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STUDIES ON THE CARBOHYDRATE METABOLISM OF THE LIVER FLUKE *FASCIOLA HEPATICA*

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SUMMARY

Fasciola hepatica utilizes carbohydrate anaerobically at a high rate. When glucose is omitted from the medium, the amount of glycogen utilized varies from 84–97 μmoles of glucose units/g wet wt./6 h. The rate of glucose utilization from the outside medium varies from 110–180 μmoles/g wet wt./6 h. Production of volatile fatty acids accounts for almost all of the carbohydrate utilized anaerobically. These acids were identified as propionic and acetic acids in an approximate ratio of 3:1. Only 4–9% of the metabolized carbohydrate is converted to lactic acid. The rates of glucose utilization, lactic acid and volatile fatty acid production are only slightly decreased in the presence of atmospheric oxygen. Oxygen is utilized by these organisms when available. The respiratory quotient varies from 1.56–2.2. Anaerobic metabolism of flukes with ligatured oral openings is identical with intact organisms. This indicates that neither the absorption of glucose from outside medium, nor the excretion of the metabolic products are carried out through the gut of the organism.

INTRODUCTION

Previous studies indicated that carbohydrate is the major, if not the exclusive source of energy for many parasitic helminths[1,2]. The end products of carbohydrate metabolism vary from one species to another. As early as 1926, WEINLAND AND VON BRAND[3] observed that the utilization of carbohydrate by *Fasciola hepatica in vitro* is associated with the production of higher fatty acids and possibly butyric acid. Their experiments were carried out on the European variety of these organisms. The experiments reported in this paper were carried out on a variety of *Fasciola hepatica* which is predominant on the Gulf Coast of this country. In these experiments, the rate of endogenous carbohydrate utilization and of glucose uptake were measured under

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aerobic and anaerobic conditions. Quantitative as well as qualitative determinations of the fermentation products of these organisms revealed that propionic and acetic acids are the main end products of carbohydrate metabolism of this parasite.

MATERIALS AND METHODS

Fasciola hepatica were obtained from the bile ducts of infected cattle at a local slaughter house. They were transferred to the laboratory in saline medium which had the same composition as that reported by DAWES[1]. Metabolic rate of the liver fluke was measured in medium which had the following composition per liter: NaCl, 121 mmoles; KCl, 4 mmoles; $CaCl_2$, 1.5 mmoles; $MgSO_4$, 1.2 mmoles; $NaHPO_4$ buffer pH 7.75, 40.0 mmoles. Penicillin (2,000 units/ml) and streptomycin (0.1 mg/ml) were added to the medium when flukes were cultured for periods exceeding 6 h.

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Flukes with empty guts were selected and washed several times before use. Batches of 6 flukes were incubated in 24 ml of salt medium at 37.5° in a Dubonoff

TABLE I

ANAEROBIC CARBOHYDRATE BALANCE OF THE LIVER FLUKES

Flukes were incubated for 6 h in saline medium. Temperature 37.5°. All results are expressed in μmoles/6 h/g wet wt. Flukes used in expts. 1, 2, 3 and 4 were starved overnight. Flukes used in expts. 5, 6 and 7 were not starved.

Expt. No.	Glucose concentration in medium	Glucose removed	Polysaccharide*		Total carbohydrate utilized	Metabolic products			Total carbohydrate accounted for
			Decrease	Increase		Lactic acid	Propionic acid	Acetic acid	
	M	μmoles			μmoles	μmoles	μmoles	μmoles	μmoles
1	—	—	18	—	18	0.7	34	11	22.9
	0.011	143	—	30	143	25.8	130	52	133.9
2	—	—	13	—	13	0.6	20	9	14.8
	0.011	138	—	36	138	27.7	117	64	140.3
3	—	—	8.3	—	8.3	0.81	30	9	20.0
	0.011	148	—	44	148.0	29.4	131	60	154.2
4	—	—	35	—	35.0	1.6	75	21	48.8
	0.011	136	—	—	136.0	12.0	195	63	135.0
5	—	—	84	—	84.0	0.75	198	63	131.0
	0.011	180	—	20	180.0	30.5	262	92	212.0
6	—	—	97	—	97.0	3.9	209	66	139.5
	0.011	195	—	36	195	33.6	245	89	219.8
7	—	—	94	—	94	1.6	175	48	112.3
	0.011	110	—	—	110	15.0	177	55	123.0

* Expressed as μmoles glucose.

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metabolic shaker. Unless otherwise stated, an atmosphere of nitrogen was used. Glucose[5], lactic acid[6] and steam volatile fatty acids[7] were determined in aliquots of the medium, before and after incubation. Volatile fatty acids were identified by chromatographic separation on celite columns according to BUEDING AND YALE[8]. For the determination of polysaccharide, at least 6 flukes were ground in 30 % KOH (2 ml/100 mg) and the mixture placed in a boiling waterbath for 20 min in a centrifuge tube covered with a marble. The polysaccharide was then precipitated according to the method described by GOOD, KRAMER AND SOMOGYI for glycogen[9]. Total carbohydrate was determined by the anthrone method of SEIFTER *et al.*[10]. Q_{O_2} and Q_{CO_2} were determined in Warburg vessels (total volume – 15 ml). Each flask contained 2 flukes in 3 ml of medium. Incubation was carried out for 3 h.

RESULTS

Anaerobic carbohydrate utilization

Anaerobic incubation of the worms in medium containing no glucose resulted in a marked decrease in the total carbohydrate content (Table I, expts. 5, 6, 7). In these experiments, the initial total carbohydrate content varied from 220–180 μmoles/g wet wt. Anaerobic utilization of polysaccharide varied from 84–97 μmoles/g wet wt./6 h. Flukes used in expts. 1, 2, 3 and 4 (Table I) were starved overnight and consequently contained approximately 50 % of the original polysaccharide concentration. Carbohydrate utilization of the starved flukes was markedly low.

Incubation of worms, which had been starved overnight in a medium containing glucose and 30 % bovine serum, resulted in an increase in the total carbohydrate which reached approximately the same level as that of the initial, Table II. These experiments indicate that the liver fluke polysaccharide can be synthesized from glucose approximately at the same rate as it is utilized during starvation.

TABLE II

POLYSACCHARIDE UTILIZATION AND SYNTHESIS BY *Fasciola hepatica*

Flukes were incubated overnight at 37.5° in media containing no glucose. The next day, half of the flukes were transferred to media containing antibiotics, glucose and 30 % serum and cultured for another 24 h. The other half of the starved flukes was used for glycogen determination. All results are expressed as μmoles glucose/g wet wt.

Expt. No.	*Initial glycogen content*	*Glycogen content after starvation*	*Glycogen content after feeding*
1	275	135	238
2	151	51	187
3	204	123	204
4	160	76	165
5	212	58	153

The carbohydrate stored by these organisms is a polysaccharide which was completely hydrolysed to glucose after boiling in 1 *N* HCl for 3 h. The nature of the product after hydrolysis was confirmed by the glucose oxidase reaction[11]. This indicates that the carbohydrate stored in *Fasciola hepatica* is glycogen.

Anaerobic glucose utilization by both the starved and the non-starved flukes was very high. Non-starved flukes utilized in 6 h, 110–195 μmoles of glucose/g of

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wet wt. Flukes which had been starved removed from the medium, 136–148 μmoles of glucose/g wet wt. in 6 h. The difference between the glucose uptake by the starved and non-starved flukes is not significant. Therefore, the carbohydrate level in these organisms does not interfere with the rate of glucose uptake under anaerobic conditions.

Anaerobic carbohydrate balances of the liver fluke

Flukes which had been starved overnight showed a very low endogenous metabolic rate when they were incubated under anaerobic conditions in media containing no glucose. The metabolic products of these flukes accounted for almost all the carbohydrate utilized. Representative anaerobic carbohydrate balances of starved as well as non-starved flukes are summarized in Table I. Synthesis of polysaccharide in some experiments accounted for approximately 28 % of the glucose removed from the medium. Volatile fatty acids accounted for 81–95 %, and lactic acid accounted for 4–14 % of the glucose removed and not converted to glycogen.

On the other hand, flukes which had not been starved, showed a high endogenous metabolic rate. The metabolic products in these experiments accounted for more than the endogenous carbohydrate utilized. This might be attributed to the metabolism of endogenous substrates by non-starved organisms. Volatile fatty acids were also the major end products when non-starved flukes were cultured in the presence of in the absence of glucose.

In all these experiments, it was noticed that the presence of glucose in the medium caused 10–13 fold increase in the lactic acid production while the formation of volatile fatty acids was increased by only 20 %. This indicates that the utilization of glucose stimulates lactic acid fermentation more than the production of volatile fatty acids.

Since WEINLAND AND VON BRAND[3] have reported the production of lipids and non-volatile fatty acids by the European strain of *Fasciola hepatica*, attempts were made to determine these substances in the culture medium. Aliquots of the medium were extracted overnight with ether at pH 2.0 in a continuous extraction apparatus. The ether extract was dried with anhydrous Na_2SO_4 and the residue heated gently on a sand bath to evaporate the volatile fatty acids. The total weight of the ether

TABLE III

ANAEROBIC PRODUCTION OF CARBON DIOXIDE BY THE LIVER FLUKE *Fasciola hepatica*

In each experiment two flukes in 3 ml of medium were incubated for 3 h in Warburg flasks with a capacity of 15 ml. At the end of that time 0.2 ml of 5 *N* H_2SO_4 was tipped into the main compartment to release all the bound CO_2 from the media. Figures represent the CO_2 produced in μmoles/g wet wt./3 h. Flukes used in expts. 1, 2 and 3 were non-starved, while in expts. 4, 5 and 6 flukes were starved overnight.

Expt. No.	*Carbon dioxide (μmoles/g wet wt./3 h)*	
	No glucose	*0.011 moles glucose*
1	76.0	62.5
2	64.0	64.0
3	77.0	62.0
4	50.0	50.0
5	61.0	49.0
6	51.5	56.0

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extract after neutralization ranged from 2.7 to 4.0 mg/g wet wt. Acids in this fraction amounted to an average of 29 μmoles/g wet wt./6 h. These results indicate that the amount of higher fatty acids produced by the liver fluke is negligible and could not account for any significant amount of carbohydrate utilized.

It was observed that flukes produced large quantities of carbon dioxide under anaerobic conditions (154–100 μmoles/g wet wt./6 h). The presence of glucose in the medium did not significantly change the Q_{CO_2} (Table III). Because of the high rate of propionic acid fermentation, it is possible that this acid is formed by a decarboxylation mechanism.

Identification of volatile fatty acids from culture media

Volatile fatty acids which have been isolated from media were chromatographed on celite columns[8]. It was found that these acids were propionic and acetic acids. The ratio of propionic to acetic was approximately 3:1. Fig. 1 illustrates representative experiments for the chromatographic separation of these acids. When the buffer of the non-mobile phase had a pH of 6.5, no acids were eluted with pure chloroform. Under similar conditions, butyric acid was eluted from a known mixture of acids. Propionic acid was eluted with chloroform containing 5 % butanol and acetic acid with 20 % butanol. Another column which was used to give a faster separation of the two acids had the buffer of the non-mobile phase at a pH of 3.5 (Fig. 2). Propionic acid was eluted from this column with pure chloroform while acetic acid was eluted with 5 % butanol in chloroform. Quantitative recoveries were obtained from both columns.

The identity of volatile acids excreted by the flukes was further confirmed by

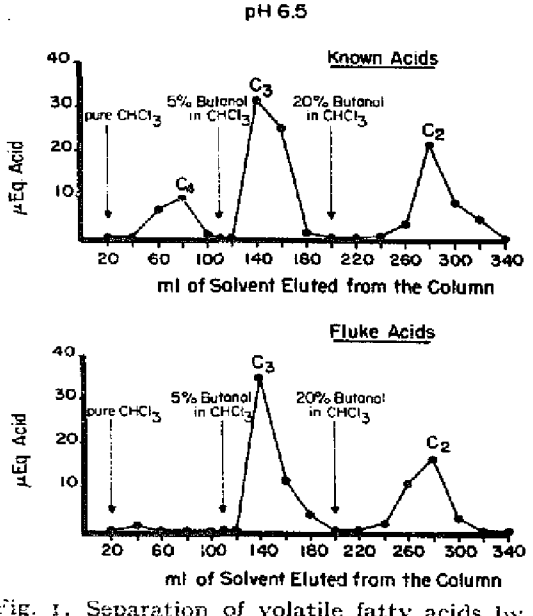

Fig. 1. Separation of volatile fatty acids by means of celite columns, 2 *M* phosphate buffer, pH 6.5.

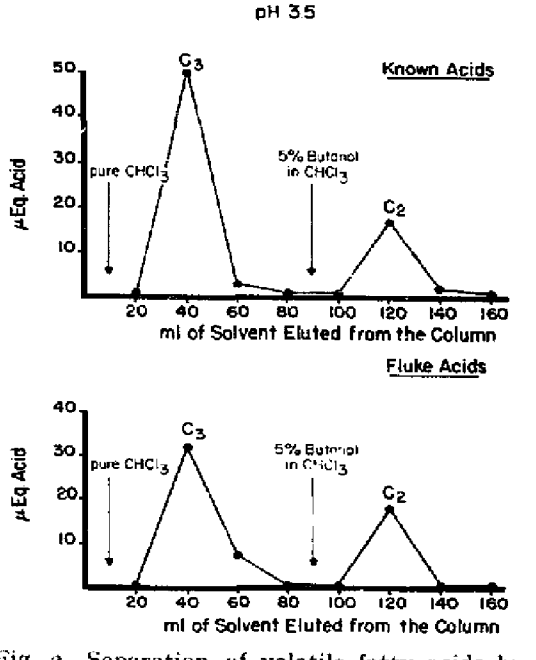

Fig. 2. Separation of volatile fatty acids by means of celite columns; 2 *M* phosphate buffer, pH 3.5.

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determination of the melting point of their derivatives. Fractions of propionic and acetic acids isolated from the columns were purified further and concentrated according to the procedure of BUEDING AND YALE[8], and the *p*-bromophenacyl esters of these fractions were prepared[12]. The melting point of the propionic acid derivative from the fluke medium was 57°–58°, and did not change when mixed with an authentic sample of *p*-bromophenacyl propionate (57°–58°). The melting point of acetic acid derivative isolated from fluke media was 81°–82°. The mixed melting point with *p*-bromophenacyl acetate (81°–82°) was 80°–81°.

Oxygen uptake and respiratory quotients of F. hepatica

Although it was found that aerobiosis has no significant effect on the metabolism of *F. hepatica*, the parasites consume oxygen available in the atmospheric air. In the absence of glucose from the medium, the oxygen uptake varied from 35.0 to 42.6 μmoles/g wet wt./3 h. The respiratory quotients varied from 2.2 to 1.65. The presence of glucose in the medium did not change the respiratory quotients nor the Q_{O_2} significantly. These experiments are summarized in Table IV. The unusually high respiratory quotients of the liver fluke suggests that these organisms do not depend on atmospheric oxygen for their metabolism. It is worth mentioning here that the amount of CO_2 produced aerobically is approximately equivalent to that produced anaerobically.

TABLE IV

RESPIRATORY QUOTIENT OF *Fasciola hepatica* IN THE ABSENCE AND IN THE PRESENCE OF GLUCOSE

In each experiment batches of two flukes in 3 ml of medium were incubated in Warburg flasks with a capacity of 15 ml for 3 h. Q_{O_2} and Q_{CO_2} were calculated as μmoles/g wet wt./3 h. Flukes used in expts. 1 to 4 were non-starved while flukes used in expts. 5 and 6 were starved.

Expt. No.	No glucose			0.011 mole glucose		
	Q_{O_2}	Q_{CO_2}	RQ	Q_{O_2}	Q_{CO_2}	RQ
1	36.8	82.0	2.2	41.3	87	2.1
2	42.6	76.0	1.67	41.0	64.0	1.56
3	40.0	69.5	1.73	36.0	66.0	1.83
4	35.0	64.0	1.83	36.8	58.6	1.60
5	40.5	67.0	1.65	38.2	70.5	1.84
6	38.0	67.0	1.76	36.8	58.6	1.60

Effect of aerobiosis on glucose utilization and production of volatile fatty acids and lactic acid

Carbohydrate balances of batches of liver flukes were determined both aerobically and anaerobically (Table V). Glucose uptake was only slightly decreased to the extent of 6–27% under aerobic conditions. Furthermore, in the presence of atmospheric oxygen, lactic acid and volatile fatty acid production were decreased only slightly. These results indicate the absence of a marked Pasteur effect.

The influence of the alimentary canal of Fasciola hepatica *on its carbohydrate metabolism*

The liver fluke ingests food through the oral opening which leads to the gut of the parasite and is surrounded by the oral sucker. Since the glucose uptake of these

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TABLE V

CARBOHYDRATE METABOLISM OF THE LIVER FLUKES UNDER ANAEROBIC AND AEROBIC CONDITIONS

Flukes were incubated for varying length of time in the standard saline medium. All results are expressed as μmoles/g wet wt.

Expt. No.	Incubation time hours	Aerobic			Anaerobic		
		Glucose uptake	Lactic acid produced	Volatile fatty acid produced	Glucose uptake	Lactic acid produced	Volatile fatty acid produced
1	5	151	29.0	369	195	19.6	378
2	5	148	15.0	296	201	20.4	426
3	5	150	16.0	435	160	17.0	356
4	3	64	8.6	157	80	13.3	172
5	3	65	13.6	140	89	7.5	183

organisms was relatively high, the question arose whether glucose has to reach the alimentary canal before it is absorbed, or whether it can be absorbed through the cuticle. To test this, experiments were designed to measure the metabolism of intact flukes as well as flukes with closed oral openings. The oral openings were closed by ligaturing the worms between the anterior and the posterior suckers. Anaerobic carbohydrate balances of ligatured batches of flukes, as well as control batches, are recorded in Table VI. It is noted that the glucose uptake of ligatured flukes is almost identical with that of the controls. Furthermore, both lactic acid and volatile fatty acids production of ligatured flukes were not significantly different from those of the control. It is concluded that neither the absorption of glucose from the outside medium, nor the excretion of these metabolic products are carried out through the gut.

TABLE VI

ANAEROBIC METABOLISM OF THE LIVER FLUKES WITH LIGATURED ORAL OPENINGS

Flukes were ligatured with cotton threads between the oral and the ventral sucker to prevent ingestion of any medium by the oral orifice. Batches of control as well as ligatured flukes were incubated for 3 h. All results are expressed as μmoles glucose/g wet wt.

Expt. No.	Type of flukes	Glucose uptake μmoles	Lactic acid μmoles	Volatile fatty acids μmoles
1	Control	56	15	152
	Ligatured	56	11	191
2	Control	68	8.0	168
	Ligatured	64	6.7	155
3	Control	51.2	7.25	163
	Ligatured	48.0	6.52	128

DISCUSSION

Anaerobic utilization of glucose and of glycogen among many parasitic helminths results in the formation of volatile fatty acids[2]. Such incomplete utilization of carbohydrate is mainly due to the inaccessability of oxygen to these organisms. The liver fluke, *Fasciola hepatica*, is a metazoan parasite which lives in the bile duct, a predominantly anaerobic environment. It is not surprising, therefore, to observe that

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these organisms cannot metabolize carbohydrate more efficiently through oxidative processes with the end products of CO_2 and water. The fact that, in the presence of atmospheric oxygen, the utilization of glucose and the production of volatile fatty acids were only reduced slightly, strongly suggests an anaerobic type of metabolism. If oxidative metabolism did contribute a large amount of energy to this organism, fermentation should be decreased to a greater extent when respiration occurs. It must be noted, however, that in the presence of atmospheric oxygen, a slight sparing effect on glucose utilization and on the production of volatile fatty acids did occur. It is, therefore, more justifiable to state that *Fasciola hepatica* may require a small portion of its oxidative metabolism.

The concentration of glycogen in the liver fluke varied from one batch to the other. This probably depends on the nutritional condition of the parasite and of the host. Glycogen in the worms was utilized at a high rate when the flukes were cultured in a medium containing no sugar. When glucose was present in the media, glycogen was conserved. In fact, in those cultures which had a low initial concentration of polysaccharide, synthesis of glycogen was demonstrated at approximately the same rate at which it was utilized during starvation. VON BRAND[1] observed that the high glycogen content of parasitic worms is related to an oxygen-poor habitat. Such correlation is satisfactory also in the case of the liver fluke. The high polysaccharide reserve in these organisms might be essential for the existence of *Fasciola* in the bile duct, which has a very low glucose content.

It has been shown that glucose is utilized at a high rate by starved as well as non-starved flukes. The average glucose uptake per six hours varied from 110–195 μmoles/g wet wt. This is lower than that reported on another trematode, *Schistosoma mansoni* (600 μmoles/g/6 h[13]).

Metabolic balance experiments have shown that the production of propionic and acetic acids accounted for almost all the carbohydrate utilized anaerobically. Only 4–8 % of the metabolized carbohydrate was converted to lactic acid. Fatty acids are formed by *Fasciola hepatica* at a much higher rate than by *Ascaris lumbricoides*. Volatile fatty acids account for at least 90 % of the metabolic products of the latter organism. The total volatile fatty acids produced by *Fasciola hepatica* in 6 h was approximately 0.3 mmoles/g wet wt., while *Ascaris lumbricoides* produces in 24 h, only 0.025–0.04 mmoles of volatile fatty acids/g wet wt.[8]. The higher metabolic rate of *Fasciola* strongly suggests higher requirements of available energy in these organisms. This might be attributed to the high muscular activity of this parasite. Stimulation of muscular activity of *Fasciola*, however, by low concentrations of serotonin (5-hydroxytryptamine) or lysergic acid diethylamide results in 2–6 fold increase in lactate production while there is little or no change in volatile fatty acid production[14,15]. It would appear that the physiological function of lactic acid fermentation in the organism is related to increased energy requirements.

WEINLAND AND VON BRAND[3], in their classical studies on the metabolism of *Fasciola hepatica*, have shown that volatile fatty acids were formed at a much lower rate than that reported in the present studies (0.007 mmole/g wet wt./6 h). This corresponds to almost 2 % of the amount produced in our experiments. It is conceivable that the European strain used by VON BRAND is biochemically different from the Gulf Coast strain used in the present study. Observations based on morphological characteristics and size strongly suggest that the Gulf Coast form is actually a hybrid

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between the European strain and the Indian *Fasciola gigantica*[16]. This hypothesis is supported by the fact that the Indian Brahman cattle harboring *Fasciola gigantica* were imported in this area on a large scale between the years 1875 and 1906.

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FORMATION OF L-XYLULOSE FROM L-GULONIC ACID IN RAT KIDNEY

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SUMMARY

Evidence is presented for the presence of an active enzyme system in rat kidney for the conversion of L-gulonolactone or L-gulonic acid to L-xylulose. Identification of the end product was established by a carrier dilution technique, specific enzymatic and colorimetric assays, and column and paper chromatographic data. Evidence is also presented for the formation of a small amount of xylitol as a further product in this reaction. A scheme for the metabolism of L-gulonolactone involving the pentose phosphate pathway is presented.

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