Aust. J. Biol. Sci., 1974, **27**, 633–43

Some Biochemical Features of White Muscle Disease in Lambs, and the Influence of Selenium

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Abstract

Typical white muscle disease has been induced in lambs that were normal at weaning but thereafter fed natural dystrophogenic fodder for 2–4 months. Protection from the disease was given by selenium when it was administered regularly as an oral drench, or incorporated into an intraruminal heavy pellet placed in the rumen at weaning.

Calcium and magnesium levels in serum were unaffected by the dystrophic condition, although urinary excretion of both ions was decreased. This lowered urinary excretion was associated with high levels of calcium in both heart and skeletal muscle, and to some extent associated with raised levels of calcium and magnesium in washed mitochondria isolated from the same tissues.

The respiratory properties of the mitochondria were examined polarographically. No differences were found between heart muscle mitochondria isolated from normal and dystrophic hearts. Skeletal muscle mitochondria from dystrophic animals showed lowered respiratory rates with palmitoyl-DL-carnitine and acetyl-DL-carnitine as substrates. The smaller differences with pyruvate and succinate were not significant. Respiratory control ratios for the dystrophic skeletal muscle organelles were always 1·0, but for selenium-supplemented animals were always $>1{\cdot}0$.

To some extent at least the abnormalities appear to be associated with the high levels of calcium in the tissues and mitochondria of dystrophic lambs.

Introduction

The importance of selenium as a trace element to the ruminant is widely recognized (Underwood 1971). Much of the work supporting its recognition has come from the study of white muscle disease (WMD) (Muth *et al.* 1958). The epidemiology of this malady, which affects mainly lambs and calves, is now fairly well understood (Hartley and Grant 1961), and the pathology of WMD also is well documented (Muth 1955); biochemical changes are not.

Since the protective role of selenium in WMD is likely to have an important bearing on its normal role in animal tissues, the experimental work reported here was aimed at delineating certain biochemical consequences of WMD that have not previously been studied in detail.

The paper describes the experimental production of nutritional muscular dystrophy in lambs that were normal at weaning, by feeding harvested dystrophogenic fodder. Biochemical changes, particularly those relating to calcium retention, are described, and the protective effects of selenium against such retention are considered. Finally, the behaviour of isolated washed mitochondria from heart and skeletal muscle is described, and the role of selenium at the level of mitochondrial function is discussed.

Materials and Methods

Animals and their Maintenance

Twenty-two normal Merino lambs (11 ewes and 11 wethers), reared at the CSIRO Field Station, O'Halloran Hill, S.A., were weaned at between 8 and 9 weeks of age. They were then offered, *ad libitum*, pelleted dystrophogenic feed selected from a selenium-responsive area on Kangaroo Island, S.A. Fodder samples had been obtained from a number of properties and analysed, and a batch was drawn from a supply that had an average selenium content of 0·019 p.p.m. The whole batch, sufficient to supply the experiment, was lightly hammer-milled and pelleted after moistening with approximately 10% water. No other addition was made to the feed.

The lambs were housed in flyproof pens either singly or in groups of two or three. Food intakes were recorded weekly for each treatment group. Distilled water was supplied to the unsupplemented animals, and rain water to the remainder.

The 22 lambs were divided into five treatment groups as follows:

A. No supplement (control; four animals).
B. No supplement; exercised daily (four animals).
C. Supplemented with selenium (2 × 3 mg selenium as an oral drench of sodium selenite each week; four animals).
D. As for C but animals were exercised daily (four animals).
E. Supplemented with selenium; each animal was given an intraruminal selenium pellet (5% elemental selenium, Kuchel and Buckley 1969) and a grinder (six animals).

Exercise consisted of 50–100 revolutions (0·25–0·5 km) daily in a treadmill. The details of the technique have been published elsewhere (Godwin 1972).

All animals received a supplement of 30 000 i.u. vitamin A and 60 000 i.u. vitamin D weekly.

Sampling and Analytical Techniques

(i) *Live animals*

Whole blood selenium levels were determined every 2 weeks. Samples taken at the same time were used for the determination of serum calcium, magnesium, phosphate and creatine kinase levels. Each animal was confined to a metabolism cage at least once during the experimental period for a 24-h collection of urine. Analysis was made for urinary calcium, magnesium, sodium and potassium, and urine volumes were noted. In some cases the collection period was extended to 48 h to check the 24-h value; good agreement was obtained.

Selenium was determined by Watkinson's method (1966), calcium and magnesium by atomic absorption spectroscopy, and sodium and potassium by flame photometry. Phosphate determinations were done either by autoanalysis using the stannous chloride–hydrazine method recommended by Technicon Instruments Corporation (Ardsley, N.Y.), or by the phosphomolybdate method of Gomori (1942). Creatine kinase was determined by scaling down the method of Neilsen and Ludvigsen (1963).

Electrocardiograms (ECGs) were taken regularly on all animals; the technique has already been described (Godwin and Fraser 1965, 1966).

(ii) *Dead animals*

Only one of the 22 lambs died with WMD and the remainder were slaughtered at pre-arranged times beginning during the eighth week of the experiment. At slaughter pieces of heart and skeletal muscle were removed to ice-cold 0·15M KCl. In each case the muscle was freed of connective tissue and cut up finely with scissors before being minced for only 10 s in a Sorvall homogenizer in a medium essentially similar to that of Chappell and Perry (1954), but with the omission of EDTA. Final concentrations in the solution were 0·1M KCl, 0·05M tris–HCl, 0·001M ATP and 0·005M $MgSO_4$. Cellular debris was spun down at 700 *g* and mitochondria at 10 000 *g*. The mitochondrial pellet was washed three times in calcium-free 0·25M sucrose. Both EDTA and EGTA [ethylenedioxybis-(ethylamine)-*N*,*N*,*N*′,*N*′-tetraacetic acid] were omitted from all solutions in order to disturb the endogenous calcium level of the mitochondria as little as possible.

Calcium and magnesium levels in mitochondrial suspensions were measured, after extraction with 1M HCl at 95°C, by atomic absorption spectroscopy using a ×10 scale expansion. Mitochondrial

protein was determined by u.v. absorption at 280 nm with pure bovine albumin as a standard, and by digestion followed by microKjeldahl determination.

The washed mitochondria were examined in a polarographic system, using a Clarke-type electrode (Titron Instruments Pty Ltd, Melbourne) connected directly to a high-impedance recorder. The volume of the reaction vessel was 2 ml and it was maintained at 30±0·1°C by a water-jacket. The medium used had the following composition: 0·225M sucrose, 10 mM phosphate buffer, 5 mM $MgCl_2$, 20 mM KCl, 20 mM tris–HCl, pH adjusted to 7·4. The substrates used were succinate, pyruvate–fumarate, palmitoyl-DL-carnitine and acetyl-DL-carnitine. Palmitoyl-DL-carnitine was synthesized by the method of Bremer (1968) and its activity in the system was checked against a small quantity of palmitoyl-L-carnitine supplied by Dr A. Snoswell (Department of Biochemistry, Waite Agricultural Research Institute, Glen Osmond, S.A.). The concentration used in the system was 0·04 mM after it was established that the DL material was inhibitory above 0·08 mM, due presumably to the presence of the D-isomer.

The integrity of the mitochondrial preparations was tested by two different methods: (1) supravital staining with a 1:10 000 dilution of Janus Green B in 0·85% saline, and (2) examination of the permeability characteristics of the mitochondrial membranes from dystrophic lambs, by the method of Chappell and Haarhoff (1967). Supravital staining was carried out on most of the preparations prior to their use in the polarograph. The examination of the permeability characteristics was made on a separate group of dystrophic lambs but did serve to confirm that mitochondria from dystrophic animals had similar swelling characteristics to those from selenium-supplemented lambs. The agents used in studying the behaviour of the mitochondria as osmometers were ammonium malate, ammonium acetate and potassium chloride.

Histological examination was made of samples of semitendinosus muscle, cardiac muscle, kidney, pancreas and liver, in order to assess the severity of WMD. Alizarin Red S was used to reveal the presence of calcium salts.

Results

Clinical signs of muscular dystrophy appeared in some lambs that were not receiving selenium as a supplement within 2 months of their being weaned on to the dystrophogenic feed; most showed clinical symptoms by 4 months. Blood selenium levels of all animals at weaning ranged from 0·10 to 0·18 μg/ml and thereafter values fell steadily in the unsupplemented lambs. Two typical curves showing the fall in blood selenium are given in Fig. 1. Values levelled off in all deficient animals at around 0·02 μg/ml, but in selenium-treated lambs the levels were maintained at around 0·20–0·30 μg/ml.

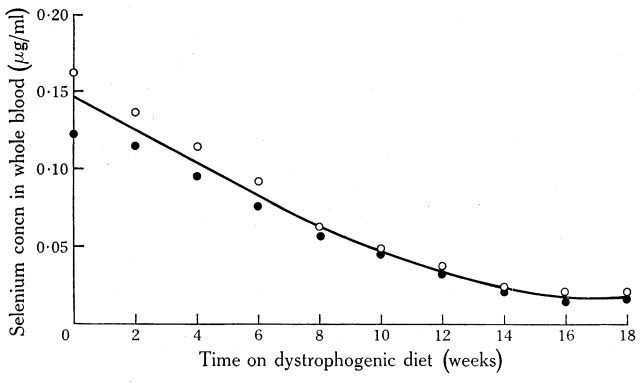

Fig. 1. Fall in whole blood selenium in two lambs fed dystrophogenic fodder pellets from weaning.

Electrocardiographic records showed the development of abnormal patterns in dystrophic lambs (Fig. 2); similar changes have been previously reported from this laboratory (Godwin and Fraser 1966; Godwin 1968). Daily exercise on the treadmill

had a moderating effect on the development of the dystrophic condition (Godwin 1972), resulting in both delayed onset as well as less severe lesions being present post-mortem. This beneficial effect was reflected in fewer abnormalities in the ECG records (Fig. 2).

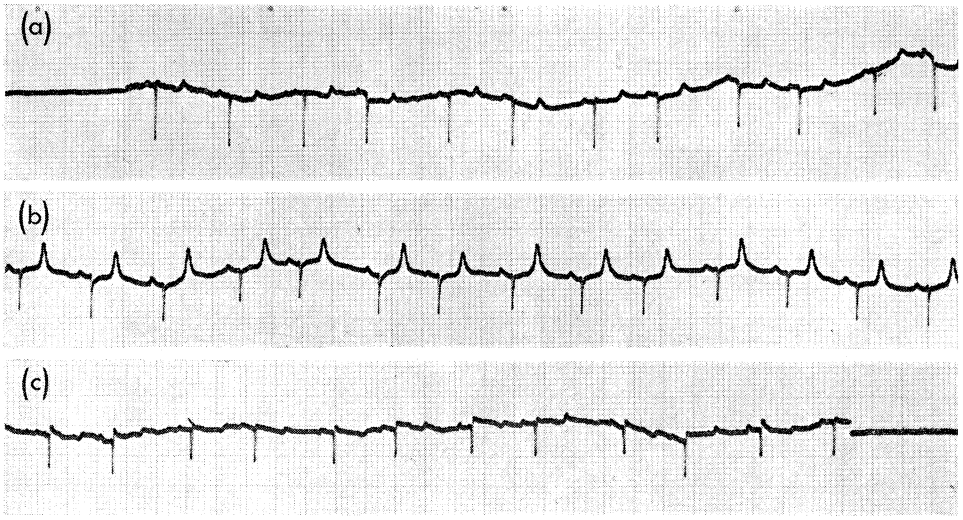

Fig. 2. ECG traces (lead II) showing development of abnormal trace in a non-exercised lamb when fed a dystrophogenic fodder, and the beneficial effect of daily treadmill exercise. (*a*) Selenium-supplemented animal—normal trace (Godwin and Fraser 1966). (*b*) Characteristic abnormality in a non-exercised dystrophic lamb. Note abnormally high *T*-wave with elevated *S–T* segment. (*c*) Animal with no selenium supplement but exercised daily. Trace is virtually normal.

Lambs receiving selenium pellets were protected from dystrophic changes. The action of the pellet in relation to the histopathological lesions occurring in WMD will be reported separately (Kuchel and Godwin, unpublished data).

Serum calcium and magnesium values were followed throughout the experimental period; levels remained very steady with no differences between treatment groups. Values for serum calcium for the four treatment groups A–D (see Methods) were 5·34, 5·09, 5·26 and 5·11 m-equiv/l (S.E. $\pm$ 0·10), and for magnesium, 1·79, 1·76, 1·76 and 1·75 m-equiv/l (S.E. $\pm$ 0·05).

Table 1. Urinary excretion of cations in lambs fed dystrophogenic fodder

Group	Excretion (m-equiv/day) of:				Urine
	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	vol. (ml)
Unsupplemented	0·66–2·50	6·63–24·5	22·2–74·1	20·9–110·0	150–910
Selenium-supplemented	6·48–20·6	22·7–37·7	11·0–76·9	77·8–134·6	250–1080
Statistical analysis	$P<0·01$	$P<0·01$	n.s.	n.s.	n.s.

Urinary excretion of calcium and magnesium, measured over 24 h, in collections taken from the fourth experimental week onward showed a marked difference between selenium-supplemented and unsupplemented groups (see Table 1). Signifi-

cantly ($P<0\cdot01$) more calcium and magnesium were excreted by the supplemented animals. No differences were observed in urinary sodium and potassium excretion nor in urinary volume. Calcium and magnesium levels in muscle are shown in Table 2, and corresponding values for mitochondria in Table 3. The wide range of values for calcium in both tables made it more appropriate to test for significance using the Wilcoxan rank sum test. This was unnecessary for the magnesium values for which Student's *t*-test was used. The difference in skeletal muscle mitochondrial calcium shown in Table 3 just failed to reach significance at the 5% level.

Table 2. Calcium and magnesium levels in tissues of lambs fed dystrophogenic fodder

Values are expressed as μ-equiv/g wet weight of tissues; the range of values is given for Ca and the mean value ± S.E. for Mg

Group		Heart	Statistical analysis[A]	Skeletal muscle	Statistical analysis[A]
Unsupplemented	Ca	1·23–157·0		1·25–9·00	
	Mg	18·1±0·47		17·3±0·80	
Selenium-supplemented	Ca	1·04–1·73	$P<0\cdot01$	0·80–1·57	$P<0\cdot01$
	Mg	18·5±0·47	n.s.	20·5±0·80	$P<0\cdot01$

[A] Ca values tested by Wilcoxan rank sum test; Mg values by Student's *t*-test.

Table 3. Calcium and magnesium levels in washed mitochondria from lambs fed dystrophogenic fodder

Values are expressed as μ-equiv/100 mg mitochondrial protein; the range of values is given for Ca and the mean value ± S.E. for Mg

Group		Heart mitochondria	Statistical analysis[A]	Skeletal muscle mitochondria	Statistical analysis[A]
Unsupplemented	Ca	1·15–21·0		2·28–10·09	
	Mg	3·51±0·25		4·70±0·24	
Selenium-supplemented	Ca	0·93–1·57	$P=0\cdot05$	2·17–4·50	just n.s.
	Mg	3·77±0·25	n.s.	4·27±0·24	n.s.

[A] Ca values tested by Wilcoxan rank sum test; Mg values by Student's *t*-test.

Functional Properties of the Mitochondria

Supravital staining of mitochondrial preparations with Janus Green B showed that preparations from dystrophic and normal muscle were comparable with respect to the density and staining properties of the mitochondria.

Further confirmation of mitochondrial integrity in dystrophic muscle was obtained by swelling experiments conducted later on a separate group of lambs. Graded concentrations of ammonium malate, potassium chloride and ammonium acetate were set up (0·2, 0·1, 0·05, 0·025 and 0·0125M) and osmolalities were measured. Based on measurements of mitochondrial protein, equivalent amounts of mitochondrial suspension were added to the above solutions and double reciprocal plots obtained of $1/E_{610\,nm}$ versus 1/osmolality, using the method of Chappell and Haarhoff (1967). Although the capacity of the skeletal muscle mitochondria to act as osmometers was less than that of liver mitochondria, there was no apparent difference between those from dystrophic and non-dystrophic muscle (Fig. 3).

In the polarograph system 0·1 ml of a suspension of washed mitochondria in 0·25M sucrose was added to 2 ml of medium in the reaction vessel. Final concentrations of substrates were 5·4 mM pyruvate, 5·4 mM succinate, 0·5 mM acetyl-DL-carnitine and 0·04 mM palmitoyl-DL-carnitine. After the addition of the mitochondria and substrate, ADP was added to give a concentration of 0·4 mM. Heart muscle mitochondria were tightly coupled and state 3 and 4 respiration values readily measured.* ADP : O ratios for the four substrates were calculated and had the following ranges: pyruvate, 2·1–3·7; succinate, 1·3–4·6; acetyl-DL-carnitine, 1·9–4·4; and palmitoyl-DL-carnitine, 1·8–5·7. No differences due to the dystrophic changes were apparent even though most of the hearts showed typical endocardial WMD plaques.

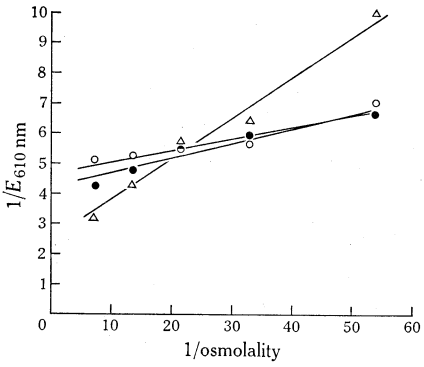

Fig. 3. Double reciprocal plot of optical density against osmolality of suspending medium, showing behaviour of muscle and liver mitochondria as osmometers. Suspending medium consisted of 7·5 μg Antimycin, 0·33 mM EDTA and 6 mM tris–HCl (pH 7·4). △ Normal liver. ○ Normal muscle. ● Selenium-deficient muscle.

Table 4. Respiration rates of isolated skeletal muscle mitochondria from dystrophic and selenium-supplemented lambs

Rates are expressed as μ-atoms oxygen per minute per milligram protein

Group	Substrate			
	Palmitoyl-DL-carnitine	Acetyl-DL-carnitine[A]	Pyruvate	Succinate
Unsupplemented	0·45±0·21	0–1·5	1·56±0·45	2·11±0·73
Selenium-supplemented	1·86±0·19	1·0–17·4	2·71±0·35	3·04±0·69
Statistical analysis	$P<0·01$	$P=0·02$	n.s.	n.s.

[A] Due to one unusually high value (17·4) amongst the supplemented group, these values were tested by the Wilcoxan rank sum test.

Respiration rates in the presence of ADP were also similar in the dystrophic and selenium-protected groups. However, when skeletal muscle mitochondrial preparations were examined in the same way the most obvious difference was the failure of the dystrophic organelles to respond to ADP. In almost all cases the addition of ADP was not followed by an increased rate of respiration. It was therefore not possible to calculate ADP : O ratios. Typical traces from the polarograph are shown in Fig. 4.

Respiration rates measured in the presence of ADP showed that dystrophic organelles had reduced rates toward palmitoyl- and acetyl-DL-carnitine (Table 4).

* State 3 respiration is where all required components are present, and the respiratory chain itself is the rate-limiting factor; state 4 is where only ADP is lacking (Lehninger 1964).

Average values for succinate, and particularly pyruvate, were low for dystrophic mitochondria but the differences were below significance level. The low respiration rates were not explained by differences in mitochondrial protein concentration, the average values for which in the dystrophic and non-dystrophic preparations were close, namely 22 and 23 mg/ml respectively.

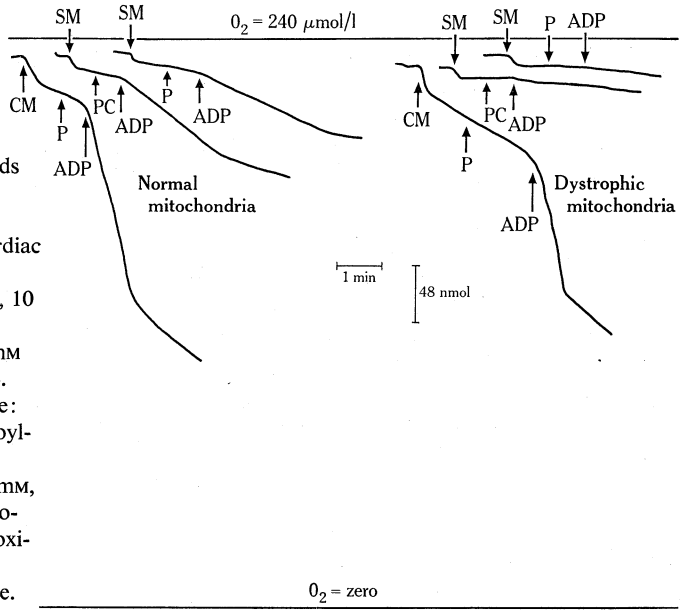

Fig. 4. Polarographic records of substrate oxidation by mitochondria isolated from skeletal muscle (*SM*) and cardiac muscle (*CM*). Medium was composed of 0·225M sucrose, 10 mM phosphate buffer, 5 mM $MgCl_2$, 20 mM KCl and 20 mM tris–HCl, pH adjusted to 7·4. Substrate concentrations were: pyruvate (*P*) 5·4 mM, palmitoyl-DL-carnitine (*PC*) 0·04 mM. ADP was added to give 0·4 mM, and 0·1 ml of suspended mitochondria, equivalent to approximately 2 mg mitochondrial protein, was used in each case.

Histological observations confirmed the presence of typical WMD in animals not protected by selenium. Calcium deposition in heart muscle as revealed by specific staining with Alizarin Red S indicates that calcium deposition is not necessarily in highest concentration where muscle is most highly damaged. Calcium deposition was frequently observed in the tubules of kidneys from dystrophic animals.

Discussion

These experiments demonstrate an uncomplicated approach to the production of nutritional muscular dystrophy in otherwise normal lambs. Previously, experimental WMD has been induced in lambs either by feeding dystrophogenic hay to their dams (Muth *et al.* 1959; Hogue *et al.* 1962) or by feeding normal lambs synthetic-type diets in which the protein source is Torula yeast (Hopkins *et al.* 1964; Godwin and Fraser 1966).

It is probable that the onset of WMD has been facilitated in the present experiments by pelleting the selenium-low fodder, this having increased the *ad libitum* intake (Wainman *et al.* 1972).

The fall in blood selenium (see Fig. 1) was rapid compared with that observed in mature ewes transferred from normal pastures to selenium-low pastures in the same area from which feed used in these experiments was harvested (Godwin *et al.* 1970). The level of 0·02 μg/ml reached in animals not slaughtered until 18 weeks confirms the observations of the New Zealand workers (Hartley 1967) that sheep suffering from

selenium-responsive disease have blood levels of selenium as low as 0·02 μg/ml. However, WMD of a quite severe kind was encountered in these experiments in animals having blood selenium levels of 0·043 and even 0·067 μg/ml at slaughter. It may be significant that the only animal to die with WMD had a blood selenium level of 0·022 μg/ml.

Daily treadmill exercise, whilst delaying the onset of muscular dystrophy (Godwin 1972), appeared not to alter the rate of decline in blood selenium levels, nor was there any apparent effect on mitochondrial behaviour. However, since the criterion for slaughter was some degree of observable dystrophic change this latter observation particularly was to be expected. The beneficial effect of exercise led to ECG traces being virtually normal in the exercised group throughout the experiment. The change in ECG pattern is a useful parameter in this type of experiment. A normal pattern may be interpreted in a misleading way, but the presence of an abnormal record is a useful indication that a terminal stage has been reached.

Deposition of calcium in muscle tissue has been recognized in descriptive accounts of WMD for some time (Muth 1955). To some extent its distribution has been studied in lambs born to ewes fed dystrophogenic feed (Schubert *et al.* 1961), and the effectiveness of selenium given as sodium selenite to the ewe in preventing calcium retention in lamb muscle has been noted. The present experiments show that calcium retention in muscle and mitochondria was prevented by the administration of selenium, either as an intraruminal pellet or as a regular oral drench of sodium selenite. There appears to be an associated increased excretion of calcium in the urine. This would seem to be present during the early period of ingestion of the dystrophogenic feed. It may therefore be legitimate to look upon the deposition of calcium in the dystrophic animal as related more directly to circulating levels of selenium rather than as the end result of dystrophic change in the muscle.

Schubert *et al.* (1961) noted that a wide range in calcium content of skeletal muscle among WMD-afflicted lambs occurred, so much so that it '. . . . precludes all but individual comparison to normal values'. They remarked on the relative constancy of magnesium values, 'irrespective of status with regard to WMD'. The present results, which show a difference in calcium in both heart and skeletal muscle ($P < 0·01$ in each case), emphasizes the effectiveness of the experimental approach. Differences in magnesium levels ($P < 0·01$) in skeletal muscle in the dystrophic animal have not been reported previously. Skeletal muscle mitochondria showed lowered respiration rates when pyruvate, palmitoyl-DL-carnitine and acetyl-DL-carnitine were used as substrates. These lowered rates seem to be associated with an increase in tissue and mitochondrial calcium, although the increase in skeletal muscle calcium was not quite significant.

There are two observations that may be pertinent. In the first place the range of values for skeletal muscle mitochondrial calcium was wide, and this could account for the lack of a significant difference statistically. This becomes apparent if the median values are given: mitochondrial calcium levels ranged from 2·28 to 10·09 μ-equiv/100 mg mitochondrial protein in dystrophic muscle (median value 5·27) and from 2·17 to 4·50 μ-equiv/100 mg mitochondrial protein in selenium-protected muscle (median value 2·84).

In the second place preliminary observations suggest that skeletal muscle mitochondria are much more readily uncoupled by the addition of calcium ions *in vitro*

than are heart mitochondria. With tissues from normal lambs it was necessary to raise the concentration of calcium to 6–8 m-equiv/l to cause uncoupling of heart mitochondria, but an increase of only 1 m-equiv/l was needed to induce uncoupling in skeletal muscle mitochondria. However, uncoupling of respiration in isolated mitochondria is associated with a rise in rate, hence the respiratory inhibition seen in these studies, characteristic of the dystrophic mitochondria, is not likely to be due simply to raised calcium levels.

Respiratory control ratios (RCRs), defined as (rate of oxygen utilization in state 3) ÷ (rate of oxygen utilization in state 4), are regarded as a rigorous test for normal mitochondrial function (Lehninger 1964). The RCR has already been shown to be the rate of respiration in the presence and absence of ADP. Respiratory inhibition invalidates RCR measurements and when ADP addition does not stimulate respiration then the resulting RCR values approach 1·0. The RCR values for skeletal muscle mitochondria from dystrophic (unsupplemented) lambs were 1·0 with either palmitoyl- or acetyl-DL-carnitine as substrate. However, in selenium-supplemented lambs values ranged from 1·4 to 10·0 (median 2·25) and from 1·0 to 4·5 (median 1·85) respectively; i.e. the values, although spread over a wide range, were all $>1\cdot0$.

The behaviour of isolated mitochondria in hereditary muscular dystrophy, both in the human (Lin *et al.* 1972) and in the experimental animal (Lochner and Brink 1967; Jacobson *et al.* 1970), has been studied. Comparable studies have not, so far, been made in nutritional muscular dystrophy, though a preliminary report of the present experiments has appeared (Godwin 1973). Defects in mitochondrial function that have been observed in hereditary muscular dystrophy have included reduced oxidation rate of fatty acids in both human (Lin *et al.* 1972) and mouse (Lin *et al.* 1970), decreased respiration rates and respiratory control ratios in cardiomyopathic hamsters (Wrogemann *et al.* 1972), and uncoupling of oxidative phosphorylation of skeletal muscle mitochondria of dystrophic hamsters (Wrogemann *et al.* 1970). The authors of this last paper suggested that the calcium levels, which were abnormally high, could account for the uncoupling observed.

Two papers that have appeared recently have a bearing on our findings. Wrogemann *et al.* (1973) have suggested that a defect in oxidative phosphorylation in mitochondria from genetically dystrophic hamsters can be partly explained by the loss of pyridine nucleotides, and is associated with high calcium levels in necrotic areas of dystrophic muscle. NADH-linked substrates exhibited impaired respiration and a lack of state 3 to state 4 transition. Levels of NAD^+ were low in dystrophic mitochondria but addition of NAD^+ did not restore respiratory control, indicating a complex abnormality.

In the studies reported in the present paper the normal osmotic behaviour of dystrophic mitochondria (Fig. 3) would argue against leakiness of the mitochondrial membranes, with consequent loss of pyridine nucleotides. The major effect found in these studies is respiratory inhibition. Uncoupling may very well occur because of excess calcium but this has not been demonstrated. Respiratory inhibition alone will account for respiratory control values of 1·0 and is itself sufficient reason to cause tissue damage.

Whanger (1973) has shown that skeletal muscle slices from dystrophic lambs convert glutamate to carbon dioxide at a slower rate than do slices from selenium-supplemented lambs. No differences were apparent with succinate or pyruvate.

The present study is important because of the similarity with comparable studies in hereditary muscular dystrophy. It seems likely that the biochemical lesion or lesions in the nutritional dystrophic condition, at the level of the isolated mitochondria, are similar to the hereditary type. This has been shown with respect to the intact body since exercise taken regularly by lambs fed dystrophogenic fodder had a moderating effect on the development of WMD (Godwin 1972). Those results were discussed in relation to the effectiveness of an exercise program in certain types of hereditary muscle disease reported clinically (Vignos and Watkins 1966).

Acknowledgments

Grateful acknowledgment is made to Mr G. L. Langsford for analyses of samples for calcium, magnesium, sodium and potassium, Mr R. A. Buckley for selenium analyses, and Mr E. J. Partick for technical assistance.

We thank Mr W. B. Hall, Division of Mathematical Statistics, CSIRO, Adelaide, for statistical treatment of results.

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Manuscript received 2 May 1974