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COMPARISON OF ADENYLATE KINASE FROM LIVER AND MUSCLE OF NORMAL MICE AND THOSE WITH AN HEREDITARY MUSCULAR DYSTROPHY

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SUMMARY

1. Adenylate kinase (EC 2.7.4.3) activity of the liver and hindleg muscle has been compared in normal and dystrophic mice.

2. The total activity and specific activity of the muscle enzyme was greatly reduced in the dystrophic animals but the properties of the enzyme as estimated by its sensitivity to pH, temperature and dithiol reagents were unaffected by the disease. The dystrophic enzyme was slightly less stable on storage probably due to changes in the dystrophic muscle.

3. The properties of the liver enzyme were very different from the muscle enzyme with respect to these criteria.

4. Electrophoresis on starch and polyacrylamide gels showed tissue specific isoenzyme patterns which remained unchanged in the dystrophic samples.

5. It is concluded that, unlike the human enzyme, mouse muscle enzyme remains qualitatively unchanged in the dystrophic animals.

6. The differences between mouse adenylate kinases and those of other species are discussed.

INTRODUCTION

Investigations of boys suffering from the sex-linked (Duchenne type) hereditary muscular dystrophy revealed that the sensitivity to thiol reagents of adenylate kinase (ATP:AMP phosphotransferase, EC 2.7.4.3) in crude muscle extracts was different from normal human muscle [1]. The dystrophic enzyme was more characteristic of that found in the liver. However, in mice with an autosomal hereditary dystrophy resembling the human dystrophy, it has been found that creatine kinase is abnormal in having less than two reactive thiol groups per molecule [2]. In this respect, the creatine kinase of human dystrophics is reported to be normal [3]. It

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Abbreviations: DTNB, 5,5′-dithiobis-(2-nitrobenzoate).

therefore became of interest to see whether or not the adenylate kinase of dystrophic mouse muscle had atypical properties as compared with that from normal mice. Data are presented for the enzymes from skeletal muscle and liver and these are compared with those from human and other animals.

MATERIALS AND METHODS

Animals

Mice were the Bar Harbor 129 strain, carrying the autosomally recessive dystrophy gene *dy*. For controls, either litter-mates of the dystrophic mice, or mice whose families had shown no dystrophy for several generations and were of a similar age to the dystrophics, were used.

Chemicals

NADP and glucose-6-phosphate dehydrogenase were obtained from the Boehringer Corp. (London) Ltd, Uxbridge Road, Ealing W5 2TZ, U.K., ADP from Miles-Seravac (PTY) Ltd, Maidenhead, Berks, U.K., hexokinase and starch (hydrolysed, Smithies) from British Drug Houses Ltd, Poole, Dorset, U.K., DTNB from Ralph N. Emmanuel Ltd, Wembley, Middx. HA0 1PY, U.K. Other chemicals were supplied by British Drug Houses or Sigma (London) Chemical Co. Ltd, London W.6. U.K.

Preparation of samples

Mice, aged 8–23 weeks, were killed by chloroform anaesthesia, and the tissues rapidly removed, rinsed with cold 0.9% (w/v) saline, blotted and weighed. Liver and muscle tissue was sometimes stored frozen at −20 °C until the day of use. Tissue samples of approx. 1 g were homogenized in 5 vol. ice-cold 0.1 M Tris–HCl, pH 8.0, in a Polytron ST 10 (for muscle tissue) or a motordriven Potter–Elvehjem homogeniser (for liver tissue). The homogenates were centrifuged at 6000 rev./min (approx. 4000 × *g*) in an MSE 18 centrifuge for 5 min and unless otherwise stated, enzyme analyses were performed on the fresh supernatants. In some experiments, the supernatants were vacuum dialyzed overnight at 4–6 °C against fresh homogenizing buffer in visking tubing that had been treated with boiling 1 mM EDTA, pH 7.4, and then rinsed with distilled water [4]. Alternatively, visking tubing that had been soaked in 10% (v/v) acetic anhydride in pyridine for 16 h and boiled in several changes of distilled water was used for dialysis against the homogenising buffer. This latter procedure reduces the effective pore size of the tubing [5]. In other experiments samples were stored under various conditions before assay (see Results).

Assay of enzyme activity

Spectrophotometric assays. Adenylate kinase was assayed by coupling with hexokinase and glucose-6-phosphate dehydrogenase using the method of Oliver [6] as described by Brownson [5]. The final concentrations, in a volume of 1 ml were: glucose, 40 mM; $MgCl_2$, 2 mM; NADP, 0.18 mM; ADP, 1 mM; glucose-6-phosphate dehydrogenase, 0.25 enzyme unit/ml; hexokinase, 0.5 enzyme unit/ml; in 0.1 M Tris–HCl, pH 8.0, at 30 °C. The assay mixture was prepared fresh each day and stored in ice until use. Between 10 and 25 μl of enzyme was added to 1 ml of assay solution at

30 °C and, after about 1 min the rate of increase in absorbance at 340 nm, in relation to a blank curette with no enzyme, was measured in a Unicam SP 800 attached to a Servoscribe recorder adjusted so that 0.2 absorbance unit gave 100% reading. The use of quartz inserts (Cary) in the 10 mm × 10 mm quartz cells allowed a 1-ml volume of assay solution to be read with a pathlength of 2 mm. With control experiments, in which hexokinase was omitted from the assay solution, no increase in absorbance was observed on addition of enzyme.

One enzyme unit is defined as the number of micromoles of ADP converted to ATP per minute by the enzyme at 30 °C.

For estimation of enzyme activity in the presence of 5,5′-dithiobis-(2-nitrobenzoate) (DTNB), extracts (containing 16.4 enzyme units/ml final volume) were incubated with the thiol in the Tris–HCl buffer, pH 8.0, for 20 min in an ice bath before assay of 10–50 μl of the mixture. Controls, with no DTNB present, were treated in a similar manner. A stock solution of DTNB, 60 mM, in 0.1 M Tris–HCl, pH 8.0, was freshly prepared each day. Protein was assayed by the method of Lowry et al. [7].

Acrylamide gel electrophoresis. 7% acrylamide gels (7.5 cm × 0.6 cm diameter) were prepared by the method of Ornstein [8] and Davis [9] as described by Smith [10]. No spacer or sample gel was used. Gels were pre-run at 1 mA/gel for 30 min to remove the persulphate used as a catalyst for gel formation. A load of 250 μg of soluble protein (after overnight vacuum dialysis at 4–6 °C) in 10% (w/v) sucrose was applied to each gel and electrophoresis performed at 1 mA/gel for 10 min and then at 2–2.5 mA/gel in Tris–glycine buffer, pH 8.3, at 4 °C until the bromophenol blue albumin marker had migrated to within 1–2 cm of the anodic end of the gel. Gels were then removed and divided longitudinally into two by forcing the gel through a fine wire across the bottom of a hole in a rubber bung. One half was stained for adenylate kinase activity as described by Scopes [11]. Gels were developed for 40 min at room temperature (approx. 20 °C) in the dark. Enzyme activity was located by the presence of purple bands of reduced *p*-nitroblue tetrazolium. The other half of the gel was stained for protein for 2 h in 0.2% naphthalene amido black in 7% (v/v) acetic acid and destained by repeated washings in 7% acetic acid.

Starch gel electrophoresis. Gels were prepared in perspex trays (21 cm × 13 cm) using 33 g of starch in stock buffer (Tris, 218 g; EDTA (disodium salt), 11.68 g; boric acid, 61.8 g in 1 l water, pH 8.6) diluted 40 times with distilled water to give 300 ml. Samples were applied on 1 or 2 paper inserts (1.3 cm × 0.3 cm Whatman 3MM paper) and the gels run horizontally at 3 mA/cm width of gel for 3.5 h at 4–6 °C using stock buffer diluted 16 times in the electrode vessels. After the electrophoresis the gel was sliced into two horizontally. One half was stained for adenylate kinase activity by the agar overlay technique as described by Brownson [5] except that *p*-nitroblue tetrazolium was used instead of thiazolyl blue. Gels were developed at approximately 30 °C for 2 h in the dark and then covered with polythene and left at 4–6 °C. Purple bands continued to develop for several days. The other half was immersed in 0.05% (w/v) naphthalene amido black in acetic acid–methanol–water (10:40:50, by vol.) to locate protein. Excess stain was removed by repeated washings in the acetic acid–methanol–water mixture.

RESULTS

Spectrophotometric assays

Unless otherwise stated, assays were performed with freshly prepared supernatants.

Total activity in samples. The adenylate kinase activities of muscle and liver from normal and dystrophic mice are shown in Table I. For muscle, both the specific activity and the enzyme activity per unit weight of tissue in the dystrophic preparations are less than half that of the normals and due to the much reduced volume of hindleg muscle in the afflicted animals, the total activity per leg in animals of similar age is only about 15% of the normal.

TABLE I

ADENYLATE KINASE ACTIVITY IN MOUSE MUSCLE AND LIVER

Adenylate kinase activity was assayed on freshly prepared supernatants as described in Materials and Methods. Results are mean ± standard deviation of the activity in the combined muscles from both hindlegs or the total liver of one mouse. Number of animals used (aged 8–23 weeks) is shown in parentheses. Significance of the results was assessed by Student's *t* test. n.s., not significant.

	Normal			Dystrophic			Dystrophic as a percentage of normal (%)
Hindleg muscle							
Enzyme units/mg soluble protein	11.5 ± 3.2	(4)	*P* 0.02	4.9 ±1.3	(4)		43
Enzyme units/g wet wt muscle	528 ± 84	(4)	*P* 0.03	255 ±68	(4)		48
Enzyme units/2 legs	629 ±181	(4)	*P* 0.01	85 ±20	(4)		13.5
Liver							
Enzyme units/mg soluble protein	0.15± 0.09	(3)	n.s.	0.11± 0.02	(3)		73
Enzyme units/g wet wt liver	13.9 ± 8.5	(3)	n.s.	12.5 ± 3.21	(3)		90
Enzyme units/total liver	13.0 ± 8.3	(3)	n.s.	5.9 ± 1.3	(3)		45

Compared to muscle, there is much less enzyme activity present in the liver. Dystrophy does not significantly affect the specific activity of the enzyme but, due to the reduced tissue size, the total activity in the liver is less in the dystrophic animals.

Effect of acid treatment. Rabbit muscle adenylate kinase is known to be stable to acid treatment [12]. The effect of low pH on muscle and liver samples from normal and dystrophic mice are shown in Table II. The activity of muscle preparations was only reduced to between 80 and 95% of the control. In contrast, the activity of the liver preparations was reduced to 23–30% of the control. There was no significant difference between the normal and dystrophic extracts for either tissue. Due to the precipitation of other protein during the procedure the specific activity of the muscle preparations increased while that of the liver preparations was only slightly reduced.

Effect of temperature. The heat stability of muscle and liver enzymes was measured by preincubation at 55 °C for varying periods before assay. The muscle

TABLE II

EFFECT OF ACID TREATMENT ON THE ADENYLATE KINASE ACTIVITIES OF MOUSE TISSUE SUPERNATANTS

Muscle and liver samples containing 10–20 enzyme units/ml were acidified with 2 M HCl to pH 2.0, left for 10 min at room temperature (20 °C) and the pH restored to 8.0 with 2 M NaOH. The precipitated protein was removed by centrifugation and the supernatant assayed for adenylate kinase activity. Control tubes, in which water, instead of acid and alkali, was added, were also assayed. Number of estimations is shown in brackets.

	Acid treated as a percentage of control (%)	
	Normal	Dystrophic
Muscle (3)		
Activity/total extract	84–92	81–87
Activity/mg protein	140	140
Liver (2)		
Activity/total extract	30	23
Activity/mg protein	70	67

enzyme for normal and dystrophic animals behaved in a similar manner (Fig. 1). For the liver enzyme, even after 1-h incubation at 55 °C the residual activity of normal and dystrophic extracts was still 12–25% of the initial value.

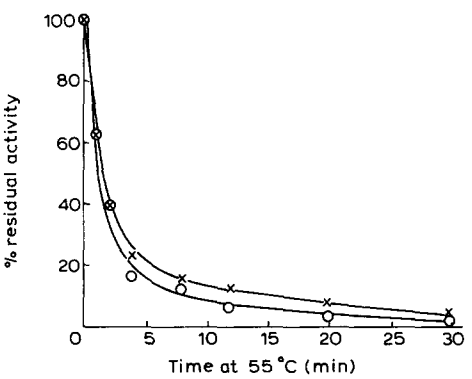

Fig. 1. Heat inactivation of muscle adenylate kinase. Extracts containing 8.4 enzyme units/ml were incubated in a water bath at 55 °C and aliquots removed at intervals before assay of adenylate kinase activity. ×, normal; ○, dystrophic.

Effect of DTNB. The effect of varying concentrations of DTNB on the activity of normal and dystrophic liver and muscle preparations are shown in Fig. 2.

The muscle enzymes from normal and dystrophic mice were inhibited to a similar extent by DTNB. At $5 \cdot 10^{-4}$ M DTNB, the muscle enzymes were completely inhibited but at a similar concentration, the liver enzyme (normal or dystrophic) was only 20–40% inhibited. At higher concentrations of DTNB the inhibition of the liver enzyme was overcome and a slight activation was observed.

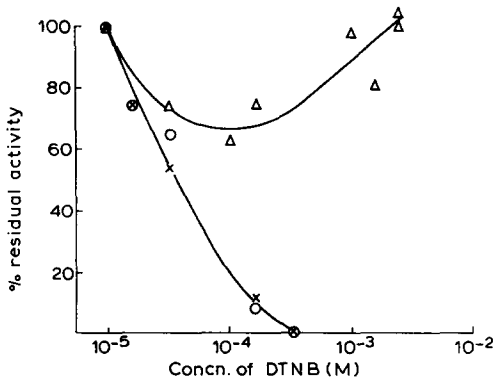

Fig. 2. Effect of DTNB on liver and muscle adenylate kinase activity. Extracts containing 16.4 enzyme units/ml final volume were incubated with DTNB in 0.1 M Tris–HCl, pH 8.0, for 20 min in an ice bath before assay of adenylate kinase activity. ×, normal muscle; ○, dystrophic muscle; △, normal liver.

TABLE III

EFFECT OF DIALYSIS AND STORAGE ON MOUSE MUSCLE ADENYLATE KINASE

Muscle extracts containing similar enzyme units/ml were stored under the conditions described before assay of adenylate kinase activity. Results are presented as a percentage of original activity before treatment expressed as indicated.

Treatment	Normal			Dystrophic		
	Activity (%/g wet wt)	Activity (%/mg protein)	Activity (%/ml)	Activity (%/g wet wt)	Activity (%/mg protein)	Activity (%/ml)
4 h at 4–6 °C	100	100		100	100	
Overnight at 4–6 °C						
No dialysis	52	51		36	44	
Vacuum dialysis	46	27		32	38	
Acetylated dialysis	82	57		86	54	
+ 1 mg/ml albumin			47			47
+ 1.5 mg/ml albumin			81			65
+ 10 mg/ml albumin			110			78
+ 15 mg/ml albumin			95			81
+ 10 mM mercaptoethanol			75–100			86
+ 100 mM mercaptoethanol			92–100			81–86
+ 10 mM mercaptoethanol + 15 mg/ml albumin			93			102
+ 100 mM mercaptoethanol + 10 mg/ml albumin			90–110			81–95
diluted 10 times with buffer			2			86
diluted 10 times with buffer + 100 mM mercaptoethanol			84			90
Overnight frozen at −15 °C			74			86

Effect of dialysis and storage. In the experiments described above, adenylate kinase was assayed on the soluble protein from freshly prepared homogenates. For starch and acrylamide gel electrophoretic experiments to be described below the preparation was sometimes concentrated by vacuum dialysis. The dialysis treatment reduced the activity of the samples to less than if the extracts had been left untreated overnight (Table III). However, when the pore size of the visking tubing was decreased by acetylation the loss in activity became less than the undialysed control (Table III).

Table III also shows the effects of other treatments on the stored activity of the samples. The enzyme activity of the liver samples was much more stable than that of muscle and completely retained its activity after overnight storage at 4–6 °C.

When muscle extracts were left for 4 h in ice there was no inactivation but after 20–24 h at 4–6 °C less than 50% of the initial activity remained. Addition of bovine serum albumin at a concentration of 1 mg/ml had little effect but at 10 mg/ml, complete activity was retained in the normals and about 90% in the dystrophics. Mercaptoethanol (100 mM) also stabilised the activity, as did the addition of both albumin (10 mg/ml) and mercaptoethanol (100 mM) together.

If the samples were diluted 10-fold with the homogenising buffer before overnight storage, the activity in the normals was reduced more than that in the dystrophics. Mercaptoethanol (100 mM) retained the activity of the diluted preparations to 80–90% of the fresh extract.

When the soluble protein samples were left at −15 °C for 20 h, the activity was reduced to 74–80% of that in the fresh extracts. Storage of the tissue itself at −15 °C for up to 8 days before preparation of the extract usually resulted in little change in activity.

Starch and acrylamide gel electrophoresis

Soluble extracts from mouse liver and muscle were analysed by starch and acrylamide gel electrophoresis as described in Materials and Methods. On starch gels, specific staining for adenylate kinase demonstrated the presence of four isoenzymes in muscle and two (possibly three) in liver (Fig. 3). The two slowest anodic

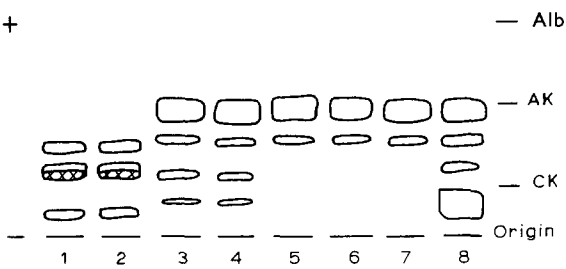

Fig. 3. Starch electrophoresis of mouse adenylate kinase. Samples were applied to the starch gel in the following order: (1) normal liver, 13 mg/ml, 2 inserts; (2) dystrophic liver, 32 mg/ml, 1 insert; (3) normal muscle, 5 mg/ml, 1 insert; (4) dystrophic muscle, 2.4 mg/ml, 2 inserts; (5) acid-treated normal muscle, 5 mg/ml, 1 insert; (6) normal muscle, 5 mg/ml treated with $2 \cdot 10^{-4}$ M DTNB, 1 insert; (7) vacuum dialysed normal muscle, 5 mg/ml, 1 insert; (8) normal muscle dialysed in acetylated dialysis sac, 5 mg/ml, 1 insert. The principle protein bands observed in the muscle extracts are shown at the side: CK, creatine kinase; AK, adenylate kinase; Alb, albumin; XXX, Haemoglobin. Experimental details are given in Materials and Methods.

migrating muscle isoenzyme at pH 8.6 only appeared after 2 to 3 days at 4–6 °C; the fastest isoenzyme was the most abundant. The isoenzyme patterns of normal and dystrophic muscle were not significantly different and the bands occurred in the same quantitative proportions. The two fastest liver isoenzymes were of similar mobility to the second and third fastest muscle isoenzymes.

Samples that had been vacuum dialysed overnight showed a general reduction in activity of all the isoenzymes, as compared to freshly prepared samples or samples dialysed in acetylated tubing. Acid treatment destroyed the two slowest muscle bands. Treatment of the extracts with $2 \cdot 10^{-4}$ M DTNB before electrophoresis reduced the intensities of the two faster migrating bands of normal and dystrophic muscle preparations but no inhibition of the liver isoenzymes was evident (Table IV). Acrylamide

TABLE IV

COMPARISON OF ADENYLATE KINASE ACTIVITIES IN MAMMALIAN MUSCLES

Animal	Muscle	Activity (μmoles/min per g wet wt)	Temperature of assay (°C)	Reference
Human	Red (various)	50– 73	25	[18]
	Leg (mixed)	60	25	[19]
Rabbit	Red (various)	30– 45	25	[20]
	White (various)	190–250	25	[20]
Rat	Red (soleus)	60	25	[21]
	Mixed, mainly white, (gastrocnemius)	1450	30	[22]
	Skeletal	172	22	[23]
	Skeletal	389	25	[24]
Mouse	Leg (mixed)	250–500	30	Present work

gel electrophoresis at pH 8.3 demonstrated the presence of three muscle isoenzymes (Fig. 4) although the fastest was not always evident. A fourth band was sometimes present. The main isoenzyme migrated anodically behind two other bands and ahead of the fourth band. Again the isoenzyme pattern of normal and dystrophic muscle extracts were similar; acid treatment had little effect on the two main isoenzymes but the presence of DTNB ($0.5 \cdot 10^{-4}$–$2.0 \cdot 10^{-4}$ M) in the assay medium reduced the intensity of both these bands and at $5 \cdot 10^{-4}$ M DTNB no activity was observed. $AgNO_3$, at a concentration of 10^{-4} M, almost completely inactivated all the muscle isoenzymes.

The two isoenzymes present in the liver migrated anodically much more slowly than the main muscle band (Fig. 4) and at approximately the same rate as the slowest muscle isoenzyme evident in some extracts. Acid treatment destroyed these bands but $2 \cdot 10^{-4}$ M DTNB had little effect.

DISCUSSION

Comparison of enzyme properties in crude tissue extracts

It is generally recognised nowadays that to determine the properties of an enzyme a purified preparation is desirable to avoid complications arising from the

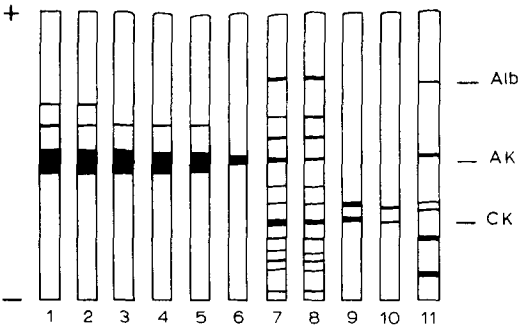

Fig. 4. Acrylamide gel electrophoresis of mouse adenylate kinase. 250 μg of soluble protein was applied to each gel as follows: (1) normal muscle; (2) dystrophic muscle; (3) normal muscle held at pH 2.0 for 10 min and then readjusted to pH 8.0 for electrophoresis; (4) normal muscle plus $5 \cdot 10^{-5}$ M DTNB; (5) normal muscle plus $2.0 \cdot 10^{-4}$ M DTNB; (6) normal muscle plus 10^{-4} M $AgNO_3$; (7) normal muscle, protein pattern; (8) dystrophic muscle, protein pattern; (9) normal liver; (10) dystrophic liver; (11) normal and dystrophic liver, protein pattern. The positions of the principle protein bands are indicated as in Fig. 3.

presence of unknown contaminants. This ideal is not always attainable or even necessary for a preliminary investigation. Thus Schirmer and Thuma [1], with experience of purified adenylate kinase from human muscle were able to compare some properties of the enzyme from crude supernatants of normal and dystrophic human muscle. However, they were unable to say whether the differences they observed in the reaction with DTNB arose from primary or secondary modification of the enzyme or from a mixture of isoenzymes in different proportions. This would require purification not possible with biopsy samples. The present work was undertaken to find out if the altered properties of adenylate kinase in crude muscle supernatants was also a feature of the hereditary mouse dystrophy. At the same time the range of investigations was extended by using dialysed samples to remove small molecules and comparing concentrated and diluted preparations so that adventitious ion effects might more readily be recognised and, by means of electrophoretic analysis, the possibility of changed proportions of isoenzymes revealed. The results showed conclusively that although there were marked quantitative differences in the adenylate kinase activities of normal and dystrophic muscle the enzymes were qualitatively unchanged and the ratios of isoenzymes essentially constant. As discussed later, one anomalous feature emerged and this was the reversal of inhibition of the liver enzyme by high concentrations of DTNB (Fig. 2). Purification will be required before this phenomenon is resolved.

Enzymes levels in normal and dystrophic tissues

Pennington [13] reported that the adenylate kinase activity in muscle of normal mice aged 12–20 weeks was about twice that of dystrophics (6.1 and 3.1 μmoles/min per mg protein) when expressed on a total non-collagen N basis. In contrast Kaldor and Gitlin [14] using the same method of expressing the results, were unable to find any difference. The results reported here, expressed on either a milligram of soluble protein basis or on a gram wet weight of muscle basis (Table I) are in substantial agreement with those of Pennington [13]. If the total amount of enzyme per leg is

considered, that in the dystrophics is very much less than in normals of a comparable age due to tissue atrophy and the much smaller size of the animal. This contrasts with the DNA and RNA levels which, per leg, remained unaltered in the diseased state [15]. Muscle adenylate kinase is also reported to be reduced in the hereditary dystrophic hamster [16].

Enzyme levels in mouse liver have not been compared before. The specific activity is very much less than that of the muscle and is slightly reduced in the dystrophics when expressed on a protein or wet weight basis. The total amount of liver enzyme activity is greatly reduced, not because of atrophy but because of the smaller size of the animal.

Over the ages studied (8–23 weeks) the specific activity of muscle adenylate kinase did not significantly change in either the normals or the dystrophics.

Comparison in mouse and human dystrophy

Muscle adenylate kinase activity from dystrophic boys is reported to be only 50% inhibited by a concentration of DTNB that completely inhibits the enzyme from normal controls [1]. This was not found with the mouse for which the normal and dystrophic muscle were indistinguishable in their response to DTNB (Fig. 2). However, it would seem that, in general, mouse adenylate kinase is more reactive towards this reagent than the human enzyme at pH 8.0. Reaction of mouse enzyme extracts containing 16.4 enzyme units/ml was carried out at 0 °C because of its relative instability (Fig. 1) and the concentration of DTNB required to cause 50% inhibition was $5 \cdot 10^{-5}$ M. For the human enzyme extracts containing 10 enzyme units/ml although the temperature used was 25 °C the concentration of inhibitor causing 50% inhibition was $2 \cdot 10^{-5}$–$3 \cdot 10^{-5}$ M.

In reaction towards DTNB the human dystrophic muscle enzyme was found to behave like the human liver enzyme [1]. The mouse liver enzyme resembles the human liver enzyme in that it is much less reactive towards DTNB but shows an additional curious feature that as the inhibitor concentration is raised above 0.1 mM the inhibition is reversed so that with 4 mM the activity is the same or greater than the initial value.

To further check that there were no structural differences between the normal and dystrophic mouse adenylate kinases the two enzymes were also compared with respect to pH and temperature stability and electrophoretic mobility on starch and acrylamide gels. Apart from the finding that there was generally less enzyme activity in the dystrophic muscle no other differences emerged and it must be concluded that, as with the creatine kinase, the mouse and human dystrophics are different in this respect.

The differences in enzyme behaviour found in Table III are probably explained by differences in the storage properties of normal and dystrophic muscle reflecting differences in tissue composition. The greater need of dystrophic extracts for a reducing agent is in accord with the finding of Hooton and Watts [17] that a small decrease in the ratio of reduced to oxidised glutathione and a marked increase in the extent of oxidation of the soluble proteins exists in dystrophic mouse muscle as compared with normals. Similarly, differences in the rate of inactivation of myofibrillar ATPase were found by Kaldor and Gitlin [14] to reflect different rates of thiol oxidation that could be overcome with a suitable reducing agent. For adenylate

kinase, albumin as well as a thiol agent are necessary to preserve complete stability (Table III). This might also be predicted on the basis of the observation by Pennington [13] of a more than 2-fold increase in the catheptic activity of dystrophic mouse muscle. In consequence, for maximum stabilization a combination of reducing agent and albumin is required (Table III).

Comparison of adenylate kinase from various sources

The activity of adenylate kinase varies considerably from one muscle to another and between different species (Fig. 4, refs 18–24). From electrophoretic studies (Fig. 4) it is the second most abundant enzyme present in the soluble protein of mouse hindleg muscle, the most abundant being creatine kinase. The activity is higher in white muscle than red (ref. 20 and present authors, unpublished).

The properties of the muscle enzyme vary with source. Thus the enzyme from rabbit retains its activity after heating at 100 °C for 20 min [25] whereas the mouse enzyme is 50% inactivated after heating at 55 °C for about 2 min (Fig. 1). The isoenzyme patterns of various rodent skeletal muscles are reported to be very different and could not be grouped phylogenetically or ecologically; each had one major band and several minor bands on starch gel electrophoresis at pH 6.7 [26]. Unlike the mouse, which was not studied by Traugott and Mossaro [26], at least five isoenzymes were usually found with the middle band showing the most activity. In the mouse, the fastest on starch gels was most abundant (Fig. 3) while the two most cathodic bands were also very slow to appear and might represent deoxy AMP kinase activity rather than adenylate kinase [27]. These two bands appeared particularly sensitive to acid treatment. The number of bands resolved was not improved by using acrylamide rather than starch gel electrophoresis. Using a different buffer system Khoo and Russell [28] found that in the human, the slowest of the three muscle enzymes on starch gel was the most abundant, ε similar pattern being observed for brain and erythrocytes [28]. This order was found for the mouse when the muscle extracts were analysed by acrylamide, as opposed to starch gel electrophoresis and perhaps argues against the occurrence here of deoxy AMP kinase. Also, in this connection it should be noted that all the bands appeared within 40 min. The reasons for these differences in electrophoretic behaviour will be a fruitful field for further study.

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