Biochemistry of Dystrophic Muscle

MITOCHONDRIAL SUCCINATE–TETRAZOLIUM REDUCTASE AND ADENOSINE TRIPHOSPHATASE

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Little is known about biochemical abnormalities which accompany or are responsible for the morphological changes which occur in the primary myopathies. A number of such diseases have been characterized in humans (Walton & Nattrass, 1954). The discovery (Michelson, Russell & Harman, 1955) of a muscular dystrophy in a strain of mice, inherited by an autosomal recessive gene, has facilitated the search for biochemical alterations in myopathy. Although this condition may not be identical with any of the types of human muscular dystrophy, its investigation may help to throw light on the biochemistry of human muscular dystrophies. It seems probable that many, at least, of the secondary biochemical changes may be common to various types of muscle disease.

A few workers (e.g. Weinstock, Epstein & Milhorat, 1958; Hazzard & Leonard, 1959; White, 1959) have reported altered concentrations of certain muscle enzymes in the mouse myopathy. In view of reported morphological abnormalities in mitochondria of dystrophic mouse muscle (Dr G. W. Pearce, unpublished work; Ross, Pappas & Harman, 1960), the activity of two mitochondrial enzyme systems, succinate dehydrogenase (measured by reduction of a tetrazolium salt) and adenosine triphosphatase, was measured in dystrophic and normal mouse muscle. Observations are also recorded on the measurement and properties of these enzyme systems in normal muscle and on methods of preparing mitochondria from skeletal muscle.

MATERIALS

Abbreviation. INT, 2-(*p*-Iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride.

Mice. These were of the inbred strain 129, bred from animals purchased from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, U.S.A., and used when 2–3 months old. The 'normal' animals include an unknown number of mice heterozygous with respect to the recessive gene responsible for the myopathy.

Chemicals. ATP was obtained as the cryst. disodium salt from C. F. Boehringer und Soehne GmbH., Mannheim, Germany; it was neutralized with KOH before use. The cryst. barium silver salt of phosphoenolpyruvic acid, from the same source, was converted into the free acid with HCl, passed through a column of Amberlite IR-120 (H^+ form) and neutralized with KOH. Cryst. pyruvate kinase (ATP–phosphopyruvate transphosphorylase) was obtained also from Boehringer as a suspension in 2·1 M-$(NH_4)_2SO_4$. It was diluted with 4 vol. of water; assay by the method of Bücher & Pfleiderer (1955) showed that the diluted preparation was stable for several weeks, at least, when stored at −20°.

Phenazine methosulphate (*N*-methylphenazonium methosulphate), neotetrazolium [2:2′-(*p*-diphenylene)-bis-(3:5-diphenyl)tetrazolium chloride] and INT were purchased from L. Light and Co. Ltd. The corresponding formazan was prepared from INT by adding a solution of ascorbic acid to a solution of INT in 5% (w/v) Na_2CO_3 and washing the precipitate thoroughly with water; it was dried *in vacuo* over P_2O_5. 2:4-Dinitrophenol was recrystallized twice from water.

METHODS

Preparation of muscle mitochondria. Mice were killed by dislocation of the neck and bled from the throat. The hind legs were removed as quickly as possible into a cold (0–2°) solution of sucrose–EDTA (sodium salt) (0·25 M-sucrose containing mM-EDTA); the preparation was subsequently kept cold. Visible fat and nerve were removed and the muscle (normally 1–2 g. from a single mouse) was chopped finely with very sharp scissors. It was homogenized in about 5 vol. of sucrose–EDTA in a Potter–Elvehjem homogenizer with Perspex pestle and glass tube (clearance, 0·1 mm. approx.). The tube was enclosed in an ice–water jacket and the pestle rotated for 6 min. at a fairly slow speed. A further 20 vol. of medium was added and, after thorough mixing, the preparation was centrifuged for 5 min. at 600*g*. The sediment was mixed thoroughly with 20 vol. of medium (by a hand-operated homogenizer) and centrifuged as before. The combined supernatants were centrifuged (600*g*; 5 min.), and the supernatant was poured through glass wool and centrifuged for 15 min. (total time) at 13 400 rev./min. in the Spinco model L ultracentrifuge (rotor no. 40; 12 000*g*). The sedimented particles were resuspended in about 5 ml. of medium by a small homogenizer pestle that fitted loosely into the centrifuge tube, recentrifuged (12 000*g*; 15 min.) and resuspended in the same medium.

Measurements of succinate–INT-reductase activity (see below) on both homogenates and mitochondria indicated a recovery of about one-third of the mitochondria of the homogenate (assuming that the succinate–INT-reductase activity of the homogenate is confined to the mitochondria). The mitochondria recovered accounted for 2–3% of the total N of the homogenate.

The preparations were examined, as a routine, by phase-contrast microscopy to verify the absence of myofibrils. The quantity of mitochondria added to the tubes in the experiments represented 3–10 μg. of N.

Preparation of liver mitochondria. These were prepared from mouse liver by the usual techniques (Hogeboom, 1955); the homogenizing medium was sucrose–EDTA.

Measurement of adenosine triphosphatase activity. Mitochondria were shaken gently in conical centrifuge tubes at 30° for 20 min. (unless stated otherwise) in a medium containing (final concn.) tris–HCl (50 mM; pH 7·3 at 30°), KCl (75 mM), $MgCl_2$ (5 mM), EDTA (mM), ATP (4 mM), sucrose (50 mM). Phosphoenolpyruvate (8 mM) and pyruvate kinase [1 unit; a unit is defined as the amount which converts 1 μmole of substrate/min. when assayed by the method of Bücher & Pfleiderer (1955)] were added in some experiments, as indicated; other modifications of the medium are described in the text and Tables. The total volume was 0·25 ml.; all tubes were duplicated. After the incubation trichloroacetic acid (2·5%; 2·25 ml.) was added and inorganic phosphate was determined by the method of Fiske & Subbarow (1925); the modification described by LePage (1949) was used, scaled down to half quantities. Where atabrine or chlorpromazine was present in the incubation mixture, the mixture, after addition of trichloroacetic acid, was extracted twice with an equal volume of $CHCl_3$ to remove these compounds, which interfered with the determination of phosphate.

Similar tubes, which were not incubated, were set up as blanks.

Measurement of succinate-2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium-reductase activity. Mitochondria were shaken gently for 15 min. at 37° in the following medium (final vol. 1·0 ml.) in duplicate 12 ml. glass-stoppered tubes: potassium phosphate buffer (pH 7·4), 50 mM; INT, 0·1%; sodium succinate, 50 mM; sucrose, 25 mM. Trichloroacetic acid (1 ml. 10%) was added and the formazan was extracted with 4 ml. of ethyl acetate and its extinction measured at 490 mμ. When phenazine methosulphate was present in the incubation mixture the ethyl acetate was washed with an equal volume of water before reading.

Measurement of succinic-oxidase activity. This was measured at 37° by standard Warburg techniques with the use of small (approx. 7 ml.) flasks and trimethylpentane (sp.gr. 0·692) as the manometer fluid to increase the sensitivity. The medium (final vol. 1·0 ml.) contained: potassium phosphate buffer (pH 7·4), 50 mM; sodium succinate, 50 mM; cytochrome *c*, 20 μM; ATP, mM; $MgSO_4$, 2 mM; sucrose, 50 mM.

Determination of nitrogen. Total N of the tissue preparations was measured with a micro-Kjeldahl method (Johnson, 1941).

RESULTS

Homogenizing of muscle and preparation of mitochondria. Muscle was finely chopped with scissors and homogenized with a Potter–Elvehjem homogenizer in a number of different media and the homogenates were examined by phase-contrast microscopy. Homogenizing in 0·25 M-sucrose led to a product with very different appearance from that obtained when the medium devised by Chappell & Perry (1954), for the preparation of mitochondria from pigeon-breast muscle, was used. This medium contains: tris (pH 7·4), 0·05 M; KCl, 0·1 M; $MgSO_4$, 5 mM; ATP, mM; EDTA, mM. The sucrose medium appeared to facilitate separation of the myofibrils without damaging them extensively; unless the homogenizing was very severe the striations were all retained. The other medium, although giving a rather larger yield of mitochondria, produced myofibrils that were more poorly separated, frequently misshapen and showed no striations. This difference was consistent and reproducible over a wide range of homogenizing speeds and clearances. When separated by centrifuging, the mitochondrial fraction of the homogenate in the tris medium contained many more irregular masses, probably remnants of broken myofibrils. In consequence, the succinate–INT-reductase activity of this mitochondrial fraction was about one-quarter less than that of the mitochondrial fraction obtained with the sucrose medium (expressed on the basis of equal N content of the fractions).

The difference between the two media could not be attributed to any single constituent of the tris medium. Inclusion of ATP (mM) and $MgSO_4$ (5 mM) in the sucrose produced a homogenate similar to that obtained with the tris medium. Sucrose with either ATP or $MgSO_4$ produced an intermediate appearance, as did the tris medium with the omission of both ATP and $MgSO_4$. Addition of EDTA (mM) to sucrose made no difference to the appearance of the homogenate. In view of its reported beneficial effect upon heart-muscle mitochondria (Slater & Cleland, 1952), EDTA was included in the medium in all subsequent experiments.

Adenosine triphosphatase

Conditions of measurement. Racker & Gatt (1959) demonstrated that the rate of formation of inorganic phosphate from ATP by liver mitochondria was increased by the inclusion in the medium of a system for rephosphorylating ATP. This was due to inhibiton of adenosine triphosphatase by adenosine diphosphate, the product of the reaction. Table 1 shows that similar results were obtained with mouse-skeletal-muscle mitochondria; the activity of the enzyme was increased approximately threefold by the addition of phosphoenolpyruvate and pyruvic kinase. The pyruvic kinase had no adenosine triphosphatase activity when tested under the same conditions. No inorganic phosphate was produced from phosphoenolpyruvate by mitochondria under the conditions of the assay.

Other experiments showed that there was no increase in the rate of splitting of ATP when its concentration was increased to 8 mM (in the presence of phosphoenolpyruvate and pyruvic kinase); a concentration of only 2 mM produced 29% less activity.

Effect of magnesium and dinitrophenol. Adenosine triphosphatase activity was greatly diminished when Mg^{2+} ions were omitted from the medium (Table 2). An effect of Mg^{2+} ions could be observed in the absence of the ATP-regenerating system; hence the known requirement of pyruvic kinase for Mg^{2+} ions could, at most, only accentuate the effect of Mg^{2+} ions in the complete system.

Table 1. *Effect of added adenosine diphosphate and of an adenosine triphosphate-regenerating system upon the adenosine triphosphatase of muscle mitochondria*

Mitochondria were incubated in a medium containing tris (pH 7·3) (50 mM), KCl (75 mM), $MgCl_2$ (5 mM), EDTA (mM), ATP (4 mM) and sucrose (50 mM). Other additions are indicated in the table. PEP, Phosphoenolpyruvate.

Expt. no.	Addition to medium	Inorganic phosphate formed (μg. of P)
1	—	18·5
	PEP (8 mM) + pyruvic kinase (1 unit)	55·5
2	—	7·3
	PEP (8 mM) + pyruvic kinase (1 unit)	20·0
	Adenosine diphosphate (8 mM)	1·8
3	PEP (8 mM) + pyruvic kinase (1 unit)	36·6
	PEP (8 mM) + pyruvic kinase (3 units)	33·2

Table 2. *Effect of* Mg^{2+} *ions and* 2:4*-dinitrophenol upon adenosine triphosphatase*

Mitochondria were incubated in a medium containing tris (pH 7·3) (50 mM), KCl (75 mM), EDTA (mM), ATP (4 mM), sucrose (50 mM). Phosphoenolpyruvate (8 mM) and pyruvic kinase (1 unit) were included except where indicated. Other additions are indicated in the table.

Expt. no.	Addition to medium	Inorganic phosphate formed (μg. of P)
1	—	6·2
	$MgCl_2$ (5 mM)	38·9
	—*	5·4
	$MgCl_2$ (5 mM)*	17·4
2	—	5·3
	$MgCl_2$ (5 mM)	26·5
	$MgCl_2$ (5 mM) + dinitrophenol (0·02 mM)	31·6
	$MgCl_2$ (5 mM) + dinitrophenol (0·1 mM)	38·2
	$MgCl_2$ (5 mM) + dinitrophenol (0·5 mM)	37·8
	Dinitrophenol (0·5 mM)	8·2
	—*	5·2
	$MgCl_2$ (5 mM)*	12·1
	Dinitrophenol (0·5 mM)*	7·1
	$MgCl_2$ (5 mM) + dinitrophenol (0·5 mM)*	16·0

* Phosphoenolpyruvate and pyruvic kinase omitted.

2:4-Dinitrophenol consistently stimulated the adenosine triphosphatase activity in the presence of Mg^{2+} ions. Table 2 shows that maximum stimulation occurred at a concentration of about 0·1 mM. Other experiments showed that the relative stimulation was not influenced by the duration of incubation (5–20 min.). The magnitude of the stimulation varied considerably between experiments but never exceeded 50%. There were no consistent differences in the degree of stimulation between mitochondria prepared in 0·25 M-sucrose, 0·25 M-sucrose–mM-EDTA, or the tris medium mentioned above. Variations in the severity of homogenizing had no reproducible effect, either.

The low adenosine triphosphatase activity obtained in the absence of Mg^{2+} ions was stimulated by dinitrophenol in three out of four experiments. The stimulated rate was, however, much less than that observed in the presence of Mg^{2+} ions; this was so even in the absence of phosphoenolpyruvate and pyruvic kinase, and hence was not due merely to the requirement of pyruvic kinase for Mg^{2+} ions. Typical results are presented in Table 2.

Effect of cations. Table 3 shows the adenosine triphosphatase activity of skeletal-muscle mitochondria in the presence of various metal ions. For these experiments the mitochondria were prepared and washed in sucrose–EDTA as usual, but were finally suspended in sucrose alone to avoid the presence of EDTA in the incubation medium. The activity of Co^{2+} and Mn^{2+} ions is comparable with that of Mg^{2+} ions; Ca^{2+} and Fe^{2+} ions are less active.

Effect of inhibitors. The effects of NaF, chlorpromazine [2-chloro-10-(3-dimethylaminopropyl)-phenothiazine] and atabrine [3-chloro-7-methoxy-9-(1-methyl-4 diethylaminobutylamino)acridine] are recorded in Table 4. Each of these compounds has been reported to inhibit the adenosine triphosphatase of liver mitochondria (Löw, 1959) and chlorpromazine inhibits also brain adenosine triphosphatase (Bernsohn, Namajuska & Boshes, 1956). Chlorpromazine is evidently a potent ininhibitor of the muscle adenosine triphosphatase; atabrine is less effective, and NaF inhibits only weakly. The inhibition by chlorpromazine or atabrine was relatively almost the same in the presence or the absence of dinitrophenol.

Other properties. Washing the mitochondria a second time with sucrose–EDTA did not alter their adenosine triphosphatase activity. The activity (in the presence of Mg^{2+} ions) showed a small (8%) increase when the mitochondria, suspended in sucrose–EDTA, were kept at 37° for 1 hr.

It has been shown by several workers (e.g. Lardy & Wellman, 1953) that the adenosine triphosphatase activity of carefully prepared liver mitochondria is stimulated several-fold by 2:4-dinitrophenol. The possibility was considered that

the smallness of the stimulation obtained in the present experiments with muscle mitochondria was due to the action upon the mitochondria of some factor in the supernatant fraction of the muscle homogenate. To test this a suspension of mouse-liver mitochondria was divided into two equal portions, one of which was centrifuged. The other portion of the suspension was added to 10 vol. of a muscle supernatant fraction, stood in ice for 1 hr., then centrifuged. The muscle supernatant was obtained by centrifuging a mouse-muscle homogenate for 1 hr. at 70 000*g*. In addition, the adenosine triphosphatase activity of some of the untreated mitochondria was measured in the presence of boiled supernatant fraction of muscle (0·07 ml.). The effect of dinitrophenol (four- to five-fold stimulation of adenosine triphosphatase) was not significantly influenced by the treatment of the mitochondria with the muscle supernatant or by the inclusion of the boiled supernatant in the medium. Treatment with the muscle supernatant caused a small increase in adenosine triphosphatase activity of the liver mitochondria; this may have been due to adsorption of soluble adenosine triphosphatase, since the muscle supernatant had adenosine triphosphatase activity (0·32 μg. of phosphate P liberated/μg. of N, measured under the usual conditions, and not influenced by dinitrophenol).

Table 3. *Effect of cations upon adenosine triphosphatase*

Mitochondria were incubated in a medium containing tris (pH 7·3) (50 mM), KCl (75 mM), ATP (4 mM) and sucrose (50 mM). Metallic salts were added as indicated.

Expt. no.	Addition to medium	Inorganic phosphate formed (μg. of P)
1	—	6·4
	$MgCl_2$ (mM)	38·3
	$MgCl_2$ (5 mM)	39·0
	$MgCl_2$ (10 mM)	33·0
	$CaCl_2$ (mM)	12·4
	$CaCl_2$ (5 mM)	16·3
	$CaCl_2$ (10 mM)	17·3
	$CoCl_2$ (mM)	36·0
	$CoCl_2$ (5 mM)	33·9
	$CoCl_2$ (10 mM)	21·0
2	—	1·6
	$MgCl_2$ (mM)	21·5
	$FeCl_3$ (mM)	5·5
	$FeCl_3$ (5 mM)	5·0
	$FeCl_3$ (10 mM)	4·3
	$MnCl_2$ (mM)	19·9
	$MnCl_2$ (5 mM)	25·5
	$MnCl_2$ (10 mM)	17·3

Table 4. *Inhibitors of adenosine triphosphatase*

Mitochondria were incubated in the medium of Table 1, with other additions as indicated. Atabrine and chlorpromazine were added as hydrochlorides.

Expt. no.	Addition to medium	Inorganic phosphate formed (μg. of P)
1	—	13·0
	NaF (mM)	13·3
	NaF (5 mM)	13·2
	NaF (25 mM)	8·2
	Atabrine (mM)	8·5
	Atabrine (5 mM)	3·2
	Atabrine (10 mM)	1·7
	Chlorpromazine (mM)	0·2
2	—	13·5
	Chlorpromazine (0·04 mM)	10·0
	Chlorpromazine (0·2 mM)	4·2
	Chlorpromazine (mM)	0·4
	2:4-Dinitrophenol (0·1 mM)	16·2
	Chlorpromazine (mM) + 2:4-dinitrophenol (0·1 mM)	0·7
	Atabrine (2 mM)	8·0
	Atabrine (2 mM) + 2:4-dinitrophenol (0·1 mM)	9·7

Dystrophic muscle. The adenosine triphosphatase activity of mitochondria (expressed on the basis of mitochondrial N) from muscle of four dystrophic mice is compared in Table 5 with that of normal mice in the absence or the presence of dinitrophenol. The mean unstimulated value for the dystrophic mice is slightly (15%) higher than the normal ($P < 0·02$). There is no significant difference in the degree of stimulation by dinitrophenol, although there was considerable variation.

Succinate–tetrazolium reductase

Shelton & Rice (1957) showed that the succinate dehydrogenase of liver homogenates reduced INT much more efficiently than neotetrazolium, although INT was less efficient than O_2 as an electron acceptor, in the presence of the complete succinate-oxidase system. In the present work with muscle mitochondria it was also found that INT was superior to neotetrazolium; on a molar basis approximately 20 times as much formazan was produced from INT under comparable conditions. Table 6 shows that, except with very small quantities of mitochondria, the formazan production was proportional to the amount of mitochondria present: moreover, it increased linearly with time. There was negligible INT reduction by the mitochondria when succinate was omitted from the medium.

In another experiment formazan production from INT was compared with the O_2 uptake of mitochondria from the same suspension. The quantity of formazan produced (during 10 min.) was 0·057 μmoles; this was calculated from a measured value of $20·1 \times 10^3$ for the molar extinction coefficient (490 mμ) of the formazan dissolved in ethyl acetate. The corresponding O_2 uptake (with

Table 5. *Mitochondrial adenosine triphosphatase from normal and dystrophic muscle*

Mitochondrial adenosine triphosphatase was measured as described in the Methods section; phosphoenolpyruvate and pyruvic kinase were included in the medium. The concentration of 2:4-dinitrophenol, where present, was 0·1 mM.

	Inorganic phosphate formed (μg. of P/μg. of mitochondrial N)			
	Normal		Dystrophic	
	Without dinitrophenol	With dinitrophenol	Without dinitrophenol	With dinitrophenol
	4·08	5·18	4·22	5·18
	3·62	4·16	4·46	5·07
	3·43	4·83	4·43	5·10
	4·07	5·33	4·32	5·00
Mean	3·80	4·88	4·36	5·09

Table 6. *Reduction of* 2-(p-*iodophenyl*)-3-(p-*nitrophenyl*)-5-*phenyltetrazolium by muscle mitochondria: variation with time and quantity of mitochondria*

Formazan production was measured as described in the Methods section, except that the time of incubation was varied as indicated.

Mitochondrial N present (μg.)	Time of incubation (min.)	*E*
1·38	15	0·058
2·76	15	0·164
5·52	15	0·342
11·04	15	0·696
22·08	15	1·378
22·08	10	0·921
22·08	5	0·450

Table 7. *Effect of phenazine methosulphate upon reduction of* 2-(p-*iodophenyl*)-3-(p-*nitrophenyl*)-5-*phenyltetrazolium*

Formazan production was measured as described in the Methods section, except that sodium succinate (50 mM) was added only where indicated. Other additions were as shown.

Addition to medium	*E*
Mitochondria added	
—	0·010
Succinate	0·630
Phenazine methosulphate (5 mM)	0·097
Succinate + phenazine methosulphate (5 mM)	1·26
Succinate + phenazine methosulphate (mM)	0·899
No mitochondria	
—	0
Phenazine methosulphate (2·5 mM)	0·076
Succinate + phenazine methosulphate (2·5 mM)	0·084
Sodium malonate (0·1 M) + phenazine methosulphate (2·5 mM)	0·452
Heated serum* (0·2 ml.) + phenazine methosulphate (2·5 mM)	0·703

* Human blood serum was diluted with an equal volume of water, heated for 5 min. in boiling water and filtered.

10 times the amount of mitochondria) was 15·1 μl. of O_2, measured during the 10 min. after a 10 min. equilibration period; the rate of O_2 uptake was constant for at least 50 min. Hence, when corrected to the same quantity of mitochondria, the O_2 uptake (μg.atoms) was approx. 2·5 times the formazan production (μmoles).

Further experiments were carried out to determine the effect of phenazine methosulphate upon the reduction of INT. Phenazine methosulphate can act as a direct acceptor of electrons in the oxidation of succinate by succinate dehydrogenase of heart muscle (Singer, 1959). It seemed possible, therefore, that this compound might increase formazan production by acting as intermediate electron carrier. Table 7 shows that this was the case. However, INT was also reduced non-enzymically in the presence of phenazine methosulphate; such reduction was rapid when malonate or heated human blood serum was also added. For this reason, phenazine methosulphate was not used in routine measurements of succinate dehydrogenase activity with INT.

Dystrophic muscle. There was no significant difference in the activity of mitochondria from normal and dystrophic muscle. The mean values (extinction/μg. of mitochondrial N) were respectively 0·060 (0·058–0·064) and 0·061 (0·055–0·071).

DISCUSSION

A number of workers have described procedures for the isolation of mitochondria from muscle tissue (e.g. Chappell & Perry, 1953, 1954; Kitiyakara & Harman, 1953; Holton, Hülsmann, Myers & Slater, 1957). Muscle presents considerable difficulties in this respect, on account of its toughness and, more important, the presence of the myofibrils. The mitochondria are arranged in rows between the myofibrils and the problem is to break up the cells and separate the myofibrils sufficiently to obtain an adequate yield of mitochondria without, at the same time, damaging the mitochondria or disrupting the myofibrils into small fragments which would contaminate the mitochondrial fraction. In my hands, with mouse skeletal muscle, the use of gentle but prolonged homogenizing of finely chopped muscle produced good separation of relatively undamaged myofibrils as judged by microscopic examination. The medium used by Chappell & Perry (1954) for preparing mitochondria from pigeon-breast muscle does not appear to be suitable for mouse skeletal muscle.

The relatively small stimulation of the adenosine triphosphatase of the muscle mitochondria by dinitrophenol is in marked contrast with the large stimulation of liver adenosine triphosphatase. A large response of liver adenosine triphosphatase

to dinitrophenol is considered by many workers to be an index of mitochondrial integrity. However, several authors (Chappell & Perry, 1953; Holton *et al.* 1957) have found that the adenosine triphosphatase of mitochondria from various types of muscle shows a relatively small response to dinitrophenol. Possibly this is simply the result of the greater difficulty in preparing undamaged muscle mitochondria. The finding of Chappell & Perry (1954) that adenosine triphosphatase of pigeon-breast-muscle mitochondria prepared in a medium containing tris, Mg^{2+} ions, ATP and EDTA showed a higher stimulation by dinitrophenol, was not paralleled in the present work with mouse skeletal muscle.

The approximate equivalence of Mg^{2+}, Co^{2+} and Mn^{2+} ions in activating the muscle adenosine triphosphatase is similar to the findings of Bronk & Kielly (1958) for liver adenosine triphosphatase. A lower activity of Ca^{2+} ions was found also by Chappell & Perry (1953) for pigeon-breast-muscle mitochondria.

The slightly higher adenosine triphosphatase activity of mitochondria from the dystrophic mouse muscle may explain a recent report (Zymaris, Saifer & Volk, 1960) that there is a lowered concentration of ATP in the dystrophic muscle. It is perhaps of interest that Michelazzi, Mor & Dianzani (1957), in contrast, found a decrease in mitochondrial adenosine triphosphatase in guinea-pig muscle after denervation. The presence of a normal level of succinate–INT-reductase activity in the mitochondria is of interest in view of the reported morphological changes in mitochondria in dystrophic muscle.

SUMMARY

1. The adenosine triphosphatase of mouse skeletal-muscle mitochondria was activated about equally by Mg^{2+}, Co^{2+} and Mn^{2+} ions; Fe^{3+} and Ca^{2+} ions were less active.

2. The enzyme was inhibited by adenosine diphosphate, chlorpromazine, atabrine and, weakly, by F^- ions.

3. Its activity was enhanced by 2:4-dinitrophenol, but never by more than about 50%.

4. INT [2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride] was less efficient than oxygen as an acceptor of electrons from the succinate-dehydrogenase system of the mitochondria.

5. Mitochondria from muscle of mice with hereditary muscular dystrophy had, on the average, a 15% higher adenosine triphosphatase activity than normal (compared on the basis of equal mitochondrial nitrogen). The succinate–INT-reductase activity was normal.

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