differences in the responses to various components but unfortunately they have all been to the advantage of the parasite. The inhibitors that we have so far studied inhibit the host enzymes more easily than the corresponding ones of the parasite.

The schistosome gut is a long diverticulum, so that what goes in through the mouth is either

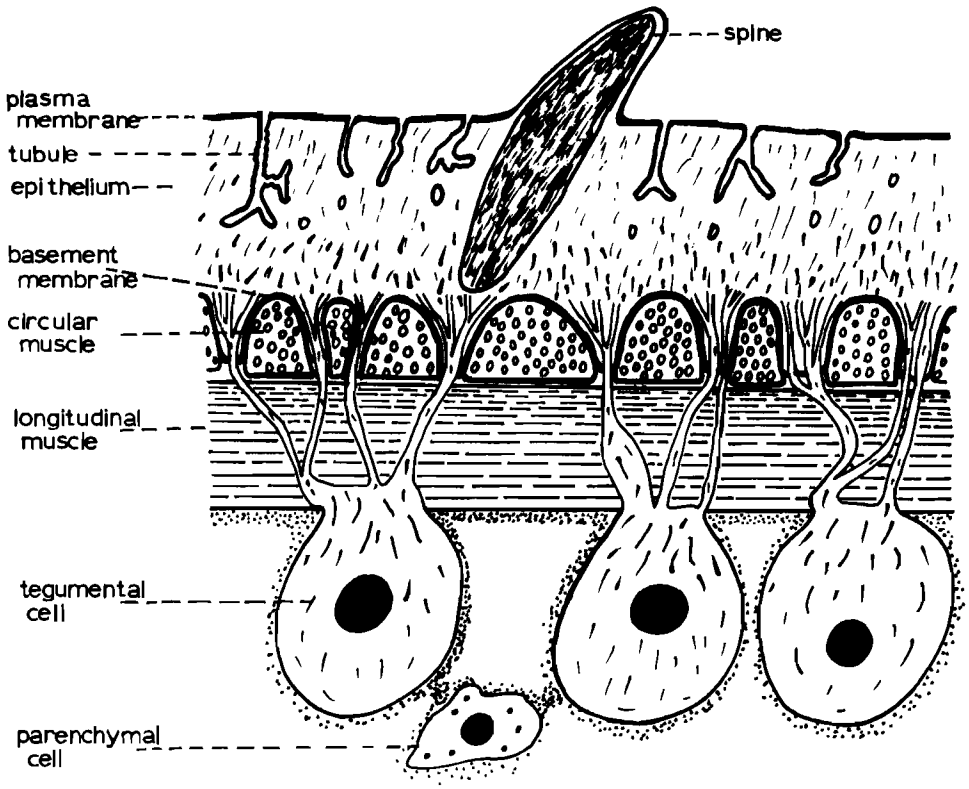

FIGURE 1
Diagram of the tegument and associated structures of *Schistosoma*.

absorbed or comes out the same way, and the turnover is slow. The peristaltic movements of the gut wall do not result in large scale expulsion of the contents, and it is difficult to see how the gut can supply the large amount of glucose that the schistosome needs, even if the gut epithelium is convoluted in order to increase its surface area. Moreover, the gut is clogged with a black sticky mass derived from the breakdown of the haemoglobin from ingested host erythrocytes, which increases even further the inability of the gut to cope with the problem of obtaining sufficient glucose.

The logical place for glucose assimilation to take place is through the outer skin, which lies bathed in glucose-rich blood, and this is in fact what happens. The tegument is not a hard impervious "cuticle", but a highly organised active syncitial structure secreted by cells situated deep below the musculature (Fig. 1).

We were able to demonstrate that glucose was actually absorbed through the tegument by incubating live flukes in a medium containing radioactive glucose, and preparing autoradiographs (Fripp 1967b). The radioactivity was strong on the dorsal and lateral outer parts of the tegument of the male, but less on the ventral parts – the gynaecophoric canal – whereas there was a more

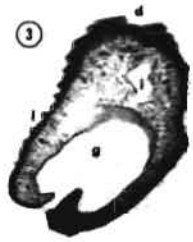

FIGURE 2
Autoradiograph (A) of dry-mounted longitudinal section (S) of male and female *S. haematobium* after incubation *in vitro* for 2 hr in medium containing I-C14 glucose. The radioactivity is concentrated in the peripheral tissues. (For details of the technique see Fripp 1967b).

FIGURE 3
Beta-glucuronidase activity in *S. mansoni* (male, T.S.) The gut (i) and margins of the gynaecophoric canal (g) are less reactive than the dorsal (d) and lateral (l) peripheral areas and the tegumental cells.

general distribution within the peripheral tissues of the females (Fig. 2).

We have also shown the presence of several enzymes in the tegument using histochemical techniques. These have included β-glucuronidase, a hydrolytic enzyme that might have had a digestive function originally, but seems to be concerned with steroid metabolism, and also the breakdown of oligosaccharides (Fig. 3), and the exopeptidase aminopeptidase (Fig. 4) (Fripp 1966; 1967a).

The presence of aminopeptidase is interesting as it indicates that the amino-acids that it splits off from the serum polypeptide chains can be incorporated into the fluke through the tegument. Alkaline phosphatase, which is often associated with areas of glucose uptake (but is probably not part of the assimilatory process itself) was found in the tegument but not in the gut epithelium.

The only enzyme so far that has been histologically demonstrated in large amounts in the gut epithelium, is acid phosphatase, but this enzyme has a wide, fairly general distribution throughout the body of both the male and female flukes. There are proteolytic enzymes in the gut that are probably associated with the breakdown of the haemoglobin. In fact, a globinolytic enzyme has been demonstrated biochemically (Timms & Bueding 1959). These proteases must be weak or highly substrate-specific enzymes as they seem to be incapable of denaturing enzymes such as β-glucuronidase in the host blood swallowed by the schistosome.

The work just described furnishes strong evidence for the existence of a division of labour between the gut and the tegument of schistosomes associated with the assimilation of food. Thus the alicyclic amino-acids derived from the red cells such as histidine and tryptophan (which is based on the indole nucleus) and also arginine, for which the schistosome has a predilection, and the strongly ketogenic amino-acid leucine are assimilated through the gut epithelium whilst other amino-acids, either free or released from peptides by aminopeptidase, and glucose enter the schistosome through the tegument.

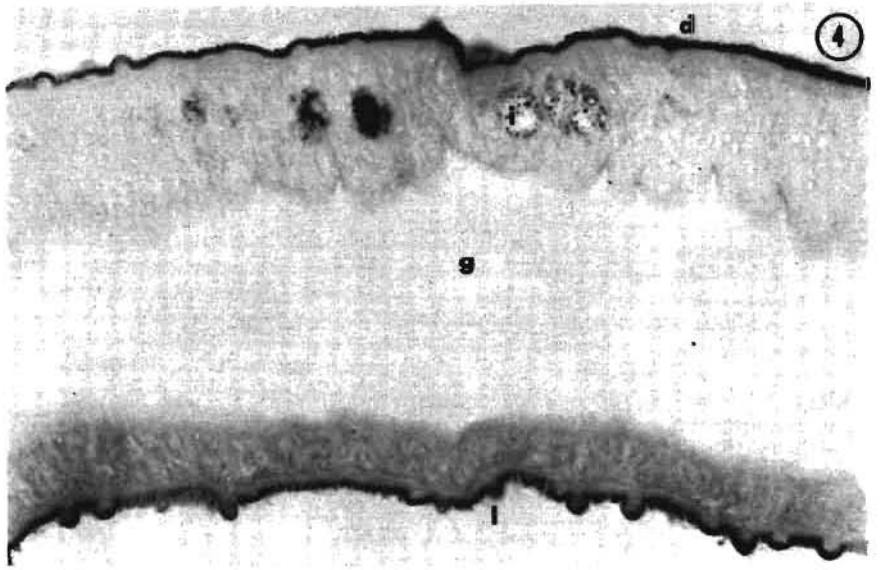

FIGURE 4
Amino-peptidase activity in *S. rodhaini* (male, L.S.) The activity is concentrated in the epithelial tissues. The black area in gut (i) is pigment. Lettering as for Fig. 3.

However, this is an over-simplification. An isolated female fluke is smaller than one clasped in the male's gynaecophoric canal, and it seems that the female can only obtain certain factors necessary for her wellbeing or development from her partner. An example of this exists in the case of proline. Proline, an amino-acid used extensively in egg production, is absorbed from the gut of the male but not from that of the female. She obtains it from the male across the floor of the gynaecophoric canal (Senft 1970). This dietary dimorphism may also explain why some drugs more readily affect the male flukes.

The male is thus not only a sexual partner for the female. In addition he supplies her with various essential factors, leaving her to concentrate on her main function, the production of eggs, which if figures can be believed are laid every few seconds. The tanning of her eggs requires oxygen and this can explain her higher oxygen consumption calculated on a weight basis which allows for the difference in size between the female and the larger male flukes.

A live schistosome is tolerated by the host, it does not stimulate a foreign body reaction by the host, but when the parasite dies, it is quickly invaded and digested by the host's leucocytes. There is evidence that this resistance has at least two facets, both active processes.

First, the schistosome continuously secretes an immunologically inert envelope that covers the tegument. We have demonstrated the presence of a mucopolysaccharide cover on the surface of these live flukes but it is not the complete story. Other compounds, perhaps heparin for instance, might also be involved.

Secondly, the schistosome can somehow incorporate protein from the host into its body thereby retaining the immunological identity of the host. The host is deceived into assuming

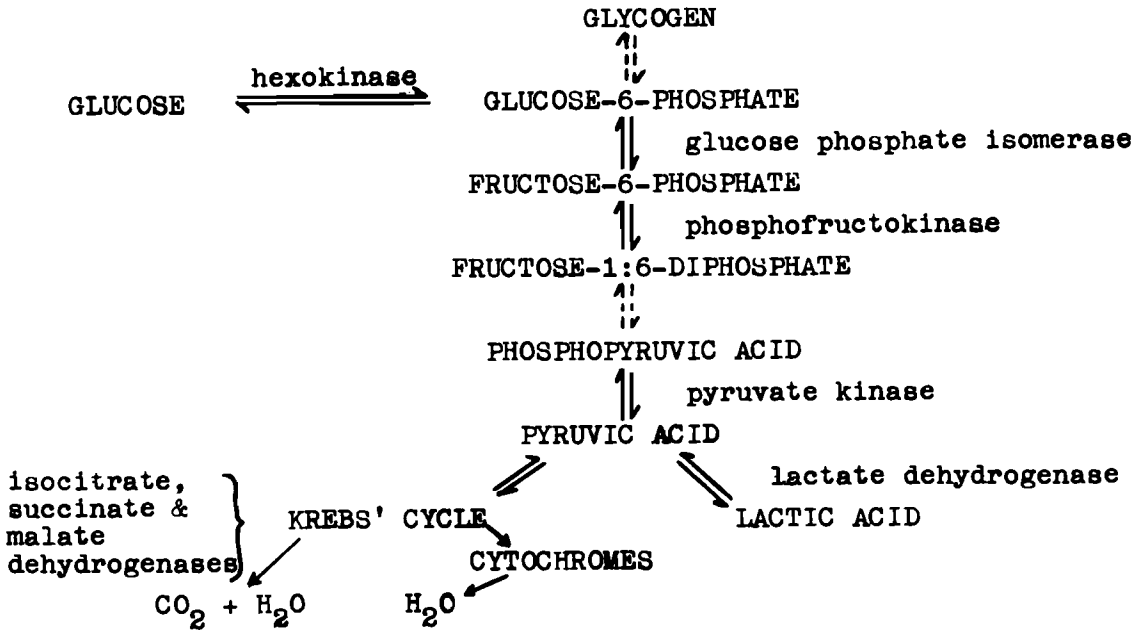

FIGURE 5
Simplified diagram of glucose metabolism to show the enzymes that have been demonstrated in schistosomes.

that the schistosome is part of itself. If the schistosome is transplanted from, for instance, a mouse to a monkey, the new host tries to reject it until the schistosome replaces the "foreign" protein with the new host protein (Smithers 1968).

A further incorporation of host material occurs with fatty acids. We have not been able to detect any schistosome lipases although we have looked hard and long. If they do not occur, it is likely that fatty acids are absorbed as such. One of our current studies has revealed that the pattern of structural fatty acids follow that of the host, that is to say, the relative proportions of the various fatty acids in the structural fats of the schistosome body tends to be similar to those of the host serum and therefore the profiles vary with those of the host. In addition, the fatty acids are also selectively absorbed and those that appear in the schistosome body are depleted in the serum (Fripp & Crawford, in press).

Lipids occur in the schistosome body, but these cannot be degraded without lipases. It is possible that lipids constitute an excretory product that can be stored or voided via the flame cells, but this is not proven, although lipids are recognised as terminal excretory products of intestinal helminths.

As a corollary to the two observations on proteins and fatty acids, can we really call a schistosome a "true" parasite since parts of its structure are host material? The enzymology of host and parasite differ, so we may with safety continue to call a schistosome a parasite, with its own identity.

REFERENCES

BUEDING, E. 1950. Carbohydrate metabolism of *Schistosoma mansoni*. *J. gen. Physiol.* 33: 475-495.

BUEDING, E. and CHARMS, B. 1951. Respiratory metabolism of parasitic helminths without participation of the cytochrome system. *Nature, Lond.* 167: 149.

FRIPP, P.J. 1966. Histochemical localization of β-glucuronidase in schistosomes. *Expl Parasit.* 19: 254-263.

FRIPP, P.J. 1967(a). Histochemical localization of leucine amino-peptidase in *Schistosoma rodhaini*. *Comp. Biochem. Physiol.* 20: 307–309.

FRIPP, P.J. 1967(b). The sites of (1-^{14}C) glucose assimilation in *Schistosoma haematobium*. *Comp. Biochem. Physiol.* 23: 893-898.

FRIPP, P.J. 1970. Separation of mammalian schistosome iso-enzymes by vertical thin-layer polyacrylamide gel electrophoresis. *J. Parasit.* 56: Sect. II, 422.

MANSOUR, T.E. and BUEDING, E. 1954. The actions of antimonials on glycolytic enzymes of *Schistosoma mansoni*. *Br. J. Pharmac. Chemother.* 9: 629-634.

SENFT, A. 1970. *De novo* or salvage pathway of purines in *Schistosoma mansoni*. *J. Parasit.* 56: Sect. II, 314-315.

SMITHERS, S.R. 1968. Immunity of blood helminths In: *Immunity to blood parasites.* ed. A.E.R. Taylor, Oxford: Blackwell.

TIMMS, A. and BUEDING, E. 1959. Studies of a proteolytic enzyme from *Schistosoma mansoni*. *Br. J. Pharmac.* 14: 68-73.