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THE EFFECT OF SEROTONIN AND RELATED COMPOUNDS ON THE CARBOHYDRATE METABOLISM OF THE LIVER FLUKE, *FASCIOLA HEPATICA*[1]

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It has been reported previously that serotonin (5-hydroxy-tryptamine) stimulated the rhythmical movement of the parasitic trematode, the liver fluke, *Fasciola hepatica* (Mansour, 1957). The stimulatory effect was found to be peripheral and not mediated through the central ganglia and to occur anaerobically as well as aerobically. *Fasciola hepatica* metabolizes carbohydrate anaerobically at a high rate (Mansour and Lago, 1958). The rate of carbohydrate metabolism is not significantly changed under aerobic conditions. Production of volatile fatty acids accounts for almost all the carbohydrate utilized anaerobically (Mansour, 1959). These acids were identified as propionic and acetic acids in an approximate ratio of 3:1. Only 4 to 9% of the metabolized carbohydrate could be accounted for as lactic acid. Previous studies indicated that carbohydrate is the major, if not the exclusive, source of energy for many other parasitic helminths (Von Brand, 1952). The stimulatory effect of serotonin on the rhythmical movement of the liver fluke strongly suggested that serotonin might affect its carbohydrate metabolism. In the present paper, the effects of serotonin and related compounds on the anaerobic carbohydrate metabolism of these worms are reported.

METHODS. *Fasciola hepatica* were obtained from the bile ducts of infected cattle at a local abbatoir. The flukes were transported to the laboratory in saline medium which had the same composition as that reported by Dawes (1954). Since regular supplies of freshly collected organisms were not available, attempts were made to culture the parasites in the laboratory as long as possible. It was possible to keep the parasites in 500-ml conical flasks containing 100 ml of the following medium: 70 ml of Dawes saline medium, 30 ml of cattle serum, 200,000 units of penicillin, 10 mg streptomycin, and 200 mg dextrose. Cattle serum was prepared in the laboratory under nonsterile conditions and was stored at −20°C; 15 to 20 flukes were kept in each flask. The parasites were transferred to fresh medium every 1 to 2 days, depending upon the turbidity of medium. Flukes kept under these conditions were found to exhibit normal motility and normal metabolic rates for as long as 2 weeks. Metabolic rates of the liver flukes were measured in a medium which had the following composition per liter: NaCl, 121 mmol; KCl, 4 mmol; CaCl, 1.5 mmol; $MgSO_4$, 1.2 mmol; Na_2HPO_4 buffer pH 7.75, 40 mmol. Flukes with empty guts were selected and washed several times before use. Carbohydrate metabolism of flukes was measured on batches of 6 flukes which were incubated in 24 ml of saline medium at 37.5°C for 6 hours under nitrogen in a Dubonoff metabolic shaker. When the incubation time was reduced to 3 hours the volume of the medium was reduced to 12 ml of salt medium per 6 flukes. Glucose (Somogyi, 1948), lactic acid (Barker and Summerson, 1941) and steam volatile fatty acids (Bueding, 1949) were determined in aliquots of the medium, before and after incubation. Volatile fatty acids were identified by chromatographic separation on celite columns (Bueding and Yale, 1951). Glycogen was isolated from the flukes according to the procedure described previously (Mansour, 1959) and glycogen was determined by the anthrone method (Seifter *et al.*, 1950).

RESULTS. *Effect of serotonin on glucose metabolism.* The anaerobic carbohydrate balances of batches of flukes in media containing dextrose were measured in the presence as well as in the absence of serotonin (table 1). Serotonin (1×10^{-4} M to 5×10^{-4} M) produced a significant increase in glucose uptake. The total increase in carbohydrate utilization varied from 80 to 150 μmol/g wet weight per 6 hours. Serotonin also produced a 4- to 7-fold increase in lactic acid

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TABLE 1

Effect of serotonin on anaerobic carbohydrate balance of the liver flukes

Flukes (6 per batch) were incubated for 6 hr in saline medium (24 ml) containing 0.01 M glucose. Temperature 37.5°C. All results are expressed as μmol per g wet weight. In experiments 5, 6, 7 and 8, incubation was carried out for 3 hr in 12 ml of medium; (—) indicates no change.

Expt. No.	Molar Concentration of Serotonin	Glucose Removed	Glycogen*		Total Carbohydrate Utilized	Metabolic Products	
			Increase	Decrease		Lactic acid	Volatile fatty acids
1	—	180	20	—	160	31	354
	5×10^{-4}	223	—	17	240	180	310
2	—	195	36	—	159	34	334
	5×10^{-4}	240	—	8	248	209	303
3	—	150	—	—	150	30	235
	5×10^{-4}	260	—	—	260	139	293
4	—	112	—	—	112	36	218
	5×10^{-4}	262	—	—	262	134	316
5	—	68	—	12	80	15	185
	5×10^{-4}	130	—	40	170	100	203
6	—	60	—	—	60	16	119
	5×10^{-4}	101	—	—	101	99	153
7	—	83	—	—	83	16	191
	1×10^{-4}	98	—	—	98	50	150
8	—	80	—	—	80	16	196
	1×10^{-5}	71	—	—	71	25	206

* Expressed as μmol of glucose per g wet weight.

production. The extra amount of lactic acid produced by the flukes in the presence of serotonin accounted for approximately 50 to 100% of the net increase of the carbohydrate utilized. Production of volatile fatty acids was either not changed significantly, or was increased slightly (expt. 4, 5). Chromatographic separation of volatile fatty acids indicated that the ratio between the amount of propionic acid and acetic acid (approximately 3:1) was not changed when serotonin was present in the medium. Serotonin in concentrations which did not affect the motility (1×10^{-5} M) did not cause a significant change on the carbohydrate metabolism. This suggests that the effect of serotonin on the carbohydrate metabolism of the flukes was related to its stimulant action on rhythmical movement. While lactic acid accounts only for a minor portion of the carbohydrate utilized by normal flukes, it is a major metabolic product

TABLE 2

Effect of serotonin on endogenous carbohydrate metabolism of the liver flukes

Flukes were incubated in medium containing no glucose for 3 hr except in experiment 4 (6 hr). Results are expressed as μmol per g wet weight.

Expt. No.	Molar Concentration of Serotonin	Glycogen* Utilized	Metabolic Products	
			Lactic acid	Volatile fatty acids
1	—	58	5	170
	1×10^{-3}	92	40	210
2	—	48	3	168
	5×10^{-4}	77	30	220
3	—	45	8	216
	5×10^{-4}	87	29	270
4	—	109	9	250
	5×10^{-4}	161	49	348
5	—	58	5	170
	1×10^{-4}	89	10	197
6	—	58	5	171
	1×10^{-5}	52	7	189

* Expressed as μmol glucose.

when rhythmical activity of the flukes is increased by serotonin.

Effect of serotonin on endogenous metabolism. The liver fluke utilized endogenous glycogen at a high rate when incubated anaerobically in medium containing no glucose. Experiments were carried out to test the effect of serotonin on the endogenous carbohydrate metabolism (table 2). Glycogen utilization was increased in the presence of concentrations of serotonin which stimulate motility (1×10^{-4} M to 1×10^{-3} M). The stimulatory effect on glycogenolysis was associated with a 2- to 10-fold increase in lactic acid production. Volatile acids were increased only to an extent of 16 to 40%. The amount of lactic acid produced by the flukes in the presence of serotonin (5×10^{-4} M) could account for 15 to 19% of the utilized carbohydrate. Control experiments showed that only 3 to 9% of the utilized carbohydrate could be accounted for as lactic acid. Production of volatile fatty acids accounted for a greater part of the extra carbohydrate utilized when motility was stimulated by serotonin. Therefore, stimulation of muscular activity of *Fasciola* in the absence of glucose is associated with marked stimulation of lactic acid fermentation by the flukes. Furthermore, when

TABLE 3

Effect of lysergic acid diethylamide on the carbohydrate metabolism of the liver fluke

Incubation was carried on for 3 hr in experiments 1, 2, 4 and 5, and for 6 hr in experiment 3. In experiments 1, 2 and 3, glucose (0.01 M) was present in the medium. In experiments 4 and 5 no glucose was present in the medium. Results are expressed as μmol per g wet weight.

Expt. No.	Molar Concentration of LSD	Glucose Removed	Glycogen* Depleted	Metabolic Products	
				Lactic acid	Volatile fatty acids
1	—	92	—	32	230
	1×10^{-6}	124	—	63	200
2	—	110	—	14	212
	1×10^{-6}	142	—	52	214
3	—	136	—	12	258
	1×10^{-6}	173	—	61	285
4	—	—	53	3	179
	1×10^{-6}	—	72	15	196
5	—	—	53	4.0	186
	1×10^{-6}	—	65	25.0	206

* Expressed as μmol of glucose.

the stimulated flukes are dependent on endogenous carbohydrate, the production of volatile fatty acids accounts for a large portion of the additional amount of utilized carbohydrate.

Effect of lysergic acid diethylamide (LSD) on the carbohydrate metabolism. LSD (1×10^{-6} M), a potent stimulant to rhythmical movement, stimulated the rate of glucose uptake by the flukes (table 3). This was accompanied by a marked increase in lactic acid production while no significant change in the production of volatile acids was observed. When glucose was omitted from the medium, LSD caused a slight increase in glycogen utilization. The production of lactic acid was increased to the extent of 5- to 6-fold, while volatile fatty acids were increased only slightly. Thus, the increase of muscular activity of the flukes produced by LSD is accompanied by a marked increase in lactic acid fermentation without a change in the production of volatile acids.

Effect of a serotonin antagonist on glucose metabolism. The question arose whether the effect of serotonin on the carbohydrate metabolism of the flukes is due to a direct action on the glycolytic systems or whether it is secondary to the increase in muscular activity. In order to investigate this problem, the action of serotonin on the carbohydrate metabolism was investigated in the presence of an agent which antagonizes its stimulant effect on the motility (Mansour, 1957), namely bromolysergic acid diethylamide (BOL). These experiments are summarized in table 4. In all these experiments, serotonin (5×10^{-4} M) caused a significant increase in glucose uptake and a 3- to 7-fold increase in lactic acid production. BOL, when present alone in a concentration which causes paralysis (1×10^{-5} M), produced no significant change in glucose or lactic acid production. BOL, in a concentration which antagonized the stimulant effect of serotonin on motility (1×10^{-5} M), also antagonized the stimulant effect of serotonin on lactic acid production. The stimulant effect of serotonin on the glucose uptake by the flukes was antagonized only partially. These experiments suggest that the increase in lactic acid production due to serotonin is related to an increase in muscular

TABLE 4

Effect of bromolysergic acid diethylamide 1×10^{-5} on the glycolytic action of serotonin 5×10^{-4} M (5-HT) on flukes

Incubation was carried on for 3 hr. Results are expressed as μmol per g wet weight. Experimental conditions are as in table 1.

Expt. No.	Additions	Glucose Uptake	Lactic Acid Production	Volatile Fatty Acid Production
1	—	67	11	180
	5-HT	100	77	136
	BOL	85	15	166
	BOL + 5-HT	100	12	164
2	—	78	28	218
	5-HT	125	77	181
	BOL	107	18	177
	BOL + 5-HT	100	13	165
3	—	58	26	131
	5-HT	131	123	192
	BOL	86	20	190
	BOL + 5-HT	106	26	137
4	—	77	19	Not determined
	5-HT	151	111	Not determined
	BOL	73	24	Not determined
	BOL + 5-HT	100	31	Not determined

activity. On the other hand, the stimulant effect of serotonin on glucose uptake in these organisms may be dependent only partially on rhythmical movement.

Effect of l-epinephrine on carbohydrate metabolism. In contrast to serotonin, epinephrine does not have any effect on the rhythmical movement of the liver fluke (Mansour, 1957). Since epinephrine plays an important role in the carbohydrate metabolism of vertebrates, its effect on this lower organism was tested (table 5). Glucose uptake of the flukes was slightly increased by epinephrine, only at very high concentrations (1×10^{-3} M). Lactic acid production, volatile acids production and glycogen depletion were not changed significantly. These results indicate that epinephrine does not have a significant effect either on glycolysis or on glycogenolysis.

DISCUSSION. The above results indicate that stimulation of muscular activity by serotonin is accompanied by an increase in glycogenolysis and glycolysis. This is characterized by a marked increase in lactic acid production. On the other hand, the production of volatile acids which are the main metabolic products of flukes (Mansour, 1959), was changed only slightly. Therefore, it appears that the stimulant effect of serotonin on muscular activity produces a shift from fatty acids to lactic acid fermentation. This suggests that in this organism, lactic acid fermentation could meet increased energy requirements more efficiently than fatty acids fermentation.

The effect of serotonin on glucose uptake and lactic acid production by the liver fluke could be due either to a direct effect of serotonin or could result from the increase in rhythmical movement. The fact that BOL could block both the effect of serotonin on motility as well as the increase in lactic acid production, suggests that the latter effect is secondary to an increase in motility. However, BOL, in concentrations which abolish the stimulatory effect of serotonin on the motility of the flukes did not abolish completely the stimulatory action of serotonin on glucose uptake. It is possible, therefore, that serotonin might have a direct effect on mechanisms concerned with the uptake or the utilization of glucose by the flukes. This is supported further by the fact that BOL, in concentrations which cause paralysis of rhythmical movement, did not cause reduction in glucose uptake by the flukes.

TABLE 5

Effect of l-epinephrine on the carbohydrate metabolism of the liver flukes

Flukes were cultured for 3 hr in saline medium. In experiments 1, 2 and 3, dextrose (0.01 M) was present in the medium. In experiments 4, 5 and 6, no dextrose was present in the medium. All results are expressed as μmol per g wet weight.

Expt. No.	Molar Concentration of l-Epinephrine	Glucose Uptake	Glycogen* Depleted	Metabolic Products	
				Lactic acid	Volatile fatty acids
1	—	73	—	27	128
	1×10^{-3}	80	—	29	147
2	—	74	—	32	136
	1×10^{-3}	87	—	24	150
3	—	61	34	18	275
	1×10^{-3}	86	45	17	191
	5×10^{-4}	50	53	9	260
4	—	—	72	8	172
	1×10^{-3}	—	59	9	175
	5×10^{-4}	—	95	8	175
5	—	—	45	3	100
	1×10^{-3}	—	55	3	110
6	—	—	42	2	96
	1×10^{-3}	—	38	3	110

* Expressed as μmol of glucose per g wet weight.

It has been reported previously that rhythmical movement of *Fasciola hepatica* is stimulated by low concentrations of serotonin and of other naturally occurring indolalkylamines (Mansour, 1957). Epinephrine and other catecholamines did not exert any stimulatory effect; neither did epinephrine have any significant action on the carbohydrate metabolism of the liver fluke. These observations, as well as our previous demonstration of the presence of serotonin in these organisms (Mansour *et al.*, 1957), suggest that serotonin or a related compound has an effect on the carbohydrate metabolism of this invertebrate similar to that of epinephrine in mammals.

SUMMARY

The major products of carbohydrate metabolism of the liver fluke are volatile fatty acids

(propionic and acetic acids in an approximate ratio of 3:1), while only 4 to 9% of the utilized carbohydrate is converted to lactic acid. Stimulation of parasite movement under anaerobic conditions by low concentrations of serotonin or of LSD results in an increase in glucose uptake, glycogen breakdown and a 2- to 10-fold increase in lactic acid production, while there is little or no change in the production of volatile fatty acids. Bromolysergic acid diethylamide, a compound which blocks the stimulant effect of serotonin on motility, also antagonized the stimulatory effect on lactic acid production and antagonized only partially the increase in glucose uptake by these organisms. Epinephrine does not exert a significant effect on the carbohydrate metabolism of the liver fluke. It is postulated that in these parasites, lactic acid fermentation can meet increased energy requirements more efficiently than fatty acid fermentation. The possible role of serotonin on the carbohydrate metabolism of these organisms is discussed.

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