Proc. Indian Acad. Sci. (Anim. Sci.), Vol. 98, No. 4, July 1989, pp. 249–257.
© Printed in India.

Mitochondrial adenosine triphosphatase of *Penetrocephalus ganapatii* (Cestoda: Pseudophyllidea) in relation to activators and inhibitors

S DHANDAYUTHAPANI* and K NELLAIAPPAN
Department of Zoology, University of Madras, Guindy Campus, Madras 600 025, India
*Present address: Central Leprosy Teaching and Research Institute, Chengalpattu 603 001, India

MS received 4 November 1988; revised 26 April 1989

Abstract. The mitochondrial adenosine triphosphatase of the cestode parasite, *Penetrocephalus ganapatii* exists as a latent form and is activated by dinitrophenol or $MgCl_2$. Irrespective of $MgCl_2$ or dinitrophenol concentrations, the enzyme shows optimum activity at pH 8. Effects of inhibitors on Mg^{2+}, dinitrophenol and Mg^{2+} + dinitrophenol activated ATPase reveal that ATPase is not highly sensitive towards oligomycin and the alteration of the redox state of the respiratory chain components by rotenone, antimycin A, azide and cyanide has little effect on ATPase activity. –SH groups of the enzyme seem to play a limited role in the hydrolysis of ATP, as the enzyme is only partially sensitive to *p*-chloromercuribenzoic acid. Generally, cations inhibit Mg^{2+} stimulated ATPase and activate dinitrophenol stimulated ATPase but most of the anions are inhibitory to dinitrophenol or Mg^{2+} stimulated ATPase.

Keywords. Mitochondria; ATPase; activators; inhibitors.

1. Introduction

The terminal step of oxidative phosphorylation in the respiratory chain is catalyzed by a complex enzyme system called mitochondrial ATPase (Panet and Sanadi 1976; Tzagaloff 1976). This ATPase complex is oligomycin sensitive and activation of ATPase by Mg^{2+} and the uncoupler 2,4-dinitrophenol (DNP) is an universal feature (Chefurka 1981a). The site for synthesis and hydrolysis of ATP is the soluble F_1 unit of this ATPase complex (Penefsky *et al* 1960; Pullman *et al* 1960; Racker 1970). The F_1 ATPase isolated and characterized from a variety of sources displays remarkable similarities in structure and properties (Panet and Sanadi 1976). The characteristics of uncoupler activated ATPase have been thoroughly studied in mammalian liver mitochondria with respect to inhibitors (Lardy *et al* 1958; Bruni *et al* 1965; Lardy and Lin 1969; Beechey *et al* 1967), dependence on cations (Amons *et al* 1968; Cereijo-Santalo 1972) and anions (Veldsema-currie and Slater 1968) and involvement of respiratory chain components (Weiner and Lardy 1974). In parasitic helminths, although a few attempts have been made to study the property of this enzyme in the nematodes *Ascaris lumbricoides* (Hayashi 1973) and *A. suum* (Van den Bossche 1972, 1974) and the cestode *Schistocephalus solidus* (Walker and Barrett 1983), no information is available in other parasitic helminths regarding the properties of this enzyme, particularly in relation to activators and inhibitors. In view of the importance of ATP synthesis and other ATP-dependent functions in these highly specialized anaerobic animals, this paper deals with the effect of activators and inhibitors on the mitochondrial ATPase of a pseudophyllid cestode parasite, *Penetrocephalus ganapatii*.

2. Materials and methods

2.1 *Preparation of mitochondria*

Live parasites of *P. ganapatii* were collected from the intestine of the host fish *Saurida tumbil.* They were washed several times in 0·85% saline before homogenization. A 10% homogenate was prepared in 0·25 M sucrose and centrifuged at 1,000 *g* for 10 min to sediment the cell debris. The supernatant fraction was recentrifuged at 10,000 *g* for 15 min. The sediment obtained was resuspended in the sucrose solution to the original volume and centrifuged again at 10,000 *g* for 15 min. The final pellet containing mitochondria was suspended in a known volume of sucrose solution and used for the enzyme assay. The whole procedure was carried out at 4°C. The presence of mitochondria in the pellet was assessed by assaying the marker enzymes succinate dehydrogenase and cytochrome oxidase.

2.2 *Enzyme assay*

Mitochondrial ATPase (EC 3·6·1·4) activity was measured following the method of Veldsema-currie and Slater (1968). The reaction mixture contained 50 mM Tris-HCl buffer pH 8, 75 mM KCl, 1 mM EDTA, 2·5 mM ATP, mitochondrial suspension and water to a total volume of 1 ml. The reaction was initiated by the addition of 0·1 ml of mitochondrial suspension (150–200 μg protein). The reaction mixtures incubated, after the addition of mitochondrial protein, for periods from 5–30 min showed that ATPase activity was linear up to 15 min. Therefore the reaction mixtures were incubated for 15 min in all cases and then terminated by the addition of 10% (w/v) ice-cold trichloroacetic acid (TCA). The mitochondrial suspension added after the addition of TCA to the reaction mixture constituted the control. After termination of reaction, the precipitate formed was removed by centrifugation at 1,000 *g* for 5 min. The resultant supernatant was analyzed to determine the amount of phosphate liberated. Quantitative estimation of phosphate was carried out following the method of Fiske and Subbarow (1925). Protein was determined according to Lowry *et al* (1951). The specific activity of the enzyme is expressed as nmol P_i liberated/min/mg protein.

2.3 *Chemicals*

ATP, oligomycin, rotenone, *p*-chloromercuribenzoic acid (*p*-CMB), malate, fumarate and 2-oxoglutarate were obtained from Sigma Chemical Co., St. Louis, Missouri, USA. Antimycin A was obtained from Cal Biochem, La Jolla, USA. Malonate was obtained from British Drug House, Poole, England.

3. Results

Freshly prepared mitochondria of *P. ganapatii* showed only low activity of ATPase from pH 7–10·5 with a maximum of 39 ± 3 nmol at pH 8 (figure 1). Various concentrations of $MgCl_2$ at different pH exhibited an activation of ATPase activity but it was found to be concentration and pH dependent (figure 1). The

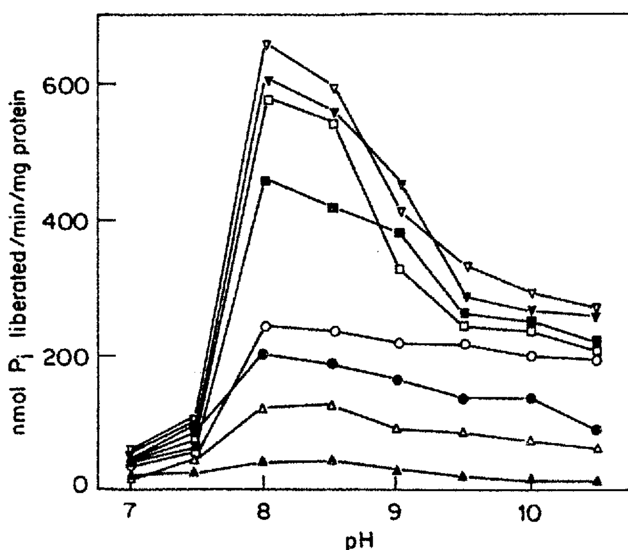

Figure 1. Effects of pH and $MgCl_2$ on the mitochondrial ATPase of *P. ganapatii*. Activities were measured in the reaction mixture described under materials and methods but varying the pH of the buffer and by adding different concentrations of $MgCl_2$.
(▲), No $MgCl_2$; (●), 1 mM $MgCl_2$; (○), 2 mM $MgCl_2$; (■), 3 mM $MgCl_2$; (□), 4 mM $MgCl_2$; (▼), 5 mM $MgCl_2$; (▽), 10 mM $MgCl_2$; (△), 100 mM $MgCl_2$.

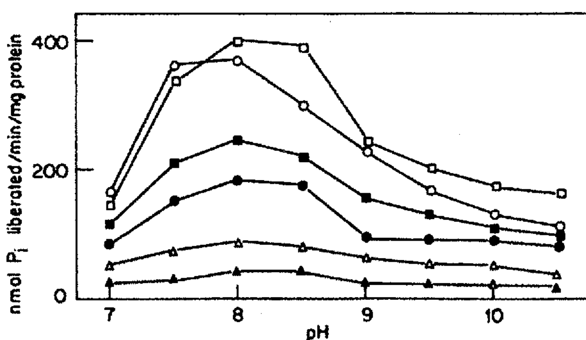

Figure 2. Effects of pH and $MgCl_2$ on the mitochondrial ATPase of *P. ganapatii*. Activities were measured in the reaction mixture described under materials and methods but varying the pH of the buffer and by adding different concentrations of DNP.
(▲), No DNP; (△), 1 μM DNP; (●), 10 μM DNP; (○), 100 μM DNP; (□), 1 mM DNP; (■), 10 mM DNP.

maximum extent of activation was noticed at 10 mM $MgCl_2$ which showed an activity of 660 ± 12 nmol at pH 8. A high concentration of 100 mM $MgCl_2$, however, exhibited a reduction in ATPase activity, which was well below of that obtained for 1 mM $MgCl_2$. Nevertheless, the activity curves showed an optimum pH of 8 irrespective of $MgCl_2$ concentrations.

The data in figure 2 represent the effect of the uncoupler DNP. Different concentrations of DNP were also effective in activating the enzyme but dependent upon

concentration of DNP and pH similar to that of $MgCl_2$. One mM DNP was found to exert maximum activation of ATPase with an activity of 399 ± 7 nmol at pH 8. However, as has been noticed for higher concentrations of $MgCl_2$, a higher concentration of 10 mM DNP showed a diminished ATPase activity.

Different concentrations of $MgCl_2$ and DNP in combinations were also tried at pH 8 of the reaction mixture to find out the optimal $MgCl_2$ and DNP concentrations required to elicit the maximal ATPase activity. The results (figure 3) indicated that the activity was enhanced by lower concentrations of $MgCl_2$ and DNP but diminished at higher concentrations. A maximum ATPase activity of 860 ± 14 nmol was obtained when 5 mM $MgCl_2$ and 100 μM DNP were present in the reaction mixture.

The effects of different inhibitors on the ATPase activity of *P. ganapatii* are shown in table 1. The energy transfer inhibitor oligomycin at concentrations of 1, 5 and 10 μg in the reaction mixture brought out significant but not effective inhibition of Mg^{2+} as well as DNP activated ATPase. However, the effect of oligomycin on the ATPase induced by both Mg^{2+} and DNP was found to be very meagre even at the highest concentration of 10 μg oligomycin. Similarly, the inhibitors of the respiratory chain viz. rotenone (site I), antimycin A (site II), sodium azide (site III) and potassium cyanide (site III) at different concentrations were also found to exert a weak inhibitory effect on the ATPase induced by either Mg^{2+} or DNP or by both. However, the inhibition obtained at 1×10^{-5} M of rotenone and 1×10^{-3} M of cyanide was observed to be substantial when compared to other inhibitors of the respiratory chain. Besides these, the inhibitor *p*-CMB, which binds with the $-SH$ group of the enzyme, had also exhibited a low inhibitory effect on the enzyme induced by Mg^{2+} as well as DNP.

Table 2 shows the effects of anions on the ATPase activity of *P. ganapatii*. With an exception of succinate, which showed a small but significant activation (17%) of the DNP activated ATPase, all other anions had either no effect or inhibitory effects. The ATPase induced by Mg^{2+} ions was strongly inhibited by 2-oxoglutarate. But the effect was seemed to be less on the DNP activated and Mg^{2+} + DNP acti-

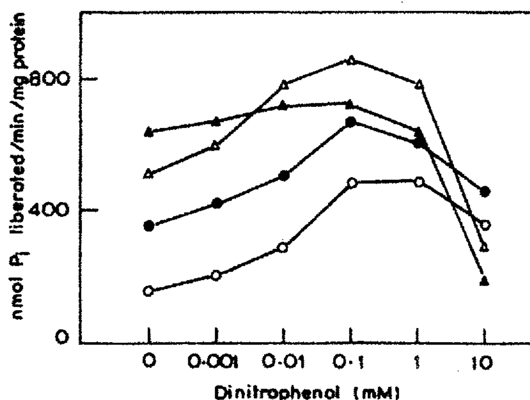

Figure 3. Effects of DNP on the $MgCl_2$ activated mitochondrial ATPase of *P. ganapatii*. Activities were measured in the reaction mixture described under materials and methods. The pH of the assay mixture was 8.
(○), 1 mM $MgCl_2$; (●), 2·5 mM $MgCl_2$; (△), 5 mM $MgCl_2$; (▲), 10 mM $MgCl_2$.

Table 1. Effect of inhibitors on Mg^{2+} and DNP activated mitochondrial ATPase of *P. ganapatii*.

	$MgCl_2$ (10 mM)		DNP (1 mM)		$MgCl_2$ + DNP (5 mM) (100 μM)	
Inhibitors[a]	ATPase[b] activity	Inhibition (%)	ATPase[b] activity	Inhibition (%)	ATPase[b] activity	Inhibition (%)
Control	660 ± 12	0	399 ± 07	0	860 ± 14	0
Oligomycin[c]						
1 μg	577 ± 15	13	297 ± 21	26	867 ± 26[d]	0
5 μg	458 ± 14	31	244 ± 17	39	825 ± 12[d]	4
10 μg	389 ± 26	41	186 ± 39	53	725 ± 19	16
Rotenone[c]						
1×10^{-7} M	608 ± 13	8	362 ± 09	9	854 ± 16[d]	0
1×10^{-6} M	556 ± 26	16	274 ± 23	31	862 ± 20[d]	0
1×10^{-5} M	465 ± 19	30	215 ± 25	46	636 ± 13	26
Antimycin A[c]						
1×10^{-6} M	587 ± 13	11	397 ± 06[d]	0	856 ± 16[d]	0
1×10^{-5} M	503 ± 27	24	377 ± 11	6	846 ± 19[d]	2
1×10^{-4} M	457 ± 17	31	332 ± 21	17	826 ± 22[d]	4
Azide						
1×10^{-4} M	548 ± 27	17	396 ± 12[d]	0	869 ± 18[d]	0
1×10^{-3} M	399 ± 25	40	379 ± 11	5	800 ± 11	17
Cyanide						
1×10^{-4} M	658 ± 12[d]	0	370 ± 10	7	856 ± 09[d]	0
1×10^{-3} M	365 ± 15	45	190 ± 26	52	578 ± 21	33
p-CMB						
1×10^{-4} M	590 ± 09	11	374 ± 16	6	856 ± 18[d]	0
1×10^{-3} M	523 ± 25	21	318 ± 21	20	847 ± 19[d]	2

[a]Inhibitors were added in the reaction mixture at concentrations given above and incubated for 15 min.
[b]The ATPase activity was assayed in the reaction mixture described under materials and methods. The activities are expressed as nmol P_i liberated/min/mg protein. Values are given as Mean ± SD for 5 determinations.
[c]Oligomycin, rotenone and antimycin A were dissolved in ethanol and added in 10 to 50 μl to achieve the desired concentrations. Control tubes received appropriate amount of ethanol alone.
[d]Values are not significantly different from control at 5% level in Student-Newman-Keuls test (Sokal and Rohlf 1969).

vated ATPase. The other anions fumarate and oxalate also showed some inhibitory effect on the ATPase activity of *P. ganapatii*.

Different divalent cations at 10 mM concentration were also used to study their effects. The results revealed that the effects were variable (table 3). When the reaction mixture contained Mg^{2+} ions, the effects of cations were mostly inhibitory falling in the order of $Zn^{2+} > Mn^{2+} > Cd^{2+} > Ni^{2+} > Ba^{2+}$. In contrast to this, when the reaction mixture contained DNP, the enzyme was found to be enhanced by many of the cations, which fall in the order of $Ba^{2+} = Mn^{2+} < Ca^{2+}$. However, there seemed to be no further activation of the enzyme if the reaction

Table 2. Effect of anions on Mg^{2+} and DNP activated mitochondrial ATPase of *P. ganapatii*.

	$MgCl_2$ (10 mM)		DNP (1 mM)		$MgCl_2$ + DNP (5 mM) (100 μM)	
Anions[a] (10 mM)	ATPase[b] activity	Activity (%)	ATPase[b] activity	Activity (%)	ATPase[b] activity	Activity (%)
Control	688 ± 14	100	402 ± 09	100	842 ± 08	100
L-Glutamate	658 ± 37[c]	95	401 ± 12[c]	100	830 ± 14[c]	99
Acetate	661 ± 24[c]	96	403 ± 14[c]	100	844 ± 13[c]	100
Oxalate	495 ± 25	72	274 ± 32	68	695 ± 19	83
Succinate	664 ± 09[c]	96	471 ± 40	117	839 ± 16[c]	100
L-Malate	598 ± 15	87	396 ± 34[c]	99	856 ± 22[c]	102
Fumarate	456 ± 21	66	238 ± 36	59	556 ± 11	66
Malonate	562 ± 26	82	382 ± 15	95	837 ± 14[c]	100
2-Oxoglutarate	048 ± 11	7	164 ± 16	40	415 ± 21	49

[a]Anions were added in the reaction mixture and incubated for 15 min.
[b]The ATPase activity was assayed in the reaction mixture described under materials and methods. The activities are expressed as nmol P_i liberated/min/mg protein. Values are given as Mean ± SD for 5 determinations.
[c]Values are not significantly different from control at 5% level in Student-Newman-Keuls test (Sokal and Rohlf 1969).

Table 3. Effect of cations on Mg^{2+} and DNP activated mitochondrial ATPase of *P. ganapatii*.

	$MgCl_2$ (10 mM)		DNP (1 mM)		$MgCl_2$ + DNP (5 mM) (100 μM)	
Cations[a] (10 mM)	ATPase[b] activity	Activity (%)	ATPase[b] activity	Activity (%)	ATPase[b] activity	Activity (%)
Control	645 ± 01	100	398 ± 11	100	851 ± 14	100
Mn^{2+}	396 ± 19	61	542 ± 31	136	731 ± 12	86
Ca^{2+}	647 ± 15[c]	100	707 ± 33	177	766 ± 16	90
Ba^{2+}	498 ± 45	77	540 ± 27	135	842 ± 21[c]	99
Cd^{2+}	429 ± 26	67	401 ± 09[c]	100	664 ± 19	78
Ni^{2+}	481 ± 15	75	403 ± 10[c]	101	846 ± 13[c]	100
Zn^{2+}	292 ± 30	45	342 ± 29	86	544 ± 24	64

[a]Cations were added in the reaction mixture and incubated for 15 min.
[b]The ATPase activity was assayed in the reaction mixture described under materials and methods. The activities are expressed as nmol P_i liberated/min/mg protein. Values are given as Mean ± SD for 5 determinations.
[c]Values are not significantly different from control at 5% level in Student-Newman-Keuls test (Sokal and Rohlf 1969).

mixture contained both Mg^{2+} and DNP, although there was some inhibitory effect by Zn^{2+} and Cd^{2+} ions.

4. Discussion

The data presented show that mitochondrial ATPase of *P. ganapatii* exists in a masked state and is unmasked by the addition of Mg^{2+} or DNP in the reaction

mixture. This property of the enzyme is in line with the observations made in a variety of mammalian and bacterial sources (Panet and Sanadi 1976). Possibly the masked state of the enzyme is due to the presence of an endogenous trypsin sensitive protein inhibitor in the ATPase complex as has been noticed for the beef heart mitochondria (Pullman and Monroy 1963).

In the present study, the Mg^{2+} activated ATPase is stimulated further by DNP, atleast in the lower concentrations (figure 3). Similar results have also been reported for the ATPase of housefly mitochondria (Chefurka 1981c) and turtle heart mitochondria (Rotermund and Previtera 1970). Probably this may be due to the different mechanisms of activation of the enzyme by these chemicals. It has been reported that Mg^{2+} acts as a chelator in the enzyme substrate interaction to enhance the activity (Lardy and Wellman 1953; Myers and Slater 1957a; Selwyn 1968), whereas the DNP is reported to activate the enzyme by uncoupling the coupled mitochondria (Lardy and Elvehjem 1945; Cooper and Lehninger 1957; Hall and Palmer 1969).

It is rather surprising to note that the mitochondria of *P. ganapatii* show relatively higher activity in the presence of Mg^{2+} than DNP. In contrast, DNP is found to be more effective in activating the enzyme of mammalian mitochondria (Myers and Slater 1957a). However, this does not seem to be peculiar to this parasite alone. Earlier studies on helminth parasites have also reported such discrepancies with regard to Mg^{2+} as well as DNP activation of the enzyme. While the mitochondrial ATPase of adult *A. lumbricoides* shows activation only by Mg^{2+} and not by DNP (Hayashi 1973), the ATPase of adult *Taenia taeniaeformis* shows no activation either by Mg^{2+} or by DNP, although activation of the enzyme by these chemicals could be noticed from the mitochondria of the larval stage (Weinbach and Von Brand 1970). It is difficult to speculate the possible reason for this discrepancy, as there are no apparent differences in the nature of the assay mixture and mitochondrial isolation medium used in these studies. However, this may indicate the differences in the permeability of the mitochondrial membrane of these helminths.

The mitochondria of *P. ganapatii* also show an unique property of having only one pH optimum of 8 for both Mg^{2+} and DNP activated ATPase. More than one optimum pH in alkaline range has been reported for the rat liver mitochondrial ATPase (Myers and Slater 1957a) and there have been distinct pH optima for Mg^{2+} (8·5) and DNP (7·4) activated ATPase of the heart mitochondria of the turtle *Chrysemys picta* (Rotermund and Previtera 1970). The ATPase of *P. ganapatii* is also different from the housefly which exhibits an optimum pH of 6·5 in the acidic range (Chefurka 1981b).

The energy transfer inhibitor oligomycin was first introduced by Lardy *et al* (1958) as an inhibitor of oxidative phosphorylation. Subsequent experiments by Weiner and Lardy (1974) have shown that 1 μM of oligomycin is sufficient to bring out the complete inhibition of ATPase activity. In the present study, it has been noticed that the inhibition is very weak even at a higher concentration of 10 μM oligomycin, suggesting that only a portion of the ATPase of *P. ganapatii* is sensitive towards oligomycin.

The present investigation also shows that the respiratory inhibitors are not effective in inhibiting the ATPase of *P. ganapatii*. Rotenone, antimycin A, azide and cyanide at concentrations that are highly inhibitory to the respiratory chain have produced only a weak or moderate inhibitory effect on the ATPase of *P. ganapatii*. Weiner and Lardy (1974) and Chefurka (1981a) have noticed that inhibition of the

ATPase by respiratory inhibitors is dependent upon the metabolic state of the mitochondria and the nature of the uncoupler used. In view of this, it may be assumed that, under the conditions of our experiments, the alteration of the redox state of the mitochondria by respiratory inhibitors has little effect on the ATPase of *P. ganapatii*. Further, *p*-CMB, the well known thiol group inhibitor, was also found to be a weak inhibitor of the ATPase of *P. ganapatii*. This indicates that −SH group may not play a major role in the hydrolysis of ATP. This observation is in contrast to liver mitochondria, where *p*-CMB is reported to have strong inhibitory effect on ATPase (Myers and Slater 1957b).

The ATPase of *P. ganapatii* induced by Mg^{2+} or DNP or by both is inhibited by some of the anions. Inhibition by anions has also been noticed in DNP activated ATPase of rat liver mitochondria (Veldsema-currie and Slater 1968). The inhibition may be because of the competition by these anions with Mg^{2+} or DNP for penetration into the mitochondria. However, the interesting observation with anions is the activation of DNP-activated ATPase by succinate. Although the reason for this activation is not known at present, this property of *P. ganapatii* mitochondrial ATPase is similar to that reported for fly muscle mitochondrial ATPase (Chefurka 1981c).

It is interesting to note that Mg^{2+} activated and DNP activated ATPase vary in their response towards the cations. While the former is inhibited by most of the cations the latter showed activation by some of the cations. However, only inhibitory effect by some of the cations was noticed when both Mg^{2+} and DNP were present in the reaction mixture. The activation of DNP-activated ATPase by metal ions may be attributed to the chelating effect of these ions in the enzyme substrate interaction similar to that of Mg^{2+} ions (loc. cit). However, the inhibitory effect of the divalent cations on the Mg^{2+} stimulated ATPase could be due to the binding of the cations with the enzyme surface (Ulrich 1964).

It would appear, from the foregoing account, that the properties of mitochondrial ATPase of *P. ganapatii* differ not only from mammalian and insect mitochondria but also from parasites, though it resembles them to certain extent.

Acknowledgement

The financial assistance by the Council of Scientific and Industrial Research, New Delhi is gratefully acknowledged.

References

Amons R, Van den Bergh S G and Slater E C 1968 The effects of monovalent cations on the dinitrophenol-induced ATPase of rat liver mitochondria; *Biochim. Biophys. Acta* **162** 452–454

Beechey R B, Roberton A M, Holloway C T and Knight I G 1967 The properties of dicyclohexylcarbodiimide as an inhibitor of oxidative phosphorylation; *Biochemistry* **6** 3867–3879

Bruni A, Luciani S and Bortignon C 1965 Competitive reversal by adenine nucleotides of atractyloside effect on mitochondrial energy transfer; *Biochim. Biophys. Acta* **97** 434–441

Cereijo-Santalo R 1972 The effects of electrolytes on the 2,4-dinitrophenol-activated ATPase of rat liver mitochondria; *Arch. Biochem. Biophys.* **148** 22–26

Chefurka W 1981a Effects of inhibitors of the respiratory chain and energy-transfer system on mitochondrial ATPase activity of houseflies; *Comp. Biochem. Physiol.* **B69** 361–369

Chefurka W 1981b The uncoupler-stimulated adenosine triphosphatase activity in housefly flight-muscle mitochondria; *Comp. Biochem. Physiol.* **B69** 377–385

Chefurka W 1981c ATPase inhibitor and apparent deficiency of uncoupler-stimulated ATPase activity in mitochondria of houseflies (*Musca domestica* L.); *Comp. Biochem. Physiol.* **B69** 371 376

Cooper C and Lehninger A L 1957 Oxidative phosphorylation by an enzyme complex from extracts of mitochondria; *J. Biol. Chem.* **224** 561 578

Fiske C H and Subbarow Y 1925 The colorimetric determination of phosphorous; *J. Biol. Chem.* **66** 375–400

Hall D O and Palmer J M 1969 Mitochondrial research today; *Nature (London)* **22** 717 723

Hayashi H 1973 ATP formation by mitochondria from *Ascaris lumbricoides* var. suis; *Jpn. J. Parasitol.* **22** 97-107

Lardy H A and Elvehjem C A 1945 Biological oxidations and reductions; *Annu. Rev. Biochem.* **14** 1 30

Lardy H A and Wellman H J 1953 The catalytic effect of 2,4-dinitrophenol on adenosine triphosphate hydrolysis by cell particles and soluble enzymes; *J. Biol. Chem.* **201** 357–370

Lardy H A, Johnson D and McMurray W C 1958 Antibiotics as tools for metabolic studies. I. A survey of toxic antibiotics in respiratory, phosphorylative and glycolytic systems; *Arch. Biochem. Biophys.* **78** 587 597

Lardy H A and Lin H C 1969 Inhibition of mitochondrial oxidative phosphorylation by aurovertin; in *Inhibitors as tools in cell research* (eds) T Bucher and H Sies (Berlin: Springer Verlag) pp 279 281

Lowry O H, Rosebrough N J, Farr A L and Randall R J 1951 Protein measurement with the Folin Phenol reagent; *J. Biol. Chem.* **193** 265 275

Myers D K and Slater E C 1957a The enzymic hydrolysis of adenosine triphosphate by liver mitochondria. I. Activities at different pH values; *Biochem. J.* **67** 558 572

Myers D K and Slater E C 1957b The enzymic hydrolysis of adenosine triphosphate by liver mitochondria. II. Effect of inhibitors and added cofactors; *Biochem. J.* **67** 572–579

Panet R and Sanadi D R 1976 Soluble and membrane ATPases of mitochondria, chloroplasts and bacteria: Molecular structure, enzymatic properties and functions; *Curr. Top. Membr. Transp.* **8** 99–150

Penefsky H S, Pullman M E, Datta A and Racker E 1960 Partial resolution of the enzymes catalyzing oxidative phosphorylation. Participation of a soluble adenosine triphosphatase in oxidative phosphorylation; *J. Biol. Chem.* **235** 3330–3336

Pullman M E and Monroy G C 1963 A naturally occurring inhibitor of mitochondrial adenosine triphosphatase; *J. Biol. Chem.* **238** 3762–3769

Pullman M E, Penefsky H S, Datta A and Racker E 1960 Partial resolution of the enzymes catalyzing oxidative phosphorylation. Purification and properties of soluble dinitrophenol-stimulated adenosine triphosphatase; *J. Biol. Chem.* **235** 3322-3329

Racker E 1970 *Membranes of mitochondria and chloroplasts* (Princeton: Van Nostrand)

Rotermund A J and Privitera C A 1970 Adenosine triphosphatase activity of heart mitochondria of the turtle, *Chrsemys picta*; *Comp. Biochem. Physiol.* **37** 1–22

Selwyn M J 1968 Model reaction for mitochondrial adenosine triphosphatase; *Nature (London)* **219** 490–493

Sokal R R and Rohlf F G 1969 *Biometry—The principles and practice of statistics in biological research* (San Francisco: W H Freeman and Company)

Tzagaloff A 1976 The adenosine triphosphatase complex of mitochondria; in *The enzymes of biological membranes* (ed.) A S Martinosi (New York: Plenum Press) Vol 4 pp 103–124

Ulrich F 1964 Kinetic studies of the activation of mitochondrial adenosine triphosphatase by Mg^{++}; *J. Biol. Chem.* **239** 3532–3536

Van den Bossche H 1972 Studies on the phosphorylation in *Ascaris* mitochondria; in *Comparative biochemistry of parasites* (ed.) H Van den Bossche (New York: Academic Press) pp 455–468

Van den Bossche H 1974 Adenosine triphosphatase activity in *Ascaris* muscle mitochondria; *Comp. Biochem. Physiol.* **B49** 71–78

Veldsema-currie R D and Slater E C 1968 Inhibition by anions of dinitrophenol-induced ATPase of mitochondria; *Biochim. Biophys. Acta* **162** 310–319

Walker R W and Barrett J 1983 Mitochondrial adenosine triphosphatase activity and temperature adaptation in *Schistocephalus solidus* (Cestoda: Pseudophyllidea); *Parasitology* **87** 307–326

Weinbach E C and Von Brand T 1970 The biochemistry of Cestode mitochondria. I. Aerobic metabolism of mitochondria from *Taenia taeniaeformis*; *Int. J. Biochem.* **1** 39–56

Weiner M W and Lardy H A 1974 Effects of respiratory chain inhibitors on mitochondrial ATPase activity; *Arch. Biochem. Biophys.* **162** 568–572