CARBOHYDRATE METABOLISM OF SCHISTOSOMA MANSONI*

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In contrast to vertebrates and to bacteria relatively little is known about the metabolism of invertebrates (1). For a better understanding of comparative biochemistry information about this subject is required. Furthermore, investigations of the biochemical characteristics of parasitic invertebrates will reveal metabolic reactions essential for survival of these organisms. Information obtained from such studies will make it possible to search for non-toxic inhibitors of essential metabolic reactions and eventually may afford a possibility of replacing the presently prevailing empirical methods with a more rational development of chemotherapeutic agents against pathogenic parasites.

Accordingly, when an investigation concerned with the chemotherapy of schistosomiasis was initiated in this laboratory, it was decided to study the metabolism of *Schistosoma mansoni*, an organism concerned with the pathogenesis of one form of this disease.

Materials and Methods

The schistosomes were removed with the aid of dissecting needles from the mesenteric and portal veins of mice infested with *Schistosoma mansoni*. The infestation had been produced by the intraperitoneal injection of 125 to 150 cercariae per mouse 6 to 10 weeks previously. The cercariae were obtained from shedding snails (*Australorbis glabratus*). The latter were either naturally infested snails collected in Puerto Rico or they had been infested by exposure to miracidia from eggs present in the livers of mice with schistosomiasis.[1] The schistosomes were placed in a medium of the following composition: 0.137 M NaCl, 0.0085 M KCl, 0.0003 M $CaCl_2$, 0.005 M $MgCl_2$, and 0.06 M sodium phosphate or glycylglycine buffer. The pH of this solution was 7.7. After several washings with this medium, 10 to 25 pairs of worms were transferred into 0.8 ml. of medium contained in a Warburg respirometer vessel (total volume of vessel: 4 to 5 ml.). Incubation was carried out at 37.5°C. for a period of 2 hours. After several

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rapid transfers into distilled water, the organisms were dried at 90°C. to constant weight. The analytical procedures for the determinations of the oxygen uptake, carbon dioxide production, glucose utilization, and lactic acid production, and of the glycogen content, were identical with those used and described in a recent paper (2). The metabolic activities of schistosomes from mice infested with various batches of cercariae varied considerably regardless of whether the cercariae were obtained from naturally or laboratory infested snails. However, within a single group of mice which had been infested by the same batch of cercariae, the variations in metabolic activities of duplicate samples from their average never exceeded ±10 per cent and in many experiments on the rates of glucose utilization and lactic acid production a deviation of less

TABLE I

Lactic Acid Production of S. mansoni in the Presence and in the Absence of Glucose

Experiment No.	After incubation in glucose-free medium (37.5°C. 90 min.)			Without previous incubation		
	0.005 M glucose		No glucose	0.005 M glucose		No glucose
	Q_G*	Q_L‡	Q_L‡	Q_G*	Q_L‡	Q_L‡
1	193	174	7	196	224	31
2	195	170	12	220	257	28
3	207	177	5	206	241	32
4	197	178	6	168	196	39
5	182	171	18	243	268	35
6	158	146	17	260	301	48
7	181	173	14	180	254	48
8	138	126	10	152	208	47
9	158	137	5	183	211	36
10	176	152	7	316	258	55

* Micrograms glucose removed per hour per milligram (dry weight).

‡ Micrograms lactic acid produced per hour per milligram (dry weight).

than ±5 per cent from the average was observed. In order to minimize the variabilities in the metabolic rates each experiment reported below was carried out with schistosomes of a single group of mice.

RESULTS

Utilization of Glucose and Production of Lactic Acid by S. mansoni.—The rate of glucose utilization by these organisms was extremely high. They removed from the medium in 1 hour an amount of glucose which is equivalent to 15 to 26 per cent of their dry weight (Table I). The main end-product of the metabolism of glucose by *S. mansoni* was found to be lactic acid. When the schistosomes were incubated in the basic medium containing glucose the production of lactic acid was higher than could be accounted for by the quantity of glucose utilized (Table I). Thus, it became apparent that lactic acid is formed by the organisms

from endogenous sources. As shown in Table I, the schistosomes formed in the absence of glucose 11 to 23 per cent of the total amount of lactic acid which was produced in the presence of this carbohydrate. However, after the organisms had been allowed to remain for 90 minutes at 37.5°C. in the basic salt medium without glucose their endogenous production of lactic acid was markedly reduced (Table I). Under these conditions, production of lactic acid accounted for 81 to 91 per cent of the glucose used by the schistosomes (Table I). As will be reported in a subsequent paper, the production of lactic acid from glucose by the schistosomes was also established by the preparation and identification of the *p*-bromphenacyl derivative of lactic acid following isolation of this substance from the basic medium in which a large number of the trematodes had been incubated.

Neither the rate of glucose utilization nor the rate of lactic acid production was changed by shifting from aerobic to anaerobic conditions (Table II). Therefore, the rate of glycolysis of *S. mansoni* is not affected by the presence or absence of atmospheric oxygen.

Oxygen Uptake and Respiratory Quotients of S. mansoni.—Although aerobiosis has no effect on the rate of glycolysis of the schistosomes, the organisms consume oxygen available in the atmospheric air. In the absence of glucose from the medium the oxygen uptake varied from 3.05 to 10.15 μl. per hour per mg. dry weight and between 6.7 and 12.8 μl. when the medium contained glucose (Q_{O_2}; Table III). Without glucose in the medium the respiratory quotient of the trematodes varied from 0.58 to 1.61. When the respiratory quotient in the absence of glucose was lower than 1, addition of this carbohydrate produced an increase in the respiratory quotient. Conversely, a respiratory quotient markedly above unity, in the absence of glucose, was decreased when schistosomes of the same group of mice were incubated in a medium containing glucose. In the two experiments (Nos. 4 and 8, Table III) in which the respiratory quotient without glucose was close to unity, addition of glucose to the medium produced no change in this value.

During the period immediately following anaerobiosis the rate of respiration of many parasitic helminths is increased temporarily (3–5). This postanaerobic increase of the oxygen uptake was not observed with the schistosomes because the rate of respiration of these organisms was found to be the same after incubation for 2 hours either in nitrogen or in air (Table IV).

When the schistosomes are incubated in the basic medium aerobically or anaerobically in the presence of glucose their rates of respiration and of glycolysis remain constant for a period of at least 6 hours.

Differences between Male and Female Schistosomes.—Female schistosomes tend to have a lower rate of glycolysis than the males (Table V). The differences in metabolic activities between male and female schistosomes were statistically significant with regard to the rates of glucose utilization and lactic

TABLE II

Aerobic and Anaerobic Carbohydrate Metabolism of S. mansoni

The figures in each box represent the metabolic activities of two different batches of schistosomes (10 pairs per batch) removed from the same group of infested mice. Anaerobiosis was produced by saturating the vessels (attached to the Warburg manometers) with nitrogen (previously passed through alkaline pyrogallol and over red hot copper wire) for 10 minutes. The stopcocks were then closed, the manometers placed in the waterbath and shaken for 120 minutes. Glucose concentration: 0.005 M. As buffer, phosphate was used in Experiments 1 to 4, while in Experiments 5 to 8, glycylglycine was used.

Experiment No.	Atmosphere	Q_G	Q_L
1	Air	230 221	262 248
	Nitrogen	223 238	236 260
2	Air	173 157	241 207
	Nitrogen	158 146	218 202
3	Air	206 200	263 253
	Nitrogen	228 205	284 258
4	Air	191 178	239 222
	Nitrogen	198 172	242 218
5	Air	179 185	218 241
	Nitrogen	175 160	225 201
6	Air	205 179	259 220
	Nitrogen	214 194	246 212
7	Air	158 170	196 214
	Nitrogen	161 186	214 232
8	Air	204 182	239 216
	Nitrogen	191 202	231 265

acid production, but not with regard to respiration. Males contain more than twice as much glycogen as the females. The glycogen content of the males

TABLE III

Respiratory Quotient of S. mansoni in the Absence and in the Presence of Glucose

The figures for Q_{O_2} * and Q_{CO_2} ‡ represent the results of duplicate determinations performed on two different batches of schistosomes (15 pairs per batch) removed from the same group of infested mice. In each experiment the respiratory quotient was calculated from the average values for Q_{O_2} and Q_{CO_2}.

Experiment No.	No glucose			0.0075 M glucose		
	Q_{O_2}*	Q_{CO_2}‡	Respiratory quotient	Q_{O_2}*	Q_{CO_2}‡	Respiratory quotient
1	5.2	3.1	0.69	7.6	6.3	0.88
	4.55	3.7		7.3	6.8	
2	5.6	8.0	1.39	7.6	9.2	1.20
	6.2	8.4		8.0	9.5	
3	9.8	5.6	0.58	12.1	9.6	0.79
	10.5	6.1		13.5	10.6	
4	7.3	6.85	1.03	9.0	8.1	1.00
	6.0	6.7		7.2	8.1	
5	6.1	7.9	1.31	9.2	11.1	1.19
	6.4	8.5		9.6	11.2	
6	5.0	3.6	0.66	11.3	10.6	0.86
	5.6	3.4		12.2	9.7	
7	5.4	8.5	1.61	7.1	9.7	1.43
	4.8	7.9		6.5	9.8	
8	7.15	7.6	1.01	7.2	7.1	0.975
	7.2	7.0		8.6	8.3	
9	2.9	3.9	1.39	6.5	7.5	1.15
	3.2	4.6		6.9	7.9	
10	5.3	3.5	0.62	8.6	6.5	0.76
	5.5	3.2		8.7	6.7	

* Microliters O_2 taken up per hour per milligram (dry weight).

‡ Microliters CO_2 produced per hour per milligram (dry weight).

varied from 12.2 to 19.6 per cent of their dry weight; that of the females from 2.4 to 4.9 per cent. During incubation in basic schistosome medium buffered with phosphate or with glycylglycine, both sexes lost glycogen, even in the presence of glucose.

TABLE IV

Oxygen Uptake of Schistosomes after Anaerobiosis

The schistosomes were incubated in basic schistosome medium (glycylglycine, 0.01 M glucose) for 2 hours in an atmosphere of nitrogen at 37.5°C. in Warburg vessels attached to manometers. Simultaneously schistosomes from the same batch were incubated in an atmosphere of air and their oxygen uptake recorded. After 2 hours the vessels were removed from the manometers and their contents saturated with air by rotating them for 2 minutes. The vessels were then attached again to the manometers and the oxygen uptake of the schistosomes was recorded.

Period after start of experiment	Schistosomes incubated in nitrogen (0 to 120 min.)		Schistosomes incubated in air (0 to 120 min.)	
	Q_{O_2}		Q_{O_2}	
	No. 1	No. 2	No. 1	No. 2
min.				
0–120	—	—	8.2	8.3
135–195	8.5	7.9	7.6	8.8
195–255	8.9	7.6	7.8	8.4

TABLE V

Oxygen Uptake and Glycolysis of Male and Female Schistosomes

15 males and 30 females from the same group of infested mice were used for each experiment. Glucose concentration: 0.005 M. The average dry weight of one male was 0.057 mg. that of one female 0.024 mg.

Experiment No.	Q_{O_2}		Q_G		Q_L	
	♂	♀	♂	♀	♂	♀
1	10.0	10.7	159	160	188	195
2	5.1	8.1	172	98	194	102
3	8.7	13.4	165	129	195	149
4	12.2	13.4	201	95	239	141
5	10.2	11.9	204	131	241	160
6	8.9	9.4	243	122	282	145
7	5.1	9.1	172	130	198	158
8	8.8	9.65	165	160	195	195
9	10.1	10.5	171	84	204	105
10	11.6	11.4	224	103	254	115
11	7.9	8.8	183	122	212	139
12	10.5	11.8	184	110	224	135
Average	9.1	10.7	187	120	219	145
Standard error	±4.8	±3.0	±65	±55	±109	±86
Critical ratio	0.57		6.1		5.3	

Effect of Ionic Environment on the Metabolism of S. mansoni.—The addition of potassium ions to a medium containing sodium chloride and sodium phosphate as the only salts produced a considerable increase in metabolic activity and motility. No significant difference in the metabolic activity was observed when the concentration of potassium varied between 2.5×10^{-3} and 3.5×10^{-2} M. Similarly, magnesium ions produced an increased metabolic rate of the schistosomes, although this effect was less marked than that of potassium. With a medium containing both potassium and magnesium, metabolic activity and motility were invariably greater than when only one of these ions was present. Addition of calcium ions to sodium chloride–sodium phosphate medium had only a slight effect, but low concentrations of calcium produced a definite increase in the rate of glycolysis and in the motility of the worms when potassium and magnesium ions were also present. In such a mixture, optimal metabolic activity and motility occurred with a magnesium concentration of 5×10^{-3} M. Lowering of the magnesium concentration resulted in decreased motility of the worms and a higher concentration of this ion reduced the rate of glycolysis. No marked difference in the metabolic activity was observed when the concentrations of sodium chloride varied between 6.8×10^{-2} and $\times 10^{-1}$ M, but a further increase produced a decrease in the rate of glycolysis and in motility. Similarly, and as pointed out previously (6), a lower buffer concentration resulted in decreased metabolic activity. The presence of sulfate (1×10^{-3} to 1×10^{-2} M), nitrate (1×10^{-3} to 1×10^{-2} M), ammonium (1×10^{-4} to 1×10^{-3} M), manganous (1×10^{-4} to 1×10^{-3} M), ferrous or ferric (1×10^{-5} to 2×10^{-4} M) ions had no effect on the metabolic activity of the worms.

Effect of pH on the Metabolism of S. mansoni.—This effect is reproduced in Fig. 1. The pH of each medium was measured before and after the experimental period (2 hours). Because of the production of large amounts of lactic acid a decrease in pH occurred regularly despite the high concentration of the buffer. However, the difference in the hydrogen ion concentration of the medium before and after the experiment never exceeded 0.25 pH unit. The average of these two values has been used in the graphs of Fig. 1.

Optimal rates of glycolysis were maintained within a pH range of 7.3 to 8.7. No difference in glycolytic or respiratory activity was observed with phosphate or glycylglycine as the buffer. A marked fall in the rate of glucose utilization and lactic acid production occurred when the pH of the medium was decreased below 7.3 and at a pH of 6.6 the schistosomes utilized three times less glucose and produced about 2.5 times less lactic acid than at the optimum pH levels between 7.3 and 8.7. The respiration of the schistosomes remained at optimal levels within a pH range of 7.0 to 8.7. Below a pH of 7.0 the oxygen uptake fell to a similarly marked degree as the rate of glycolysis. An increase in pH above 8.7 resulted in a less sharp reduction in the rate of glycolysis than a fall below 7.3.

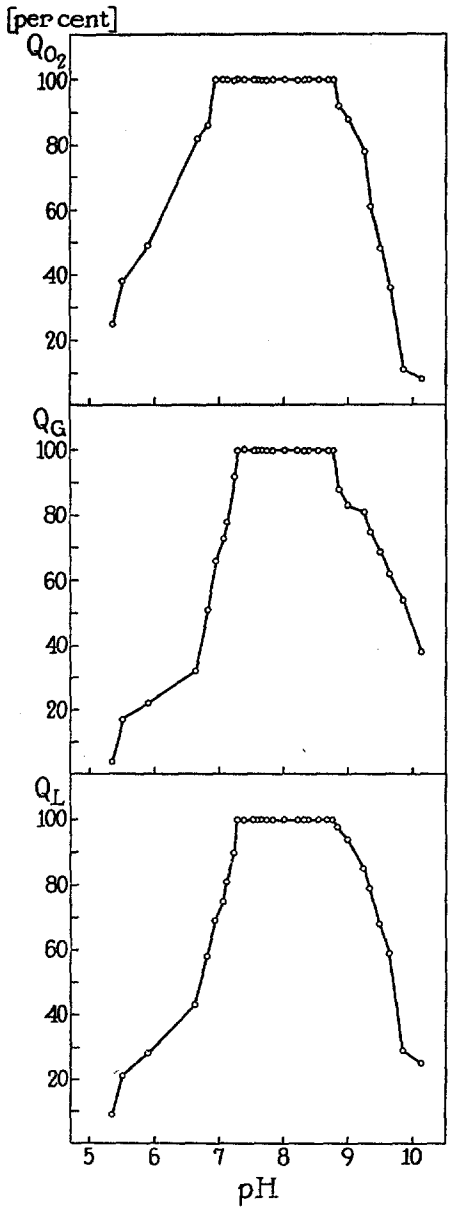


FIG. 1. Effect of pH on the metabolism of *S. mansoni*. The rates of oxygen consumption (Q_{O_2}; upper curve), of glucose utilization (Q_G; middle curve), and of lactic acid production (Q_L lower curve) are recorded as the percentages of the respective metabolic activities occurring at the pH optimum.

Fig. 1 and the foregoing discussion of these curves has been based on the results of experiments with 23 different groups of schistosomes removed from mice which had been infested with cercariae from laboratory or naturally infested snails. However, in two groups of schistosomes (one originating from cercariae of laboratory infested snails, the other from naturally infested snails) the rate of glycolysis began to decline at a pH below 7.9 with glycylglycine and at a pH below 7.5 with phosphate as the buffer. The cause for this atypical behavior of these two groups of worms cannot as yet be explained.

TABLE VI

Effect of Arsenite and of Fluoride on the Metabolism of Schistosomes

Basic schistosome medium (phosphate buffer); glucose concentration: 0.0075 M.

Experiment No.	Sodium arsenite	Previous incubation* with sodium arsenite	Sodium thioglycollate	Glutathione	NaF	Q_{O_2}	Q_G	Q_L
1							186	222
	1×10^{-3} M						83	106
	1×10^{-3} M		1×10^{-2} M				79	110
	1×10^{-3} M			1×10^{-2} M			87	104
2							192	223
		1×10^{-3} M					113	119
		1×10^{-3} M	1×10^{-2} M				120	108
		1×10^{-3} M		1×10^{-2} M			109	122
3						8.2	186	220
					1×10^{-2} M	8.4	184	215
					2×10^{-2} M	5.4	87	90
					4×10^{-2} M	3.2	40	46

* Previous to the experiment the schistosomes had been incubated in basic schistosome medium (0.0075 M glucose) with or without sodium arsenite at 37.5°C. for 40 minutes.

The motility of worms generally was not decreased until the pH fell below 6.5. The organisms usually lost their motility when the pH was raised above 8.3. Motility was maintained better when glycylglycine instead of phosphate was used as the medium.

Inhibitors of the Metabolism of S. mansoni.—

Arsenite: As illustrated in Table VI, sodium arsenite inhibited glycolysis of the schistosomes. This inhibition was not prevented by thioglycollate or glutathione in concentrations which were 10 times higher than that of the arsenite. When the schistosomes were incubated for 40 minutes in a medium containing sodium arsenite (1×10^{-3} M), and then were transferred into a medium containing no arsenite, their rate of glycolysis remained low, even

in the presence of thioglycollate or glutathione (Table VI). Furthermore, the latter two compounds did not reverse the inhibitory effect of *p*-chloromercuric benzoate on the rate of glycolysis of schistosomes. Similar observations were made with another parasitic helminth, the filarial worm *Litomosoides carinii* (2).

Fluoride: In contrast to its selective effect on enolase, the enzyme catalyzing the conversion of phosphoglycerate to phosphopyruvate, of mammalian tissues and of yeast, fluoride produces a generalized depression of respiration, of glucose utilization, and of lactic acid production of the schistosomes (Table VI).

Quinacrine ("Atabrine"): It has been reported that low concentrations of quinacrine produce an inhibition of the respiratory metabolism of malaria parasites (7). Subsequently, it was shown that quinacrine inhibits competitively the catalytic action of adenine nucleotides, the prosthetic group of flavoproteins (8, 9). Quinacrine inhibits the respiration of schistosomes only in very high concentrations (1×10^{-3} M). However, even low concentrations of this compound stained the worms deeply, indicating that quinacrine was taken up by the organisms, but failed to affect their oxygen uptake. Similarly, high concentrations of quinacrine in the medium were required to inhibit glycolysis of the schistosomes. These observations are consistent with the fact that quinacrine is chemotherapeutically ineffective in schistosomiasis.

Cyanine Dyes: Recently it has been reported (10, 11) that various cyanine dyes exert marked chemotherapeutic activity in the filariasis of the cotton rat and that they inhibit in extremely low concentrations the respiratory metabolism of the filarial worm, *Litomosoides carinii*. The oxygen uptake of *S. mansoni* also is inhibited by these compounds. In a concentration of 2.6×10^{-6} M (1 to 1,000,000), active cyanine dyes produce a decrease in the oxygen uptake of these organisms. This depression becomes gradually more pronounced and reaches a maximum (80 to 90 per cent) after a period of 3 hours (Fig. 2). The same effect can be observed when the schistosomes remain in a serum medium containing a cyanine dye for only 30 minutes, are washed several times, and then are transferred into the same medium containing no cyanine dye (Fig. 3). Apparently, the cyanines are adsorbed strongly by these organisms and recovery from their effect is not observed, at least during an incubation period of 6 hours.

This property of the cyanines would make these compounds good chemotherapeutic agents against schistosomiasis if, *in vivo*, a similar concentration of a cyanine dye in the blood could be maintained for a period of at least 30 minutes and secondly, provided respiration was essential for the survival of *S. mansoni*. The first condition was met in the work of Peters and Welch (12) who developed a method of maintaining in rabbits a blood concentration of an active cyanine dye (1'-ethyl-3,6-dimethyl-2-phenyl-4-pyrimido-2'-

cyanine chloride; Chemotherapy Center No. 863) for a period of 60 minutes between a level of 5.2×10^{-6} M (1: 500,000) and of 2.6×10^{-6} M (1:1,000,000). This was done by the intravenous injection of the dye followed by its slow intravenous infusion on alternate days during a period of 3 weeks. Yet, several days after completion of such a dosage schedule, live schistosomes were found

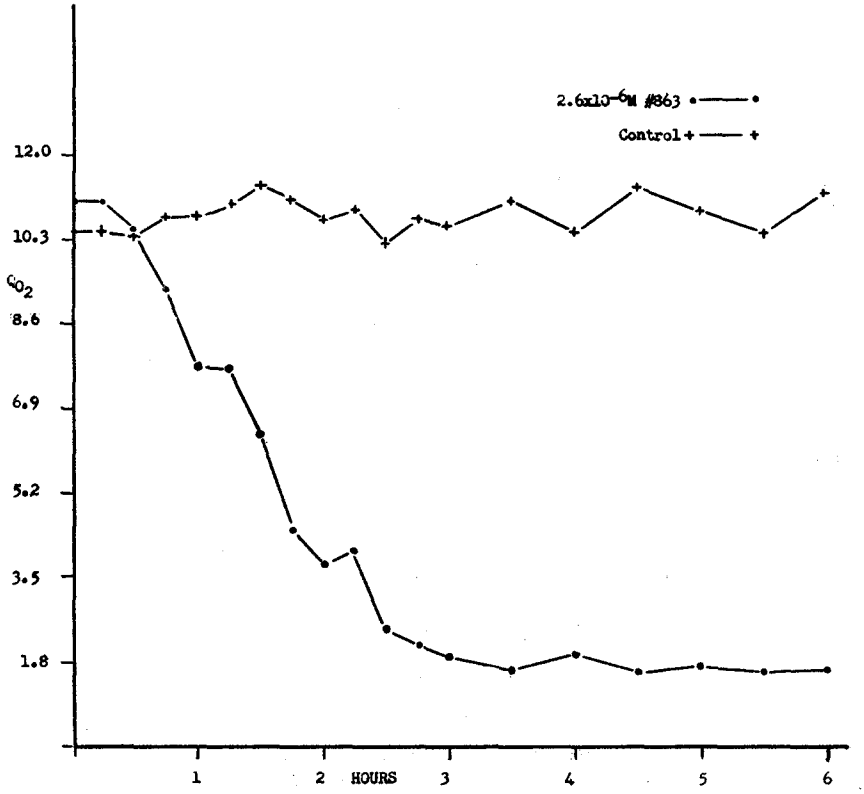


FIG. 2. Effect of cyanine dye 863 (1′-ethyl-3,6-dimethyl-2-phenyl-4-pyrimido-2′-cyanine chloride) on the oxygen uptake of *S. mansoni*.

in the mesenteric and portal veins, as well as in the liver of the host (13). The oxygen uptake of these organisms was decreased to an extent of almost 80 per cent (13), indicating that the dose of the cyanine dye administered was sufficient to produce *in vivo* the same effect on the respiration of the schistosomes as had been observed *in vitro*.

As illustrated in Table VII, the cyanines did not inhibit the rate of glycolysis of the schistosomes. In these experiments the organisms were at first incubated in the basic medium at 37.5° C. for 1 hour without (controls) and

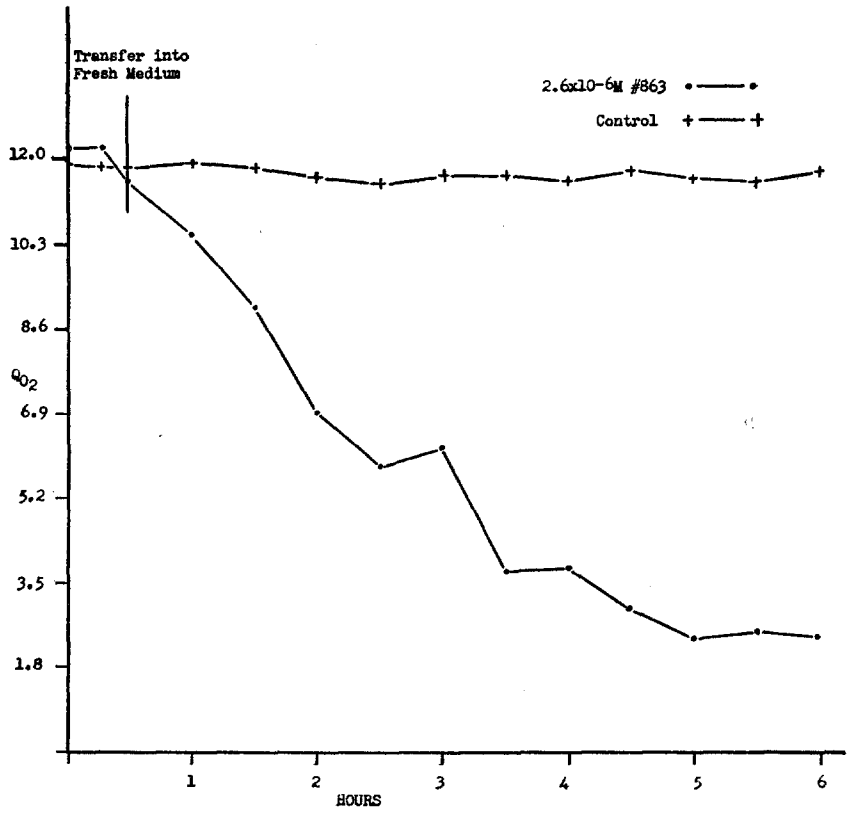


FIG. 3. Oxygen uptake of *S. mansoni* after incubation for 30 minutes with cyanine dye 863 (2×10^{-6} M).

TABLE VII

Effect of 1'-Ethyl-3,6-Dimethyl-2-Phenyl-4-Pyrimido-2'-Cyanine Chloride (Chemotherapy Center No. 863) on the Metabolism of S. mansoni

Molar concentration of cyanine dye	Q_{O_2}	Q_G	Q_L
—	9.3	201	226
	9.0	208	233
2.6×10^{-6}	0.8	206	228
	0.5	199	240

with the cyanine dye (concentration: 2.6×10^{-6} M) (experimental organisms). They were then transferred into the medium in which their metabolic activities were determined. The preliminary incubation period with the cya-

nine dye insured a more complete inhibition of the oxygen uptake during the experimental period. Although under these conditions inhibition of the respiration of the trematodes by the cyanine dye was practically complete their rate of glucose utilization and of lactic acid production was the same in the absence and in the presence of the dye (Table VII).

In spite of the marked inhibition of their respiration by the cyanines, the schistosomes are able to survive. Since these dyes do not affect glycolysis, this metabolic reaction might be more essential for the survival of the parasite than oxidative processes.

Fuadin: Organic antimonials, *e.g.* sodium antimony biscatechol disulfonate (fuadin), exhibit chemotherapeutic activity in schistosomiasis, but their therapeutic index is low; *i.e.*, they possess relatively high toxicity for the mammalian host. Fuadin inhibited glycolysis of the schistosomes; however, the degree of this effect varied considerably from one group of worms to another and, as pointed out previously (13), in almost every experiment a given concentration of fuadin inhibited glycolysis to a lesser degree than the oxygen uptake of the trematodes (Table VIII). Thus, fuadin is quite effective in depressing the respiration of the schistosomes, a metabolic reaction which appears to be non-essential for their survival. On the other hand, inhibition of glycolysis *in vivo* may occur only at levels which are toxic to the host.

2-Methyl-1,4-Naphthoquinone: The experiments described above suggested that inhibitors of glycolysis of *S. mansoni* having low mammalian toxicity might be better potential chemotherapeutic agents against schistosomiasis than compounds affecting primarily oxidative reactions. Since certain naphthoquinones inhibit the growth of lactic acid–producing bacteria (14), as well as their rate of glycolysis (15), the effect of 2-methyl-1,4-naphthoquinone (vitamin K), a relatively non-toxic substance, on the rate of glycolysis of the schistosomes was tested. In a protein-free salt solution, low concentrations of this naphthoquinone inhibited glycolysis of the parasites (Table IX). On the other hand, the oxygen uptake of the organisms was decreased to a smaller extent than the rates of utilization of glucose and of production of lactic acid. Therefore, in contrast to fuadin, 2-methyl-1,4-naphthoquinone inhibited glycolysis primarily.

Usually, but not consistently, the antiglycolytic effect of 2-methyl-1,4-naphthoquinone was less marked when the pH of the medium was increased (Table X). Since many naphthoquinones interact with serum proteins (16) the effect of 2-methyl-1,4-naphthoquinone on the metabolism of the schistosomes in dialyzed human serum was studied. This was necessary in order to evaluate whether or not this compound would maintain its ability to inhibit glycolysis of the trematodes *in vivo*. It is shown in Table IX that about 5 times higher concentrations of the naphthoquinone in dialyzed human serum than in a protein-free salt solution were required to produce the same

degree of inhibition of glycolysis. No difference in the inhibitory effect of 2-methyl-1,4-naphthoquinone was found regardless of whether the serum was dialyzed or not. It is evident, therefore, that this quinone is inactivated to a considerable extent by human serum proteins, but that it does not lose completely its antiglycolytic activity in this medium. Since the same degree of

TABLE VIII

Inhibitory Effect of Fuadin on Metabolism and Motility of S. mansoni

All figures represent the decrease (in per cent) compared to the corresponding control values in the same media without fuadin. All media contained 0.005 M glucose.

Experiment No.	Molar concentration of fuadin	Per cent inhibition of			Motility*	
		Oxygen uptake	Glucose utilization	Lactic acid production	♂	♀
1	1×10^{-4}	100	33	32	0	0
	1×10^{-5}	64	12	21	0	+
	1×10^{-6}	20	0	0	+	++
2	1×10^{-2}	96	68	77	0	0
	1×10^{-5}	92	27	37	+	+
	1×10^{-6}	68	0	6	++	++
3	1×10^{-2}	100	62	57	0	0
	1×10^{-4}	100	32	55	0	±
	1×10^{-5}	90	15	24	±	±
4	1×10^{-5}	93	64	35	0	±
	1×10^{-6}	47	38	25	0	±
5	1×10^{-4}	88	85	56	0	±
	1×10^{-5}	81	57	45	0	±
	1×10^{-6}	35	10	10	±	++
6	1×10^{-4}	68	43	42	0	0
	1×10^{-5}	69	32	30	±	±
	1×10^{-6}	30	0	0	++	++

* Motility at the end of the experimental period is expressed by the following symbols: ++, very high; +, high; ±, fair to sluggish; 0, no motility.

inactivation occurs in a medium containing 4 per cent human serum albumin (Table IX) it appears that interaction of this serum protein fraction with the quinone causes its lower inhibitory effect on the carbohydrate metabolism of the schistosomes.

Aldehydes: dl-Glyceric aldehyde,[2] a known inhibitor of glycolysis of mammalian tissues (17), was found to inhibit glucose utilization and lactic acid production of *S. mansoni* (Table XI). Propionic aldehyde and acetaldehyde

[2] I am indebted to Dr. H. O. L. Fischer for a supply of this compound.

were much weaker inhibitors while the activity of formaldehyde was about equal to that of d,l-glyceric aldehyde. Butyric aldehyde proved to be a less potent inhibitor than formaldehyde, but was more active than propionic aldehyde (Table XI). In view of these observations it appeared of interest

TABLE IX

Effect of 2-Methyl-1,4-Naphthoquinone on the Metabolism of S. mansoni

Glucose concentration: 0.005 M.

Experiment No.	Medium	Molar concentration of 2-methyl-1,4-naphthoquinone	Per cent inhibition of		
			Oxygen uptake	Glucose utilization	Lactic acid production
1	Salt*	2×10^{-5}	0	36	27
		5×10^{-5}	12	76	47
		1×10^{-4}	51	100	88
		3×10^{-4}	78	100	93
2	Salt‡	3×10^{-5}	3	43	37
		5×10^{-5}	7	68	57
		1×10^{-4}	33	92	78
3	Dialyzed serum§	3×10^{-5}	0	8	0
		1×10^{-4}	0	27	23
		5×10^{-4}	21	85	74
4	Dialyzed serum§	1×10^{-4}	0	39	45
		4×10^{-4}	7	67	64
		8×10^{-4}	28	85	76
5	Albumin‖	1×10^{-4}	0	29	24
		4×10^{-4}	19	64	56
6	Albumin‖	3×10^{-5}	0	4	7
		1×10^{-4}	0	38	31
		4×10^{-4}	24	69	65

* Basic schistosome medium (phosphate buffer).

‡ Basic schistosome medium (glycylglycine buffer).

§ Human serum dialyzed against basic schistosome medium (phosphate buffer).

‖ 4 per cent human serum albumin in basic schistosome medium (phosphate buffer).

to measure the effect of some aromatic aldehydes on the metabolism of the worms (Table XII), because many of these compounds are metabolized only slowly by mammalian organisms (18) and thus should be fairly stable *in vivo*. In a concentration of 5×10^{-3} M, benzaldehyde produced only a slight decrease in the rate of glycolysis of the schistosomes (Table XII). Introduction of an hydroxyl group in the paraposition (*p*-hydroxybenzaldehyde, vanillin) led to a decrease in or loss of activity, while the presence of an ortho-hydroxy

TABLE X

Effect of pH on the Antiglycolytic Activity of 2-Methyl-1,4-Naphthoquinone

Basic schistosome medium (phosphate or glycylglycine buffer); glucose concentration: 0.005 M.

Experiment No.	pH	Molar concentration of 2-methy-1,4-naphthoquinone	Per cent inhibition of	
			Glucose utilization	Lactic acid production
1	7.43	3×10^{-5}	42	35
	8.82	3×10^{-5}	7	0
2	7.45	1×10^{-4}	89	73
	8.82	1×10^{-4}	74	54
3	7.36	5×10^{-5}	67	57
	8.82	5×10^{-5}	65	45
4	7.75	5×10^{-5}	85	47
	8.82	5×10^{-5}	6	8
5	7.78	5×10^{-5}	88	62
	8.85	5×10^{-5}	60	49

TABLE XI

Effect of Aliphatic Aldehydes on the Metabolism of S. mansoni

Basic schistosome medium (phosphate buffer); glucose concentration: 0.005 M.

Compound	Molar concentration	Per cent inhibition of		
		Oxygen uptake	Glucose utilization	Lactic acid production
d-l-Glyceraldehyde	5×10^{-3}	31	79	56
	1×10^{-3}	0	29	22
Formaldehyde	2.5×10^{-2}	78	100	100
	5×10^{-3}	43	53	50
	5×10^{-4}	0	0	0
Acetaldehyde	2.5×10^{-2}	11	26	28
	5×10^{-3}	0	0	0
Propionaldehyde	2.5×10^{-2}	0	31	33
	5×10^{-3}	0	0	0
Butyraldehyde	2.5×10^{-2}	24	62	68
	5×10^{-3}	0	23	26

group (salicylaldehyde) produced a marked increase in the antiglycolytic activity. Introduction of a chlorine atom in position 5 of salicylaldehyde resulted

in a further increase in antiglycolytic activity (2 hydroxy-5-chlorobenzaldehyde). In contrast to *p*-hydroxybenzaldehyde, *p*-nitro- and *p*-methoxybenzal-

TABLE XII

Effect of Aromatic Aldehydes on the Metabolism of S. mansoni

Glucose concentration: 0.005 M.

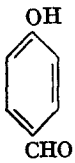
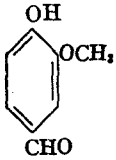
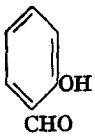
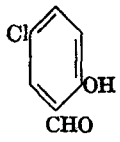
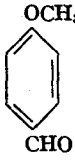
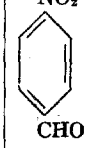
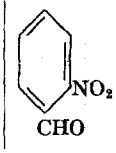
Compound	Structure	Medium	Molar concentration	Per cent inhibition of		
				Oxygen uptake	Glucose utilization	Lactic acid production
Benzaldehyde		Salt*	2×10^{-3}	31	12	16
		Salt*	5×10^{-3}	41	24	22
		Serum‡	5×10^{-3}	32	0	0
p-Hydroxybenzaldehyde		Salt*	5×10^{-3}	0	0	3
		Salt*	5×10^{-3}	0	0	0
Vanillin		Salt*	5×10^{-3}	12	11	0
		Serum‡	5×10^{-3}	6	0	0
Salicylaldehyde		Salt*	2×10^{-3}	26	30	21
		Salt*	5×10^{-3}	59	61	60
		Salt*	5×10^{-3}	50	62	58
		Serum‡	5×10^{-3}	29	39	29
		Serum‡	5×10^{-3}	43	26	28
2-Hydroxy-5-chlorobenzaldehyde		Salt*	2×10^{-3}	47	49	25
		Salt*	5×10^{-3}	62	95	76
		Serum‡	5×10^{-3}	25	34	16
Anisaldehyde		Salt*	2×10^{-3}	18	25	27
		Salt*	5×10^{-3}	40	47	43
		Serum‡	5×10^{-3}	24	29	16

TABLE XII—*Concluded*

Compound	Structure	Medium	Molar concentration	Per cent inhibition of		
				Oxygen uptake	Glucose utilization	Lactic acid production
p-Nitrobenzaldehyde		Salt*	5×10^{-4}	16	15	11
		Salt*	2×10^{-3}	30	35	28
		Salt*	5×10^{-3}	58	70	70
		Serum‡	5×10^{-3}	56	59	50
		Serum‡	5×10^{-3}	46	56	43
m-Nitrobenzaldehyde		Salt*	2×10^{-3}	21	28	12
		Salt*	5×10^{-3}	36	50	52
		Serum‡	5×10^{-3}	25	24	12

* Basic schistosome medium (phosphate buffer).

‡ Human serum dialyzed against basic schistosome medium (phosphate buffer).

dehyde (anisaldehyde) inhibited glycolysis of the schistosomes to a greater degree than the unsubstituted benzaldehyde. *M*-Nitrobenzaldehyde was less active than *p*-nitrobenzaldehyde. Human serum proteins decreased markedly the activity of *o*-hydroxybenzaldehydes, but a much less pronounced inactivation was observed under these conditions with *p*-nitrobenzaldehyde. No inhibition of glycolysis or of respiration was observed with pterine-(6)-aldehyde. Generally the aldehydes inhibited glycolysis of the schistosomes to about the same extent as the oxygen uptake of the organisms.

DISCUSSION

A comparison of the respiratory quotients of schistosomes in the absence and in the presence of glucose suggests that these organisms oxidize glucose completely to carbon dioxide and water. However, only a very small proportion of the total amount of glucose utilized by these trematodes can be accounted for by oxidation. The increase in the oxygen uptake produced by the addition of glucose to the medium varied between 1.7 and 6.5 μl. or 0.08 and 0.29 micromole of oxygen per hour and mg. (dry weight). This would account for the oxidation of 0.013 to 0.048 micromole of glucose since 6 micromoles of oxygen are required for the complete oxidation of 1 micromole of glucose to CO_2 and water. On the other hand, the schistosomes utilize in 1 hour 152 to 260 μg. or 0.84 to 1.44 micromoles of glucose per hour and mg. Therefore, the increase in the oxygen uptake produced by the addition of glucose accounts only for 1.5 to 3 per cent of the total amount of glucose utilized by the worms.

Several metabolic characteristics of *S. mansoni* are similar to those of the

filarial nematode *Litomosoides carinii* whose metabolism has been the subject of a recent investigation (2): (1) With both helminths, lactic acid is the main end-product of fermentation. Most other parasitic invertebrates convert carbohydrate predominantly to formic, acetic, propionic, butyric, valeric, and higher fatty acids (19 *a*). (2) In contrast to all other invertebrates whose respiration has been measured before and after anaerobiosis (19 *b*) no post-anaerobic increase in the oxygen uptake was observed with *S. mansoni* or *L. carinii*. (3) The respiration of both organisms is markedly inhibited by low concentrations of cyanine dyes. On the other hand, the metabolism of the two parasitic helminths differs basically in other respects. (1) Under conditions where the rate of glycogen synthesis by *L. carinii* was extremely high no synthesis of polysaccharide could be demonstrated with *S. mansoni*. (2) Anaerobically the filarial worms convert a much larger proportion of the carbohydrate utilized by them to lactic acid than under aerobic conditions. On the other hand, aerobiosis has no effect on the rate of glucose utilization and lactic acid production of *S. mansoni*. (3) Inhibition of the respiration of *L. carinii* by the cyanine dyes results in a compensatory increase in the rate of glycolysis and is followed by the death of the worms (10). The rate of glycolysis of *S. mansoni* is not affected and the organisms survive even if their oxygen uptake is inhibited to an extent of over 75 per cent as a result of the action of a cyanine dye. Therefore, in contrast to the filarial worm, *L. carinii*, anaerobic reactions appear to be sufficient to supply the major portion of the energy necessary for survival of *S. mansoni*. Indications are available that other parasitic helminths can survive under anaerobic conditions (20). It is conceivable that respiration is merely a rudimentary function of the schistosomes which has been carried over either from a previous stage of the life cycle of the parasite or from a different, but phylogenetically related species. Such an hypothesis is consistent with the fact that glycolysis of *S. mansoni*, an anaerobic metabolic process, is not affected by the respiratory metabolism of the worms. If oxidative reactions did supply a considerable amount of the energy essential for the organisms, it should be expected that anaerobic metabolism would decrease when respiration occurs; conversely, anaerobiosis or inhibition of respiration would result in a compensatory increase in the rate of glycolysis. On the other hand, the possibility cannot be excluded that *S. mansoni* may require a small proportion of its normal oxidative metabolism. Although this organism survives when almost 80 per cent of its respiration is inhibited by a cyanine dye, death may occur eventually if respiration were inhibited completely.

The chemotherapeutic effects of fuadin and of 2-methyl-1,4-naphthoquinone in experimental schistosomiasis suggest that glycolysis is essential for the survival of the pathogenic parasite. Fuadin inhibits glycolysis of the schistosomes in higher concentrations than those required to affect their respiration. If the chemotherapeutic activity of this antimonial is due to its inhibitory action

on glycolysis of *S. mansoni* such an effect could not be obtained unless relatively large doses of fuadin are administered to the host. This is consistent with the fact that fuadin is curative in experimental and clinical schistosomiasis only in doses bordering upon toxic levels for the host.

In contrast to fuadin, 2-methyl-1,4-naphthoquinone inhibits glycolysis of the schistosomes to a much greater extent than the respiration of the worms. In plasma, the physiological habitat of the parasites, the inhibitory effect of 2-methyl-1,4-naphthoquinone on glycolysis is markedly reduced and its action on the respiration is almost completely abolished because of interaction of the quinone with albumin. If glycolysis is essential for the survival of *S. mansoni* this compound should have slight chemotherapeutic activity in schistosomiasis. Such an effect has been observed experimentally (6, 13).

None of the aldehydes whose inhibitory effect on the rate of glycolysis of the trematodes was tested had sufficiently high activity *in vitro* to warrant a chemotherapeutic trial *in vivo*. However, the relative activities of these compounds suggest that phenylglyceraldehydes with a substituent nitro- or methoxy group in the ortho-position might prove to be more potent inhibitors of glycolysis.

SUMMARY

1. *Schistosoma mansoni* utilizes in 1 hour an amount of glucose equivalent to one-sixth to one-fifth of its dry weight. Over 80 per cent of the metabolized glucose is converted to lactic acid by this organism.

2. The rates of glucose utilization and of lactic acid production by *S. mansoni* are the same under aerobic and under anaerobic conditions.

3. A high rate of lactic acid production and the absence of a postanaerobic increase in the oxygen uptake differentiate *S. mansoni* from most other parasitic helminths whose metabolism has been studied.

4. Arsenite and *p*-chloromercuric benzoate inhibit in low concentrations the oxygen uptake and the rate of glycolysis of *S. mansoni*. This inhibition is not prevented or reversed by an excess of glutathione or of thioglycollate.

5. Fluoride inhibits the removal of glucose and the production of lactic acid by *S. mansoni* to the same degree.

6. Low concentrations of quinacrine (atabrine) do not affect the respiration or the carbohydrate metabolism of the schistosomes.

7. The inhibitory effect of aldehydes on the metabolism of *S. mansoni* has been measured. Among this group of compounds *dl*-glyceraldehyde and *o*-nitrobenzaldehyde are the most effective inhibitors of glycolysis.

8. In a concentration of 2.6×10^{-6} M (1:1,000,000) a cyanine dye inhibits almost completely the respiration of the schistosomes, but has no effect on their rate of glycolysis. The oxygen uptake of the worms is inhibited by fuadin to a greater degree than their rate of glycolysis. 2-methyl-1,4-napthoquinone is a

much more effective inhibitor of glycolysis than of the respiration of *S. mansoni*. The latter compound interacts with plasma albumin and, therefore, its inhibitory action on the metabolism of the schistosomes is greatly reduced in human serum or plasma.

9. Evidence is discussed which indicates that, in contrast to glycolysis, respiratory metabolism is not essential for the survival of *S. mansoni*.

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