Some Biochemical Effects of Triamcinolone Acetonide on Rat Liver and Muscle

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1. The powerful anti-inflammatory glucocorticoid triamcinolone acetonide, administered to rats at 20 and 2.5 mg/kg, leads to a decrease in the incorporation *in vivo* of [^3H]uridine and [^{32}P]orthophosphate into hind-limb skeletal muscle. 2. At the higher dose, this decrease in the rate of incorporation of precursors into RNA precedes a decrease in the incorporating ability of muscle ribosomes, which commences about 4–5 h after drug administration, but is unaccompanied by any changes in the concentration of tissue ATP or free amino acids. 3. The ribosomal dysfunction extends to polyribosomes, which can only be successfully isolated from the muscle of triamcinolone-treated animals after the addition of α -amylase to the tissue homogenate to remove glycogen. 4. The specific radioactivity of muscle protein labelled *in vivo* with ^{14}C -labelled amino acids does not decrease progressively after triamcinolone administration. After 2 h there is an apparent stimulation of incorporation which leads to an overall discrepancy between measurements of protein-synthetic activity made *in vivo* and *in vitro*. 5. There is a significant increase in muscle-glycogen concentration between 8 and 12 h after the administration of triamcinolone acetonide (20 mg/kg), although a significant decrease occurs after 4 h. The fall in glycogen concentration may be due to a decrease in the rate of synthesis of protein essential for glucose uptake into the tissues. 6. As judged by (a) incorporation of ^{14}C -labelled amino acids into protein, (b) [^3H]uridine and [^{32}P]orthophosphate incorporation into RNA, (c) the rate of induction of tryptophan pyrrolase and (d) changes in the pool sizes of taurine and tryptophan, the responses in liver followed the same time-course as those in muscle after administration of the drug.

Triamcinolone acetonide is used to produce myopathy in rats as a model for the study of the antimyopathic action of anabolic steroids. In previous studies, skeletal muscle isolated from rats receiving high doses of triamcinolone acetonide possessed decreased ability to incorporate amino acids into protein *in vitro* (White, 1967; Bullock, White & Worthington, 1968). The work described here was designed to examine some of the factors that might be responsible for this decreased ribosomal activity. A preliminary account has appeared (White, Peters, Richardson & Small, 1969).

MATERIALS AND METHODS

Radioactive compounds. L-[U- ^{14}C]Leucine (specific radioactivity 311 mCi/mmol), L-[U- ^{14}C]phenylalanine (504 mCi/mmol), U- ^{14}C -labelled protein hydrolysate (specific radioactivity 54 mCi/mg-atom of C), [5- ^3H]uridine (specific radioactivity 24 Ci/mmol) and sodium [^{32}P]phosphate (injection B.P.) were 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 (cetyl alcohol-polyoxyethylene condensate) was purchased from Imperial Chemical Industries Ltd., Manchester, U.K. α -Amylase (twice crystallized from hog pancreas) was obtained from the Worthington Biochemical Corp., Freehold, N.J., U.S.A. Triamcinolone acetonide was purchased from E. R. Squibb and Sons Ltd., Speke, Liverpool, U.K.

Animals. White Wistar-strain male albino rats (275–300 g) were used. These were either bred in these laboratories or purchased from Scientific Products Farm, Ash, Canterbury, Kent, U.K.

Administration of triamcinolone acetonide and radioactive compounds. The drug was administered by intraperitoneal injection as a fine suspension in 0.9% NaCl. For experiments involving doses of 2.5 and 20 mg/kg, suspensions containing 1.25 and 10 mg/ml were used respectively.

Labelled substances were injected intraperitoneally in

0.5 ml of 0.9% NaCl. Each animal received 5 μ Ci of [U - 14 C]leucine, 5 μ Ci of 14 C-labelled protein hydrolysate, 200 μ Ci of [5 - 3 H]uridine or 200 μ Ci of sodium [32 P]orthophosphate.

Method of obtaining blood. Animals were killed by decapitation and blood was drained from the neck into tubes containing a small crystal of heparin.

Preparation, incubation and analysis of ribosomes. These were prepared from hind-leg muscle. The procedure was either as described for the isolation of P ribosomes from psoas muscle (Bullock *et al.* 1968) or a modification of this method in which the KCl concentration was changed to 0.25 M (Heywood, Dowben & Rich, 1967) during homogenization. The concentrated salt solution added to the post-nuclear supernatant contained 2.0 M-KCl and the concentration of salts in the cushioning medium during the final centrifugation was not altered.

To prepare glycogen-free ribosomes, amylase (approx. 620 units) was added to 13 ml of the 3000g supernatant and the suspension was kept for 10 min at 0°C. α -Amylase (1 unit) frees 1 μ mol of maltose/min from soluble starch at 25°C. Subsequent operations were unchanged. Ribosomes were incubated and analysed on continuous sucrose density gradients as described by Bullock *et al.* (1968).

Preparation of muscle and liver cell sap and plasma for analysis of amino acid pool sizes. Fresh skeletal muscle (2.0 g) was homogenized with the Ultra-Turrax homogenizer for 1 min. In 10 ml of the medium used for the preparation of ribosomes. Liver was homogenized by using the same proportion of tissue to medium but with a Potter-Elvehjem Teflon homogenizer. The homogenate was centrifuged for 90 min at 35000g. These supernatants (4.0 ml) or plasma (4.0 ml) were mixed with 10% (w/v) sulphosalicylic acid (4.0 ml) and the precipitate of protein was removed by centrifugation. The resulting supernatant was heated for 5 min at 90°C and centrifuged before analysis of 0.5 ml for ninhydrin-positive substances on a Beckman amino acid analyser. The instrument was programmed by the standard method for the analysis of physiological fluids. Recoveries were corrected by including [U - 14 C]leucine in the original homogenizing medium. Glutamine and glutamic acid cannot be reliably isolated by this procedure, since they decompose in sulphosalicylic acid. Other amino acids are stable.

Liver and muscle RNA. This was prepared from 10 g of tissue by established procedures (Kirby, 1956, 1962). The E_{260}/E_{280} ratio of the isolated RNA varied between 2.0:1 and 2.2:1.

Phosphorus determination in RNA. The method of Berenblum & Chain was used as modified by Martin & Doty (1949).

Glycogen. This was determined on 2 ml samples from a solution of 500 mg of tissue in 30% (w/v) KOH (5 ml) by using anthrone as described by Hassid & Abraham (1957).

Isolation of protein from muscle and liver for determination of specific radioactivity. Tissue (0.5 g) was homogenized in water (5.0 ml) by using the Ultra-Turrax at 160 V for 1 min. Protein was precipitated with 10% (w/v) trichloroacetic acid (20 ml) and the mixture was heated for 10 min at 90°C and centrifuged. The precipitate was washed twice in 10 ml of ethanol-ether (3:1, v/v). The protein was dissolved in 2 M-NaOH (5 ml), reprecipitated with 10% (w/v) trichloroacetic acid (25 ml), washed with 5% (w/v) trichloroacetic acid (10 ml), heated, centrifuged,

washed with ethanol (10 ml) and dissolved in 2 M-NaOH (2.0 ml). The alkaline protein solution was diluted 200-fold for the determination of protein (Lowry, Rosebrough, Farr & Randall, 1951).

Blood sugar. This was determined by the glucose oxidase method of Huggett & Nixon (1957) by using a Boehringer Test Kit.

Tryptophan pyrrolase (total enzyme). This was assayed in liver by the method of Knox & Piras (1966).

Muscle ATP. Animals were killed by a blow on the head and one hind leg was immediately cut off to avoid, as far as possible, involuntary flexing of the muscles. The gastrocnemius muscle was rapidly dissected out and homogenized in 5.0 ml of 0.12 M-HClO₄ within 45 s of the animal being killed. ATP was measured in the acid supernatant by the method of Lamprecht & Trautschold (1963).

Measurement of radioactivity. This was carried out in a Packard model 3003 liquid scintillation spectrometer.

The radioactivity of RNA labelled with 32 P was measured by Cerenkov radiation in 10 mm-KH₂PO₄ by the method of Clausen (1968) by using the 3 H channel. The efficiency for counting 32 P by this method is about 20%.

RNA labelled with 3 H was dissolved in 1.0 ml of 0.2 M-NaOH and its radioactivity measured in 10 ml of scintillation fluid (Bray, 1960) containing Cab-O-Sil (4%). The counting efficiency for 3 H was about 40%.

Protein labelled with 14 C from experiments involving labelling *in vivo*, and from determinations of ribosomal activity, was dissolved in 2 M-NaOH. The radioactivity of the alkaline solution (0.5 ml or 1.0 ml) was measured in scintillation fluid (Bray, 1960) containing Cab-O-Sil (4%). The counting efficiency for 14 C was 65%. 8000–10000 counts were accumulated for each sample.

RESULTS

Incorporation of radioactive precursors into protein and RNA. In preliminary experiments with drug-treated and normal animals, the specific radioactivity of protein and RNA from muscle and liver increased for 5 h after a single injection of radioactive precursors. To show the correlation between events in muscle and liver at two drug doses, original results are not quoted directly (Figs. 1 and 2) but are related to control values. When triamcinolone acetonide was administered at 2.5 mg/kg there was little change in the rate of incorporation of radioactive precursors into protein of liver and muscle (Fig. 1), suggesting that rates of protein synthesis in these tissues were not greatly changed. However, there was a large increase of 32 P incorporation into liver RNA, which reached a maximum after 6 h, and a decrease in the incorporation of 32 P into muscle RNA between 2 and 8 h after the drug had been given. Weber, Srivastava & Singhal (1965) studied the incorporation of [6 - 14 C]orotic acid into RNA in liver and obtained similar results, but they did not report a second stimulation between 8 and 12 h, and in their studies maximum stimulation occurred after 8 h rather than 6 h. This slight difference may have been because triamcinolone acetonide is more

Fig. 1. Percentage changes in incorporation *in vivo* of ^{14}C -labelled protein hydrolysate into muscle protein ($\blacktriangle$) and liver protein (Δ) and incorporation *in vivo* of sodium $[^{32}\text{P}]$ phosphate into muscle RNA ($\bullet$) and liver RNA ($\circ$) after a single injection of triamcinolone acetonide (2.5 mg/kg). Liver and muscle RNA were isolated from different sets of animals in two separate experiments. Liver and muscle protein were from the same animals in a third experiment. Each point represents pooled tissue from three animals. The labelled precursors were given 2 h before the animals were killed. The specific radioactivities of liver and muscle protein from control animals (time zero) were 148.4 and 80.6 c.p.m./mg respectively. The specific radioactivities of liver and muscle RNA from control animals (time zero) were 74.8 and 98.3 c.p.m./ μmol of P respectively.

Fig. 2. Percentage changes in incorporation *in vivo* of $[\text{U-}^{14}\text{C}]$ leucine into muscle protein ($\blacktriangle$) and liver protein (Δ) and incorporation *in vivo* of $[\text{5-}^3\text{H}]$ uridine into muscle RNA ($\bullet$) and liver RNA ($\circ$) after a single injection of triamcinolone acetonide (20 mg/kg). Liver and muscle protein and liver and muscle RNA were derived from the same animals in two separate experiments. Each point represents pooled tissue from three animals. The labelled precursors were given 2 h before the animals were killed. The specific radioactivities of liver and muscle protein from control animals (time zero) were 455.2 and 59.3 c.p.m./mg respectively. The specific radioactivities of liver and muscle RNA from control animals (time zero) were 12.7 and 12.6 c.p.m./ μmol of P respectively.

rapidly absorbed than the diacetate used by Weber *et al.* (1965).
When triamcinolone acetonide was given at 20 mg/kg there was an initial increase in the rate of

Table 1. Changes in the incorporation *in vitro* of $[\text{U-}^{14}\text{C}]$ leucine into protein by a muscle ribosomal preparation after administration of a single injection of triamcinolone acetonide (20 mg/kg) at zero time.

Each result was obtained from pooled muscle tissue of three animals. Ribosomes were prepared as described in the Materials and Methods section by using 0.25 M-KCl. They were not treated with amylase. Incubations were in duplicate and each incubation tube contained 30–40 μg of ribosomal RNA.

Time (h)	Incorporation	
	(d.p.m./ μg of RNA)	(% of control)
0	80.1	100
2	77.7	97
3	80.0	100
4	72.1	90
5	74.3	93
6	68.2	85
7	68.6	86
12	60.0	75

incorporation of precursors into muscle protein and then a decrease that began after 2 h and continued up to 8 h (Fig. 2). Between 12 and 24 h results were different for each experiment. Sometimes the rate of amino acid incorporation returned towards normal values as shown in Fig. 2 and on other occasions amino acid incorporation continued to decrease between 12 and 24 h. However, the shape of the curve up to 8 h was highly reproducible. A continuous decrease in the rate of incorporation of $[^3\text{H}]$ uridine into muscle RNA was observed from zero time when triamcinolone acetonide was given at 20 mg/kg. Without measurements on nuclear polymerase activity in muscle and knowledge of the pool sizes of RNA precursors and their rate of entry into the nucleus we cannot be sure that there is a decrease in the rate of synthesis of muscle RNA immediately after the injection of triamcinolone acetonide, but results of experiments with $[^3\text{H}]$ -uridine and $[^{32}\text{P}]$ orthophosphate support this conclusion.

From Fig. 2 and Table 1, the activity of ribosomes isolated from muscle at various times after the administration of triamcinolone acetonide can be compared with the apparent rate of total protein synthesis as measured by the incorporation of $[\text{U-}^{14}\text{C}]$ leucine. One obvious difference occurs after 2 h when, in the whole animal, the rate of protein synthesis apparently increased whereas ribosomal activity remained the same as, or slightly less than, the control value. Between 2 and 6 h the incorporation of precursors into protein *in vivo* and ribosomal activity decreased to about the same value (Fig. 2 and Table 1) and thereafter there was reasonable agreement between assessments of protein-synthetic

activity made *in vitro* and *in vivo* up to 24h (Bullock *et al.* 1968).

The incorporation of [^{14}C]phenylalanine into protein by ribosomes from triamcinolone-treated animals decreased, like that of [^{14}C]leucine, to 71% of the control value.

Glycogen and blood glucose. Glycogen concentration in muscle decreased significantly ($P < 0.05$) 4h after the administration of triamcinolone acetonide (20mg/kg) and increased significantly to about

2.5 times the control value between 8 and 12h (Fig. 3). Blood-glucose concentration increased significantly ($P < 0.05$) after 4h.

Free amino acid concentration. In muscle and plasma, the concentrations of individual amino acids were virtually unchanged up to 8h after the administration of triamcinolone acetonide (20mg/kg). In liver, the only significant changes were in the concentration of taurine, which increased from 1.2 to 4.5 $\mu\text{mol/g}$ of tissue, and in that of tryptophan which decreased from 0.1 to less than 0.03 $\mu\text{mol/g}$ of tissue.

Muscle ATP. The mean control concentration from five individual animals was 9.93 (s.e.m. ± 0.68) $\mu\text{mol/g}$. This value decreased insignificantly to 8.83 (s.e.m. ± 0.34) $\mu\text{mol/g}$ 2h after the administration of triamcinolone acetonide (20mg/kg). ATP concentrations 4, 6, 8 and 12h after the administration of the drug likewise showed no significant change from control values.

Activity and yield of muscle ribosomes. Because of the large increase in muscle glycogen that occurred after the administration of triamcinolone acetonide, we thought that ribosomes isolated from treated animals must be contaminated with glycogen and that this might be the cause of the atypical sucrose density gradient exhibited by these ribosomes (Bullock *et al.* 1968), and also the cause of their decreased activity. Ribosomes from animals treated with triamcinolone acetonide (Fig. 4b)

Fig. 3. Changes in concentrations of muscle glycogen (○) and blood glucose (●) after administration of a single injection of triamcinolone acetonide (20mg/kg) at zero time. Each result is the mean of measurements made on five individual animals ($\pm$ s.e.m.).

Fig. 4. Sucrose-density-gradient profiles of ribosomal preparations isolated from skeletal muscle. (a) Amylase-treated muscle ribosomes from control animals; (b) untreated muscle ribosomes from animals 12h after a single injection of triamcinolone acetonide (20mg/kg); (c) amylase-treated muscle ribosomes from animals 12h after a single injection of triamcinolone acetonide (20mg/kg). Amylase treatment was carried out on the post-nuclear suspension as described in the Materials and Methods section. 0.25M-KCl was used in the homogenizing medium for the preparation of ribosomes.

Table 2. *Effect of triamcinolone acetone on the activity of muscle polyribosomes*

Triamcinolone acetone (20 mg/kg) was administered to rats 12 h before they were killed. The homogenization of muscle was carried out in 0.25 M-KCl. The post-nuclear supernatant was treated with amylase as described in the Materials and Methods section and ribosomes were harvested by centrifugation for 2 h through buffer containing 0.3 M-sucrose (Bullock *et al.* 1968), 1.0 M-sucrose or 1.5 M-sucrose.

Treatment	Concn. of sucrose layer (M)	RNA yield (μ g/g wet wt. of muscle)	Incorporation	
			(d.p.m./ μ g of RNA)	(% of control)
Control	0.3	121	177	100
Triamcinolone acetone	0.3	139	149	84
Control	1.0	77	370	100
Triamcinolone acetone	1.0	89	284	77
Control	1.5	52	479	100
Triamcinolone acetone	1.5	47	352	74

Table 3. *Changes in tryptophan pyrrolase activity in rat liver after administration of a single injection of triamcinolone acetone (20 mg/kg) at zero time.*

Each result was obtained from pooled liver tissue of three animals.

Time (h)	Tryptophan pyrrolase activity	
	(unit/g)	(% of control)
0	0.051	100
2	0.096	188
4	0.250	490
6	0.285	559
8	0.243	476

showed a broad and slightly retarded monomer peak with no discrete peaks over the remaining part of the gradient. However, by treating another batch of the same post-nuclear supernatant with amylase, we prepared ribosomes with a sucrose-density-gradient profile (Fig. 4c) indistinguishable from that of the control (Fig. 4a) but still with decreased amino acid-incorporating ability (Table 2). When the incorporating abilities of amylase-treated ribosomes from muscle of control and triamcinolone-treated animals were compared after fractionation through a discontinuous sucrose density gradient the differences in their relative activities were retained (Table 2). Since the same cell sap was used for control and test ribosomes this difference in amino acid-incorporating ability was not due to pool size differences or to differential rates of charging to tRNA. The low activity of ribosomes from the triamcinolone-treated animals was not due to a low polyribosome/monoribosome ratio, since the activity differential was retained in ribosomes that sedimented through 1.5 M-sucrose and that therefore contained virtually no single ribosomes (Wettstein, Staehelin & Noll, 1963).

In all the experiments in which 0.25 M-potassium chloride was used in the isolation of ribosomes the yield of ribosomal RNA/g wet wt. of tissue ranged between 60 and 130 μ g. Previously, Bullock *et al.* (1968) found that the normal yield of P ribosomes varied between 20 and 40 μ g of ribosomal RNA/g wet wt. of tissue. There was no evidence that triamcinolone acetone treatment resulted in a decreased yield of muscle ribosomes or polyribosomes (Table 2).

Tryptophan metabolism. Tryptophan pyrrolase activity in the liver increased extremely rapidly after the administration of triamcinolone acetone (Table 3) and this increase was accompanied by a decrease in the size of the liver tryptophan pool mentioned above. No changes in muscle or blood tryptophan concentrations were detected over the 4 h period. These results suggest that the increase in liver tryptophan pyrrolase activity is not induced by the entry of tryptophan into liver from muscle, a possibility arising from the demonstration by Labrie & Korner (1968) that the administration of tryptophan to adrenalectomized animals causes a significant actinomycin-sensitive increase in the activity of liver tryptophan pyrrolase. It appears that tryptophan pyrrolase activity is limiting for tryptophan metabolism and that the increased rate of breakdown of the amino acid after the increase in enzyme activity causes its concentration to be decreased in the tissue extract.

DISCUSSION

The experiments on the incorporating ability of isolated muscle ribosomes have extended previous findings (Bullock *et al.* 1968). It was shown that the decrease in their activity that becomes significant 4–5 h after the administration of the glucocorticoid, triamcinolone acetone, is neither due to the contamination of the ribosomes with glycogen nor to an

increase in their monoribosome/polyribosome ratio, since ribosomes that contain predominantly polyribosomes still differ appreciably in activity from control polyribosomes. Changes in the overall energy content of the tissue (Manchester & Young, 1959; Manchester, 1966), as judged by the ATP concentration, also seem unlikely to be the cause of the ribosomal dysfunction.

Liver protein synthesis is influenced at the ribosomal level by the flow of amino acids to the liver (for references see Jefferson & Korner, 1969). Since significant changes in the pool size of a number of amino acids occur in muscle 4 h after the administration of cortisone to starved adrenalectomized rats (Betheil, Feigelson & Feigelson, 1965) it was thought that changes in amino acid-pool sizes in muscle after triamcinolone acetonide administration might lead to the decrease in ribosomal activity. Again this seems an unlikely mechanism, since activities of free amino acids in tissue extracts were virtually unchanged 8 h after the administration of the drug.

Since there was a decrease in the rate of incorporation of two labelled precursors, [^{32}P]orthophosphate and [^3H]uridine, into RNA before the decrease in ribosomal activity, this activity may be due to a decrease in the rate of RNA synthesis in muscle occurring as an almost immediate response to triamcinolone injection. This suggestion agrees with results of experiments with isolated thymus cells, where hybridization techniques were used to show that the production of ribosomal RNA was diminished 3 h after the injection *in vivo* of a number of powerful anti-inflammatory glucocorticoids, including triamcinolone acetonide (Drews, 1969). However, it contradicts the finding of Ferguson & Wool (1962) that the incorporation of [^{14}C]adenine into RNA of isolated diaphragm was unaffected by treating intact rats for 4 days with cortisol, in spite of the fact that the rate of incorporation of amino acids into protein decreases by 50% in this time (Wool & Weinshelbaum, 1959). More work is indicated on the early effects of corticosteroids on nuclear metabolism in muscle, but because of the high degree of contamination with various species of adenosine triphosphatase we failed to demonstrate changes in the activity of RNA polymerase in isolated nuclei. The changes in the specific radioactivity of pulse-labelled muscle protein after triamcinolone administration show the total effect of the drug on ribosomal activity, the number of active ribosomes and the rate of uptake of amino acids into the tissue. We therefore expected this method of labelling protein to show a greater percentage decrease in the specific radioactivity of muscle protein than that seen in the protein made by ribosomes *in vitro*. However, this was not so, owing to a big discrepancy after 2 h, when there appeared to be a stimulation of amino acid incorporation *in vivo* and no change *in vitro*.

There was no decrease in the pool size of free amino acids over the first 8 h of triamcinolone acetonide treatment, so that within the tissue the specific radioactivity of amino acids entering the protein could be expected to be the same in control and drug-treated animals. At present we cannot explain this lack of agreement.

Since the hepatic output of glucose is decreased immediately after glucocorticoid administration (Lecocq, Mebane & Madison, 1964), the increase in blood glucose concentration implies a decrease in the uptake of glucose by peripheral tissue beginning about 4 h after the administration of triamcinolone acetonide. Of these peripheral tissues adipose tissue is almost certainly affected over this time (Munck & Koritz, 1962; Munck, 1962), as judged by the administration of cortisol *in vivo* to adrenalectomized rats, but this effect might not apply to muscle. However, the fact that muscle glycogen concentration falls significantly 4 h after the administration of triamcinolone acetonide suggests that glucose uptake into muscle is decreased after treatment with this more powerful glucocorticoid. The entry of glucose into the muscle cell follows Michaelis-Menten kinetics (Morgan, Henderson, Regen & Park, 1961), indicating the initial binding of glucose to a specific membrane site. If this site contains a protein that is rapidly turning over, then a decrease in the amount of this protein due to a general decrease in protein synthesis would lower the rate of glucose entry into the cell. The timing of the effect of triamcinolone on muscle protein synthesis suggests that inhibition of glucose uptake may be a result and not a cause of it. Evidence for a similar mechanism by which amino acid uptake into muscle is diminished by corticosteroids was found by Kostyo & Redmond (1966), who showed that puromycin and actinomycin D are corticomimetic in that they inhibit the uptake of amino acids into isolated diaphragm *in vitro* after 2–3 h.

Skeletal muscle is generally thought to be non-glyconeogenic (Scrutton & Utter, 1968), and the increase in glycogen concentration that occurs in this tissue between 8 and 12 h after the administration of triamcinolone acetonide is almost certainly due to the inhibition of glucose utilization. Among the most likely causes of decreased glycolysis is a rise in the tissue concentration of metabolites from the increased oxidation of free fatty acids (Randle, Newsholme & Garland, 1964; Williamson, 1967). Fatty acids would be expected to be mobilized rapidly from adipose tissue by triamcinolone.

The effects that glucocorticoids are capable of exerting on amino acid uptake in isolated diaphragm (Kostyo, 1965) suggest that muscle can respond directly to glucocorticoids. However, the pronounced changes in liver metabolism caused by these compounds indicate that it may be impossible to

understand their physiological effects completely without considering the liver. This was realized previously in studies of the mechanism of thymus involution (Feigelson, 1965; Hofert & White, 1965) and to some extent in studies on muscle (Bethel *et al.* 1965; Uete, 1966a,b). However, our results provide no evidence for a time lag between events taking place in liver and muscle and indicate that the two tissues respond to the drug equally quickly. The increases in liver tryptophan pyrrolase activity and incorporation of precursors into liver RNA start almost immediately after the administration of the steroid, as does the decrease in the incorporation of RNA precursors into muscle. Thus this glucocorticoid could affect many sites simultaneously. One of these sites may be the nuclear mechanism for the production of muscle RNA and ribosomal activity may fall because of this interaction.

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