The Effects of Catabolic and Anabolic Steroids on Amino Acid Incorporation by Skeletal-Muscle Ribosomes

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1. A method is described for the routine isolation of ribosomes from small quantities of skeletal muscle that have been homogenized with the Ultra-Turrax tissue disintegrator. 2. Ribosomes prepared by this method from rats receiving triamcinolone acetate or rabbits receiving cortisone acetate show a marked fall in their ability to incorporate amino acids when compared with ribosomes from control animals. 3. This fall in activity can be partially prevented in rats by pretreating the animals with an anabolic steroid, steroid 36644-Ba. 4. Testosterone (5mg./kg.) administered to rabbits in conjunction with cortisone acetate is not effective in maintaining ribosomal activity. However, steroid 36644-Ba at one-tenth of an equiandrogenic dose (0.05mg./kg.) is extremely effective. 5. The results with ribosomes isolated from rabbits support the concept that steroid 36644-Ba and possibly all anabolic steroids have an ability to counteract the catabolic action of corticosteroids that is greater than their androgenic activity would suggest.

It has been known for some years that androgens (anabolic steroids) are clinically useful for counteracting and controlling the undesirable effects of chronic corticosteroid therapy. The subject has been reviewed by Reifstein (1956). One of the undesirable side effects is muscle myopathy, which is an important component of the catabolic state induced by glucocorticoids. To investigate the biochemical events underlying the androgenic reversal of glucocorticoid-induced myopathy a system was required *in vitro* that would show a change in a measurable parameter after the administration of these compounds *in vivo*.

Muscle shows decreased ability to synthesize protein *in vitro* when isolated from animals receiving high doses of cortisol and cortisone (Manchester, Randle & Young, 1959; Wool & Weinshelbaum, 1959; Shimizu & Kaplan, 1964), and it was shown by White (1967) that the amino acid-incorporating ability of ribosomes (called in the present paper P ribosomes) isolated from the psoas muscle of rats fell after Δ^1 -9 α -fluorohydrocortisone 16 α ,17 α -acetonide (triamcinolone acetonide) had been given *in vivo*, and that pretreatment of the animals with the anabolic compound 17 β -hydroxy-17 α -methyl-androsta-1,4-dien-3-one (methandionone or methandrostenolone) enabled full ribosomal activity to be retained. In the present paper these results have been extended by the examination of another anabolic steroid (steroid 36644-Ba) composed of

17 β -hydroxy-7 α ,17 α -dimethyl-A-nor-B-homo-oestrane-3,6-dione (I) in equilibrium with its enol form (II).

One of the most important questions in the clinical use of anabolic steroids is whether their ability to antagonize the catabolic effects of glucocorticoids is due solely to their androgenicity. On the basis of the levator ani assay (Hershberger, Shipley & Meyer, 1953), which uses the castrated male rat, steroid 36644-Ba, administered subcutaneously, was 150–500 times more efficient than methyltestosterone in restoring the weight of the levator ani muscle to normal, but only 2.5–10 times more potent than methyltestosterone in restoring the weight of the seminal vesicles and ventral prostate (Desaules & Schär, 1967). Thus the compound can be said to have an anabolic to androgenic ratio between 15 and 200.

To see whether steroid 36644-Ba has an anti-catabolic to androgenic ratio in a similar range, experiments were carried out with ribosomes from rabbits that had been pretreated with cortisone acetate. A regimen for drug administration was used (Little, 1966) in which cortisone acetate was administered to rabbits for 2 weeks followed by a 2-week period in which the animals received cortisone acetate together with either testosterone or different amounts of steroid 36644-Ba. Under these conditions it was established that, when the two compounds were administered to different animals

(I), 17 β -hydroxy-7 α ,17 α -dimethyl-A-nor-B-homo-oestrane-3,6-dione; (II), 6,17 β -dihydroxy-7 α ,17 α -dimethyl-A-nor-B-homo-oestr-5-en-3-one.

in approximately equiandrogenic dosage, steroid 36644-Ba had a greater ability to restore ribosomal activity to normal than testosterone.

A simpler method for the preparation of muscle ribosomes than the one reported by Breuer, Davies & Florini (1964) was devised. The characteristics of the amino acid-incorporating activity of these ribosomes with regard to cell sap, energy and incubation times have been reported (White, 1967). In the present paper the preliminary work that led to the establishment of the simplified procedure is described.

Two methods for the disintegration of muscle were compared. The first method used a homogenizer essentially of the Potter type as modified by Aldridge, Emery & Street (1960), and the second used the Ultra-Turrax tissue disintegrator successfully used by Muscatello, Andersson-Cedergren, Azzone & Decken (1961) for preparation of fragments from muscle mitochondria, and by Widnell, Hamilton & Tata (1967) for preparation of rat heart nuclei.

Differential centrifugation of the muscle homogenates and examination of various fractions by electron microscopy showed that the contents of the subfractions varied considerably with the methods of homogenization, and that a much larger yield of ribosomes was obtained when the Ultra-Turrax was used in preference to the Perspex homogenizer. This was confirmed by direct comparison of ribosomal yields.

MATERIALS AND METHODS

Radioactive amino acid. L-[U-¹⁴C]Leucine (specific radioactivity 150–170 mc/m-mole) was obtained from The Radiochemical Centre (Amersham, Bucks.) as a sterile aqueous solution containing 2% ethanol (0.05 mc/ml.). This solution was used directly.

Chemicals and enzyme. Potassium phosphoenolpyruvate and pyruvate kinase were obtained from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany. The dipotassium salt of ATP, the sodium salt of GTP, tris and the ammonium salt of polyuridylic acid were products of the Sigma Chemical Co., St Louis, Mo., U.S.A. Sodium

deoxycholate was 'Mann Assayed' from Mann Research Laboratories Inc., New York, N.Y., U.S.A., and Lubrol W (cetyl alcohol polyoxyethylene condensate) was purchased from Imperial Chemical Industries Ltd., Manchester. All other components of various media were A.R. chemicals and all solutions were prepared in freshly glass-distilled water.

Cortisone acetate was obtained from British Drug Houses Ltd., Poole, Dorset, and triamcinolone acetonide from E. R. Squibb and Sons Ltd., Speke, Liverpool. Testosterone was obtained from Organon Laboratories Ltd., London, W.C. 2.

Animals. The rats used were Wistar-strain male albinos weighing 275–300g., bred in these Laboratories. New Zealand white rabbits were used.

Administration of steroids. These were injected intraperitoneally as saline suspensions.

Preparation of P ribosomes from psoas muscle. After the required muscles had been dissected out, subsequent operations were carried out at 4°. Psoas muscles removed from a group of rats were chilled in medium A containing 9mM-MgCl₂, 75mM-KCl, 35mM-tris-HCl buffer, pH 7.4 at 37°, and 0.15M-sucrose (Leon, Arrhenius & Hultin, 1962), and minced with a small precooled hand mincer. A portion of mince (4g.) was then homogenized in medium A with an Ultra-Turrax homogenizer (Janke und Kunkel Kg., Staufen i. Br., W. Germany). In this instrument the tissue is cut by means of a steel knife that rotates at high speed at the end of a stainless-steel tube. At 240v the speed of the motor is 24000 rev./min. For the present work 80v was supplied through a variable resistance. Homogenization was continued for 1min. with 20 passes of the probe through the muscle suspension, and then debris and nuclei were removed from the homogenate by centrifugation at 1000g for 10min. The supernatant (13ml.) was pipetted into a beaker and stirred with a magnetic stirrer during the addition of 4ml. of a solution containing 2.5M-KCl and 10mM-MgCl₂, 2ml. of a solution of 10% (w/v) sodium deoxycholate and 1ml. of 10% (w/v) Lubrol (Rendi & Hultin, 1960). The resulting suspension was centrifuged at 14000g for 20min., and the supernatant was then layered in two 10ml. portions over 5ml. of medium B containing 9mM-MgCl₂, 0.6M-KCl, 35mM-tris-HCl buffer, pH 7.4 at 37°, and 0.3M-sucrose, and centrifuged at 100000g for 2hr. in an MSE Superspeed 50 centrifuge with the 8 × 25ml. angle-head rotor. Clear pellets were obtained with slightly raised protein/RNA ratios ranging from 1.4:1 to 1.8:1, due probably to the presence of small quantities of mitochondrial fragments released by the addition of detergent to the 1000g supernatant. Ribosomes

prepared in this way were previously shown to require energy and cell sap for the incorporation of amino acids into protein (White, 1967). Linear incorporation continued for 30 min.

Preparation of *M* ribosomes from *psaos* muscle. Psoas muscles (12–16g.) from a control or drug-treated group of rats were minced, and 8g. was homogenized in two 4g. portions as described for P ribosomes. The homogenate was centrifuged at 14000g for 10 min., and microsomes were harvested by centrifuging the supernatant at 40000g for 30 min. The pellet was washed once in 10 ml. of medium A by resuspension and centrifugation, and then resuspended in 13 ml. of medium A. This suspension was then treated in the same way for the preparation of M ribosomes as the 1000g supernatant was for the preparation of P ribosomes.

Preparation of *P* ribosomes from *rectus femoris* muscle. Rectus femoris muscles from a single rabbit were minced and homogenized with the Ultra-Turrax homogenizer in 4-g. portions. P ribosomes were prepared in the same way as from rat *psaos* muscle.

Cell sap and pH5 fraction. Liver (10g.) from normal rabbits and rats was homogenized in 25 ml. of medium A in a glass homogenizer with a Teflon pestle. After centrifugation of the homogenate at 14000g for 6 min. the mitochondrial supernatant was centrifuged for 1 hr. at 100000g. Clear cell sap was siphoned off with a hypodermic syringe (Campbell & Kernot, 1962).

Incubation of ribosomes. Ribosomes were always incubated at 36° at two concentrations in duplicate tubes. Each incubation tube therefore contained, in a total volume of 0.5 ml., either 0.1 ml. (10–20 µg. of RNA) or 0.05 ml. of ribosomal suspension, MgCl₂ (5 µmoles), KCl (50 µmoles), pyruvate kinase (8 µg.), potassium phosphoenolpyruvate (0.5 µmole), ATP (0.5 µmole), GTP (0.1 µmole), sucrose (60 µmoles), tris-HCl buffer, pH 7.4 at 37° (13 µmoles), L-[U-¹⁴C]leucine (3.3 mµmoles, 0.5 µCi), and cell sap that had been diluted tenfold with medium A (0.3 ml.). Control tubes contained all except ribosomes.

Gradient-sedimentation analysis of ribosomes. Sucrose density gradients (20 ml.) were prepared from 15% (w/v) and 30% (w/v) sucrose in medium A by the method of Britten & Roberts (1960). Ribosomal suspensions (0.5 ml.) were layered on the gradients, which were then centrifuged for 2 hr. at 128000g in the 3 × 23 ml. MSE swing-out rotor.

A close-fitting Teflon cap, equipped with two thin stainless-steel tubes, was pushed into the centrifuge tube, and the gradient was pumped out from the bottom with 40% (w/v) sucrose over 40 min. by using a slow-infusion apparatus (Arnold Palmer Ltd., London, S.W. 2). The *E*₂₆₀ of the suspension was directly recorded by means of a Uvicord (LKB Instruments Ltd., Selsdon, Surrey).

Chemical analyses. RNA in suspension of ribosomes was estimated by washing 0.1 ml. or 0.05 ml. of the suspension twice with 1.0 ml. of ice-cold 0.2N-HClO₄, and extracting once with 1N-HClO₄ at 70°. The *E*₂₆₀ of the extract was measured after removal of the protein by centrifugation.

Suspensions (0.8 ml.) of the pellets obtained from homogenized muscle were treated with 0.8 ml. of ice-cold 0.4N-HClO₄. The precipitates were washed twice with 1.0 ml. of 0.2N-HClO₄ and extracted with 1N-HClO₄ as for ribosomes. DNA was estimated on 0.5 ml. of the extract by the method of Burton (1956). However, DNA was found in only one fraction (Table 1, Ultra-Turrax fraction A),

in an amount that would have contributed less than 10% of the *E*₂₆₀ of the 1N-HClO₄ extract. For this reason the *E*₂₆₀ of the 1N-HClO₄ extracts was taken as a satisfactory measure of the RNA present in them.

The quantity of RNA in 1N-HClO₄ extracts was estimated by using ϵ_{260} 10800 (Ogur & Rosen, 1950).

Protein was measured by the modified biuret method (Lowry, Rosebrough, Farr & Randall, 1951).

Measurement of radioactivity. All solutions of trichloroacetic acid contained L-leucine (1 mg./ml.).

At the end of the incubation 0.3 ml. of undiluted cell sap was added to each incubation tube and proteins were precipitated with 1 ml. of 10% (w/v) trichloroacetic acid. The tubes were treated for 10 min. at 90° and centrifuged for removal of the supernatant. The proteins were warmed again with 1 ml. of (10% w/v) trichloroacetic acid for 10 min. and then dissolved in 0.5 ml. of 0.5N-NaOH. After the proteins were reprecipitated with 1 ml. of 10% (w/v) trichloroacetic acid, they were washed three times with 1 ml. of hot 5% (w/v) trichloroacetic acid before being dissolved in 0.5 ml. of 0.5N-NaOH for counting in a Packard Tri-Carb liquid-scintillation counter, with polypropylene or glass vials and a scintillation fluid (Bray, 1960) containing Cab-O-Sil (3%). A total of 8000–10000 counts was accumulated and the efficiency was determined by the addition of 0.1 ml. of Na₂¹⁴CO₃ standard (The Radiochemical Centre) to each vial. The standard contained 20000 disintegrations/min.

Preparation of pellets from muscle homogenates. Muscle was homogenized with the Ultra-Turrax homogenizer as described above for the preparation of P ribosomes, or 4g. portions were homogenized in 20 ml. of medium A in a Perspex-and-glass homogenizer of the type described by Aldridge *et al.* (1960). Fifteen passes were made with each of three pestles, rotating at 850 rev./min. The widths of the annuli between the pestles and the tube were 0.015 in., 0.010 in. and 0.005 in. respectively.

Homogenates prepared by these methods were centrifuged to give pellets sedimenting between: 0 and 1000g (10 min.); 1000g and 14000g (15 min.); 14000g and 40000g (30 min.); 40000g and 75000g (30 min.); 75000g and 105000g (60 min.). Each pellet was suspended in medium A (1.0 ml.). Of this suspension, 0.8 ml. was taken for DNA and RNA analysis and 0.2 ml. for protein analysis.

RESULTS

In Table 1 are shown the recoveries of RNA and protein in fractions of *psaos* muscle obtained after different homogenization procedures and after treatment of the animals with triamcinolone acetonide. DNA (2.5 µg./g.) could be detected only in fraction A from muscle disintegrated with the Ultra-Turrax homogenizer. Nuclei were therefore largely unbroken in both procedures and were effectively retained in the 0–1000g fractions. The yields of total RNA differed, but probably not significantly, in the two methods. Fraction A always had the largest amount of RNA compared with fractions B, C and D, and when the Perspex homogenizer was used there was a significant difference between the RNA contents of fractions

Table 1. *Composition of fractions from psoas-muscle homogenates*

Psoas muscles were pooled from 12 control rats and from 12 rats that had been injected with triamcinolone acetonide (20mg./kg.) 24hr. previously. Portions (4.0g.) of each batch of minced muscle were homogenized with the Ultra-Turrax or the Perspex homogenizer, and fractions sedimenting at different *g* values were prepared as described in the Materials and Methods section. Yields of protein and RNA are expressed per g. wet wt. of minced muscle.

Fraction	Conditions of separation	Yields							
		Control				Triamcinolone acetonide-treated			
		Ultra-Turrax homogenizer		Perspex homogenizer		Ultra-Turrax homogenizer		Perspex homogenizer	
		Protein (mg./g.)	RNA (μg./g.)	Protein (mg./g.)	RNA (μg./g.)	Protein (mg./g.)	RNA (μg./g.)	Protein (mg./g.)	RNA (μg./g.)
A	1000g–14000g	4.52	21.23	2.94	33.85	1.16	32.22	1.34	38.63
B	14000g–40000g	3.72	20.70	4.77	14.70	0.97	28.58	0.87	18.73
C	40000g–75000g	0.83	6.83	0.95	11.10	0.88	12.10	0.89	8.50
D	75000g–105000g	0.51	3.70	0.48	13.00	0.35	9.20	0.48	4.05
Total		9.58	52.46	9.14	72.65	3.36	90.31	3.58	76.90

A and B. To maximize the ribosomal yield, only the debris and nuclei (0–1000g) were removed before treatment of the homogenate with deoxycholate to release the ribosomes. When this was done ribosomal yields were 35–50% higher when the Ultra-Turrax homogenizer was used in preference to the Perspex homogenizer. Electron micrographs of the cell fractions, including the 0–1000g fraction, showed that intact mitochondria were always present in this heavy fraction but were absent from fraction A when the Ultra-Turrax homogenizer was used. However, the Perspex homogenizer produced altogether less disruption of the tissue, and a large number of relatively intact mitochondria were then present in fraction A. It therefore appeared that ribosomes were released with greater ease from fractions derived from more severely disrupted tissue. Presumably when the Perspex homogenizer was used ribosomes became entrained with material that sedimented during the 14000g centrifugation of the deoxycholate-treated 1000g supernatant. The total protein content of the control fractions was nearly 2.5 times the content of fractions derived from triamcinolone-treated animals. This was undoubtedly a reflexion of the catabolic state initiated by the drug.

Ribosomes prepared from the post-nuclear supernatant of muscle disintegrated with the Ultra-Turrax homogenizer are called P ribosomes.

In experiments involving the bulbocavernosus muscle the amount of tissue used was decreased from 4g. to 2g., and homogenization was carried out in 10ml. of medium A.

The continuous fall in P-ribosomal activity over the 24hr. after the injection of triamcinolone

Fig. 2. Activity of P ribosomes from psoas muscle isolated at different times after a single injection of triamcinolone acetonide (20mg./kg.). P ribosomes were prepared as described in the Materials and Methods section. There were four animals in each group. Triamcinolone acetonide (10mg./ml.) was administered in 0.9% NaCl.

acetonide (20mg./kg.) is shown in Fig. 1. The marked fall in activity at 24hr. agrees with the results of White (1967), as do the results of the three experiments shown in Table 2: P-ribosomal activity was decreased by 22–29% after administration of triamcinolone acetonide at 1.0mg./kg. for 2 days. The pretreatment of the animals with steroid 36644-Ba (0.1mg./kg.) resulted in the preservation or partial preservation of P-ribosomal activity. In a total of eight experiments there was only one in which there was no difference between the activity of P ribosomes from triamcinolone-treated animals and that of animals that had been treated with triamcinolone together with steroid 36644-Ba. In all other cases P ribosomes from animals receiving the two compounds were more

Table 2. *Amino acid-incorporating ability of P ribosomes isolated from psoas muscle of rats treated with steroid 36644-Ba and triamcinolone acetonide*

P ribosomes were isolated as described in the Materials and Methods section. Triamcinolone acetonide (0.5mg./ml.) and steroid 36644-Ba (0.05mg./ml.) were administered in 0.9% NaCl. In the experiment with steroid 36644-Ba, triamcinolone acetonide was administered on the third and fourth days from commencement simultaneously with steroid 36644-Ba. There were four animals in each group.

Treatment	Dose (mg./kg./day)	Time of administration (days)	Incorporation (disintegrations/ min./ μ g. of RNA)		
			Expt. 1	Expt. 2	Expt. 3
None			71.9	77.2	193.7
Triamcinolone	1.0	2	56.3	56.2	136.7
Steroid 36644-Ba	0.1	4			
+			80.2	81.5	152.3
Triamcinolone	1.0	2			

Table 3. *Amino acid-incorporating ability of P ribosomes from the psoas muscle of rats treated with steroid 36644-Ba and triamcinolone acetonide*

Triamcinolone acetonide (10mg./ml.) and steroid 36644-Ba (0.05mg./ml.) were administered in 0.9% NaCl. In the experiments with steroid 36644-Ba, triamcinolone acetonide was administered on the second or fourth day from commencement, simultaneously with steroid 36644-Ba. There were four animals in each group.

Treatment	Dose (mg./kg./day)	Time of administration (days)	Incorporation (disintegrations/ min./ μ g of RNA)	
			Expt. 1	Expt. 2
None			49.4	64.9
Triamcinolone	20	1	15.3	43.6
Steroid 36644-Ba	1.0	2		
+			25.6	54.1
Triamcinolone	20	1		
Steroid 36644-Ba	1.0	4		
+			22.9	39.3
Triamcinolone	20	1		

active. The differences ranged from 11% to 61%, based on the activity of the ribosomes from animals that had received triamcinolone acetonide. Similar experiments carried out at a steroid 36644-Ba concentration of 0.01mg./kg. gave equivocal results, from which it was concluded that the drug had no ability to antagonize the effects of triamcinolone acetonide at this concentration.

There was only a partial preservation of P ribosomal activity (Table 3) on administration of triamcinolone acetonide (20mg./kg.) to animals that had been pretreated with steroid 36644-Ba (1.0mg./kg.), and in contrast with experiments with methandrostenolone (White, 1967) there was no advantage in increasing the time for which steroid 36644-Ba was administered before the animals were given triamcinolone acetonide. In Fig. 2 the effects of steroid 36644-Ba on the spontaneous return of ribosomal activity to normal

are shown. In these two studies six groups of rats were involved in each experiment. There were four animals in each group. Two groups were controls. Two groups received one injection of triamcinolone acetonide (20mg./kg.) at zero time and were killed either 24hr. or 72hr. afterwards. Two groups received steroid 36644-Ba (1.0mg./kg.) daily, commencing 1 day before a single injection of triamcinolone acetonide (20mg./kg.). Again, as in Table 3, pretreatment with the anabolic steroid preserved P-ribosomal activity to some extent, although not completely. However, the rate of recovery of ribosomal activity was no greater in the case of animals receiving steroid 36644-Ba than in controls.

Attempts were made to discover why P ribosomes from the muscle of animals treated with triamcinolone acetonide had decreased activity. The difference between the sucrose-density-gradient

Fig. 2. Comparison between the activity of P ribosomes from control animals and that of animals receiving triamcinolone acetonide either alone or with steroid 36644-Ba. ○, Groups of animals given triamcinolone acetonide (20mg./kg.) at zero time. ●, Groups of animals given either two or four daily injections of 36644-Ba (1mg./kg.) commencing 24 hr. before triamcinolone acetonide (20mg./kg.) at zero time. —, Expt. 1; —, Expt. 2. Control groups (.....) were killed at the same time as the groups receiving steroids. There were four animals in each group. Triamcinolone acetonide (10mg./ml.) and steroid 36644-Ba (0.5mg./ml.) were administered in 0.9% NaCl.

profiles of control ribosomes and those of ribosomes from treated animals is immediately obvious (Fig. 3). The loss of discrete peaks in the profile was highly characteristic of P ribosomes from triamcinolone-treated animals and was seen in a number of experiments. However, the areas under the two curves in Fig. 3 are almost identical, indicating that the low incorporating ability of P ribosomes prepared from triamcinolone-treated animals was not due to an increase in the relative number of ribosomes with low sedimentation values. This view is supported by preliminary studies (A. M. White, unpublished work) in which the polyuridylic acid-directed incorporation of phenylalanine (Nirenberg & Matthaei, 1961) was investigated. These suggest that an increased proportion of monomer ribosomes was probably not the reason for the decreased activity of ribosomes from triamcinolone-treated animals.

The effects of steroid 36644-Ba on the activity of ribosomes from different muscles are shown in Table 4. The activities of ribosomes from psoas muscle and bulbocavernosus muscle did not differ significantly from control values when steroid 36644-Ba was given at 0.1mg./kg. for 4 days. However, at 1.0mg./kg. there was a slight stimulation of protein-synthetic activity in ribosomes from the bulbocavernosus muscle. This is in agreement with the 25–30% increase in weight

Fig. 3. Centrifugation profiles in sucrose density gradients (15–30% sucrose in medium A) of P ribosomes from psoas muscle. -----, Control ribosomes; —, ribosomes from animals treated with triamcinolone acetonide (20mg./kg.) 18 hr. before being killed. The same amount of ribosomal RNA (273 μ g.) was present on both gradients. There were seven animals in each group. Triamcinolone acetonide (10mg./ml.) was administered in 0.9% NaCl.

that occurred in these muscles over the 4-day period.

M ribosomes isolated from washed muscle microsomes as described in the Materials and Methods section did not have decreased activity when they were isolated from the muscle of animals that had received triamcinolone acetonide at 20mg./kg. (Table 5).

The experiments with rabbits (Table 6) fall into a different category. In these studies single animals were given cortisone acetate (15mg./kg./day) for about 2 weeks, followed by the same dose of cortisone acetate together with testosterone or steroid 36644-Ba for a further 2 weeks. At the end of this time the animals were killed, and the comparative activities of populations of P ribosomes from the rectus femoris muscles were determined. The results in Table 6 show that animals given testosterone at 5.0mg./kg. yielded ribosomes whose activity was significantly below that of controls, and that steroid 36644-Ba at a roughly equiandrogenic dose (0.5mg./kg.) enabled ribosomal activity to return to near normal values. The same can be said for 0.05mg. of steroid 36644-Ba/kg., but at 0.005mg./kg. steroid 36644-Ba was ineffective.

Only one result could be obtained for cortisone acetate alone because of the difficulty of maintaining the rabbits for 4 weeks on this compound.

Table 4. *Effect of steroid 36644-Ba at different concentrations on the activity of P ribosomes from bulbocavernosus and psoas muscles*

Steroid 36644-Ba (0.05 mg./ml. or 0.5 mg./ml.) was administered in 0.9% NaCl. There were four animals in each group.

Treatment	Dose (mg./kg./day)	Time of administration (days)	Incorporation (disintegrations/min./ μ g. of RNA)					
			Psoas				Bulbocavernosus	
			Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
None			75.4	47.8	91.1	76.5	45.8	41.8
Steroid 36644-Ba	0.1	4	68.7	45.9			49.8	41.1
	1.0	4			80.0	83.5	60.3	68.9

Table 5. *Amino acid-incorporating ability of different populations of ribosomes*

P ribosomes and M ribosomes, prepared as described in the Materials and Methods section, were isolated from the psoas muscle of rats to which triamcinolone acetonide (20 mg./kg.) had been given 24 hr. previously. Animals that did not receive triamcinolone acetonide were used as controls. N.E., Not estimated.

Expt. no.	Treatment	Incorporation			
		P ribosomes		M ribosomes	
		(disintegrations/ min./ μ g. of RNA)	(% of control)	(disintegrations/ min./ μ g. of RNA)	(% of control)
1	None	N.E.		137.9	
	Triamcinolone	N.E.		127.1	92.2
2	None	78.7		95.5	
	Triamcinolone	49.3	63	108.2	111
3	None	104.8		156.7	
	Triamcinolone	73.7	70	135.5	87

DISCUSSION

The method described for the homogenization of muscle allows the routine examination of protein-synthetic activity in ribosomal populations from 4 g., and even as little as 2 g., of tissue. The experiments with ribosomes from the bulbocavernosus muscle illustrate the use of the smaller quantity of tissue. In addition, ribosomes prepared in this way (P ribosomes) have decreased activity when they are isolated from the muscle of animals under treatment with large doses of two anti-inflammatory glucocorticoids. There is no stimulation of P ribosomes at any time up to 24 hr. after an acute dose of triamcinolone acetonide is given. This is in contrast with the effect of triamcinolone on liver, where both RNA turnover (Weber, Srivastava & Singhal, 1965) and ribosomal activity (Leon *et al.* 1962) markedly increase over this time. The behaviour of muscle with regard to protein synthesis thus bears a strong resemblance to that of the thymus (Pena, Dvorkin & White, 1966) after the administration of cortisol.

P ribosomes consist of ribosomes that were free in the tissue before homogenization and ribosomes that were bound to a reticulum. M ribosomes consist almost entirely of ribosomes of the latter type. Since the activity of M ribosomes is virtually unaffected when the animals from which they are isolated have received triamcinolone it must be the free-ribosomal component of P ribosomes that shows such a marked decrease in activity under these conditions. From an examination of the sucrose-density profiles of P ribosomes from triamcinolone-treated animals, it appears that particles are present that have a range of *S* values similar to normal ribosomes but differing in weight by amounts that do not correspond to discrete ribosomal units. It is probably the presence of these particles, perhaps degraded ribosomes, that causes a decrease in the activity of P ribosomes per unit of ribosomal RNA. Whether the situation *in vitro* reflects a similar one *in vivo* cannot at present be decided.

The decreased activity of ribosomes from animals receiving cortisone or triamcinolone provides a

Table 6. Amino acid-incorporating ability of ribosomes isolated from the rectus femoris muscle of rabbits under different treatments

Treatment	Dose (mg./kg./day)	Expt. 1 Females		Expt. 2 Males		Expt. 3 Females		Expt. 4 Males	
		Administration time (days)	Incorporation (disintegration/min./ μ g. of RNA)	Administration time (days)	Incorporation (disintegration/min./ μ g. of RNA)	Administration time (days)	Incorporation (disintegration/min./ μ g. of RNA)	Administration time (days)	Incorporation (disintegration/min./ μ g. of RNA)
None			46.9		37.5		84.2		48.8
Cortisone	15	27	37.8	29	17.9	27	38.8	28	31.8
Cortisone	15			14		13		14	
Testosterone	5.0			29		27		28	
Cortisone	15			14		13		14	
Steroid 36644-Ba	0.5			29	31.6	27	95.1	28	48.6
Cortisone	15	27	50.7	29	29.5				
Steroid 36644-Ba	0.05	12		14					
Cortisone	15			29	10.4				
Steroid 36644-Ba	0.005			14					

P ribosomes were prepared from the muscle of single animals as described in the Materials and Methods section. Cortisone acetate (60 mg./ml.) and steroid 36644-Ba (2.0 mg./ml., 0.2 mg./ml. and 0.02 mg./ml.) were administered in 0.9% NaCl.

measurable parameter for assessing under precise experimental conditions compounds that may have an anticatabolic or antiglucocorticoid effect.

A new modified androgen, steroid 36644-Ba, has been tested in this way. Although this compound is certainly able to reverse the effects of cortisone acetate in rabbits at extremely low concentrations (0.05 mg./kg.), its activity in rats, particularly against triamcinolone acetonide at 20 mg./kg., was only slight. Methandrostenedione (White, 1967) at 5.0 mg./kg. enabled full activity in ribosomes from psoas muscle to be retained when it was administered daily to rats for 5 days before triamcinolone acetonide, whereas steroid 36644-Ba at 1.0 mg./kg. (a very high dose in view of its androgenicity) was only partially effective. Also, in contrast with experiments with methandrostenedione (5.0 mg./kg.), increasing the pretreatment time before the administration of triamcinolone acetonide did not enhance the ability of steroid 36644-Ba to protect ribosomal function.

The most interesting findings are those that concern the comparison between steroid 36644-Ba and testosterone in the rabbit. It appears that testosterone is completely unable to counteract the decrease of muscle ribosomal activity by cortisone, whereas steroid 36644-Ba is highly effective in this respect at one-hundredth of the testosterone dose. In view of this it seems unlikely that a purely androgenic component of steroid 36644-Ba is bringing about the antagonism of cortisone, for, if this were so, then one would expect testosterone at the concentrations given to have at least some activity in this direction.

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