Molecular and Biochemical Parasitology, 13 (1984) 301–307
Elsevier

MBP 00488

THE ENERGY METABOLISM OF *FASCIOLA HEPATICA* DURING ITS DEVELOPMENT IN THE FINAL HOST

ALOYSIUS G.M. TIELENS, JOSEPHUS M. VAN DEN HEUVEL and SIMON G. VAN DEN BERGH

Laboratory of Veterinary Biochemistry, State University of Utrecht, P.O. Box 80177, 3508 TD Utrecht, The Netherlands

(Received 21 May 1984; accepted 19 June 1984)

Mature liver flukes, *Fasciola hepatica*, of different ages were isolated from the bile ducts of experimentally infected rats. Their energy metabolism was studied during aerobic incubation with [6-^{14}C]glucose. The results showed that the aerobic potentials of the parenchymal liver flukes are not lost immediately after arrival in the bile ducts, but in a later phase. During the development of the newly excysted juvenile into the mature adult the major part of ATP production in aerobic incubations is successively contributed by three different pathways of glucose breakdown. The Krebs cycle, which is by far the main energy-yielding pathway of the juvenile fluke, is gradually replaced by aerobic acetate formation and, finally, by the anaerobic dismutation reactions of the adult liver fluke. This observed decrease in Krebs-cycle activity per mg protein is not the result of a decrease in activity per individual fluke. The Krebs-cycle activity per fluke actually increases enormously during its whole development. This indicates that the aerobic potential of adult *F. hepatica* is not just a remnant of earlier aerobic stages but that classical, mammalian type mitochondria are produced during the entire development of the fluke. Calculations are presented which demonstrate that the Krebs-cycle activity of the developing *F. hepatica* is directly proportional to the surface area of the fluke. This supports our view that Krebs-cycle activity is limited by the diffusion of oxygen and can only occur in the outer layer of the liver fluke during its entire development in the final host.

Key words: *Fasciola hepatica*; Energy metabolism; Glucose breakdown; Bile-duct stage; Aerobic-anaerobic switch

INTRODUCTION

Both the newly excysted fluke and the early liver-parenchymal stage of *Fasciola hepatica* have a predominantly aerobic energy metabolism. In the presence of oxygen, juveniles oxidize glucose mainly to carbon dioxide, but they can also survive prolonged periods of anaerobiosis during which they excrete propionate and acetate [1,2]. A functioning Krebs cycle is a true characteristic of the juvenile stage of *F. hepatica*, but this activity slowly decreases during the development of the fluke in the liver parenchyma [2]. Concomitantly, acetate becomes the major end product of the aerobic degradation of glucose in vitro [2]. This formation of acetate requires oxygen and is probably the most important source of energy for the developing fluke. Flukes

in the bile ducts have an anaerobic energy metabolism. They ferment glucose mainly to acetate and propionate, both under aerobic and anaerobic conditions [3]. This anaerobic dismutation starts to play a significant role in the energy metabolism during the last phase of development in the liver parenchyma, but the aerobic production of acetate is then still the most important pathway of the breakdown of glucose [2].

After arrival in a bile duct the very low oxygen content of bile [4] and probably also the size of the fluke will force the parasite to a permanently anaerobic energy metabolism. It is unknown, however, whether the aerobic potential is lost immediately. To investigate this, liver flukes of different ages were isolated from the bile ducts of experimentally infected rats and incubated with [6-^{14}C]glucose in the presence of oxygen to study their aerobic activities.

The results show that the aerobic potential of the parenchymal *F. hepatica* is not lost immediately upon arrival in the bile duct but in a later phase. It is demonstrated that the Krebs-cycle activity per individual fluke actually increases during the entire development in the host. Calculations show that the Krebs-cycle activity in aerobic incubations is all the time directly proportional to the surface area of the fluke. This supports our view that Krebs-cycle activity is restricted to the outer layer of the fluke.

MATERIALS AND METHODS

Isolation of flukes. Liver flukes were isolated from the main bile duct of male Wistar rats (about 180 g at the time of infection) that had been orally infected with about 30 metacercariae each. On days 50, 77 or 114 after infection the flukes were isolated and washed with fresh medium [2], whereupon their incubations were started immediately.

Incubations. Aerobic incubations of the flukes were carried out as before [2], which is as described in [1] but with D-[6-^{14}C]glucose instead of D-[U-^{14}C]glucose. Flukes of 50 days were incubated for 14 h, whereas incubations of 77- and 114-day-old flukes were terminated after 4 h.

Assays and materials. All assays and materials were identical to the ones used in our earlier studies [1,2].

RESULTS

End products of glucose breakdown. Within 50 days after the administration of metacercariae, mature liver flukes were present in the bile ducts, as was observed earlier [5], also on rats. For the experiments adult liver flukes were isolated from the main bile duct on days 50, 77 and 114 after infection. All three bile-duct stages were mature as they excreted eggs during incubation. The mean protein content per fluke at

day 50, 77 and 114 was: 1.3, 10.4 and 23.4 mg, respectively. A 114-day-old fluke in the bile-duct stage thus contained 100 000 times as much protein as a newly excysted juvenile [2].

The isolated liver flukes were incubated aerobically with [6-^{14}C]glucose. The radioactive end products of glucose breakdown, excreted by the parasites into the medium, were determined and the results are shown in Table I. The products called 'rest' in Table I were not identified [1,2] and their amounts were calculated on the assumption that they had half the specific activity of glucose. The formation of acetate, propionate and lactate was calculated using a specific activity half that of glucose. The specific activity of carbon dioxide was assumed to be one-sixth of that of glucose. In Fig. 1 the formation of carbon dioxide, acetate and propionate is expressed as a percentage of the amount of glucose catabolized to form these end products.

The total recovery of labelled end products of the incubations of 50- and 77-day-old flukes was 92–99% of the radioactive glucose consumed. The incubations of 114-day-old flukes resulted in a mean recovery of 78%. This low recovery was also always observed in incubations of adult flukes of undefined age, obtained at a local slaughter-house (not shown). These low recoveries are probably the result of a further utilization of acetate and/or propionate by the parasite. It has been suggested that acetate can be used in fatty acid chain elongation by the adult liver fluke (Van Vugt, F. (1977) Over het energie metabolisme van de volwassen leverbot, *Fasciola hepatica*. Thesis, Utrecht, The Netherlands). The low recovery implies that the acetate and

TABLE I

End products of glucose breakdown excreted by *Fasciola hepatica*

Age of flukes (days)	Excretion of end products (nmol h^{-1} mg^{-1} protein)				
	CO_2	Acetate	Propionate	Lactate	'Rest'[a]
50	30	110	29	13	10
	39	128	25	11	8
77	19	102	164	22	13
	19	93	132	16	9
114	11	60	125	8	7
	11	68	132	12	9

The flukes (7–47 mg protein per Erlenmeyer flask) were incubated at 38°C with D-[6-^{14}C]glucose (0.2–0.4 Ci mol^{-1}). The radioactive end products in the fluke-free medium were analysed. All values are corrected for blank incubations. Results of separate incubations are presented. For total recovery of labelled end products, see text.

[a] See text.

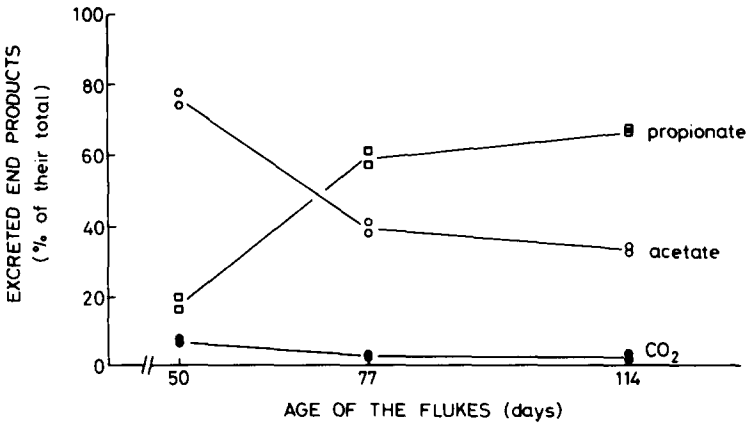

Fig. 1. Excreted end products in aerobic incubations of *Fasciola hepatica* in the bile-duct stage. The formation of CO_2, acetate and propionate is expressed as percentage of the amount of glucose catabolized to form these end products (calculated from the results in Table I).

propionate values obtained with 114-day-old flukes are unreliable, and it is not certain that these dismutation products were in exact redox balance, as might be concluded from Fig. 1.

Contribution of the different pathways of glucose catabolism to ATP production. Theoretical yields of ATP [2] of the three pathways of glucose breakdown (Krebs cycle, aerobic acetate formation and anaerobic dismutation) were used to calculate the contribution of each pathway to ATP production in aerobic incubations. In Fig. 2 the formation of ATP in each pathway is expressed as a percentage of the total ATP production in the three pathways for all the individual stages of *F. hepatica* examined

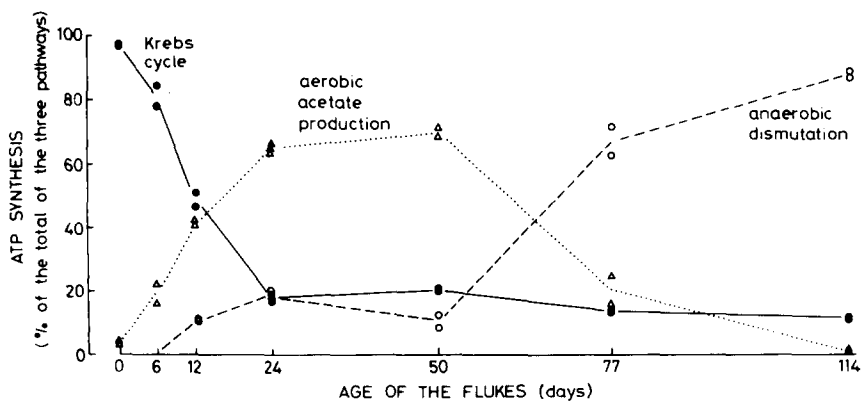

Fig. 2. Contribution of the three pathways of glucose breakdown to ATP synthesis during aerobic incubations of *Fasciola hepatica*.

by us, thus including earlier data [2]. The calculations were based on the assumption that all the excreted propionate was formed by the anaerobic dismutation pathway and that this pathway produced propionate and acetate in a molar ratio of 2:1.

DISCUSSION

It should be emphasized that all data reported in this paper result from aerobic in vitro incubations and probably do *not* reflect the in vivo situation. In vivo, the availability of oxygen in the bile will be limiting for aerobic functioning, and hence these aerobic incubations reflect capacities which are not utilized in vivo.

During the development of *F. hepatica* in the liver parenchyma a steady decrease in Krebs-cycle potential occurs [2], which continues after arrival in the bile duct (Table I and Fig. 1). Fig. 1 shows that liver flukes, shortly after arrival in the bile duct, still possess the capacity of aerobic acetate formation [2] if oxygen is present. This capacity decreased after a longer stay in the anaerobic bile duct as is shown by the results of incubations of 77- and 114-day-old flukes (Fig. 1).

Fig. 2 shows that during aerobic incubations the major part of ATP is successively produced in three different pathways of glucose breakdown: in the early parenchymal stage Krebs-cycle activity dominates, which is followed by aerobic acetate production during the rest of the parenchymal stage and the beginning of the bile-duct stage, and, finally, anaerobic dismutation becomes the main ATP-producing process. This reflects the development in aerobic capacities, whereas *F. hepatica* shows no development in anaerobic capacities: the ability to catabolize glucose anaerobically to propionate and acetate is present immediately after in vitro excystment and persists during maturation [2,3].

The steady decrease in Krebs-cycle potential of *F. hepatica* during its development in the vertebrate host could be the result of a decrease in the number of mitochondria which possess Krebs-cycle activity. This would be the case if mitochondria with Krebs-cycle activity are remnants of an earlier, aerobic stage: the (meta)cercariae.

To investigate this possibility we calculated the Krebs-cycle activity per individual fluke in the various stages of development. These calculations (Table II) show that the Krebs-cycle activity per fluke increased more than thousand-fold during development, which indicates that the number of mitochondria with Krebs-cycle activity increased in each fluke. Therefore, Krebs-cycle activity is a true characteristic of *F. hepatica* and not a remnant from earlier aerobic stages; classical mitochondria are synthesized during the entire development of the fluke.

The observed decrease in Krebs-cycle activity per mg protein and, concomitantly, its increase per individual fluke could be explained if it is assumed that during the development of *F. hepatica* in the liver parenchyma, growth limits the oxygen diffusion to the tissues so that in the inner layers an aerobic energy metabolism is no longer possible. Therefore, Krebs-cycle activity will be limited to the outermost layer

TABLE II

Excreted end products in aerobic incubations of juvenile *Fasciola hepatica* expressed per individual fluke

Age of flukes (days)	Excretion of end products (nmol h^{-1} per fluke)		
	CO_2	Acetate	Propionate
0	0.067	0.0015	-
6	0.23	0.045	-
12	1.50	1.37	0.53
24	6.17	25.77	11.0
50	46.5	160.5	38.0
77	195	1027	1559
114	254	1493	2995

The values were calculated from the means of the incubations presented in [2] and Table I.

of the fluke. This model should not be taken too rigorously: Krebs-cycle activity will not be the unique pathway up to a certain depth with an abrupt transition inwards. A gradient of Krebs-cycle activity will exist and cells in the intermediate layer will switch from one pathway to another and back, depending on the availability of oxygen and on their energy requirement. The thickness of the 'Krebs-cycle layer' is not necessarily equal all over the fluke, but will depend on the metabolic rate. This theory would imply that Krebs-cycle activity is proportional to the surface area of the fluke.

A simplified model was used to check whether the Krebs-cycle activity in aerobic incubations was proportional to the surface area throughout the development of *F. hepatica* in the vertebrate host. It was assumed that: (1) the liver fluke is rectangular and that the length and breadth of the fluke are large compared to its thickness, so that the surface of the lateral faces can be ignored; (2) the protein content of the fluke is directly proportional to its volume; (3) during growth of *F. hepatica* the dimensions increase by the same factor in all three directions. Using these assumptions the surface area of an individual fluke in each stage was calculated with the thickness of the newly excysted juvenile as unity. In Fig. 3 for individual flukes of each stage the log of these calculated surface areas was plotted against the log of the measured production of labelled carbon dioxide (Table II). The slope of the line is almost exactly 45°, which shows that throughout the entire development of the fluke the production of carbon dioxide was directly proportional to the calculated surface area. This indicates that Krebs-cycle activity occurs exclusively in the outer layer of *F. hepatica*.

If it is also assumed that the newly excysted juvenile has the maximal thickness for fully aerobic functioning, the total volume of the aerobic layer can be calculated at all ages. In the adult stage this aerobic volume is far less than 1% of the total volume. This explains why the adult *F. hepatica* has always been regarded to have a completely anaerobic energy metabolism during aerobic incubations in vitro. However,

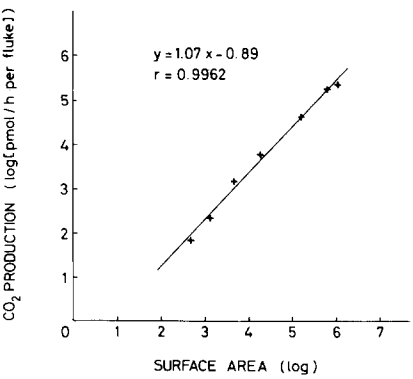

Fig. 3. Relationship between measured carbon dioxide production and calculated surface area per fluke during the development of *Fasciola hepatica* in the final host.

throughout its development in the final host, *F. hepatica* maintains its Krebs-cycle potential; it is only restricted to a very thin outer layer.

ACKNOWLEDGEMENTS

The authors thank Dr. H.J. Over and the Parasitology Department of the Central Veterinary Institute (Lelystad) for the generous supply of metacercariae. This work was supported by the Netherlands Foundation for Chemical Research (S.O.N.) with financial aid from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

REFERENCES

1 Tielens, A.G.M., van der Meer, P. and van den Bergh, S.G. (1981) The aerobic energy metabolism of the juvenile *Fasciola hepatica*. Mol. Biochem. Parasitol. 3, 205–214.

2 Tielens, A.G.M., van den Heuvel, J.M. and van den Bergh, S.G. (1982) Changes in energy metabolism of the juvenile *Fasciola hepatica* during its development in the liver parenchyma. Mol. Biochem. Parasitol. 6, 277–286.

3 Lahoud, H., Prichard, R.K., McManus, W.R. and Schofield, P.J. (1971) Volatile fatty acid production by the adult liver fluke *Fasciola hepatica*. Comp. Biochem. Physiol. 38B, 379–391.

4 Moss, G.D. (1970) The excretory metabolism of the endoparasitic digenean *Fasciola hepatica* and its relationship to its respiratory metabolism. Parasitol. 60, 1–19.

5 Boray, J.C., Happich, F.A. and Andrews, J.C. (1967) Studies on the suitability of the albino rat for testing anthelmintic activity against *F. hepatica*. Ann. Trop. Med. Parasitol. 61, 104–111.