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THE ACTIONS OF ANTIMONIALS ON GLYCOLYTIC ENZYMES OF *SCHISTOSOMA MANSONI*

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It is generally assumed that the chemotherapeutic action of organic antimonials is brought about by mechanisms similar to those of arsenicals—that is, by interaction with, and inactivation of, sulphydryl enzymes (Findlay, 1950). Both arsenicals and antimonials have chemotherapeutic activity against the same parasitic protozoa, such as certain species of trypanosomes and leishmania. On the other hand, antimonials are effective in schistosomiasis whereas arsenicals are not. In *Schistosoma mansoni*, glycolysis proceeds at an exceedingly rapid rate, and this metabolic process is more essential for the survival of the worms than is respiration. It has been found previously that glycolysis in intact worms is inhibited by organic antimonials (Bueding, 1950). In the present paper the effects of antimonials on glycolysis of worm homogenates, and on some of their glycolytic enzymes, are reported.

METHODS

The adult worms were obtained as in previous studies (Mansour and Bueding, 1953). The parasites were homogenized in cold potassium glycylglycine buffer (0.01M, *p*H 7.5) in an all-glass homogenizer; 1 ml. of buffer was used for each 100 pairs of worms. The final molar concentrations of the constituents in the medium, optimal for glycolysis of the homogenates, were as follows: potassium glycylglycine buffer, 5×10^{-2} (*p*H 7.5); glucose, 8×10^{-3}; adenosine triphosphate (ATP), 5×10^{-3}; diphosphopyridine nucleotide (DPN), 7×10^{-5}; nicotinamide, 3×10^{-3}; magnesium chloride, 8×10^{-3}. Schistosome homogenates were shaken in this medium at 38° C. for 1 hr. The mixtures were deproteinized by the addition of barium hydroxide and of zinc sulphate (Somogyi, 1945a). For the determination of preformed lactic acid control samples were deproteinized before incubation. Lactic acid was determined according to Barker and Summerson (1941). The same procedure was used for measuring the rate of glycolysis in the presence of two other substrates, fructose-6-phosphate (F-6-P) and fructose-1,6-diphosphate (HDP). Potassium arsenate (final concn. 1×10^{-3}M) was present in the medium when HDP was used as the substrate. Glucokinase, fructokinase and mannokinase activities were measured by incubating the enzyme preparations of the worms with the hexose and with ATP in the presence of magnesium chloride, for a period of 10 min. at 38° C. The final molar concn. of the constituents optimal for this assay were: glucose or mannose, 1.5×10^{-3}; fructose, 1.6×10^{-2}; ATP, 7×10^{-3}; $MgCl_2$, 1×10^{-2}; potassium glycylglycine buffer (*p*H 7.5), 5×10^{-2}. The hexose was added to the experimental samples before incubation and to the control samples immediately after incubation. Protein and phosphate esters were precipitated with barium hydroxide and zinc sulphate (Somogyi, 1945a). The concentration of the non-phosphorylated hexose was determined in the filtrate (Somogyi, 1945b). Hexose utilization was calculated from the difference between the free hexose concn. of the control (reaction mixture incubated without the hexose) and that of the experimental sample. Purification of glucokinase and of phosphohexose isomerase was carried out according to Bueding and MacKinnon (unpublished methods). Phosphohexose isomerase activity was measured by incubating (38° C., 15 min.) the enzyme preparation of the worms in media containing glucose-6-phosphate (molar concn. 1.25×10^{-2}) and potassium glycylglycine buffer (*p*H 7.5; molar concn. 5×10^{-2}). Proteins were precipitated with one volume of trichloroacetic acid (15% w/v). The concn. of the fructose ester formed was determined by the method of Roe (1934) as modified by Higashi and Peters (1950). Phosphofructokinase activity was determined by incubating the enzyme in a medium containing potassium glycylglycine buffer (*p*H 7.5; 5×10^{-2}M), magnesium chloride (8×10^{-3}M), ATP (8×10^{-3}M) and F-6-P (1×10^{-2}M) for 20 min. at 38° C. Proteins were precipitated in the experimental tubes after, and in the control tubes before, incubation, by adding 9 volumes of trichloroacetatic acid (5% w/v). Enzymatic activity was determined by the rate of disappearance of acid-labile phosphate resulting from the transfer of phosphate from ATP to F-6-P: after heating the protein-free filtrate for 7 min. in 1N-HCl in a boiling water-bath, phosphate was determined according to Gomori (1942). Phosphofructokinase from rat brain was prepared by the method of Muntz (1953).

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RESULTS

Effect of Antimonials on Lactic Acid Production from Glucose.—Two trivalent antimonials, stibophen and antimony potassium tartrate, had a marked inhibitory effect when glucose was used as the substrate (Table I). On the basis of these observations an attempt was made to determine which enzyme or enzymes were responsible for the reduction of glycolysis produced by the antimonials.

TABLE I

EFFECT OF ANTIMONIALS ON LACTIC ACID PRODUCTION BY EXTRACTS OF *SCHISTOSOMA MANSONI*

Antimonial	Molar Concn.	μMoles Lactic Acid/mg. Protein/1 Hr.		% Inhib.
		Control	With Antimonial	
Antimony potassium tartrate	1×10^{-3}	2·75	0·15	95
	1×10^{-4}	2·5	0·7	72
	4×10^{-5}	2·5	2·1	15
Stibophen	1×10^{-3}	2·75	0	100
	5×10^{-4}	2·75	0·0375	98
	8×10^{-5}	2·5	0·65	74

Effect on Hexokinases.—The first enzyme tested was glucokinase, which catalyses the initial step in the utilization of glucose, namely, its phosphorylation to glucose-6-phosphate by ATP. Both the purified and the crude enzyme preparations were relatively insensitive to high concentrations of antimonials (Table II). At a concentration of

TABLE II

EFFECT OF ANTIMONIALS ON GLUCOKINASE ACTIVITY OF *SCHISTOSOMA MANSONI*

Activities are expressed in μmoles of glucose phosphorylated per mg. protein in 10 min. (37° C.)

Enzyme Preparation	Molar Concn.	Control	Antimony Potassium Tartrate		Stibophen	
			Activity	% Inhib.	Activity	% Inhib.
Purified enzyme	1×10^{-2}	2·2	1·55	28	1·06	51
	3×10^{-3}	3·2	2·94	8	3·2	0
	1×10^{-3}	1·2	1·6	0	1·85	0
Schistosome homogenate	$1·6\times10^{-2}$	0·63	0·76	0	0·43	31
	3×10^{-3}	0·75	0·75	0	0·65	13
	1×10^{-3}	0·75	0·62	17	0·86	0

1×10^{-3}M both stibophen and antimony potassium tartrate produced complete inhibition of glycolysis but did not affect hexokinase activity. Other hexokinases, fructokinase and mannokinase, which are present in this parasite (Bueding and MacKinnon, 1953), were also insensitive to high concentrations of the antimonials. Therefore, inhibition of hexokinase cannot account for the inhibition of glycolysis of schistosomes by antimonials. On the other hand, the purified enzyme preparations, as well as homogenates of the worms, were markedly inhibited by low concentrations of oxophenarsine (mapharsen) and of other sulphydryl inhibitors, such as *p*-chloromercuric benzoate. For example, at a concentration of 5×10^{-4}M, oxophenarsine inhibited the activity of purified glucokinase to an extent of 83%.

Phosphohexose Isomerase.—This enzyme catalyses the second step in glycolysis, namely, the reversible conversion of glucose-6-phosphate to fructose-6-phosphate. Neither stibophen nor antimony potassium tartrate, at concentrations below 1×10^{-2}M, inhibited enzymatic activity significantly (Table III). The above observations excluded the first two reactions of glycolysis as the site of the inhibitory action of these drugs.

TABLE III

EFFECT OF ANTIMONIALS ON THE ACTIVITY OF PURIFIED PHOSPHOHEXOSE ISOMERASE OF *SCHISTOSOMA MANSONI*

Activities are expressed as μmoles of fructose-6-phosphate produced per mg. protein in 15 min. (37° C.)

Molar Concn. of Antimonial	Control μmole F-6-P	Antimony Potassium Tartrate		Stibophen	
		μmole F-6-P	% Inhib.	μmole F-6-P	% Inhib.
1×10^{-2}	520	390	25	415	20
5×10^{-3}	470	425	10	450	3
1×10^{-3}	520	435	16	480	8
5×10^{-4}	470	470	0	490	0

Effect of Antimonials on Lactic Acid Production from F-6-P and HDP.—In order to localize the effect of the antimonials, their action on lactic acid production from HDP and from F-6-P was measured. When HDP was used lactic acid production was not affected even by high concentra-

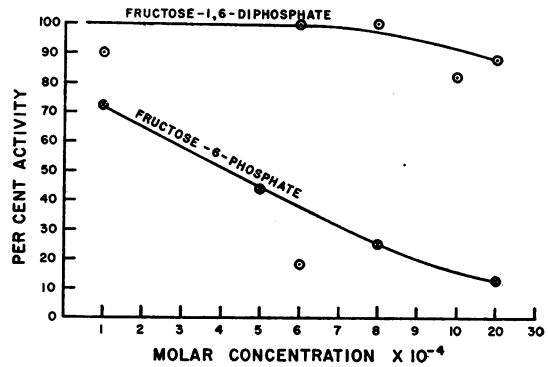

FIG. 1.—Effect of antimony potassium tartrate on lactic acid production from F-6-P and from HDP by schistosome extracts. Abscissa: molar concn. of antimonial. Ordinate: activity (% of control).

tions of antimony potassium tartrate (Fig. 1). On the other hand, with F-6-P as the substrate the activity of the extract was markedly decreased at molar concentrations above 5×10^{-4}. Similar results were obtained with stibophen (Fig. 2). These data indicated that the inhibition of glycolysis by antimonials is brought about by blocking the

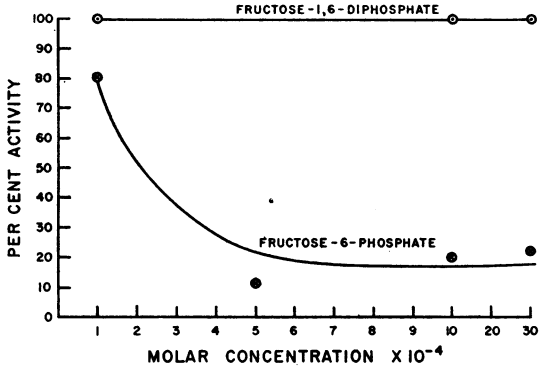

FIG. 2.—Effect of stibophen on lactic acid production from F-6-P and HDP by schistosome extracts. Abscissa: molar concn. of stibophen. Ordinate: activity (% of control).

formation of HDP from F-6-P. The latter reaction is brought about by the action of phosphofructokinase, which catalyses the phosphorylation of F-6-P to HDP by ATP.

Effect of Antimonials on Phosphofructokinase.—The data summarized in Table IV demonstrate that the activity of the phosphofructokinase of schistosomes was inhibited markedly by low concentrations of trivalent antimonials. On the other hand, the

TABLE IV

INHIBITION OF PHOSPHOFRUCTOKINASE ACTIVITY OF *SCHISTOSOMA MANSONI* AND OF RAT BRAIN BY ANTIMONIALS

Molar Concn. of Antimonial	Antimony Potassium Tartrate		Stibophen	
	Worm Enzyme % Inhib.	Brain Enzyme % Inhib.	Worm Enzyme % Inhib.	Brain Enzyme % Inhib.
1×10^{-2}		100		0
4×10^{-3}	100	54	100	
1×10^{-3}	100	32	100	0
5×10^{-4}	100	4	100	
3×10^{-4}	100	0	85	
1×10^{-4}	70	0	44	0
5×10^{-5}	56	0	19	
3×10^{-5}	32	0	0	
1×10^{-5}	2	0	0	0

enzyme which catalyses the same reaction in the host is much less sensitive to the same antimonial compounds (Table IV). Concentrations of tartar emetic 80 times higher than those necessary to inhibit the worm enzyme were required to produce the same degree of inhibition of the brain enzyme. With stibophen the difference was more striking still because even the highest concentration used had no effect on the mammalian enzyme. Oxophenarsine (5×10^{-4}M) completely inhibited the activity of phosphofructokinase of schistosomes. As with antimonials, the enzyme of rat brain was much less sensitive to oxophenarsine (Table V).

TABLE V

EFFECT OF OXOPHENARSINE ON PHOSPHOFRUCTOKINASE ACTIVITIES OF *SCHISTOSOMA MANSONI* AND OF RAT BRAIN

Molar Concn.	Worm Enzyme % Inhib.	Brain Enzyme % Inhib.
2×10^{-3}	—	88
5×10^{-4}	100	0
1×10^{-4}	73	0
4×10^{-5}	36	—
1×10^{-5}	17	—

In contrast to the two trivalent antimonials, high concentrations (4×10^{-3}M) of a pentavalent antimonial, sodium stibogluconate (Schmidt, 1948), had no effect on the activity of the worm enzyme.

DISCUSSION

It is evident that in schistosomes low concentrations of trivalent antimonials inhibit one reaction of glycolysis, the phosphorylation of F-6-P to HDP. By contrast, a pentavalent antimonial did not affect the activity of phosphofructokinase of the worms. This is in agreement with the finding of Lee and Chung (1935) that pentavalent antimonials do not exert a direct lethal action on schistosomes *in vitro*. It would appear that, as with arsenicals, pentavalent antimonials are reduced by the tissues of the host before chemotherapeutic activity is obtained. According to Chen (1948) trivalent antimonials as well as trivalent arsenicals have an inhibitory effect on three glycolytic enzymes of *Trypanosoma equiperdum*—hexokinase, adenosine triphosphatase and 3-phosphoglyceraldehyde dehydrogenase. This multiple action on trypanosome enzymes might explain the effectiveness of these compounds as chemotherapeutic agents in African trypanosomiasis. Whereas the activities of the schistosome hexokinases are inhibited by low concentrations of oxophenarsine, these enzymes are not affected by trivalent antimonials, even at high concentrations. Therefore, it appears that inhibition of enzymatic activity by these two groups of compounds is not brought about by the same mechanism. It should be noted also that enzymes catalysing the same reactions in different species are not affected by the same inhibitors. In contrast to

the schistosome hexokinases, hexokinase of *T. equiperdum* is inactivated by low concentrations of antimonials (Chen, 1948). Furthermore, the phosphofructokinase of the host is much less sensitive to trivalent antimonials and to trivalent arsenicals than is the phosphofructokinase of schistosomes. These observations, as well as those reported previously (Mansour and Bueding, 1953, and Mansour, Bueding and Stavitsky, 1954), indicate that enzymes which have the same catalytic function in the parasite and in the host are not necessarily identical. Although the selective action of trivalent antimonials on phosphofructokinase of *S. mansoni* explains the inhibition of the rate of glycolysis of the worms, it remains to be determined whether this effect accounts for the mechanism of the chemotherapeutic action of antimonials in schistosomiasis.

Summary

1. The action of trivalent antimonials and of oxophenarsine on the glycolytic enzymes of *Schistosoma mansoni* was investigated.

2. Stibophen and antimony potassium tartrate had little effect on the parasite's hexokinase, which is very sensitive to oxophenarsine.

3. Trivalent antimonials did not inhibit the activity of the parasite's phosphohexose isomerase, or the production of lactic acid from hexose diphosphate by schistosome extracts, but they had a marked inhibitory action on the phosphofructokinase of the worms.

4. The sensitivity of phosphofructokinase to trivalent antimonials accounts for the inhibitory effect of these compounds on the rate of glycolysis of *S. mansoni*.

5. Phosphofructokinase of rat brain was much less sensitive to trivalent antimonials and to oxophenarsine.

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