REVIEW ARTICLE

MECHANISMS OF ACTION OF SCHISTOSOMICIDAL AGENTS*

BY ERNEST BUEDING, M.D.

Professor of Pharmacology, Louisiana State University, School of Medicine, New Orleans, Louisiana, U.S.A.

Visiting Professor of Pharmacology, University of Oxford

MORE than 25 years ago, A. J. Clark[1] pointed out that drugs may bring about their effects on living cells by affecting enzymes. In the present article the validity of this hypothesis will be considered by analysing the biochemical actions of organic trivalent antimonials on the adult stages of the parasitic worm, *Schistosoma mansoni*. In addition, the metabolic effects of another group of schistosomicidal agents will be reviewed.

Schistosomes invade the portal and mesenteric veins, the veins of the urinary bladder and the liver sinuses of man and of other mammals. They undergo a life cycle involving certain snails as intermediate hosts and penetrate the mammalian skin when the latter comes in contact with water containing the larvae which have been shed by the snails. Schistosomiasis is caused by three species, *Schistosoma mansoni*, *Schistosoma haematobium* and *Schistosoma japonicum*, and is endemic in Egypt, other parts of Africa, Central and South America, the Middle East, China, Japan and some Pacific islands. It has been estimated[2] that over 100 million human beings suffer from schistosomiasis.

Schistosomes do not depend primarily on respiratory metabolism for survival [3–6] although the oxygen tension of their environment is relatively high[7,8]. When schistosomes are cultured under completely anaerobic conditions, they remain alive for a period of at least five days[9]. Furthermore, administration of cyanine dyes to the host causes an almost complete inhibition of the oxygen uptake of the parasites; yet, these dyes have no chemotherapeutic activity against schistosomes[6]. Survival and reproduction of schistosomes depend almost entirely on the anaerobic utilisation of carbohydrate. The rate of this utilisation is extremely high. In one hour, schistosomes metabolise an amount of glucose equal to one-fifth of their dry weight[4]. In contrast to other helminths, schistosomes convert glucose quantitatively to lactic acid[4]. In this respect the metabolism of the parasite resembles that of the host; as in vertebrate tissues, lactic acid is formed *via* the Embden-Meyerhof scheme of phosphorylating glycolysis (Table I) and the occurrence of enzymes involved in this series of reactions can be demonstrated in the parasite[10–14].

ANTIMONIALS

So far, no completely satisfactory chemotherapeutic agent against infections produced by schistosomes is known. However, trivalent

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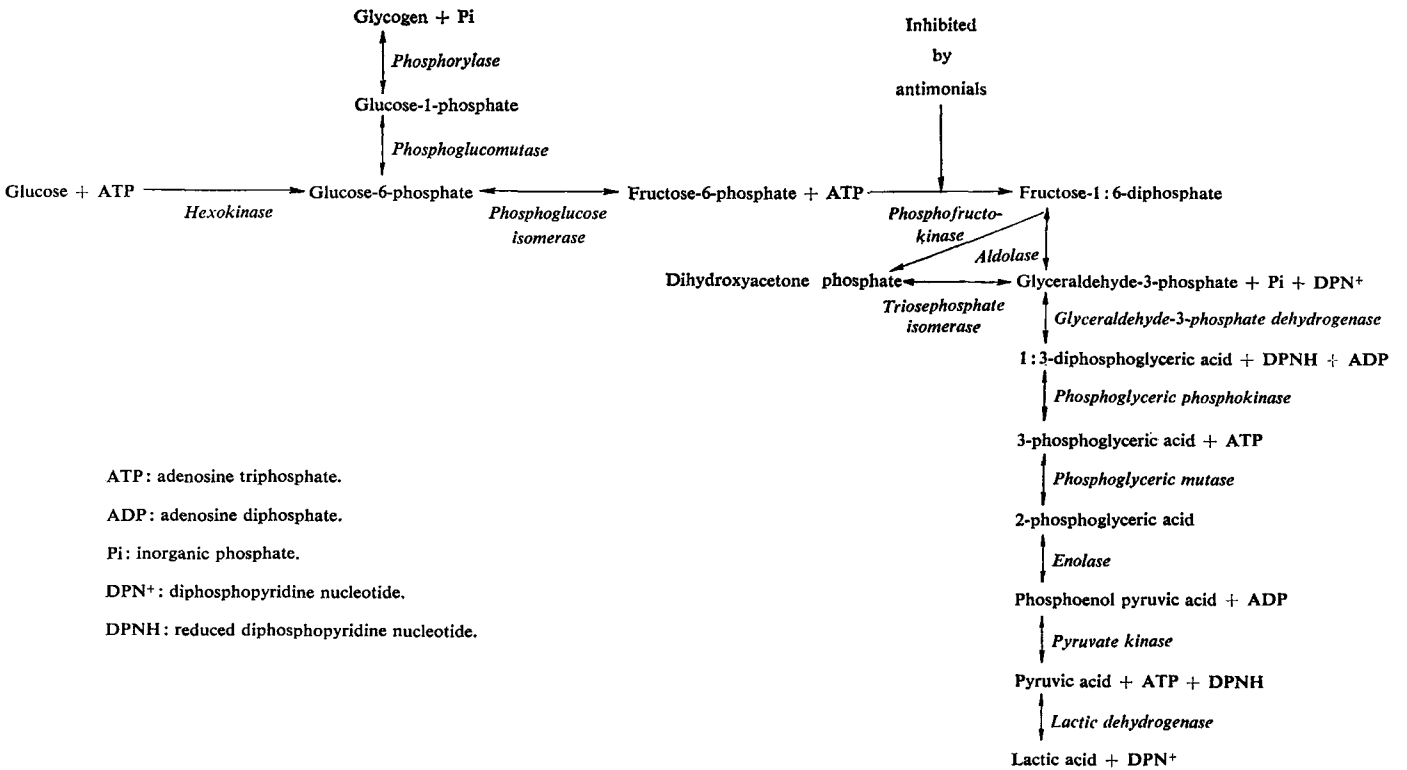
TABLE I

REACTIONS AND ENZYMES INVOLVED IN GLYCOLYSIS OF GLUCOSE AND OF GLYCOGEN



ATP: adenosine triphosphate.

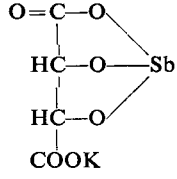
ADP: adenosine diphosphate.

Pi: inorganic phosphate.

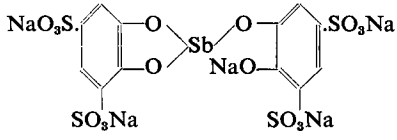
DPN+: diphosphopyridine nucleotide.

DPNH: reduced diphosphopyridine nucleotide.

organic antimonials are quite effective in the treatment of schistosomiasis although they are rather toxic[15–17]. Among these compounds potassium antimony tartrate (tartar emetic, I) and sodium antimony biscatechol disulphonate (stibophen, "fuadin," II) are used most frequently.



(I) Antimony potassium tartrate



(II) Stibophen

Low concentrations of these compounds markedly reduce the rate of glycolysis of the intact worms[4] and of homogenates of the parasites. Stibophen inhibits glycolysis of worm extracts[11] when glucose, glucose-6-phosphate or fructose-6-phosphate are used as the substrate. However, lactic acid formation from hexosediphosphate is not affected by this compound. Similar results have been obtained with antimony potassium tartrate. These observations indicate that antimonials inhibit glycolysis by blocking the formation hexosediphosphate from fructose-6-phosphate. This reaction is catalysed by phosphofructokinase and is the result of the phosphorylation of fructose-6-phosphate by adenosine triphosphate (Table I). Direct measurements of the effects of antimonials on schistosome phosphofructokinase have shown that these compounds markedly inhibit the activity of this enzyme[11]. It should be noted that the phosphofructokinase of the host has a much lower sensitivity to antimonials than the enzyme of the parasite. For example, the concentration of potassium antimony tartrate required to inhibit the mammalian enzyme to an extent of 50 per cent is 80 times higher than that which has a similar effect on the enzyme of the parasite[11]. Even with the highest concentration of stibophen used (1×10^{-2} M) no inhibition of the mammalian enzyme was observed. This selective effect of antimonials demonstrates that the enzymes which have the same catalytic function in the parasite and in the host are not identical with each other; in addition, the toxicity of antimonials for the host cannot be ascribed to an inhibition of phosphofructokinase activity. Differences at various levels in the nature of homologous glycolytic enzymes of *S. mansoni* and of its mammalian host have been demonstrated also in the cases of hexokinase[12], of phosphoglucose isomerase[13] and of lactic dehydrogenase[10,18,19]. Such differences suggest possibilities for interfering with the functional integrity

of enzymes of the parasite without affecting those catalysing the same reactions in the host.

If, in schistosomes, the rate of the phosphofructokinase reaction were limiting the rate of glycolysis of the parasite, inhibition of the activity of phosphofructokinase by antimonials would account for the inhibitory action of the latter on glycolysis. Since addition of purified rabbit muscle phosphofructokinase[20] causes an increase in the rate of glycolysis of schistosome homogenates, it is evident that the reaction catalysed by phosphofructokinase is determining the glycolytic rate of these preparations. Furthermore, the inhibitory effect of stibophen and of antimony tartrate on lactic acid production by schistosomes is abolished by the addition of mammalian phosphofructokinase[14]. Thus, lactic acid production from glucose by schistosome extracts is susceptible to inhibition by trivalent antimonials and this metabolic defect is corrected specifically by the addition of an excess of mammalian phosphofructokinase. This suggests that the reaction catalysed by phosphofructokinase might be the rate-limiting step of glycolysis in schistosome homogenates. Yet, under optimal conditions the rate of conversion of fructose-6-phosphate to fructose-1 : 6-diphosphate is more rapid than the rate of the next step in glycolysis[14], the formation of 2 moles of triosephosphate from 1 mole of fructose-1 : 6-diphosphate, a reaction catalysed by aldolase. Therefore, the rate of the reaction catalysed by phosphofructokinase is not the limiting factor. In view of these results the possibility has been explored whether the concentration of the product of the phosphofructokinase reaction could affect the rate of glycolysis. This was tested by determining the effect of the concentration of fructose-1 : 6-diphosphate on the activity of schistosome aldolase. Optimal activity is observed at a relatively high concentration of the substrate. If the concentration of the substrate is reduced below a certain critical level, a very sharp decline in the activity of aldolase occurs[14]. For example, if during glycolysis of schistosomes the molar concentration of fructose-1 : 6-diphosphate does not exceed 5×10^{-4}, even a slight decrease below this concentration, due to inhibition of phosphofructokinase activity by antimonials, would markedly reduce the activity of aldolase, resulting in an inhibition of glycolysis. Conversely, addition of mammalian phosphofructokinase would increase the rate of hexosediphosphate formation giving rise to a significant increase in aldolase activity, thereby increasing the rate of lactic acid production. Finally, inhibition of hexosediphosphate formation by antimonials could be abolished by supplying an excess of phosphofructokinase. These changes are in agreement with those observed experimentally[14]. Therefore, antimonials reduce the rate of glycolysis of schistosomes by an inhibition of the activity of phosphofructokinase; this inhibition brings about a decreased formation and thus a lower concentration of fructose-1 : 6-diphosphate which in turn results in a decrease in the activity of aldolase.

These observations were made using cell-free preparations of the worms; thus, the problem arises whether similar mechanisms operate also in the intact parasite. Utilisation of glucose and production of lactic acid by

intact schistosomes are reduced by antimonials in concentrations similar to those which inhibit the activity of the parasite's phosphofructokinase[4,14]. If antimonials were producing in intact worms an increase in the concentration of the substrate and a decrease in the concentration of the product of the phosphofructokinase reaction, this would supply evidence for the inhibition of this enzyme. Potassium antimony tartrate, in a concentration of 1×10^{-4} molar, markedly inhibits the activity of schistosome phosphofructokinase and the rate of glycolysis of worm extracts. With the same concentration of the antimonial, survival of the worms *in vitro* is reduced from 30 days to less than eight hours, and a definite reduction in the motility becomes evident after one to two hours. At this period the concentration of fructose-1:6-diphosphate in the worms is reduced markedly while fructose-6-phosphate accumulates[14]. These changes indicate an inhibition of phosphofructokinase activity during the relatively brief exposure of the parasite to potassium antimonyl tartrate. A similar reduction in the concentration of di- and an increase in that of the monophosphate ester of fructose is observed in worms obtained from mice which have received subcurative doses of stibophen[14], that is, a dosage regime producing a slight shift in the distribution of the worms from the mesenteric to the portal veins[21]. These alterations in the concentrations of phosphate esters in schistosomes indicate an inhibition of phosphofructokinase activity after exposure of the worms to antimonials within the host.

Criteria for Drug Enzyme Inhibition

The selective action of trivalent antimonials on phosphofructokinase of *S. mansoni* raises the question about the relationship between this drug-enzyme interaction and the chemotherapeutic effect of antimonials in schistosomiasis. While a multitude of enzymes are affected by drugs, it has been demonstrated only in a relatively few instances that such effects are responsible for the pharmacological or chemotherapeutic action of a particular drug. This in no way invalidates Clark's drug-enzyme theory, but can be ascribed to many inherent experimental difficulties and to frequent neglect in relating the effects of drugs on isolated biological systems to their action on the intact organism. In a discussion of this problem, Hunter and Lowry[22] have directed attention to certain requirements which must be met before rigorous proof can be accepted that a drug acts by inhibiting a particular enzyme. These criteria will be applied to the inhibitory effect of antimonials on phosphofructokinase of schistosomes.

1. *The enzyme concerned should be inhibited in the intact cells.* Exposure of schistosomes to low concentrations of potassium antimonyl tartrate *in vitro* or administration of subcurative doses of stibophen to the host produces an accumulation of the substrate and a reduction in the concentration of the product of the phosphofructokinase reaction, indicating that the activity of the enzyme is inhibited within the intact schistosomes.

2. *The inhibition of the enzyme should quantitatively explain the effects of the drug.* Inhibition of phosphofructokinase is responsible for the

reduction in the rate of glycolysis of *S. mansoni.* Since glycolysis supplies the major, if not exclusive, source of energy for the schistosomes it is quite conceivable that inhibition of glycolysis accounts for the death of the worms.

3. *Enzyme inhibition must occur with an amount of drug no greater than that necessary to produce the drug action.* This criterion has been met also. Survival of the parasites *in vitro* is reduced from 30 days to eight hours by exposure to antimonials in concentrations which produce an inhibition of schistosome phosphofructokinase activity to an extent of over 50 per cent.

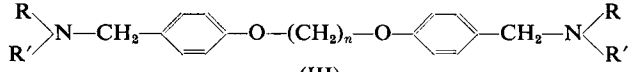
4. *If an isolated enzyme is inhibited by a concentration as low as that producing an effect on the intact organism it must be established that other cell constituents do not bind a substantial fraction of the drug.* Given concentrations of an antimonial inhibit phosphofructokinase activity of crude schistosome homogenates to the same degree as do those of purified preparations of this enzyme; therefore, it appears that there is no significant binding of antimonials by other constituents of schistosome cells.

On the basis of these considerations it is concluded that inhibition of phosphofructokinase activity can account for the schistosomicidal activity of trivalent antimonials. While the possibility cannot be excluded that these drugs may interfere also with other mechanisms essential for the survival of the parasite, these observations have revealed, within the metabolism of schistosomes, a vulnerable point which is susceptible to inhibition by a group of chemotherapeutic agents.

Alkyldibenzylamines

While antimonials have a marked inhibitory effect on a glycolytic enzyme of schistosomes another series of schistosomicidal agents exert their action through a different mechanism.

McCowen, Callender, Rennel, and Lawlish[23] have reported the amoebicidal activity of a series of alkyldibenzylamines of general formula III.



(III)

In an attempt to determine the antiparasitic spectrum of this series of compounds, it was noted that fairly low concentrations of these substances markedly reduce the survival of *S. mansoni in vitro*[24]. Incubation of schistosomes with these compounds produces paralysis of the worms; this is preceded by marked hyperactivity of the worms. On the basis of this observation the antischistosomal properties *in vitro* of some alkyldibenzylamines have been determined.

Secondary amines have considerably higher antischistosomal activity than the corresponding tertiary amine analogs. Another structural factor which has a significant effect on antischistosomal activity is the length of the central carbon chain. Optimal activity is observed with 6 carbons and a progressive decrease in activity occurs with either shortening or lengthening of the central carbon chain. When the latter

contains 6 carbons the nature of the substituent group of the secondary amine has no appreciable effect on activity. Quaternisation of the nitrogens abolishes activity.

Because of the significance of glycolysis as a source of energy for the parasite, the possibility has been investigated whether the action of dibenzylamines on the worms is associated with an interference in carbohydrate metabolism. To this end glucose utilisation and lactic acid production by schistosomes have been determined during incubation for one hour with sub-effective concentrations of alkyldibenzylamines, that is with a concentration below the one which produces changes in muscular activity during the period in which carbohydrate utilisation is measured. In the presence of a diamine, glucose utilisation is reduced significantly, but lactic acid formation is inhibited to a much lesser degree[25,26], indicating that under these conditions lactic acid must have been produced from an endogenous source. This has been confirmed by the observation that changes in the motility of schistosomes, produced by dibenzylamines, are preceded by a marked increase in glycogenolysis of the worms[26]. Following the formation of glucose-6-phosphate the pathways and enzymes concerned with the production of lactic acid are identical for glucose and for glycogen (Table I). As formation of lactic acid from glycogen is not inhibited, it appears that alkyldibenzylamines interfere either with the formation of glucose-6-phosphate from glucose or with the uptake of glucose by schistosomes. Even high concentrations of alkyldibenzylamines have no inhibitory effect on the activity of hexokinase or on the rate of glycolysis of cell-free homogenates or extracts of schistosomes; nor do they stimulate the activities of phosphorylase, of phosphoglucomutase or ATPases of the worms[26]. Because of the absence of any direct effect of high concentrations of alkyldibenzylamines on enzymes involved in the carbohydrate metabolism of the parasite it is concluded that these compounds interfere with the active transport of glucose into the worm and that the increased glycogenolysis of intact schistosomes produced by these compounds is secondary to the lack of utilisable exogenous glucose. It is noteworthy that the rate of glycolysis of cell free extracts of schistosomes is three to five times higher than that of the intact organism. Therefore, the rate of glycolysis of the latter is limited by the rate of its glucose uptake.

Because of the dependence of schistosomes on a high rate of carbohydrate metabolism, the parasite must be vulnerable to interference with glucose transport. Since antimonials interfere with another phase of the carbohydrate metabolism of the worms, i.e. with their phosphofructokinase activity, the susceptibility of schistosomes to *simultaneous* inhibition at two levels of their carbohydrate metabolism has been tested. During exposure of the worms to low concentrations (1 μg./ml.) of both an alkyldibenzylamine and of stibophen, survival of the parasite is reduced to a much greater degree in the presence of both these compounds than with the same concentration of each compound alone[26]. Therefore, the schistosomes are vulnerable to simultaneous interference at two distinct and critical levels of their carbohydrate metabolism.

Conclusions

Investigations of the metabolic effects of two groups of schistosomicidal agents have revealed that trivalent organic antimonials interfere specifically with the activity of a single glycolytic enzyme of the parasite while alkyldibenzylamines exert an inhibitory effect on the transport of glucose into the intact worm. It is concluded that a biochemical approach to chemotherapeutic problems can provide a better understanding of the mode of action of drugs and eventually may contribute to the rational, as opposed to the empirical, development of effective pharmacological and chemotherapeutic agents.

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