Biochemical Pharmacology, Vol. 20, pp. 943–953. Pergamon Press, 1971. Printed in Great Britain

RAPID MITOCHONDRIAL ENLARGEMENT IN MUSCLE AS A RESPONSE TO TRIAMCINOLONE ACETONIDE AND ITS RELATIONSHIP TO THE RIBOSOMAL DEFECT

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(*Received* 20 *July* 1970; *accepted* 8 *September* 1970)

Abstract—A study of the morphology of the predominently white *m. vastus lateralis* of the male rat (250 g) showed that 2 hr after treating the animals with a high dose (20 mg/kg) of triamcinolone acetonide and 12 hr after a low dose (0·2 mg/kg) there was pronounced enlargement and proliferation of the mitochondria in a zone adjacent to the subsarcolemmal zone. There was, in addition, some destruction of mitochondria in deeper zones and some evidence of fragile membranes in the enlarged mitochondria.

The mitochondrial enlargement has been quantified with the use of a modified Altmann stain and time and dose responses to triamcinolone acetonide constructed.

Methandienone, (17α-methyl-Δ^1-dehydrotestosterone) was shown to block the mitochondrial enlargement completely although ribosomes from pooled *m. vastus lateralis*, *m. vastus medialis* and *m. gluteus medius* still partially retained a defect which significantly reduced their ability to incorporate amino acids into protein *in vitro*. Testosterone had virtually the opposite effect from methandienone at the same dose levels but its ability to allow ribosomes to function normally was to some extent dependent on the age of the animals.

The above finding taken together with a disparity in the dose–response curves of mitochondrial enlargement and the ribosomal defect after triamcinolone acetonide treatment supports the view that these phenomena do not have a common cause and must be independent responses of the muscle cell to the action of the steroid.

TRIAMCINOLONE, either uncombined or as its diacetate, has frequently been used to produce steroid myopathy in experimental animals as a model for this condition in humans. These animal studies have generally involved the daily administration of the glucocorticoids for periods ranging from 5 days in rats[1] to 3 weeks in rabbits[2] or 5 weeks in dogs.[3]

While these models are of value for light and electron microscopic comparison with human myopathy they can contribute little to the understanding of the biochemical mechanisms responsible for the myopathic state since only the long-term effects of steroid administration are seen. For this reason we have mainly studied changes in various biochemical parameters following a single dose of triamcinolone acetonide.[4, 5]

The most pronounced morphological changes seen in the long-term experiments are those relating to the mitochondria.[1] Proliferation of enlarged organelles occurs in regions where white fibres predominate and in some areas there is considerable loss of interfibrillar mitochondria.[1, 6]

In the work described here it is established that morphological changes of this type are also a feature of acute experiments with single doses of triamcinolone acetonide. Moreover, evidence is produced which is consistent with the idea that mitochondrial enlargement and the previously described ribosomal defect[4, 5] are independent phenomena. In order to undertake the investigation a quantitative procedure has been

devised for counting the number of enlarged mitochondria in samples of skeletal muscle. Use has also been made of the differential blocking of triamcinolone acetonide with testosterone and methandienone (17α-methyl-Δ^1-dehydrotestosterone).*

MATERIALS AND METHODS

Radioactive compounds. L-[U-^{14}C] Leucine (specific radioactivity 311–344 m/m-mole) was obtained from The Radiochemical Centre, Amersham, Bucks, U.K.

Chemicals and enzymes

Potassium phosphoenolpyruvate and pyruvate kinase were obtained from the Boehringer Corp. (London) Ltd., London, W.5, U.K. The dipotassium salt of ATP, the sodium salt of GTP and tris were obtained from Sigma (London) Chemical Co. Ltd., London, S.W.6, U.K. Sodium deoxycholate was Mann Assayed from Mann Research Laboratories Inc., New York, N.Y., U.S.A., and Lubrol W (cetylalcohol-polyoxyethylene condensate) was purchased from Imperial Chemical Industries Ltd., Manchester, U.K. Triamcinolone acetonide was purchased from E.R. Squibb and Sons Ltd., Speke, Liverpool, U.K. Histological stains were obtained from Hopkins and Williams Ltd. Special embedding medium for electron microscopy was obtained from Taab Laboratories Ltd., Emmer Green, Reading, U.K.

Administration of triamcinolone acetonide, testosterone and methandienone. These substances were administered by intraperitoneal injection as fine suspensions in 0·9% NaCl. For experiments involving doses of 0·2, 1, 5 and 20 mg/kg, suspensions containing 0·1, 0·5, 2·5 and 10 mg/ml were used respectively.

Animals. White Wistar strain male albino rats (250 g) were used. These were either bred in these laboratories from stock originally obtained from Scientific Products Farm, Ash, Canterbury, Kent, U.K. (CIBA rats in Results and Discussion) or obtained directly from Carworth Europe, Alconbury, Huntingdon, U.K. (CFHB rats in Results and Discussion).

Preparation of ribosomes from skeletal muscle. The method used was derived through modification of procedures previously published.[4, 5, 7] Pooled *m. gluteus medius, m. vastus lateralis* and *m. vastus medialis* from one animal were used. The total weight of these was about 4 g. After dissecting out the muscles and the removal of fat and connective tissue, all operations were carried out at 4°.

The trimmed muscles were placed in medium (20 ml) containing sucrose (0·15 M), tris–HCl buffer (0·1 M, pH 7·4 at 37°), KCl (0·185 M) and $MgCl_2$ (0·009 M). After chopping the muscle with scissors, homogenisation was carried out using the Ultra Turrax rotating at 10,000 rev/min. The speed was controlled with a variable resistance. Homogenisation was continued for 1 min with 20 passes of the probe through the suspension of broken tissue which was then centrifuged at 2000 *g* for 15 min to remove debris and nuclei. An aliquot (6·5 ml) of the supernatant was pipetted into a beaker and stirred with a magnetic stirrer during the addition of 2·0 ml of a solution containing KCl (2 M) and $MgCl_2$ (0·01 M), 1 ml of 10% (w/v) sodium deoxycholate and 0·5 ml of 10% (w/v) Lubrol. The resulting suspension was centrifuged at 20,000 *g* for 15 min. An aliquot (5·0 ml) of the supernatant was layered over medium (2 ml) containing sucrose (0·3 M), tris–HCl buffer (0·035 M), KCl (0·6 M) and $MgCl_2$ (0·009 M) and centrifuged for 90 min at 156,000 *g* in an MSE Superspeed 50 centrifuge using the

* Dianabol.®

10 × 10 angle rotor. The resulting pellet of ribosomes was washed superficially with medium containing tris–HCl buffer (0·035 M), KCl (0·1 M) and $MgCl_2$ (0·009 M), transferred to a small Teflon/glass Potter–Elvehjem homogeniser with 2 × 0·5 ml of the same medium and resuspended by homogenisation. Ten passes were made with the pestle (clearance 0·004 in.) rotating at 800 rev/min.

Incubation of ribosomes. This was carried out using diluted liver cell sap and 0·1 ml of ribosome suspension according to the method previously described.[4]

Direct visual estimation of enlarged or aggregated muscle mitochondria. One intact *m. vastus lateralis* from each animal was fixed in Helly's fluid for 48 hr and then transferred to a 3% (w/v) solution of potassium dichromate which was renewed daily for 5 days. They were washed thoroughly with water and after taking the samples through ascending grades of alcohol and clarifying in xylene they were impregnated with paraffin wax. Four sections (4 μm thick) were cut from each muscle sample at 24 μm intervals and were fixed to slides with an albumin–gelatin adhesive.

The staining procedure was a modification of the description of the Altmann procedure.[8] The sections were dewaxed in xylene and taken through descending grades of alcohol to water. Lugol's iodine was then poured on to the slides and they were allowed to stand for 5 min. The iodine was washed off with water, followed by 5% (w/v) sodium thiosulphate solution. The sections were then taken through ascending grades of alcohol, flooded with aniline–fuchsin made in freshly distilled aniline, gently heated by directly flaming the slides and allowed to stand for 5 min. The aniline–fuchsin was washed off with water and two differentiations were carried out in picric acid. For the first differentiations the picric acid solution contained saturated ethanolic picric acid–20% ethanol (1:4, v/v) and for the second differentiaton saturated ethanolic picric acid–20% ethanol (1:7, v/v) was used. The differentiation was controlled microscopically and usually took 15–20 min.

Photography and counting of mitochondria. Each slide was photographed (magnification × 200), with a superimposed scale, using Polaroid roll film and an MP3 Polaroid camera mounted on a Zeiss microscope. Four photographs, each representing 0·6 mm^2 of tissue, were taken per slide. The number of single mitochondria or aggregates greater than 15 μm in length were counted on a total of 16 photographs from each sample of muscle.

Electron microscopy of the vastus lateralis muscle. The following fixation techniques were used:

(a) The whole muscle was removed and a transverse slice was cut from the middle. This was cut into small pieces along the length of the fibres and fixed in 3% (w/v) glutaraldehyde in 0·1 M cacodylate buffer (pH 7·2) for 3–5 hr at 4°. After thorough washing in buffer, it was then post-fixed in 1% (w/v) OsO_4 (Millonig's) for 1·5 hr at 4° before dehydration. This will be referred to in the remainder of the text as double fixation.

(b) The muscle was treated as in (a) except that the glutaraldehyde fixative was made 2 mM with respect to $CaCl_2$.

(c) The muscle, after removal from the animal, was stretched by tying it to wooden sticks and immersed in ice-cold Millonig's 1% (w/v) OsO_4 for 2 hr. Small pieces of blackened fibres were then removed from the middle region of the muscle for further processing.

Subsequently all samples were dehydrated through a series of graded ethanols and

embedded in special embedding medium via propylene oxide. Sections of the polymerised material were cut on a Porter Blum MT-I microtome using diamond knives and were stained with 5% (w/v) uranyl acetate for 5 min and Reynold's lead citrate for 2 min. They were viewed in an AEI EM6 B microscope at 60 kV.

RESULTS

Normal mitochondria. The tissue taken for electron microscopical examination was the *m. vastus lateralis.* This muscle is composed predominantly of white fibres and is therefore of the fast reacting type. In such a muscle, cellular ATP levels are maintained to a considerable extent by anaerobic glycolysis and mitochondria are fewer in number than in a so-called red muscle. The mitochondria which are present cover a wide range of sizes and appear in longitudinal sections as round or slightly ovoid structures (Figs. 1 and 5). They are distributed fairly evenly between the fibrils but small bundles, generally of organelles somewhat larger than the mean, appear under the sarcolemma.

When either double fixation or OsO_4 on its own is used for skeletal muscle, we have found that basically the muscle morphology appears very similar by both methods. The disposition of the cristae in the mitochondria appears the same and the appearance of the fibrils and of the longitudinal (L) system of tubules is also very similar. The only essential differences are that less background material or cell matrix is retained after OsO_4 fixation and the internal structure of the transverse (T) system of tubules is vesiculated. These differences are well described in the literature and would not appear to have any bearing on the differences in mitochondrial structure seen after double or OsO_4 fixation without Ca^{2+} added or after triamcinolone acetonide treatment. Our criterion of good fixation of the tissue was taken as lack of swelling in the T-system and close apposition of the nuclear membranes.

Enlarged and fragile mitochondria. Under the action of a high dose (20 mg/kg) of triamcinolone acetonide morphological changes in the *m. vastus lateralis* were seen as early as 2 hr after drug administration. Some enlarged mitochondria were already apparent, and after 12 hr there were a large number present (Fig. 7). The enlarged mitochondria were on the side of the subsarcolemmal mitochondrial layer distal from the sarcolemma principally in the perinuclear and pericapillary regions. A narrow band of maximal enlargement extended a short way into the fibre and inside the band another was discernible where some mitochondria, frequently in association with a lipid-like droplet, were collapsing (Fig. 6). Extensive sheets of muscle with no mitochondria at all could be found quite frequently. The subsarcolemmal mitochondria showed no response to the steroid with the exception of a few which developed myelin-like figures as a result of double-fixation (see below).

Different fixation techniques indicated some differences in mitochondrial integrity. When the muscle was double-fixed the presence of whorls and myelin figures suggested focal necrosis of some mitochondria in the zone of maximal enlargement and of a few elsewhere (Figs. 2 and 3). Similar deductions were drawn by Tice *et al.*[1] from long-term studies. No evidence of this was seen in control material. The addition of $CaCl_2$ (2 mM) to the primary glutaraldehyde fixative reduced the focal necrosis to negligible proportions (Fig. 4), although some swelling of the mitochondria was produced. Under conditions of either double fixation or osmium fixation on its own the enlarged mitochondria were definitely not swollen and were therefore not considered to be so *in vivo.*

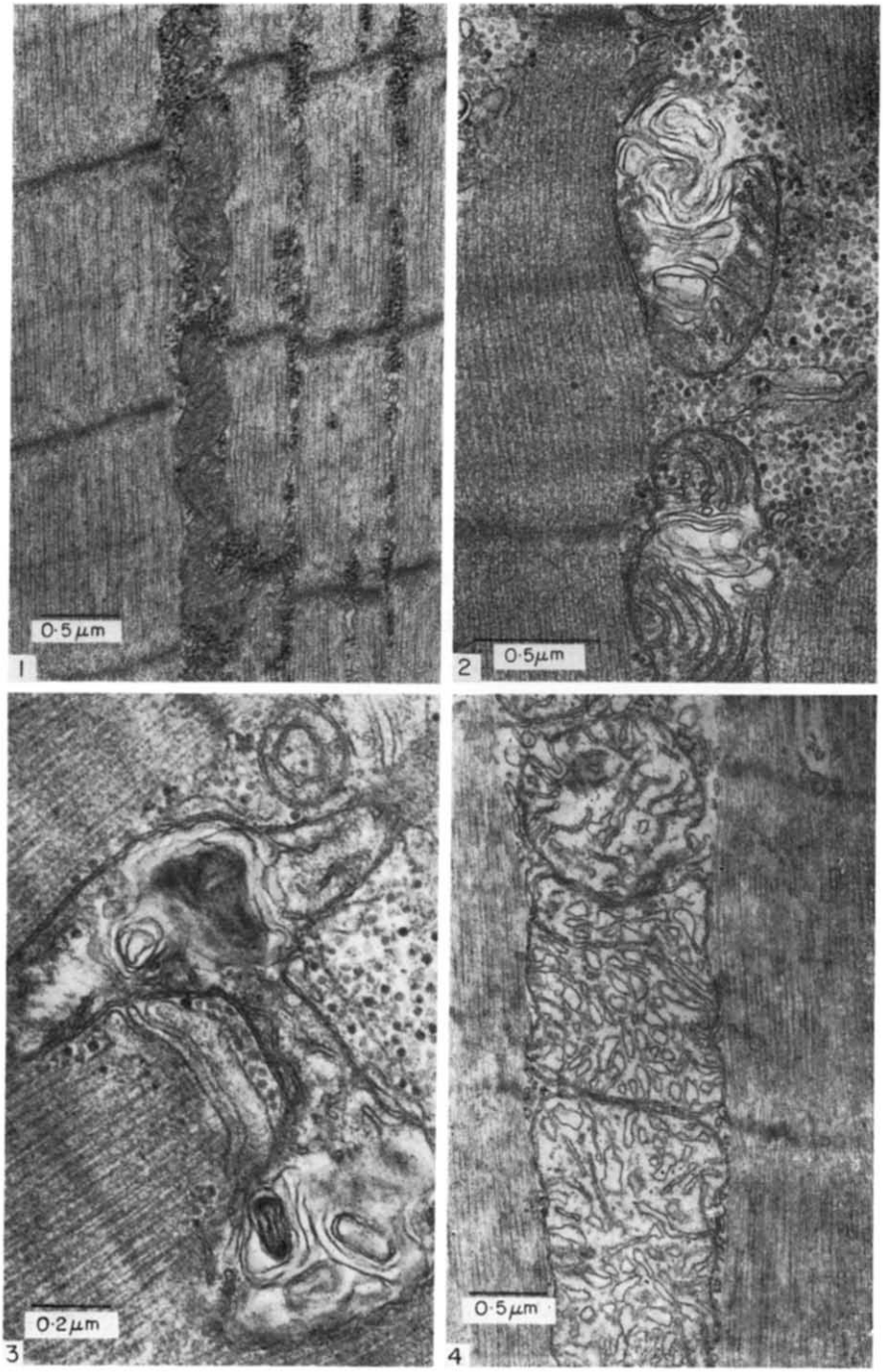

FIG. 2. *M. vastus lateralis* of a rat treated with triamcinolone acetonide 20 mg/kg, 12 hr prior to sacrifice. Focal necrosis of enlarged mitochondria and intact T-system are seen. (Mag. × 29,500.)

FIG. 3. Focal necrosis of a mitochondrion after triamcinolone acetonide treatment as in Fig. 2. Again the T-system is very well preserved. (Mag. × 48,000.)

FIG. 4. Double fixation of treated tissue with $CaCl_2$ added to the glutaraldehyde to give 2 mM final concentration. The enlarged mitochondria are slightly swollen. (Mag. × 21,600.)

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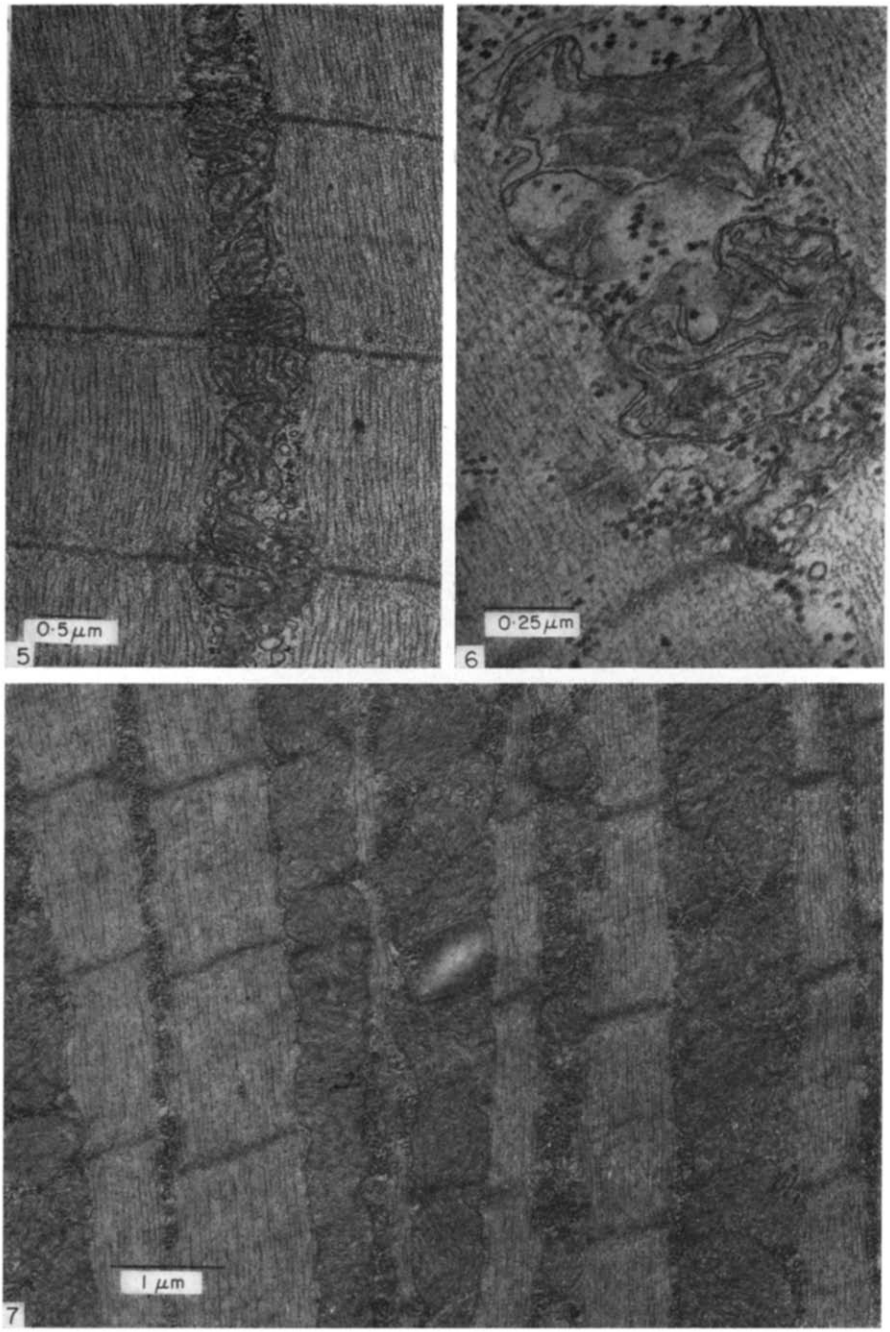

FIG. 5. Normal *m. vastus lateralis* after OsO_4 fixation. (Mag. × 18,650.)

FIG. 6. *M. vastus lateralis*, OsO_4 fixation. This illustrates an area where the mitochondria were seen to be collapsing 12 hr after triamcinolone acetonide administration. (Mag. × 34,730.)

FIG. 7. Zone of maximal mitochondrial enlargement 12 hr after treatment with triamcinolone acetonide. OsO_4 fixation. (Mag. × 16,600.)

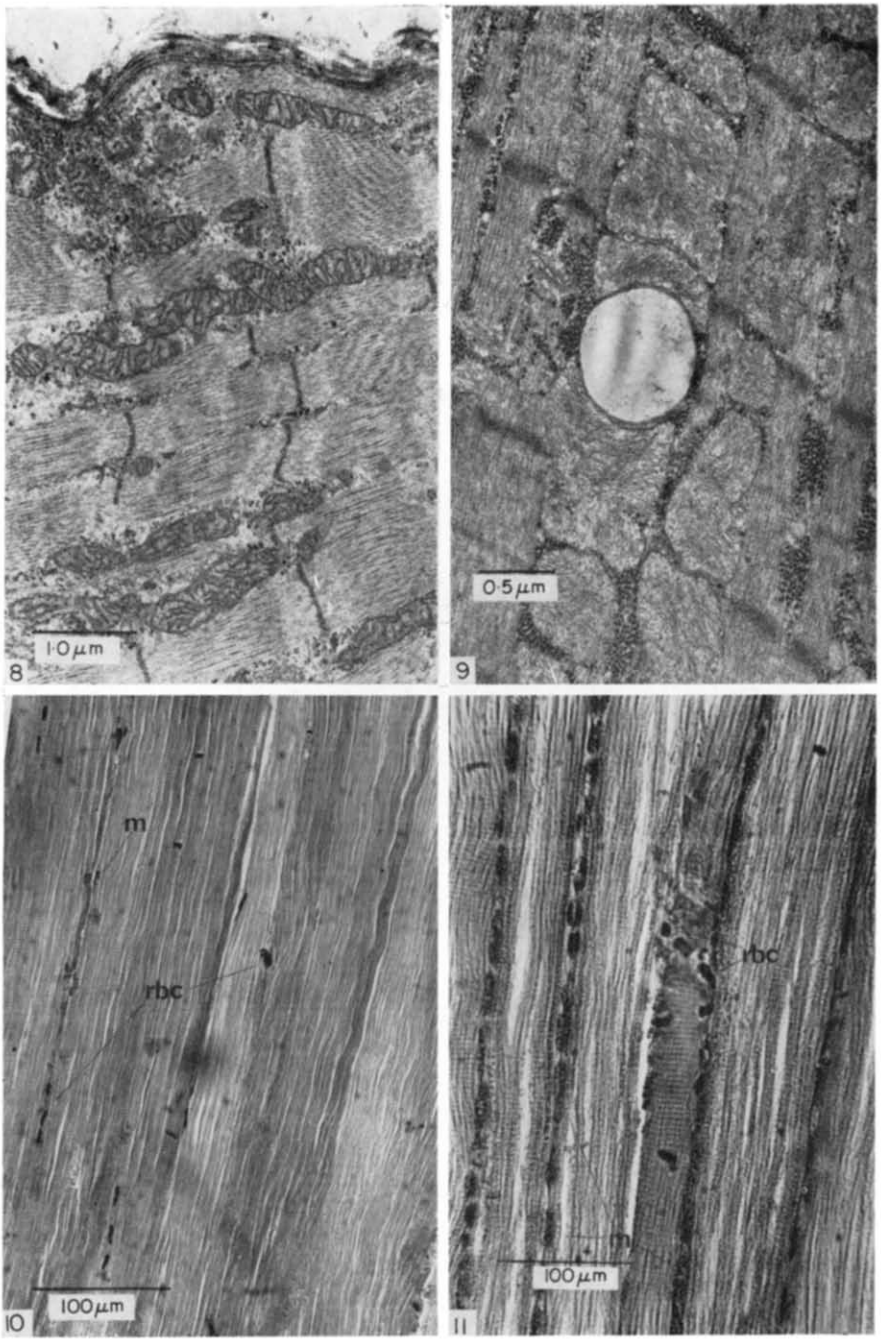

FIG. 8. Mitochondrial structure after three treatments with testosterone 5 mg/kg/day. The mitochondria appear slightly elongated and have fewer cristae per unit mitochondrial volume than normal, OsO_4 fixation. (Mag. × 16,500.)

FIG. 9. Mitochondrial structure when pretreatment with 5 mg/kg testosterone was followed by 20 mg/kg triamcinolone acetonide 12 hr prior to sacrifice. The membranes are ill-defined and many of the cristae appear to be free-floating in the organelles. OsO_4 fixation. (Mag. × 25,000.)

FIG. 10. Normal *m. vastus lateralis* after the modified Altmann staining technique. (M = mitochondria, RBC = red blood cells.) Few mitochondria are visible. (Mag. × 160.)

FIG. 11. Triamcinolone acetonide treatment. The areas staining with the modified Altmann stain have increased considerably 12 hr after giving the drug. A slight graininess is visible in the rod-shaped structures (M) but not in the red blood cells (RBC). (Mag. × 160.)

The quantitation of mitochondrial enlargement. In Fig. 10 a section of the *m. vastus lateralis* from a normal animal is shown, stained by the modified Altmann technique. Most of the structures taking up stain are red blood cells and mitochondria are only occasionally visible. At the higher magnification used for the counting procedure the biconcavity of the red blood cells became clearly visible and hence they were not confused with stained mitochondria.

After triamcinolone acetonide (12 hr, 20 mg/kg) there was a considerable increase in the amount of stain taken up by the tissue and this extra stain was concentrated in rod-like structures lying in longitudinal rows (Fig. 11). In transverse sections these structures appeared to lie on the outside of the fibres, apparently in the sub-sarcolemmal regions. However, the subsarcolemmal bundles of mitochondria do not take up the stain to any appreciable extent and moreover they appear, by electron microscopy, to be unaffected by the steroid. Therefore, it seems reasonable to suppose that under drug treatment it is the band of enlarged mitochondria beneath the subsarcolemmal layer but still towards the periphery of the fibre which is staining by the Altmann procedure. However, the appearance of the stained tissue suggests that individual organelles are not distinguished and that groups of mitochondria give rise to the stained rod-shaped units. These units we have called aggregates. A preliminary scan of muscle sections from untreated animals showed that by far the majority of the stained mitochondrial aggregates were less than 15 μm in length. We therefore considered that a count of aggregates >15 μm in length would correlate with the number of enlarged mitochondria in tissue from steroid-treated animals.

Comparison between the development of mitochondrial aggregates and the ribosomal defect at various times after the administration of triamcinolone acetonide. It can be seen from Table 1 that the number of mitochondrial aggregates increased over the first 12 hr of drug action and decreased to levels approaching normal 24 hr after a single injection of triamcinolone acetonide. It is not known whether the maximum effect occurred at the 12 hr point or later, but a maximum at 12 hr would appear

TABLE 1. THE NUMBER OF MITOCHONDRIAL AGGREGATES > 15 μm IN LENGTH AT VARIOUS TIMES AFTER A SINGLE INJECTION OF TRIAMCINOLONE ACETONIDE (20 mg/kg) OR SALINE TO 10–12 WEEK OLD CIBA RATS

Time (hr)	Treatment	Aggregates/0·6 mm² tissue (± SEM)
0	None	25·8 ± 0·9
2	Saline	23·90 ± 1·0
2	Triamcinolone acetonide	59·45 ± 5·2
4	Triamcinolone acetonide	83·20 ± 7·4
6	Triamcinolone acetonide	93·8 ± 7·5
12	Triamcinolone acetonide	104·3 ± 8·9
24	Triamcinolone acetonide	35·95 ± 1·2

Each group consisted of five animals.

likely. At 2 hr there was a highly significant rise in the number of large aggregates which was confirmed under the electron microscope. No mitochondrial aggregates were seen 2 hr after giving saline, hence they clearly appear as a result of drug administration and not as a result of trauma or injection of the saline vehicle.

By comparison, ribosomes, when isolated from the same strain of animal, do not start to function abnormally *in vitro* until 4–5 hr after the administration of the drug.[5] The possibility therefore arises that the ribosomal defect is not a direct effect of the drug but has its origin in earlier changes in the cell. Of these changes, mitochondrial hypertrophy is an obvious candidate.

Relationship between numbers of mitochondrial aggregates and the extent of the ribosomal defect at different doses of triamcinolone acetonide. At 0·2 mg/kg of triamcinolone acetonide there was a significant increase ($P < 0{\cdot}001$) in the number of mitochondrial aggregates (Table 2). This increase continued up to a dose level of 20 mg/kg although there was no significant change between 0·2 mg/kg and 5 mg/kg.

TABLE 2. THE NUMBER OF MITOCHONDRIAL AGGREGATES > 15 μm IN LENGTH 12 hr AFTER THE ADMINISTRATION OF TRIAMCINOLONE ACETONIDE TO 6 WEEK OLD CFHB RATS

Dose (mg/kg)	Aggregates/0·6 mm² of tissue ± SEM
0	25·95 ± 1·71
0·2	50·9 ± 2·85
1·0	50·7 ± 1·54
5·0	63·1 ± 6·19
20·0	128 ± 6·19

Each group consisted of five animals.

TABLE 3. THE INCORPORATION *in vitro* OF [U-^{14}C] LEUCINE INTO PROTEIN BY MUSCLE RIBOSOME PREPARATIONS 12 hr AFTER SINGLE INJECTIONS OF TRIAMCINOLONE ACETONIDE TO INTACT RATS

Dose (mg/kg)		Disintegrations/min/μg ribosomal RNA ± SEM	Per cent of control	P
None	(5)	159·4 ± 2·00	100	< 0·01
20	(5)	126·3 ± 2·72	79·0	
None	(5)	191·3 ± 4·44	100	< 0·001
5	(4)	150·6 ± 2·70	78·7	
None	(5)	251·1 ± 7·37	100	< 0·05
1	(5)	194·2 ± 4·80	77·3	
None	(5)	153·5 ± 3·54	100	< 0·05
0·2	(5)	143·1 + 1·53	93	

The number of individual animals from which ribosomes were isolated are shown in parentheses.

Mitochondrial enlargement was considered maximal at 20 mg/kg because at higher dose levels the tissue lost some of its organisation and quantitative appraisal of mitochondrial numbers became impossible.

The dose response relating to the ribosomal defect after triamcinolone acetonide is shown in Table 3. A small, but significant effect, occurred at 0·2 mg/kg but the maximum effect was already seen at 1 mg/kg.

When the two dose responses are compared using a log dose scale (Fig. 12) it is seen that they have entirely different shapes. This result suggests that the two phenomena are likely to be independent.

Effect of methandienone or testosterone on the formation of mitochondrial aggregates by triamcinolone acetonide. When animals were pretreated for 3 days with methandienone (5 mg/kg/day) and then given triamcinolone acetonide (20 mg/kg) no mito-

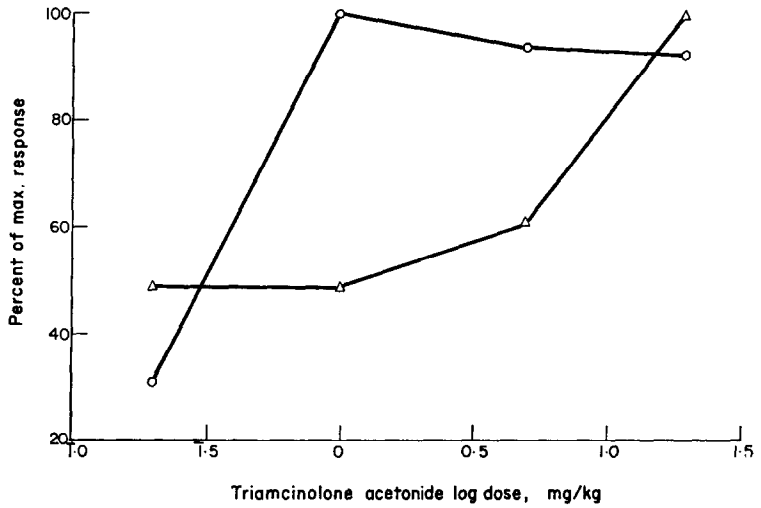

FIG. 12. Dose–response curves of mitochondrial enlargement (–△–△–) and the ribosomal defect (–O–O–) in muscle 12hr after the administration of triamcinolone acetonide.

chondrial enlargement was seen when samples of *m. vastus lateralis* were examined under the electron microscope 12 hr later. This was confirmed quantitatively by showing that the mean number of stained mitochondrial aggregates in 0·6 mm^2 of tissue from five different animals was 24·0 ± 0·9. A similar result (26·8 ± 1·34) was obtained when methandienone (5 mg/kg) was given simultaneously with triancinole acetonide (5 mg/kg) 12 hr before killing the animals. These figures do not differ significantly from the control value. Similar experiments conducted with testosterone were difficult to interpret quantitatively by the staining procedure because of a granularity which masked the outlines of the enlarged stained mitochondria and made counting difficult. However, very large numbers of enlarged mitochondria were seen under the electron microscope (Fig. 9). This was considered adequate evidence that they were present under this drug treatment. These enlarged mitochondria showed considerable differences from those seen after triamcinolone acetonide alone in that the membranes were diffuse and many of the cristae appeared to be free in the

matrix. This morphological difference may be connected with the difficulty experienced in the quantitative staining technique. Neither testosterone nor methandienone alone when administered for 3 days at 5 mg/kg had any effect on the number of mitochondrial aggregates and methandienone did not alter their appearance under the electron microscope. Testosterone, however, had an effect on the mitochondria which is shown in Fig. 8. There appeared to be a reduction in the number of cristae per unit volume of each mitochondrion and the membranes appeared extremely well defined compared with their appearance when triamcinolone acetonide was given as well (Fig. 9).

Effect of testosterone or methandienone on the ribosomal defect produced by triamcinolone. When CIBA rats (250 g, 10–12 weeks old) were given testosterone (5 mg/kg) for 3 days followed by a single injection of triamcinolone acetonide (20 mg/kg) and examined individually, their skeletal muscle ribosomes had only 84 per cent ($P < 0{\cdot}01$) of the *in vitro* activity of control ribosomes. The mean activity of ribosomes from this strain and age of animal treated with triamcinolone acetonide (20 mg/kg) alone, 12 hr before killing, was 76 per cent of control (mean of 11 experiments). There was therefore a considerable effect produced by triamcinolone acetonide even after testosterone pretreatment, a result which confirmed in single animals one previously obtained on pooled muscle from five animals.[9] However, when CFHB rats (250 g, 6 weeks old), were given the same drug treatment, the effect of triamcinolone acetonide on the activity of isolated ribosomes was virtually abolished (Table 4). This

TABLE 4. THE INCORPORATION *in vitro* OF [U ^{14}C] LEUCINE INTO PROTEIN BY MUSCLE RIBOSOME PREPARATIONS 12 hr AFTER THE ADMINISTRATION OF TRIAMCINOLONE ACETONIDE (20 mg/kg)

Treatment	Counts/min/μg RNA	Per cent control	P value
None	162·2 ± 1·3	100	<0·05
Triamcinolone acetonide + Testosterone	153·4 ± 3·2	94	
None	180·5 ± 2·9	100	<0·01
Triamcinolone acetonide + Methandienone	156·8 ± 4·4	87	

These animals had, in addition, three daily injections of either testosterone (5 mg/kg) or methandienone (5 mg/kg), the last of these injections being given 24 hr before killing. There were five animals in each group.

discrepancy between the two types of rat was probably due to different ages rather than different strains, since CFHB rats (10–12 weeks old, 375 g), also showed a similar lack of response to the blocking action of testosterone when it was administered prior to triamcinolone acetonide, the activity of muscle ribosomes remaining depressed at 81 per cent ($P < 0{\cdot}01$) of control level. When testosterone (5 mg/kg) and triamcinolone acetonide (5 mg/kg) were given simultaneously, the effect of the glucocorticoid on the ribosomal defect was completely blocked (Table 5). This type of blockade did not

depend on the age of the animals since ribosomes isolated from 375 g CFHB rats treated in a similar manner still retained 95 per cent ($P < 0.05$) of the activity of control ribosomes. When the dose of triamcinolone acetonide was raised to 20 mg/kg simultaneous administration of testosterone (5 mg/kg) had little effect on the ribosomal defect.

TABLE 5. THE INCORPORATION *in vitro* OF [U-^{14}C] LEUCINE INTO PROTEIN BY MUSCLE RIBOSOME PREPARATIONS 12 hr AFTER THE ADMINISTRATION OF DIFFERENT LEVELS OF TRIAMCINOLONE ACETONIDE TOGETHER WITH TESTOSTERONE (5 mg/kg) OR METHANDIENONE (5 mg/kg)

	Triamcinolone acetonide (mg/kg)	counts/min/μg RNA	Per cent control	P value
Testosterone	None	130·7 ± 1·2	100	>0·5
	5	132·3 ± 2·0	100	
	None	155·8 ± 6·7	100	<0·01
	20	126·7 ± 4·8	81	
Methandienone	None	145·1 ± 1·5	100	<0·01
	5	123·7 ± 3·1	85	
	None	163·0 ± 5·8	100	<0·001
	20	133·8 ± 4·5	82	

There were five animals in each group.

When methandienone was used as an agent to block the effect of triamcinolone acetonide (20 mg/kg or 5 mg/kg) the age and strain of the animals did not affect the outcome. Results with CFHB rats are given in Tables 4 and 5. It can be seen that the ribosomal defect was never completely abolished by methandienone when assayed in individual animals. This contrasts with the result obtained previously from a study in which tissue was pooled from a number of animals.[9]

Neither testosterone nor methandienone alone had any effect on the *in vitro* activity of isolated skeletal muscle ribosomes when given daily (5 mg/kg) for periods of up to 3 days.

DISCUSSION

The pattern in white muscle of mitochondrial enlargement and proliferation, together with some mitochondrial loss, was previously seen by Tice *et al.*[1] in experiments with female rats lasting 5–15 days, and involving the daily administration of triamcinolone acetate (1·5 mg/kg). The examination of animals in the period up to 5 days was not reported. From our experiments it is apparent that this pattern of changes may be an extremely rapid response to the steroid since we have seen it as early as 12 hr after a high dose (20 mg/kg) of triamcinolone acetonide. One important aspect of it, namely mitochondrial enlargement, was seen as early as 2 hr after this level of the drug and 12 hr after a lower dose (0·2 mg/kg).

The enlarged mitochondria produced by triamcinolone acetonide alone are not swollen and from their appearance after OsO_4 fixation there is every reason to believe that they are tightly coupled. However, the requirement for Ca^{2+} in glutaraldehyde

fixation to prevent focal necrosis of these mitochondria does suggest that some may possess defective membranes and therefore, until experimentally checked, their oxidative efficiency must remain in doubt. Of itself, mitochondrial enlargement of this type gives little information as to the functional state of the organelles. For example, enlarged mitochondria are seen in the muscle of both thyroidectomized and hyperthyroid animals and although in both cases isolated mitochondria were shown to be tightly coupled, the Q_{O_2} of organelles from thyroidectomized animals was considerably reduced whereas it was elevated in the mitochondrial population from hyperthyroid animals.[10]

The cause of the proliferation of enlarged mitochondria through steroid action is unknown. Under defined experimental conditions, the phenomenon has been seen in muscle, using hyper- and hypothyroid rats,[10] in the liver of rats deficient in essential fatty acids[11] and after 6 days cortisone treatment[12] and in the adrenal after hypophysectomy[13] or after the administration of the adrenal inhibitor, amino glutethimide.[14] Kimberg *et al.*[12] showed that the average mitochondrial volume throughout the liver increased fourfold but that there was a compensatory decrease in the number of mitochondria per cell so that the total volume occupied by the mitochondria in each cell remained unchanged. Racela *et al.*[14] showed in a long-term study that mitochondrial enlargement occurred after a period in which there was mitochondrial loss.

In our experiments with triamcinolone acetonide, mitochondrial enlargement in the outer region of the muscle cell may have been a compensation for loss of mitochondria in more central regions. Methandienone, through its ability to completely block mitochondrial enlargement, obviously provides a valuable tool for further investigation of the phenomenon.

The reproducibility of the ribosomal defect which occurs as a result of triamcinolone acetonide administration strongly suggests that it forms an intrinsic part of the myopathic action of the steroid. The reason for the ribosomal dysfunction, in molecular terms, is still unknown, but it appears that it may be an intrinsic property of the 80S particle, since the effect is not lost when the ribosomes are centrifuged through increasing sucrose concentrations.[5] Physiologically the sensitivity of the protein synthetic machinery to triamcinolone acetonide in young rats seems to be remarkably decreased by androgens since we have shown that the ribosomal defect does not occur when these animals are simultaneously treated with the glucocorticoid (5 mg/kg) and an equal amount of testosterone or when they are pretreated with testosterone (5 mg/kg) daily for 3 days and then given triamcinolone acetonide (20 mg/kg). This confirmed our other findings that castrated male rats of this age are much more sensitive to triamcinolone acetonide with respect to the ribosomal dysfunction than intact animals.[15] Nevertheless, this action of testosterone is rather variable. As already mentioned, age of the animals is an important factor and this may explain why testosterone was unable to affect the reduction in the *in vitro* activity of muscle ribosomes caused in rabbits by the long-term administration of cortisone.[4]

The enlargement of mitochondria and the ribosomal defect appear to be independent phenomena. These dose–response curves are quite different (Fig. 12) and it is possible to block mitochondrial enlargement with methandienone while the ribosomal defect remains unaffected. In contrast the ribosomal defect may be blocked with testosterone while mitochondrial enlargement persists. One possible mechanism for the reduced

activity of the ribosomes, namely that they come from areas of enlarged mitochondria and are affected by some metabolic consequence of this can therefore be ruled out. A number of other possibilities remain to be investigated.

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