Aberrant energy-linked reactions in mitochondria isolated from the livers of rats infected with the liver fluke *Fasciola hepatica*

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The respiratory properties of mitochondria isolated from the livers of rats infected with the parasite *Fasciola hepatica* were examined. Oligomycin-sensitive ATPase activity was also examined during the acute stage (2–4 weeks post-infection). At 2, 4 and 6 weeks post-infection, mitochondrial respiration *in vitro* (supported by site I and site II substrates) was completely uncoupled. Limited respiratory control had returned by 11 weeks post-infection, but complete recovery was not observed even at 21 weeks post-infection. At 4 weeks post-infection, uncoupled respiration (from all three energy-conserving sites) was also markedly attenuated (to the greatest extent with NADH-linked substrate). Except for pyruvate-supported respiration, this attenuation was not apparent at any other stage of the infection. The attenuation of pyruvate-supported respiration declined, but was still present, at 6 weeks post-infection. In addition to these perturbations in mitochondrial respiratory properties, mitochondrial ATPase activity at 4 weeks post-infection was insensitive to oligomycin, indicating a change in the structural integrity of the ATPase complex.

INTRODUCTION

A number of parasitic diseases appear to affect the bioenergetic metabolism of the infected host tissue. Michejda & Boczon (1972) observed that isolated rat skeletal muscle mitochondria were uncoupled during experimental trichinosis (*Trichinella spiralis*). This uncoupling *in vitro* could be reversed by addition of bovine serum albumin to mitochondria isolated after 2 weeks of infection but by 4 weeks post-infection the albumin had little effect. While investigating fish infected with *Anasakis simplex*, Boczon & Bier (1986) observed that mitochondria isolated from muscle were uncoupled, as evidenced by ADP-independent respiration and elevated oligomycin-sensitive ATPase activity. Oxidative phosphorylation and capacity for gluconeogenesis are also compromised in the livers of mice infected with the malarial parasite *Plasmodium berghei* (Geoffrion *et al.*, 1985).

A report by Van den Bossche *et al.* (1980) provided indications that mitochondria isolated from the livers of rats infected with *Fasciola hepatica* were uncoupled. In addition, Van den Bossche *et al.* (1983) showed increased mitochondrial ATPase activity following infection. Two features of the experimental procedures adopted by Van den Bossche *et al.* (1980, 1983) led us to re-examine the influence of fluke infestation on energy-linked reactions in the subsequently-isolated mitochondria. The first was the relatively high centrifugal forces used to obtain the mitochondria and the second was that the ATPase activity was measured in liver slices by employing a histochemical stain, and therefore it was not a quantitative measurement of the change.

In this investigation we show that the respiratory behaviour of the mitochondria isolated from fluke-infected liver is aberrant but that the nature of this aberration is more complex than previously reported by

Van den Bossche *et al.* (1980). Moreover, we provide evidence that the ATPase activity of the mitochondria becomes insensitive to oligomycin within a few weeks of infection.

EXPERIMENTAL

Rats

Outbred male Wistar rats were obtained from the animal breeding facility, Australian National University. All rats were 4–6 weeks of age at the time of infection. Uninfected rats of the same age were used as controls. Control and infected rats were kept in cages and provided with food and water *ad libitum*.

Metacercariae of *F. hepatica*

Metacercariae of *F. hepatica* maintained in the snail *Lymnaea viridis* were obtained from Dr. J. C. Boray, New South Wales Department of Agriculture, Glenfield, N.S.W. The metacercariae, suspended in water, were kept refrigerated and were suspended in a suitable volume of 1% carboxymethylcellulose before infecting the rats. The viability of the metacercariae was determined visually under a light microscope. Metacercariae were regarded as viable if the refractile excretory granules were present (Boray, 1969). This determination often involved removal of the metacercarial outer coat to ensure a confident judgment.

Infections

Thirty viable metacercariae were administered via a stomach tube to rats lightly anaesthetized with anaesthetic ether. The resulting infection was characterized and monitored by following the increase in serum glutamate dehydrogenase activity and the liver/body-

Abbreviations used: TMPD, *NNN'*-tetramethyl-1,4-phenylenediamine dihydrochloride; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; OSCP, oligomycin-sensitivity-conferring protein.

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weight ratio, two responses routinely observed in fascioliasis.

Subcellular fractionation of rat liver

Rats were anaesthetized with sodium pentobarbitone (approx. 50 mg/kg of body weight). The macroscopic damage to the exposed liver was noted for all infected rats. The bile ducts of rats infected for 6 or more weeks were opened and the mature flukes were removed and counted. Livers from both control and infected rats were isolated, weighed, diced with scissors and washed three times in an isolation medium containing 250 mM-mannitol, 10 mM-KCl, 5 mM-Hepes/KOH and 0.5 mM-EGTA (pH 7.4). The diced liver was then homogenized in a Thomas size C homogenizer with three passes of a teflon pestle rotating at 900 rev./min. The homogenate was made up to an approx. 10% (v/v) suspension by addition of isolation medium. All centrifugation steps were carried out in a Sorvall RC-5B refrigerated centrifuge (4 °C) with an SS-34 angle rotor and were of 5 min duration. The homogenate was centrifuged at 755 *g*. The resultant supernatant was centrifuged at 5090 *g*, while the pellet was resuspended in a wash medium whose composition was identical to the isolation medium but without the EGTA; this medium was used in all subsequent steps. The resuspended pellet was then centrifuged at 755 *g*. The 5090 *g* pellet was kept on ice while the second 755 *g* supernatant was centrifuged at 5090 *g*. The two higher-speed pellets were resuspended, combined and then centrifuged at 7710 *g* to produce the final mitochondrial pellet. This pellet contained approx. 80 mg of protein/ml. All procedures were carried out on ice (approx. 4 °C).

Protein determination

Protein was determined by a modification of the method of Lowry *et al.* (1951), in which samples were allowed to stand at 60 °C for 4 min in the presence of Lowry reagent C and for 8 min after addition of Folin reagent. Samples were cooled to room temperature before reading. Bovine serum albumin was used as standard.

Oxygen uptake studies

Oxygen uptake was followed polarographically with a Clark-type oxygen electrode in a medium containing 50 mM-KCl, 2 mM-MgSO₄, 15 mM-Hepes/KOH, 100 mM-mannitol, 0.5 mM-EDTA and 2 mM-KH₂PO₄. Unless otherwise indicated, samples were added at a final concentration of 2.0 mg of protein/ml. The final volume of the electrode chamber was 2.5 ml and the temperature was 25 °C. Either sodium pyruvate (5 mM) and malate (1 mM), or sodium succinate (5 mM), or ascorbate/*NNN'*-tetramethyl-1,4-phenylenediamine dihydrochloride (TMPD) (5 mM/1 mM) was used as oxidizable substrate. Where indicated, ADP (100 μM), NADH (0.8 mM) carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) (2 μM), rotenone (2 μM), antimycin A (2 μM) and oligomycin (4 μg/ml) were added.

Cytochrome oxidase activity was assayed polarographically using ascorbate/TMPD as substrate. Where indicated, exogenous cytochrome *c* was present at 0.5 mg/ml.

ATPase activity

ATPase activity in freeze-thawed mitochondria from both control and infected livers was measured at 37 °C

in a 2 ml reaction mixture (pH 7.4) containing 5 mM-MgSO₄, 5 mM-Hepes/KOH and 5 mM-ATP. When required, oligomycin was added at 0.5 μg/ml. Freeze-thawed mitochondria (1.5 mg of protein/ml) were added 2 min before the reaction was started by the addition of ATP. At 0, 0.5, 1, 2, 3 and 5 min after initiation of the reaction 0.25 ml samples were taken from the reaction mixture and added to 0.25 ml of 20% (w/v) trichloroacetic acid to stop the reaction. The precipitated protein was separated by centrifugation for 2 min in an Eppendorf microfuge and the supernatant P_i levels were measured by the method of Baginski *et al.* (1967).

Expression of data

Unless otherwise indicated, all data are expressed as means ± S.E.M. for $n \geq 3$ experiments. The statistical significance *P* was determined with Student's *t* test.

Materials

CCCP, oligomycin, antimycin A, rotenone, ADP and TMPD were obtained from the Sigma Chemical Co., St. Louis, MO, U.S.A. NADH, ATP and cytochrome *c* were obtained from Boehringer Mannheim, Germany.

RESULTS

Effect of fascioliasis on the mitochondrial respiratory response to CCCP and to oligomycin

The data in Fig. 1 are typical traces that show both the rates of respiration by mitochondria from livers of control rats (*a*) and from those infected for 2 (*b*), 4 (*c*) and 6 (*d*) weeks. The traces also show the responses of these mitochondria to additions of ADP, the chemical uncoupler CCCP and the ATP synthase inhibitor oligomycin. By contrast with their influence on control mitochondria, neither CCCP nor oligomycin affected respiration in mitochondria isolated from infected livers. The data also show that the rate of respiration in mitochondria from livers of 4-week-infected rats is significantly lower than at any other stage of infection (see below). The respiratory-chain inhibitors, rotenone and antimycin A, had normal effects on mitochondria from both control and 6-week-infected rat livers (results not shown). This was the case for mitochondria obtained at all stages of the infection examined in this study (see below).

Mitochondrial respiration during the course of the infection

The respiratory response of the mitochondria to ADP stimulation throughout the infection is summarized in Fig. 2(*a*). Mitochondria from 2-, 4- and 6-week-infected rats exhibited acceptor control ratios of 1.0. Those from livers of rats infected for 11 or more weeks displayed a small degree of respiratory control. For example, at 11 weeks post-infection, mitochondria utilizing site I or site II substrates exhibited acceptor control ratios of 1.25 ± 0.25 and 1.44 ± 0.23 respectively. There was no significant improvement in respiratory control between 11 and 21 weeks post-infection. At 21 weeks post-infection, acceptor control ratios of 1.64 ± 0.10 and 1.66 ± 0.05 were obtained with site I and site II substrates, respectively.

The ADP/O ratios approached zero at 2, 4 and 6 weeks post-infection in the presence of either site I or site II substrates (Fig. 2*b*). Throughout the course of infection

Fig. 1. Influence of fascioliasis on the mitochondrial respiratory response to CCCP and oligomycin. (a) Control, (b) 2 weeks post-infection, (c) 4 weeks post-infection and (d) 6 weeks post-infection

Mitochondrial respiration was measured, using pyruvate/malate as substrate, as described in the Experimental section. ADP, CCCP and oligomycin were added at 100 μ M, 2.0 μ M and 4 μ g/ml, respectively. The traces are typical of those obtained in experiments on ten control, three two-week-infected, four four-week-infected and three six-week infected rats.

the ADP/O ratio followed the same pattern as the respiratory control ratios. With either site I or site II substrates, the ratio had increased by 11 weeks post-infection, but did not return to control values over the next 10 weeks. The ADP/O ratios of control mitochondria did not change with age. On average, the ADP/O ratios were 3.26 ± 0.11 and 2.1 ± 0.09 for site I and site II substrates respectively.

These mitochondrial lesions appear to depend on the fluke burden of the host. The data in Fig. 3 show that, for succinate-supported respiration, both the acceptor control and ADP/O ratios were depressed in mature infections (those lasting 11 or more weeks) where three or more adult flukes were found in the bile duct. Infections involving three or more flukes exhibited an average acceptor control ratio of 1.42 ± 0.14 and an average ADP/O ratio of 1.26 ± 0.07 . On the other hand, infections of less than 3 flukes exhibited an average acceptor control ratio of 3.43 ± 0.16 and an ADP/O ratio of 2.0 ± 0.06 . A similar pattern of depression in these parameters was obtained with site I substrate.

The ability of the mitochondria to utilize each of the substrates (pyruvate/malate, succinate or ascorbate/TMPD) during the course of the infection is shown in Fig. 4. The most significant observation, which is con-

Fig. 2. Effect of fascioliasis on (a) the acceptor control ratio and (b) the ADP/O ratio of isolated liver mitochondria during 21 weeks post-infection

Liver mitochondria (5.0 mg of protein) from control and infected rats were incubated (25 °C) in a Clark-type oxygen electrode in the presence of 100 mM-mannitol, 50 mM-KCl, 2 mM-MgSO₄, 15 mM-Hepes/KOH, 0.5 mM-EDTA, 2 mM-KH₂PO₄ and either 5 mM-pyruvate plus 1 mM-malate (∇) or 5 mM-succinate ($\blacktriangledown$) in a final volume of 2.5 ml. The rate of oxygen uptake was monitored in the presence or absence of ADP (100 μ M) and the parameters described in this Figure were calculated from those rates. Each data point is the mean $\pm$ S.E.M. of two to four experiments.

sistent with the data presented above, is the depression in absolute mitochondrial oxygen uptake at 4 weeks post-infection. The rates of oxygen uptake with pyruvate (Fig. 4a), succinate (Fig. 4b) and ascorbate plus TMPD (Fig. 4c) were all depressed at this stage of the infection. The degree of attenuation was greatest for mitochondria utilizing site I substrate (11% of the control rate), followed by mitochondria utilizing site II (20%) and site III (35%) substrates. State 4 rates of oxygen uptake in control mitochondria remained constant with age for both pyruvate- (approx. 12 nmol of oxygen/min per mg of protein) and succinate- (approx. 20 nmol of oxygen/min per mg of protein) supported respiration. Although the control rates fluctuated from week to week in the presence of ascorbate and TMPD, it is nonetheless evident that, at 4 weeks post-infection, the rates were depressed relative to the age-matched control rates for this substrate. With pyruvate or succinate as substrate, mitochondria at 2 weeks post-infection respired at rates similar to those elicited by the uncoupler CCCP. The attenuation seen at 4 weeks post-infection is subsequently lost so that mitochondrial respiration at 6 weeks post-infection approaches that of 2 weeks post-infection and is retained throughout the remainder of the time

Fig. 3. Relationship between fluke burden and (a) the acceptor control ratio (▼) and (b) the ADP/O ratio (▽) in mature infections

The bile ducts of rats infected for 11 or more weeks were opened and the flukes removed and counted prior to preparation of liver mitochondria. Mitochondrial respiration with succinate as substrate was measured as described in the Experimental section and the acceptor control and ADP/O ratios were calculated. The values are derived either from a single experiment, or are the mean of two experiments.

course (up to 21 weeks post-infection). The attenuation at 4 weeks post-infection therefore represents a major reversible change to the condition of the liver mitochondria. A major lesion at this stage of the infection is consistent with the observation of maximum liver damage occurring immediately prior to the entry of flukes into the bile duct (Thorpe, 1965).

The possibility was examined that the attenuation of respiratory response in the mitochondrial fraction from infected livers is due to a redistribution of the organelle during isolation into either the pre- or post-mitochondrial fractions. However, experiments showed that the sub-cellular distribution of cytochrome *c* oxidase activity was identical in both control and 4-week-infected livers (results not shown) although the activity in the infected mitochondria was 120 ± 8.0 nmol of oxygen/min per mg of protein compared with 185 ± 11.0 in the control.

Further evidence for an aberration in mitochondrial energy-linked functions

The data in Table 1 show the effect of exogenous NADH on respiration by both normal and sonicated, control and 'infected' mitochondria. At 4 weeks post-infection, the rate of NADH-supported respiration by sonicated mitochondria was only 31% of the sonicated control mitochondria. This attenuation, paralleling that observed for pyruvate-supported respiration, suggests that inhibited pyruvate oxidation is not responsible for the lesion. In addition, non-sonicated mitochondria isolated from infected livers showed enhanced respiration in the presence of NADH. This result clearly suggests that exogenous NADH has access to the NADH dehydrogenase whose active site is normally orientated towards the matrix space.

The F_1F_0 -ATP synthase is an essential component of the energy-transducing assembly of the inner mitochondrial membrane. Van den Bossche *et al.* (1983) showed qualitatively that the total ATPase activity was

Fig. 4. The effect of fascioliasis on the absolute rate of mitochondrial state 4 respiration

The respiratory rates of mitochondria isolated from livers of age-matched control rats (□) and rats infected for the periods indicated (▨) were measured, as described in the Experimental section, in the presence of either (a) 5 mM-pyruvate plus 1 mM-malate, (b) 5 mM-succinate, or (c) 1 mM-TMPD plus 5 mM-ascorbate. The values are the means $\pm$ S.E.M. of results from two to five control or infected livers in each case.

elevated in liver slices from rats infected for 13 weeks; inhibition by oligomycin was not tested. In the present study, we re-examined ATPase activity in mitochondria isolated from control and infected livers. The total latent ATPase activity was unchanged at 4 weeks of infection (results not shown). This does not correlate with the depressed cytochrome *c* oxidase activity. However, there was an almost complete loss of oligomycin sensitivity in the ATPase activity at 3 and 4 weeks post-infection (Table 2). This indicates the onset of a major lesion at about 3 weeks post-infection, and it appears that the oligomycin-sensitivity conferring protein or the assembly of the ATP synthetase complex may be affected in some way.

Table 1. The effect of NADH on oxygen uptake by mitochondria isolated from rat livers at 4 weeks post-infection

Liver mitochondria isolated from both uninfected and 4-week-infected rats were sonicated as described in the Experimental section. Respiration was measured polarographically without added substrate both before (basal rate) and after the addition of NADH (0.8 mM). The values are the means $\pm$ S.E.M. ($n = 3$ rats in each group).

	Oxygen uptake (nmol/min per mg of protein)			
	Control		Infected	
	Normal	Sonicated	Normal	Sonicated
Basal rate	10.7 $\pm$ 0.2	11.1 $\pm$ 1.4	5.9 $\pm$ 0.9	4.8 $\pm$ 0.5
+ NADH	12.9 $\pm$ 0.4	56.7 $\pm$ 11.6*	16.2 $\pm$ 3.3	17.9 $\pm$ 4.2*

* Values are significantly different; $P < 0.005$.

Table 2. The effect of fascioliasis on the sensitivity to oligomycin of latent mitochondrial ATPase activity

Freeze-thawed liver mitochondria (3 mg of protein) from uninfected (0 weeks post-infection) and 2-, 3- and 4-week-infected rats were preincubated for 2 min at 37 °C in a medium containing 5 mM-MgSO₄ and 5 mM-Hepes/KOH (pH 7.4), with or without oligomycin (0.5 μ g/ml), before initiating the reaction with 5 mM-ATP (pH 7.4). The final volume of the reaction mixture was 2.1 ml and the incubation time was up to 5 min. The inorganic phosphate content of the reaction mixture was determined as described in the Experimental section. The percentage of total ATPase activity that was oligomycin-sensitive was calculated from the rates of inorganic phosphate production.

Weeks post-infection	Oligomycin-sensitive activity (% of total ATPase activity)
0	81.7 ($n = 4$)
2	88.2 ($n = 3$)
3	9.8 ($n = 3$)
4	2.1 ($n = 5$)

DISCUSSION

This work has revealed lesions in mitochondrial respiratory chain activity and ATPase function that, together with the confirmation of uncoupling of oxidative phosphorylation, demonstrate major lesions in host liver bioenergetic metabolism during fascioliasis.

It was necessary to confirm the results of Van den Bossche *et al.* (1980) because of the accumulated evidence indicating that the rat-liver mitochondrial population is heterogeneous and can be resolved, by differential centrifugation, into fractions of distinct functional integrity (Gear *et al.*, 1965a,b; Katyare *et al.*, 1970; Satav *et al.*, 1976; Bygrave *et al.*, 1978). The procedure used by the previous workers would not be expected to differentiate this functionally heterogeneous population, and it was reassuring to reproduce their results using a fractionation procedure known to produce a functionally intact, homogeneous, mitochondrial pellet.

In addition to finding depressed respiratory control and ADP/O ratios, we also observed that respiration was insensitive to the actions of CCCP and to oligomycin.

Collectively these data point to a major deficiency in the ability of the infected mitochondria to synthesize ATP. The data further indicate that the mitochondrial inner membrane is permeable to protons although this was not directly tested in the present work.

The putative increase in inner membrane permeability to protons could arise through structural damage to the inner membrane or through the presence of an endogenous proton-translocating agent. Structural damage is a likely explanation for the enhancement of basal mitochondrial respiration in the presence of exogenous NADH at 4 weeks post-infection (Table 1). Membranes are normally impermeable to NADH, which is oxidized in mitochondria at the matrix-orientated active site of the NADH dehydrogenase complex. Enhanced respiration following the administration of exogenous NADH usually indicates a loss of mitochondrial inner membrane integrity (Lehninger, 1964). The uncoupling of oxidative phosphorylation at 2, 4 and 6 weeks post-infection is therefore probably also due to this loss of inner membrane structural integrity. In addition, the attenuation of respiratory-chain activity from site I, site II and site III substrates and the loss of oligomycin-sensitive ATPase activity provide further evidence of a disruption of inner-membrane-associated functions.

Although the attenuation of respiration from all three energy-conserving sites suggests a generalized fascioliasis-induced injury, it is apparent that sites I and II are the most vulnerable. The extra sensitivity of these two complexes might be due to their lower concentration in the inner membrane compared with that of cytochrome *c* oxidase. The ratios of complex IV/complex I and complex IV/complex II are 7:1 and 3–4:1, respectively (Hatefi *et al.*, 1985).

The cause of the fascioliasis-induced attenuation has not yet been deduced, but some possibilities can be eliminated. We have shown that the general attenuation in respiratory-chain activity is not due to loss of mitochondria from the mitochondrial pellet. There was no change in the sedimentation properties of the mitochondria during differential centrifugation. The distribution of cytochrome *c* oxidase showed that neither was there a redistribution of inner membrane, and therefore of respiratory chain, into either the nuclear or post-mitochondrial fractions, nor was there a sedimentation of non-mitochondrial protein into the mitochondrial pellet. In addition, the observation that NADH-supported respiration in sonicated mitochondria was

inhibited at 4 weeks post-infection (Table 1) suggests that inhibited substrate utilization (due to inhibited tricarboxylic acid cycle activity or blocking of the entry of pyruvate into the matrix, for example) was not a cause of attenuated respiration in unsonicated mitochondria.

Further indications of a fascioliasis-induced general breakdown of inner-membrane-associated functions is the loss of oligomycin-sensitive ATPase activity at 4 weeks post-infection. Although latent ATPase activity was not changed, it had lost all sensitivity to oligomycin. The oligomycin-sensitivity-conferring protein (OSCP), which is associated with F_1F_0 -ATPase and is located between the F_0 and F_1 subunits, facilitates the binding of oligomycin to F_0 which blocks proton flow through the complex, thereby inhibiting ATP synthesis or hydrolysis. The OSCP also plays a role in the binding of F_1 to F_0 , meaning that oligomycin sensitivity is diagnostic of correct F_1F_0 -ATPase assembly. Loss of oligomycin sensitivity could be attributed to an alteration in either a function of the OSCP, such as its role in binding F_1 to F_0 , or in the structure or function of the F_0 subunit to which oligomycin binds. Although the following proposal is yet to be tested, the loss of oligomycin sensitivity, coupled with the maintenance of full latent ATPase activity, is most readily explained by the release of F_1 subunits from the membrane-bound F_0 subunits. Detached F_1 particles will catalyse oligomycin-insensitive ATPase activity in the presence of high concentrations of ATP (Nicholls, 1982). The detachment of the F_1 part of the ATPase complex from the surface of the inner membrane would be consistent with the evidence presented and could indicate a generalized type of damage to the mitochondrial inner membrane during the acute stage of fascioliasis, particularly at 4 weeks post-infection.

When working with an isolated cell fraction there is always the risk of damaging the organelle. The mitochondrial inner membrane is particularly sensitive to isolation-induced disruption. For this reason, such a disruption of the organelle's integrity cannot be ruled out as a cause of the lesions described above. However, the isolation procedure normally produces functionally intact control mitochondria, so it is apparent that the presence of liver flukes either causes complete disruption of inner membrane structure and function or sensitizes the membrane to preparative damage. Although the inner membrane lesions described are greatest at 4 weeks post-infection, the insensitivity of respiration to added ADP manifests as early as 2 weeks post-infection. At this stage, often the only signs of liver infection are small migration tracks (less than 1 mm wide) indicating entry of flukes into the parenchyma. Flukes isolated from the parenchyma at this stage of the infection are 1–2 mm long and 0.5–1 mm wide (Rajasekariah, 1977) and are physically insignificant compared with the bulk of the liver tissue. A metabolic lesion at 2 weeks post-infection is not likely to be caused by the physical activities of the flukes. Rather, it is more probable that the effect is an indirect one. This supposition has also been made for a number of other fascioliasis-induced lesions, including bile duct epithelial hyperplasia and haemolytic anaemia (Modavi & Isseroff, 1984; Coffin *et al.*, 1984). Also, remote or indirect effects of other parasites on liver metabolism have been reported. For example, the intestinal parasite *Nippostrongylus brasiliensis* impairs the hepatic microsomal drug-metabolizing system (Tekwani *et al.*, 1987). Some potential factors involved in fascio-

liasis would include toxin secretion by the flukes or tissue damage mediated by the immune response.

Van den Bossche *et al.* (1983, 1985) have proposed that the uncoupling of liver mitochondria is caused by fluke excretory/secretory products, but there is little evidence to support this hypothesis at present. Other factors may also be responsible for the lesions *in vitro* described in this work. It is clear that the effects of the host's immune response on the infected liver should be investigated.

There is increasing evidence that host-mediated reactions account for a certain proportion of total tissue damage in parasitic disease (Clark *et al.*, 1986; Beutler & Cerami, 1987). This damage is thought to be, in the main, initiated by oxygen-derived free radicals (Clark *et al.*, 1986), but there is also evidence that implicates host-derived tumour necrosis factor (cachectin) in parasite-induced tissue damage (Beutler & Cerami, 1987).

A number of indirect findings suggest that oxidative damage may contribute to the lesions described in this study. First, it is apparent that liver tissue is under oxidative stress during fascioliasis. Galtier *et al.* (1987) reported that between 3 and 6 weeks post-infection, cytosolic Ca^{2+} increases by 90% while tissue concentrations of reduced glutathione decline significantly. Also, tissue levels of the antioxidant, ascorbic acid, are reduced during fascioliasis (Gameel, 1982). Raised cytosolic Ca^{2+} concentration is a common phenomenon during oxidative stress (Orrenius *et al.*, 1985). The second element of indirect evidence supporting the hypothesis of oxidative stress is the important fact that both major hepatic lesions reported to date, depressed biotransformation activity (Facino *et al.*, 1981, 1985; Galtier *et al.*, 1986a,b, 1987) and altered respiratory-chain or ATPase activity, use membrane-bound enzyme systems that would be especially vulnerable to the effects of lipid peroxidation. Essential protein thiols are also at risk in tissues undergoing oxidative stress.

Although there is indirect evidence supporting a role for host-mediated oxidative damage to the respiratory chain, the ATPase and the inner mitochondrial membrane during the acute stage of fascioliasis, the proposal remains to be tested. Other factors may also be responsible for the lesions. The role of fluke excretory/secretory products in these metabolic lesions is yet to be clarified.

The uncoupling of respiration and the attenuation of respiratory activity are major alterations to host liver bioenergetic metabolism. Potentially the lesions would cause a major decline in cellular ATP levels through the inability to conserve energy. This has vast implications for many aspects of host physiology, including attenuated anabolism and depressed capacity to detoxify foreign compounds. It is not possible to determine from these experiments whether respiration *in vivo* is compromised during liver fluke infection. Further work is indicated to enable assessment of the function of liver mitochondria *in vivo* during fascioliasis.

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