Biochemical Pharmacology, 1962, Vol. 11, pp. 17–28. Pergamon Press Ltd., Printed in Great Britain.

EFFECTS OF BENZYLIC DIAMINES ON *SCHISTOSOMA MANSONI**

ERNEST BUEDING

Department of Pharmacology, School of Medicine,
Louisiana State University, New Orleans, Louisiana,
and
Department of Pathobiology, School of Hygiene and Public Health,
The Johns Hopkins University, Baltimore, Maryland

(*Received* 13 *July* 1961; *accepted* 29 *July* 1961)

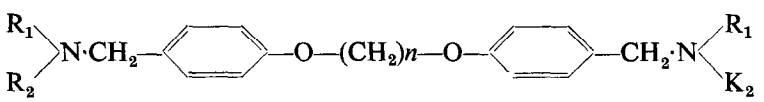
Abstract—(1) A series of benzylic diamines have marked activity against *Schistosoma mansoni in vitro*. Secondary amines are more active than tertiary amines. Optimal activity is observed when there are six carbons in the central alkyl chain.

(2) Reduction in the motility of the worms produced by these compounds is preceded by an inhibition of glucose utilization and by increased glycogenolysis. Since even considerably higher concentrations of these diamines do not affect schistosome enzymes concerned with the carbohydrate metabolism of the worms, it is concluded that the antischistosomal activity of these compounds is brought about by an inhibition of the transport of glucose into the parasite.

(3) The onset of paralysis of schistosomes produced by benzylic diamines is delayed by betaine and carnitine. This effect is enhanced by Mg^{2+} ions.

(4) Low concentrations of a diamine potentiate the anti-schistosomal activity of stibophen *in vitro*. It is concluded that schistosomes are more vulnerable to *simultaneous* inhibition of their phosphofructokinase activity (produced by organic antimonials) and of glucose transport than to interference with either of these two mechanisms alone.

McCOWEN *et al.*[1] have reported that a series of symmetrically substituted bis-aminomethylphenoxyalkanes of the general structural formula



have amebacidal activity. In an attempt to determine the antiparasitic spectrum of these compounds, it was noted that fairly low concentrations of these compounds markedly reduce the survival of *Schistosoma mansoni in vitro*. On the basis of this finding, some observations were made regarding the relationships between chemical structure and antischistosomal activity *in vitro*; in addition, the biochemical effects of one of the most active substances of this series on *S. mansoni* were investigated. Evidence has been obtained that this series of compounds interferes with the transport of glucose into the worm.

METHODS AND MATERIALS

Adult schistosomes were obtained as in earlier studies.[2,3] Individual worm pairs were incubated in 3 ml of horse serum (75 per cent w/v) at 37 °C and their motility

* Supported by research grants (E-668 and E-3515) from the National Institutes of Health, U. S. Public Health Service, and from the Eli Lilly Company.

observed under a dissecting microscope every 20 to 30 min. Antischistosomal activity of a diamine was determined by the formula C_S/C_T, where C_S is the concentration of a standard diamine, the amebacidal compound, 1:2-bis-[*p*-(N-hexylmethylaminomethyl) phenoxyl] ethane dihydrochloride[1] (compound III, Table 2), required to immobilize the worms after a given time interval, and C_T is the concentration of the compound tested producing paralysis after the same period of time. The concentrations of the standard and of the tested compounds were selected in such a manner that paralysis of the parasites occurred no sooner than after 1 hr, and not later than after 6 hr. Within these time limits the calculated activities of a given diamine were in close agreement. Paralysis of the schistosomes usually was observed after 1 hr with a concentration of 2×10^{-3} M of the standard compound, and after 3 and 6 hr with concentrations of 8×10^{-4} and 4×10^{-4} M, respectively. In most instances paralysis was preceded by marked hyperactivity of the worms, characterized by an increase in amplitude and in frequency of muscular movements. Usually immobilization of the females occurred earlier than that of the males. The average value obtained for the male and the female worms was taken as the end point. All compounds used in this study were synthesized and supplied by Dr. Koert Gerzon.

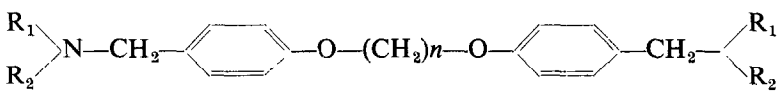
The rates of glycolysis of the intact parasites and of cell-free extracts of the organisms, the glycogen content of the worms, and hexokinase activity of schistosomes were determined as described previously.[3-6]

Phosphorylase activity of schistosomes was assayed as follows: the worms were homogenized at 0 °C in a medium containing sodium glycerophosphate (0·035 M; pH, 6·5) buffer, neutralized cysteine (0·03 M), and potassium fluoride (0·1 M); 1 ml of medium was used per sixty worm pairs. Of this homogenate, 0·3 ml were incubated at 30 °C for 10 min in a medium the constituents and final concentrations of which are listed below: glucose-1-phosphate (purified according to Sutherland and Wosilait[7]), 0·02 M; glycogen, 1 per cent; potassium fluoride, 0·07 M; sodium glycerophosphate buffer (pH 6·5), 0·03 M; the final volume was 0·7 ml. Aliquots of 0·2 ml were removed immediately after addition of the homogenate and at the end of the incubation period and placed into 1·4 ml of an ice-cold trichloroacetic acid solution (5 per cent). After centrifugation (3000 g, 15 min, 2 °C) inorganic phosphate was determined in the supernatant solutions according to Fiske and Subarrow.[8] Every assay was carried out in the presence and absence of adenylic acid (final concentration, 0·001 M). In no instance did this compound stimulate phosphorylase activity. Phosphoglucomutase activity was determined by measuring the rate of conversion of glucose-1-phosphate to glucose-6-phosphate at 25 °C during a period of 4 min. Formation of glucose-6-phosphate was measured by recording the change in optical density at 340 mμ as a result of the reduction of TPN, due to the oxidation of glucose-6-phosphate, catalyzed by glucose-6-phosphate dehydrogenase. Ten worm pairs were homogenized in 0·3 ml of a solution containing imidazole buffer (0·04 M, pH 7·4) and $MgCl_2$ (0·001 M).[9] The reaction mixture contained in a total volume of 0·8 ml: 0·03 ml of homogenate, 10 μmoles of glucose-1-phosphate, 40 μmoles of imidazole buffer[9] (pH 7·5), 1 μmole of $MgCl_2$, 0·15 μmole of magnesium fructose-1:6-diphosphate (General Biochemicals Corp.) (as a source of glucose-1:6-diphosphate), 0·4 μmole of TPN and 0·05 units[10] of glucose-6-phosphate dehydrogenase (Boehringer).

For the determination of adenosine triphosphatase (ATPase) activities of schistosomes, the parasites were homogenized in glycylglycine buffer (0·01 M; pH 9·0)

(fifty pairs per ml of buffer). It had been found previously that schistosomes contain at least two and possibly three ATPases.[11] One of them is particulate and is activated by Mg^{2+}, one is activated by Ca^{2+} and is soluble on centrifugation of the homogenate at 30,000 g for 30 min. There also is some evidence for the presence in the worm of a third ATPase which is not activated by either metal. ATPase activities were measured under predetermined optimal conditions. For the measurement of the Ca^{2+}-activated ATPase activity, the concentration of the substrate was $2{\cdot}6 \times 10^{-2}$, and that of $CaCl_2$, 4×10^{-3} M. Mg^{2+}-activated ATPase activity was determined in a final molar concentration of ATP of $1{\cdot}4 \times 10^{-2}$ and 5×10^{-3} $MgCl_2$. When ATPase activity was tested in the absence of either metal, the concentration of the substrate was 7×10^{-3} M. In all three cases the buffer was glycylglycine (pH 9·0; final molar concentration: 0·05 M) and the incubation was carried out at 30 C °for 6 min. At the beginning and the end of this period, aliquots of the reaction mixture were placed into an ice-cold solution of perchloric acid (4 per cent w/v). After centrifugation at 4000 rev/min at 0°, inorganic phosphate was determined in the supernatant solution according to Lowry and Lopez.[12]

TABLE 1. ANTISCHISTOSOMAL ACTIVITY *in vitro* OF SECONDARY BENZYLIC DIAMINES AND OF THEIR TERTIARY AMINE ANALOGS



n	R_1	R_2	Antischistosomal activity
2	C_6H_{13}	H	10
		CH_3	1
5	CH_3	H	6
		CH_3	3
5	C_2H_5	H	3
		CH_3	1·5
5	$CH(CH_3)_2$	H	6
		CH_3	3
6	$CH(CH_3)_2$	H	10
		CH_3	2

RESULTS

Relationship between structure and antischistosomal activity in vitro *of alkyldibenzylamines*

Secondary amines were 2–10 times more active than their tertiary amine analogs (Table 1). Another structural factor which had a significant effect on antischistosomal activity was the length of the central carbon chain. Optimal activity was observed with 1:6-bis-(*p*-(N-methylaminomethyl) phenoxy) hexane (compound XVI, Table 2), containing six carbons, and a progressive decrease in activity occurred

with either lengthening or shortening of the central carbon chain (Fig. 1). N:N'-dimethyl-substituted diamines (compounds X and XIV, Table 2) had higher activity than their ethyl analogs (compounds XI and XV, Table 2). The activity of N:N'-dimethyl-substituted diamines with a short central chain ($n = 2$ or 3; compounds I and IV, Table 2) was increased appreciably by substituting larger groups, such as *iso*propyl, *n*-butyl, or *n*-hexyl groups (compounds VI, V, and II, Table 2). With compounds containing a longer central carbon chain ($n = 4$ to 10) a similar increase in activity did not occur (compounds VIII, IX, XII, XIII, XVII, XIX, and XXI, Table 2). Quaternization of the nitrogens abolished antischistosomal activity.

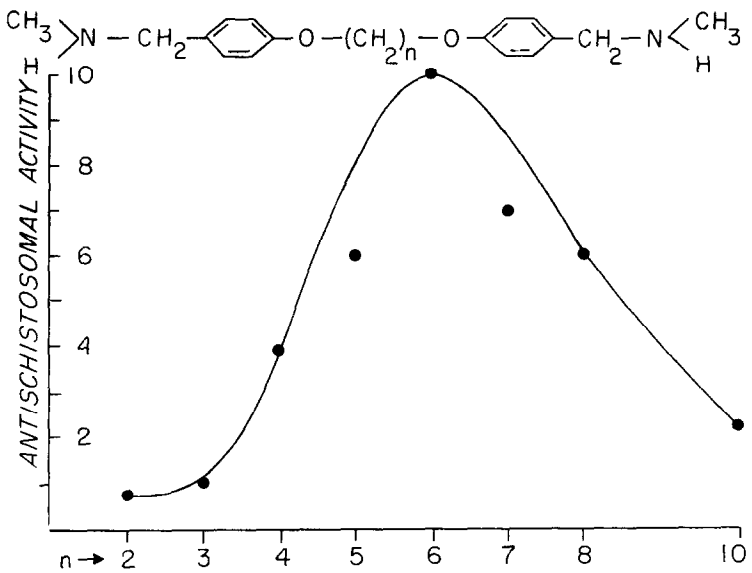


FIG. 1. Effect of length of the central carbon chain of benzylic diamines on antischistosomal activity. Abscissa: number of carbon atoms in central alkyl chain. Ordinate: antischistosomal activity.

When schistosomes were removed from the medium containing a diamine shortly before complete paralysis had occurred, recovery was observed consistently. Therefore, to some degree the effects of these compounds were reversible. In a number of cases male worms even recovered when removed from exposure to a diamine immediately after complete immobilization had set in. Reversibility was observed more readily with N-*iso*propyl-substituted diamines than with non-branched alkyl substituents.

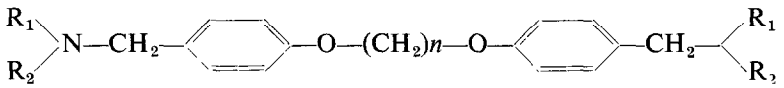
Effect of betaine and carnitine

The onset of paralysis of schistosomes produced by diamines was delayed markedly by betaine and by carnitine (Table 3); this effect was enhanced by $MgCl_2$. In the absence of betaine or carnitine, no significant antagonism by Mg^{2+} ions of the paralytic action of diamines on the worms was observed. The concentrations required to delay the paralysis were rather critical. A mere two-fold increase above, or decrease below, the optimal concentration (0·012 M) of betaine or of carnitine considerably reduced the antagonism to the paralytic action of diamines, and with more pronounced

changes beyond such concentrations these antagonistic effects were abolished. Similarly, the Mg^{2+}-concentration exhibited an almost equally sharp optimum.

In comparison with betaine, the action of choline in antagonizing the effects of diamines was only very slight. The following methylated compounds, tested over a wide range of concentrations, failed to delay the paralyzing actions of diamines on schistosomes, either in the presence or absence of Mg^{2+}: methionine, methionine sulfoxide, dimethylglycine, dimethylthetine, dimethyl propiothetine and creatine. In

TABLE 2. RELATIONSHIP BETWEEN CHEMICAL STRUCTURE AND ANTI-SCHISTOSOMAL ACTIVITY *in vitro* OF BENZYLIC DIAMINES



Compound number	n	R_1	R_2	Antischistosomal activity
I	2	$-CH_3$	H	0·75
II	2	$-C_6H_{13}$	H	10
III	2	$-C_6H_{13}$	CH_3	1
IV	3	$-CH_3$	H	1
V	3	$-C_4H_9$	H	3
VI	3	$-C(CH_3)_2CH_3$	H	1·5
VII	4	$-CH_3$	H	4
VIII	4	$-C(CH_3)_2CH_3$	H	4
IX	4	$-C_6H_{13}$	H	6
X	5	$-CH_3$	H	6
XI	5	$-C_2H_5$	H	3
XII	5	$-CH(CH_3)_2$	H	6
XIII	5	$-C_6H_{13}$	H	8
XIV	5	$-CH_3$	CH_3	3
XV	5	$-C_2H_5$	CH_3	1·5
XVI	6	$-CH_3$	H	10
XVII	6	$-CH(CH_3)_2$	H	10
XVIII	8	$-CH_3$	H	6
XIX	8	$-CH(CH_3)_2$	H	6
XX	10	$-CH_3$	H	3
XXI	10	$-CH(CH_3)_2$	H	3

addition, neither high concentrations of glucose nor insulin proved antagonistic to the paralysing effects of diamines on schistosomes. Paralysis of the worms produced by diamines also was not delayed by three compounds which markedly enhance the motor activity of schistosomes, i.e. the ganglionic blocking agent mecamylamine, 5-hydroxy-tryptamine (5-HT), and amphetamine. The stimulatory effects of the latter two compounds were blocked by 2-brom-lysergic acid diethylamide in equimolar and even in five to ten times lower molar concentrations. It has been reported that both 5-HT and amphetamine increase the motor activity of another parasitic trematode, the liver fluke *Fasciola hepatica*.[13, 14]

TABLE 3. EFFECT OF BETAINE AND OF CARNITINE IN DELAYING THE PARALYZING ACTION OF 1:6-*bis*-[p-(N-METHYLAMINOMETHYL) PHENOXY] HEXANE DIHYDROCHLORIDE ON *Schistosoma mansoni*

(The figures represent the period of time (in hours) during which motility of the worms was maintained. The worms were incubated at 37 °C in 75 per cent horse serum containing the diamine with or without betaine (0·012 M), carnitine (0·012 M) or $MgCl_2$ (0·02 M). Figures in parentheses indicate the number of experiments.)

Molar concentration of diamine		No addition	$MgCl_2$	Betaine	Betaine plus $MgCl_2$	Carnitine	Carnitine plus $MgCl_2$
1 × 10^{-4}	Limits	2·4–3·7 (15)	2·6–3·5 (15)	4·6–8·5 (15)	7·5–10·5 (15)	4·8–8·0 (15)	7·0–10·5 (15)
	Average	2·75	3·1	5·8	8·9	5·95	8·8
2·5 × 10^{-4}	Limits	1·3–2·0 (17)	1·3–2·5 (17)	2·7–5·8 (14)	3·5–8·5 (17)	2·5–5·7 (14)	4·3–5·7 (17)
	Average	1·6	1·6	4·05	6·1	3·9	5·8
5 × 10^{-4}	Limits	1·0–1·5 (12)	1·0–1·25 (12)	1·75–5·9 (12)	2·7–6·5 (12)	1·5–4·9 (12)	2·7–6·5 (12)
	Average	1·15	1·2	3·25	4·7	3·1	4·6

Effect of diamines on the carbohydrate metabolism of Schistosoma mansoni

In schistosomes, glycolysis proceeds at an extremely rapid rate[2] and this metabolic process represents a major source of energy for this parasite.[2, 3, 13] Therefore the possibility was investigated that the action of diamines on the parasite was associated with an interference with the carbohydrate metabolism of the worms. To this end,

TABLE 4. EFFECT OF 1:2-*bis*-[*p*-(N-METHYL-AMINOMETHYL)-PHENOXY)]-HEXANE DIHYDROCHLORIDE ON THE CARBOHYDRATE METABOLISM OF *Schistosoma mansoni*

(0·8 ml of buffered 75 per cent horse serum (0·035 M sodium phosphate buffer; pH 7·5) was used per 10 worm pairs. Incubation period: 60 min — 37 °C. At the end of the incubation period no difference in motility between the control and experimental groups was noticeable.)

Expt. no.	Molar concentration of diamine	Glucose utilization		Lactic acid production		Glycogen disappearance
		μM*	Per cent inhibition	μM*	Per cent inhibition	μM†
1	—	1·10	—	2·28	—	0·06
	1×10^{-4}	0·58	47	2·04	10	0·39
2	—	0·96	—	2·15	—	0·04
	1×10^{-4}	0·43	55	1·83	15	0·54
3	—	1·17	—	2·40	—	0·05
	1×10^{-4}	0·48	59	2·06	14	0·58
4	—	1·04	—	2·22	—	0·03
	1×10^{-4}	0·59	43	1·98	11	0·44

* Per mg (dry weight).
† Glucose units per mg (dry weight).

glucose utilization and lactic acid production by schistosomes were determined during incubation for 1 hr with a subeffective concentration of a diamine, that is, with a concentration below the one which produced changes in muscular activity in the period during which carbohydrate utilization was measured. In the presence of such concentrations of diamines, glucose utilization was reduced significantly, but lactic acid formation was inhibited to a much lesser degree (Table 4). Therefore, under these conditions some lactic acid must have been produced from endogenous sources. This was confirmed by the observation that changes in the motility, produced by the diamine, were preceded by a marked increase in the rate of glycogenolysis of the worms (Table 4). Similar changes in carbohydrate metabolism occurred during incubation of schistosomes with subeffective concentrations of other diamines. In protein-free salt media, glucose uptake was inhibited in the presence of much lower concentrations of diamines than in 75 per cent serum. Thus, it appears that one or several components of serum partially inactivate these compounds or prevent their interaction with the parasite.

In contrast to the marked effects of diamines on the carbohydrate metabolism of *intact* schistosomes, these compounds did not significantly affect the rate of glycolysis or the activities of glucokinase, of phosphorylase, or of phosphoglucomutase of

TABLE 5. EFFECT OF 1:2-*bis*-[*p*-(N-METHYL-AMINOMETHYL)-PHENOXY]-HEXANE DIHYDROCHLORIDE ON ENZYMATIC ACTIVITIES OF SCHISTOSOME HOMOGENATES

(All activities are expressed in terms of micromoles (produced or removed) per mg of protein.)

Experiment no.	Molar concentration of diamine during pre-incubation of the worms	Molar concentration of diamine during assay	Glycolytic activity, μmoles of lactic acid produced (60 min; 37 °C)	Hexokinase activity, μmoles glucose phosphorylated (10 min; 37 °C)	Phosphorylase activity, μmoles inorg. P liberated (10 min; 30 °C)	Phosphoglucomutase activity, μmoles glucose-6-phosphate produced (4 min; 25 °C)
1	—	—	2·7	0·74	1·75	1·18
	—	5×10^{-4}	2·6	0·71	1·79	1·13
	$2{\cdot}5 \times 10^{-4}$	—	2·6	0·70	1·78	1·24
	$2{\cdot}5 \times 10^{-4}$	5×10^{-4}	2·75	0·75	1·69	1·16
2	—	—	2·35	0·79	1·39	1·06
	—	5×10^{-4}	2·5	0·84	1·36	1·11
	$2{\cdot}5 \times 10^{-4}$	—	2·45	0·82	1·31	1·02
	$2{\cdot}5 \times 10^{-4}$	5×10^{-4}	2·4	0·78	1·37	1·08

homogenates of the parasites (Table 5). The effects of these compounds on cell-free extracts of the worms were tested using concentrations which were up to ten times higher than those which produced an inhibition of glucose uptake by intact schistosomes. Furthermore, no inhibition of these enzymatic activities could be observed with homogenates of schistosomes which had been incubated with a diamine until motility was markedly reduced (Table 5).

No stimulation of ATPase activities of schistosomes by benzylic diamines could be observed either on preincubation of the intact worms with these compounds or on addition of them to homogenates incubated with ATP. Furthermore, when schistosomes were exposed to diamines until a reduction in motility occurred, no increase in the inorganic phosphate concentration of the worms could be detected; this observation suggested also that there was no activation of ATPase activities in the intact organisms under these conditions.

The effects of diamines on schistosomes were comparable to those of phloretin (Table 6). The latter compound inhibited the rate of glucose uptake and, to a lesser extent, the rate of lactic acid formation by intact schistosomes, in concentrations which produced paralysis of the worms within 5–8 hr and which did not affect the rate of glycolysis of schistosome homogenates.

TABLE 6. EFFECT OF PHLORETIN ON *Schistosoma mansoni*

Molar concentration of phloretin	Intact worms percentage inhibition of		Worm homogenates percentage inhibition of		Time (hr) required until onset of complete paralysis*
	Glucose uptake*	Lactate production*	Glucose uptake	Lactate production	
2×10^{-4}	69	28	0	2	5·2
8×10^{-5}	50	16	0	0	8·8

* In glucose-containing buffered salt medium (60 min; 37 °C).

TABLE 7. EFFECT OF BETAINE AND OF CARNITINE ON GLUCOSE UPTAKE OF *Schistosoma mansoni*

(The worms were incubated at 37 °C for 1 hr in a glucose-containing buffered salt medium.)

Molar concentration of		Glucose utilization (μmoles)*		
Betaine	Carnitine	Experiment no.		
		1	2	3
—	—	1·06	0·86	0·97
$1{\cdot}2 \times 10^{-2}$		0·78	0·45	0·61
6×10^{-3}		0·94	0·71	0·80
$2{\cdot}4 \times 10^{-3}$		1·08	0·83	0·93
	$1{\cdot}2 \times 10^{-2}$	0·83	0·49	0·63
	6×10^{-3}	0·97	0·69	0·86
	$2{\cdot}4 \times 10^{-3}$	1·04	0·81	1·02

* Per mg of dry weight.

Effect of betaine and of carnitine on the carbohydrate metabolism of schistosomes

Incubation of schistosomes with betaine or carnitine resulted in a decreased rate of glucose uptake (Table 7). This was associated with a glycogen-sparing effect of these compounds; after exposure of the worms to a glucose-free salt medium, their glycogen content was reduced to a greater extent in the absence, than in the presence of either betaine or carnitine in a concentration of 0·012 M. It should be noted that reduction of the glucose uptake of the organisms was observed when the concentration of these compounds equalled those which optimally antagonized the paralysing action of diamines on schistosomes. With lower concentrations of betaine or carnitine, the reduction in or the lack of their ability to delay the paralytic action of diamines was paralleled by little or no inhibition of the glucose uptake of the worms.

Potentiation by a diamine of the antischistosomal action of stibophen in vitro

Exposure of schistosomes to low concentrations of a benzylic diamine and of the organic antimonial, stibophen, resulted in a much greater reduction of survival in the presence of both these compounds than with the same concentration of each compound alone (Table 8).

TABLE 8. EFFECT OF A DIAMINE* AND OF STIBOPHEN ON THE SURVIVAL OF *Schistosoma mansoni* IN 75% HORSE SERUM

(Each figure represents the average of 10 observations.)

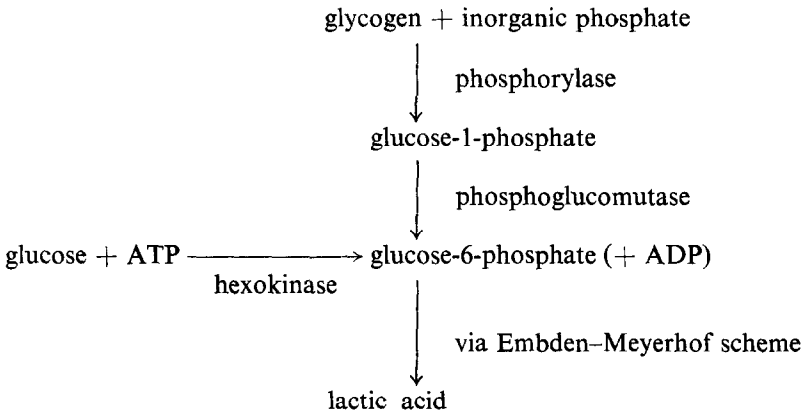
Diamine* molar concentration	Stibophen molar concentration	Average survival (days)	
		Males	Females
—	—	28·8	28·3
$5{\cdot}6 \times 10^{-6}$	—	13·9	13·5
—	1×10^{-6}	18·5	11·1
$5{\cdot}6 \times 10^{-6}$	1×10^{-6}	3·2	2·2
$5{\cdot}6 \times 10^{-6}$	—	13·9	13·5
—	5×10^{-7}	27·7	27·7
$5{\cdot}6 \times 10^{-6}$	5×10^{-7}	3·3	3·3
$2{\cdot}8 \times 10^{-6}$	—	24·5	21·0
—	1×10^{-6}	18·5	11·1
$2{\cdot}8 \times 10^{-6}$	1×10^{-6}	6·7	5·2

* 1:6-bis-[*p*-(N-methylamino-methyl) phenoxyl]-hexane dihydrochloride.

DISCUSSION

Reduction of the glucose uptake associated with a decrease of the glycogen stores of schistosomes could be brought about by benzylic diamines by: (a) inhibition of a glycolytic enzyme; (b) stimulation of glycogenolysis; (c) interference with the hexokinase reaction, or (d) inhibition of glucose transport. In the following these possibilities will be considered. (a) The rate of glycolysis of schistosome homogenates is more rapid than that of the intact organisms; yet diamines, in concentrations far in excess over those producing an inhibition of glucose uptake by *intact* worms, did not affect the rate of glycolysis of cell-free schistosome extracts. Therefore, diamines have no inhibitory effect on schistosome enzymes catalyzing the intra-cellular conversion

of glucose to lactic acid. (b) In the intact worms, inhibition of glucose uptake, produced by diamines, was associated with an increased rate of glycogenolysis. Stimulation of this process conceivably could result in an inhibition of glucose uptake because glucose-6-phosphate might accumulate as a result of an increased breakdown of glycogen; this phosphate ester inhibits the activity of schistosome glucokinase.[6]



The possibility that diamines inhibit glucose uptake of schistosomes by means of a primary stimulatory effect on glycogenolysis has been ruled out because neither phosphorylase nor phosphoglucomutase activities were stimulated by diamines; furthermore, neither of these two enzymes was activated during exposure of schistosomes to paralyzing concentrations of diamines. (c) The hexokinase reaction could be interfered with by diamines through an inhibition of hexokinase activity or by a decreased supply of ATP. Hexokinase activity of schistosomes was not affected, either in the presence of high concentrations of diamines or following incubation of the intact organisms with these compounds, under conditions producing a marked decrease in glucose uptake. Under the same conditions no increases in the activities of schistosome ATPases or in the concentration of inorganic phosphate could be observed in the worms.

Because of the absence of any direct effect of diamines 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 increased glycogenolysis is secondary to a reduced availability of exogenous glucose. These biochemical effects of diamines were demonstrable prior to the onset of changes in the motility of the parasites. Therefore, it would appear that paralysis of the worms was the result of an interference with glucose transport. Once the endogenous carbohydrate stores are depleted, inhibition of glucose transport should result in a lowered supply of metabolic energy, causing paralysis and eventually the death of the worm. It should be noted that female schistosomes, the glycogen stores of which are much lower than those of the males,[5] consistently proved more susceptible to the paralysing effects of diamines than did the male worms. Furthermore, the effects of diamines on the carbohydrate metabolism and on the motility of the worms were identical with those of phloretin, a known inhibitor of glucose transport into mammalian cells.[16]

Loss of motility of schistosomes produced by diamines was delayed in the presence of either betaine or carnitine. Possibly this effect is related to a carbohydrate-sparing

action of these compounds, because there was a close parallelism between concentrations which reduced the glucose utilization of the worms on one hand, and which antagonized the paralysing action of diamines on the other. This might be due to the fact that betaine and carnitine serve as substrates in the metabolism of schistosomes.

Low concentrations of the diamines discussed above potentiated the antischistosomal actions *in vitro* of the organic antimonial, stibophen. Trivalent organic antimonials interfere with the activity of phosphofructokinase of the parasite.[10] Since the observations reported in this paper indicate that alkyldibenzylamines inhibit another phase of carbohydrate metabolism, i.e. the transport of glucose into *S. mansoni*, it appears that the parasite is more vulnerable to *simultaneous* inhibition of two phases of its carbohydrate metabolism than to interference at only one of these levels. This phenomenon suggests that inhibition of glucose transport into schistosomes within their host might provide an opportunity to decrease the dosage of antimonials below the presently used chemotherapeutically effective levels, without reduction of their chemotherapeutic effectiveness.

Acknowledgements—The author wishes to express his appreciation to Dr. Koert Gerzon of the Eli Lilly Research Laboratories for generously supplying him with the diamines used in this study and for his most helpful suggestions, and to Mrs. Noel Banowicz for very valuable technical assistance.

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