Time course of the effect of catabolic doses of corticosterone on protein turnover in rat skeletal muscle and liver

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The time course of the response of protein synthesis in muscle and liver to catabolic doses of corticosterone (10 mg/day per 100 g body wt.) was studied *in vivo* in growing rats over a 12-day period. The rate of protein synthesis in muscle and liver and the rate of actomyosin synthesis in muscle were measured by the phenylalanine-flooding technique, and 3-methylhistidine (*N*^ε-methylhistidine) synthesis was measured by injection of labelled histidine. 3-Methylhistidine concentrations in tissue free pools and urinary excretion were also measured to compare directly with the rate of muscle protein degradation determined as the difference between synthesis and growth each day during the treatment. The overall rate of protein synthesis in muscle fell gradually over the first 4 days, reaching a rate after 5 days that was 36% of the initial rate, and this lower rate was then maintained for the following week. This decrease in the overall rate was accompanied with changes in the relative rate of synthesis in muscle proteins, since during the first 4 days there was a disproportionate decrease in the rate of actomyosin synthesis, and specifically 3-methylhistidine synthesis. In the latter case the synthesis rate was decreased to only 4% of its initial rate after 4 days. These changes in protein synthesis in muscle were accompanied by a transient increase in the rate of protein degradation, which was more than doubled on days 2 and 3 of treatment but which returned to the original rate on day 5, and a similar pattern of response was indicated by urinary 3-methylhistidine excretion, which also exhibited a transient increase. Thus in this case 3-methylhistidine excretion and measured rates of protein degradation in muscle do correlate. The transient effects of the glucocorticoids on degradation compared with the sustained effect on synthesis suggest that these two responses are achieved by different mechanisms. The hepatic size and protein mass were increased by the treatment, and protein synthesis was well maintained until after 12 days, when the rate was suppressed. Although the fractional synthesis rate was transiently increased for 24 h, it is argued that the enlarged liver most likely reflects a decrease in protein degradation resulting from the increased amino acid supply to the liver. This would result from the cessation of muscle growth while dietary supply was maintained.

We have previously reported that glucocorticoids given to rats for 5–6 days suppressed protein synthesis in skeletal muscle, but did not markedly alter rates of protein degradation in this tissue (Millward *et al.*, 1976a; Odedra & Millward, 1982). Similar results have been reported by Kelly & Goldspink (1982). In contrast, measurements of 3-methylhistidine (*N*^ε-methylhistidine) excretion in-

dicated increased degradation in response to catabolic doses of corticosterone (Tomas *et al.*, 1979; Santidrian *et al.*, 1981a,b). We have criticized the assumption that increased 3-methylhistidine excretion necessarily indicates increased skeletal-muscle protein breakdown, since in the rat non-muscle sources of 3-methylhistidine in the urine are considerable (Millward *et al.*, 1980; Millward &

Bates, 1983). However, a closer inspection of the results of Tomas *et al.* (1979) and particularly those of Santidrian *et al.* (1981b) showed that the marked increase in 3-methylhistidine excretion was an early response to treatment, but that after 4 days excretion rates were more or less normal. Thus the differences between our results and those implied from changes in 3-methylhistidine excretion may have been more apparent than real, reflecting the timing of the measurements. We have therefore made daily measurements of the effects of a catabolic dose of the hormone to determine the time course of its effects on muscle protein synthesis and degradation and urinary 3-methylhistidine excretion. It would appear that increased muscle protein degradation and 3-methylhistidine excretion are early transient responses to the treatment, but after a few days degradation returns to normal values, and suppression of protein synthesis is then the main reason for muscle growth suppression.

Since we have previously observed that in response to starvation, a condition involving increases in glucocorticoids, there is a disproportionate suppression of the synthesis of the contractile proteins (Bates & Millward, 1983; Bates *et al.*, 1983), we have measured the synthesis of actomyosin and 3-methylhistidine in the present study. It would appear that actin synthesis is markedly and disproportionately suppressed by treatment with corticosterone.

In contrast with their catabolic effect on peripheral tissues, glucocorticoid hormones have long been known to increase the protein content of the visceral tissues, particularly the liver (see Munro, 1964). Furthermore, there are several reports indicating increased rates of synthesis of fixed (i.e. non-exported) liver and export proteins, particularly after 24 h of treatment by the hormone (see Waterlow *et al.*, 1978). We have reported that the effect of triamcinolone treatment is to increase the rates of fixed liver protein synthesis, particularly in malnourished rats (Millward *et al.*, 1976b). However, most of the reported studies have involved methods which are not completely reliable, so that the mechanism of the increase in liver protein content is not clear. We have therefore followed the changes in protein synthesis in the liver with a more reliable method and show that protein synthesis is indeed well maintained in response to the treatment, at least for the first week. Since the main factor that regulates hepatic protein balance is dietary amino acid supply (Waterlow *et al.*, 1978; Garlick, 1980; Millward, 1980), it is quite likely that the changes in hepatic protein balance may be associated with an effect of the hormone on amino acid supply to the liver, and thus may be, at least in part, an indirect effect. Our measurements of food intake show that, although growth is suppressed, food intake does not

fall, with the necessary consequences that amino acid intake is surplus to requirements for growth and that amino acid supply to the liver for oxidation is increased.

Experimental

Materials

L-[4-³H]Phenylalanine, obtained from The Radiochemical Centre, Amersham, Bucks., U.K. (28 Ci/mmol), was made up to 150 mM with unlabelled phenylalanine so that the final solution contained 50 μ Ci/ml. L-[2,5-³H]Histidine (57.5 Ci/mmol), also obtained from The Radiochemical Centre, was used directly as supplied (1 mCi/ml). Corticosterone was obtained from Sigma (London) Chemical Co., Poole, Dorset, U.K.

Experimental design

The objective of these experiments was to follow the time course of changes in muscle protein mass and protein synthesis in rats treated with a catabolic dose of corticosterone (10 mg/100 g body wt. per day), so that the changes in degradation could be measured daily rather than at a single time as we previously described (Odedra & Millward, 1982). The gastrocnemius muscle was chosen, since it is reasonably large and easy to dissect quantitatively as well as being reasonably representative of the mixture of fibre types present in rodent muscle (Ariano *et al.*, 1973). An attempt was made to define as accurately as possible the growth rate of the protein mass of this muscle by measuring the changes on the previous and, in most cases, subsequent days to that when the synthesis rate was measured. Thus rats were treated every day for up to 12 days and killed in groups on days, 0, 1, 2, 3, 4, 5, 6 and 12, and synthesis rates were measured in every group except for the group killed on day 6. This group served to provide information about the rate of change of muscle protein mass on the day after the measurement of synthesis on day 5, so that growth rate (and hence degradation rate) could be calculated accurately on day 5. The growth rates of the gastrocnemius protein were calculated for each group of rats from the changes in body weight and in the muscle-protein/body-weight ratios. The change in body weight was calculated as the percentage increase for each rat, and mean values were calculated for each group. Mean values of muscle-protein/body-weight ratios were then calculated and percentage values for changes combined with body-weight changes. Thus only mean values for each group were obtained, and variability is probably best indicated by the variability in whole-body growth rates, since this was the largest single component.

The measurements of synthesis included that of mixed muscle proteins and actomyosin extracted from the same muscle, but, since no attempt was made to quantify actomyosin, degradation rates of this fraction were not measured. We also measured the relative labelling of histidine and 3-methylhistidine isolated from actomyosin in order to assess the synthesis rate of 3-methylhistidine. Since measurements of the synthesis rate of 3-methylhistidine require relatively large amounts of 3-methylhistidine to be isolated, actomyosin was also extracted from the whole skinned eviscerated carcass of the same rats and analysed for the labelling of 3-methylhistidine and histidine.

All rats were fed on a purified diet free of 3-methylhistidine and containing 20% protein (see Millward *et al.*, 1974) and housed individually. Food intake was measured daily throughout the experiments for all rats. A total of 42 rats were injected subcutaneously with 10 mg of corticosterone/100 g body wt. each day in a vehicle described previously (Odedra & Millward, 1982) and killed in groups of six after 1, 2, 3, 4, 5, 6 and 12 days, and 15 rats served as controls and were injected with vehicle alone, being killed on days 0, 5 and 12. Values for body-weight growth and food intake are the mean values for all the animals, so that the number in each group became progressively smaller as rats were killed while the experiments proceeded. In addition, two groups were similarly treated with corticosterone or vehicle alone except that they were housed in metabolic cages for the collection of urine for 12 days.

Measurement of protein synthesis

Measurements of rates of mixed protein and actomyosin synthesis were made by the phenylalanine flooding technique (Garlick *et al.*, 1980). This involves measurement of incorporation over 10 min after an injection of a dose of labelled phenylalanine equal to 10–20 times the whole-body free pool. This results in the rapid achievement of similar labelling in intracellular and extracellular pools, and this falls only very slowly over 10 min. In the same way as others have reported (Kelly & Goldspink, 1982), we also found in other experiments that the fall in labelling between 2 and 10 min in muscle and liver was insignificant, so that all animals in each group were killed 10 min after injection of phenylalanine. Synthesis rates were calculated from the labelling of protein-bound (S_p) and free (S_f) phenylalanine in gastrocnemius muscle and liver at 10 min assuming that the average value for S_f over 10 min was the same as the S_f measured at 10 min.

Measurements of the relative rates of synthesis of

3-methylhistidine and mixed protein were made by comparing the relative rates of incorporation of histidine into protein-bound histidine and 3-methylhistidine isolated from actomyosin.

At 50 min before the injection of phenylalanine (1 ml/100 g body wt.) 0.1 ml of histidine was injected intravenously. Thus incorporation of the labelled histidine was measured over 60 min.

Rats were killed by decapitation, and blood was collected from the neck. Gastrocnemius muscle and liver were quickly removed, weighed and frozen in liquid N_2 . Rats were then skinned and eviscerated and the carcass was weighed. One half of each gastrocnemius and the whole liver were analysed for protein and RNA as described previously (Millward *et al.*, 1974). The labelling of phenylalanine was measured in the initial acid supernatant and in the hydrolysate of the protein isolated after nucleic acid extraction, by the method described by Garlick *et al.* (1980).

Actomyosin was extracted from the other half of the gastrocnemius and from the whole carcass as described previously (Bates & Millward, 1983). Although no specific analysis of the purity of actomyosin was performed in these experiments, previous examination by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis has shown that this fraction contains predominantly actin, myosin and other contractile proteins (Bates *et al.*, 1983). Histidine and 3-methylhistidine were isolated from the actomyosin extracted from the carcass by ion-exchange chromatography as described previously (Bates *et al.*, 1983).

Since incorporation of labelled phenylalanine into the carcass may have continued for about 1–1½ min after death, because of the time taken for the carcass to cool down in the ice/water, no attempt was made to measure the phenylalanine labelling in this fraction. It was assumed that any continued incorporation of histidine after death would not alter the relative rates of histidine and 3-methylhistidine labelling.

Measurements were also made of free 3-methylhistidine in the gastrocnemius-muscle supernatant and plasma by reverse-phase chromatography of a fluorescent derivative of 3-methylhistidine, based on the method of Jones *et al.* (1982) as described previously (Millward & Bates, 1983). 3-Methylhistidine in hydrolysed actomyosin was also measured by this method, whereas tyrosine content was measured by the method of Waalkes & Udenfriend (1957). Measurements of urinary 3-methylhistidine were made by ion-exchange chromatography as described previously (Millward *et al.*, 1980).

Plasma insulin was measured by a charcoal separation radio-immunoassay (Herbert *et al.*, 1965) with human insulin standards (Wellcome).

Results and discussion

Effects of corticosterone treatment on growth, body composition and food intake

As shown previously (Odedra & Millward, 1982), the hormone treatment suppressed growth, so that after 12 days body weight was unchanged from the initial value (Table 1). However, there was a transient increase in body weight during the first 24 h of treatment, so that the growth suppression was not immediate. This initial gain in body weight was then lost on day 2 of treatment, after which time body weight was stable. These changes were also observed for muscle protein, except that the initial increase on day 1 of treatment was more marked, with gastrocnemius protein increasing by 12% compared with the 4% increase in body weight. Judging by the changes in carcass weight, these changes in gastrocnemius muscle were a good guide to changes in the musculature as a whole. The liver mass increased by 29% in the first 24 h and further increased after 5 days by 61% above the initial value. Similar changes were reported by Tomas *et al.* (1979).

The food intake in these rats (Table 2) was initially 14.4 g/100 g body wt., falling by about one-third as the control rats doubled their body weight. This initial food intake was maintained in the treated rats and even increased for a short period after the first week of treatment.

Effects of corticosterone on muscle protein synthesis and degradation

Rates of protein synthesis (Table 3) in the well-fed animals were very consistent with values that we

have previously obtained by the constant-infusion method with tyrosine. In particular, the RNA activity values (the rate of protein synthesis per unit of RNA; Table 4) were not significantly different from those that we have previously reported in well-fed rats of this strain, whether intact (Odedra *et al.*, 1982) or adrenalectomized (Odedra & Millward, 1982). The fall in overall synthesis rate

Table 2. *Food intake and plasma insulin concentrations in corticosteroid-treated rats*

Values for food intake are the mean values for all the rats studied (described in the legend of Table 1). Plasma insulin values were determined on the plasma of each rat killed at the various time points. Results are the means $\pm$ 1 S.E.M. for each group.

Treatment duration (days)	Food intake (g/100 g/body wt. per day)	Plasma insulin (μ units/ml)
Control		
0	14.4 $\pm$ 0.4	28.5 $\pm$ 2.5
5	12.4 $\pm$ 0.3	36.8 $\pm$ 3.5
12	9.05 $\pm$ 0.1	34.0 $\pm$ 2.9
Corticosterone-treated		
1	15.2 $\pm$ 0.3	56 $\pm$ 13
2	15.6 $\pm$ 0.5	59 $\pm$ 3.5
3	14.5 $\pm$ 0.6	67 $\pm$ 5.2
4	15.1 $\pm$ 0.6	67 $\pm$ 2.3
5	15.4 $\pm$ 0.8	61 $\pm$ 1.6
8	18.3 $\pm$ 1.0	
9	17.1 $\pm$ 2.9	
12	14.8 $\pm$ 1.5	72 $\pm$ 6

Table 1. *Effect of corticosterone treatment on body weight and composition*

Measurements were made on a total of 57 rats, all of which were fed on a 20%-protein purified diet free of 3-methyl-histidine. The rats were divided into ten groups, seven of which were treated with 10 mg of corticosterone/100 g body wt. by daily subcutaneous injection, and three groups which were treated with vehicle alone. The groups were killed at the times indicated. Carcass weight is the weight of the skinned eviscerated rat, and the gastrocnemius weight and protein refer to that in a single muscle. Results are means $\pm$ 1 S.E.M. for each group.

Treatment duration (days)	Body wt. (g)	Carcass wt. (g/100 g body wt.)	Gastrocnemius wt. (mg/100 g body wt.)	Gastrocnemius protein (% of wet wt.)	Liver wt. (g/100 g body wt.)
Control					
0	102 $\pm$ 0.7	41.2 $\pm$ 0.3	423 $\pm$ 26	16.5 $\pm$ 0.1	4.88 $\pm$ 0.08
5	146 $\pm$ 2.9	42.3 $\pm$ 0.2	431 $\pm$ 11	16.6 $\pm$ 0.2	4.81 $\pm$ 0.03
12	205 $\pm$ 4.6	43.7 $\pm$ 1.0	491 $\pm$ 14	16.9 $\pm$ 0.1	4.83 $\pm$ 0.10
Corticosterone-treated					
1	106 $\pm$ 0.9	43.4 $\pm$ 1.7	447 $\pm$ 4	16.8 $\pm$ 0.1	6.08 $\pm$ 0.31
2	103 $\pm$ 0.9	41.6 $\pm$ 0.3	417 $\pm$ 0.3	17.5 $\pm$ 0.1	6.39 $\pm$ 0.40
3	102 $\pm$ 1.0	41.1 $\pm$ 0.2	397 $\pm$ 3	17.9 $\pm$ 0.1	6.96 $\pm$ 0.20
4	99 $\pm$ 1.2	41.2 $\pm$ 0.9	398 $\pm$ 5	17.4 $\pm$ 0.2	6.78 $\pm$ 0.20
5	99 $\pm$ 1.7	41.1 $\pm$ 0.7	402 $\pm$ 6	17.6 $\pm$ 0.2	8.11 $\pm$ 0.50
6	97 $\pm$ 2.4	42.3 $\pm$ 0.3	409 $\pm$ 12	17.6 $\pm$ 0.1	7.05 $\pm$ 0.18
12	100 $\pm$ 5.2	40.1 $\pm$ 0.86	402 $\pm$ 9	17.4 $\pm$ 0.2	7.42 $\pm$ 0.40

Table 3. *Rates of protein synthesis in gastrocnemius muscle from corticosterone-treated rats*

Rates of protein synthesis were determined *in vivo* by the phenylalanine flooding technique over 10 min as described in the text. Gastrocnemius muscle was divided into two portions, for the isolation of either free and mixed protein-bound phenylalanine, or phenylalanine in actomyosin isolated as described in the text. Protein synthesis was determined assuming that labelling of the free pool observed at 10 min was similar to the average value of the precursor over the incorporation period. This was verified in preliminary experiments. Values are means $\pm$ 1 S.E.M. for the groups treated as in Table 1, except that these measurements were not made on the group treated for 6 days with the hormone.

Treatment duration (days)	Phenylalanine labelling (d.p.m./nmol)			Protein synthesis (%/day)	
	Intracellular	Mixed protein	Actomyosin	Mixed protein	Actomyosin
Control					
0	600 $\pm$ 23	0.777 $\pm$ 0.049	0.651 $\pm$ 0.025	18.5 $\pm$ 0.2	15.5 $\pm$ 0.4
5	573 $\pm$ 8	0.554 $\pm$ 0.010	0.484 $\pm$ 0.020	13.5 $\pm$ 0.9	11.8 $\pm$ 0.2
12	649 $\pm$ 20	0.505 $\pm$ 0.023	0.412 $\pm$ 0.008	10.9 $\pm$ 0.7	8.9 $\pm$ 0.3
Corticosterone-treated					
1	574 $\pm$ 30	0.549 $\pm$ 0.021	0.433 $\pm$ 0.042	13.3 $\pm$ 0.1	10.5 $\pm$ 0.5
2	588 $\pm$ 1	0.427 $\pm$ 0.018	0.271 $\pm$ 0.003	10.1 $\pm$ 0.2	6.4 $\pm$ 0.3
3	573 $\pm$ 23	0.388 $\pm$ 0.031	0.252 $\pm$ 0.003	9.4 $\pm$ 0.3	6.1 $\pm$ 0.3
4	563 $\pm$ 35	0.320 $\pm$ 0.030	0.197 $\pm$ 0.002	7.9 $\pm$ 0.8	4.87 $\pm$ 0.27
5	583 $\pm$ 22	0.277 $\pm$ 0.063	0.202 $\pm$ 0.008	6.6 $\pm$ 0.75	4.82 $\pm$ 0.75
12	595 $\pm$ 6	0.264 $\pm$ 0.039	0.201 $\pm$ 0.008	6.3 $\pm$ 0.50	4.79 $\pm$ 0.56

Table 4. *RNA concentration and activity in muscle from corticosterone-treated rats*

Values for RNA/protein ratio are calculated from the concentrations of RNA and protein measured in the gastrocnemius muscle as described in the text. Values for the RNA activity are calculated from the RNA/protein ratios and fractional synthesis rates shown in Table 3 measured in the same muscle. Values are means $\pm$ 1 S.E.M. for the groups treated as described in Table 1.

Treatment duration (days)	RNA/protein (μ g/mg)	RNA activity (g of protein synthesis/day per g of RNA)
Control		
0	13.4 $\pm$ 0.4	14.2 $\pm$ 0.30
5	9.33 $\pm$ 0.46	14.4 $\pm$ 0.80
12	7.97 $\pm$ 0.28	13.7 $\pm$ 1.21
Corticosterone-treated		
1	11.7 $\pm$ 0.8	11.6 $\pm$ 1.0
2	8.75 $\pm$ 0.44	11.6 $\pm$ 0.3
3	8.00 $\pm$ 0.36	11.9 $\pm$ 1.2
4	7.20 $\pm$ 0.30	11.1 $\pm$ 1.0
5	5.80 $\pm$ 0.52	11.6 $\pm$ 0.9
12	5.18 $\pm$ 0.06	12.1 $\pm$ 1.3

over the 12 days of the experiment (Table 3), owing to the fall in RNA concentration (Table 4), illustrates the marked developmental changes that occur at this particular age (Bates & Millward, 1981). The rate of synthesis after 5 days of treatment with corticosterone fell to values similar to those that we have previously reported (Odedra *et al.*, 1982; Odedra & Millward, 1982). The

suppression of protein synthesis was observed after 1 day of treatment with a fall of 27%, and with a continued fall after this until the plateau value was reached after 5 days. Thus the fall in synthesis was not as rapid as that observed on complete starvation (Millward & Waterlow, 1978). Part of the reason for this is the fact that the RNA activity in the treated animals, although lower than in the controls, was better maintained than in starved or diabetic rats (Pain & Garlick, 1974; Millward & Waterlow, 1978; Odedra *et al.*, 1982), possibly as a result of the high circulating concentrations of insulin (Table 2) induced by the steroid treatment. Thus the major component of the fall in protein synthesis was the loss of RNA to less than half the original value (Table 4).

The rate of synthesis of actomyosin was on average 84% of the overall rate for mixed muscle proteins in the control rats. However, after treatment with corticosterone the synthesis rate of the actomyosin was more depressed than the overall rate. After 2 days actomyosin synthesis was decreased by 60%, compared with 45% for the overall rate. Since actomyosin comprises about 70% of the total protein, this must mean that the synthesis rate of soluble proteins was much less affected by the treatment. On the assumption that soluble proteins account for 30% of the total, the synthesis rate of the soluble protein fraction can be calculated and shown to fall from an initial value of 25.5%/day to 19.8, 18.7, 17.1, 15.0, 10.8 and 9.8%/day after 1, 2, 3, 4, 5 and 12 days of treatment. Thus the relative synthesis rate of the soluble fraction and actomyosin increased from a ratio of 1.65 in the fed state

Table 5. *3-Methylhistidine-synthesis rate in muscle from corticosterone-treated rats*

The relative labelling of histidine and 3-methylhistidine isolated from actomyosin was determined 60 min after the injection of trace amounts of [^{14}C]-histidine in the same animals in which actual rates of actomyosin synthesis were determined (as described in Table 3). However, the actomyosin for these measurements was extracted from the homogenized carcass and is therefore representative of the total musculature. The 3-methylhistidine-synthesis rate is calculated as the product of the actomyosin-synthesis rate shown in Table 3 and the 3-methylhistidine/histidine ratio. Values are means $\pm$ 1 S.E.M. of the groups treated as in Table 1.

Treatment duration (days)	3-Methylhistidine/histidine (relative labelling)	3-Methylhistidine synthesis (%/day)
0	0.473 $\pm$ 0.122	7.44 $\pm$ 1.65
1	0.320 $\pm$ 0.100	3.14 $\pm$ 1.03
2	0.165 $\pm$ 0.007	1.08 $\pm$ 0.08
3	0.135 $\pm$ 0.005	0.80 $\pm$ 0.03
4	0.063 $\pm$ 0.039	0.31 $\pm$ 0.15
5	0.059 $\pm$ 0.029	0.36 $\pm$ 0.12

(similar to values previously reported by us in rats of this age; Bates & Millward, 1983) to 3 after 2–4 days of treatment. This once again demonstrates the fact that, when protein synthesis is depressed in muscle, there is a disproportionate decrease in the synthesis of the contractile proteins (Bates & Millward, 1983; Bates *et al.*, 1983).

We have previously shown that actin synthesis is particularly sensitive to starvation (Bates *et al.*, 1983), so in the present studies the synthesis rate of 3-methylhistidine was measured, since most of the 3-methylhistidine in muscle is in actin. The relative labelling of 3-methylhistidine and histidine after a 1 h injection of histidine is shown in Table 5. As long as there is no substantial lag in the methylation of histidine after peptide-bond synthesis, this ratio should be equal to the relative synthesis rate of protein-bound 3-methylhistidine and mixed muscle proteins. Since we have recently measured the 3-methylhistidine/histidine ratio at 1 and 4 h and observe the same ratio (P. C. Bates & D. J. Millward, unpublished work), we can be reasonably confident of the validity of the assumption described above. Thus by comparing the ratio of 3-methylhistidine and histidine labelling with the overall rate of actomyosin synthesis shown in Table 3, the actual rate of 3-methylhistidine synthesis can be calculated. As shown in Table 5, this rate was 7.44%/day in the control rats and fell very markedly to only about 0.3%/day after 4 days of treatment. This change was probably not due to the suppression of actin

methylation, since the 3-methylhistidine/tyrosine ratio of the actomyosin did not change over the treatment period according to our measurements. The ratio of tyrosine/3-methylhistidine was 42.2 ± 6.3 in control rats measured on day 0 and 40.2 ± 2.8 in 5-day-treated rats.

Actin comprises about 28% of natural actomyosin (see Waterlow *et al.*, 1978), with myosin accounting for about 60%. Thus with this information, and the assumption that marked changes in the composition of natural actomyosin do not occur with this treatment, the synthesis rates of the non-actin component of the contractile proteins can be calculated, which should mainly reflect myosin synthesis. In the control rats the rate was 18.6%/day, falling to 13.4, 8.5, 8.2, 6.6 and 6.6%/day after 1, 2, 3, 4 and 5 days treatment, i.e. a pattern of response very similar to that for the overall rate. Thus there would appear to be a hierarchy of sensitivity to corticosterone treatment, with actin most sensitive and soluble proteins least sensitive, and non-actin contractile proteins responding in the same way as the average for the muscle.

The mechanism of these relative changes in synthesis rate is not known. We have previously argued (Bates & Millward, 1983) that, since the difference in relative synthesis rate appears to be related to the extent of suppression of the overall rate, particularly associated with a fall in RNA activity, the changes were an accompaniment to decreased rates of translation. However, in the present results the RNA activity was not as markedly suppressed as observed in, say, starved or diabetic rats, when the value can fall to half the value observed here (Millward & Waterlow, 1978; Odedra *et al.*, 1982). Furthermore, it did not change progressively throughout the treatment period, in contrast with the progressive fall in 3-methylhistidine/histidine labelling ratio (Table 5). Thus the 3-methylhistidine/histidine labelling ratio was not correlated with the RNA activity, but was in fact better correlated with the RNA concentration. This could mean that the changes in relative synthesis rate may occur as a result of differential losses of mRNA. How this could occur is not known, but clearly this problem deserves more investigation.

In Table 6 rates of change of the gastrocnemius muscle protein are shown, which can be compared with the synthesis rates, enabling degradation rates to be calculated. The growth rates are calculated from the rate of change of body weight over the 24 h before the measurement, together with changes in muscle protein content per unit body weight. For the control rats measurements of the growth rates were made every day for the 3 days before measurement and average values were taken.

In the control rats the growth of the gastrocnemius protein was initially a little faster than that of

Table 6. *Effect of corticosterone treatment on muscle protein degradation*

Values for muscle protein growth were determined with measurements of the rates of change of body weight, muscle weight and protein content of the gastrocnemius muscle. Since the calculation of degradation was made by comparing rates of protein synthesis measured at a fixed time point with growth rates over 24 h, the rates of muscle protein growth and protein synthesis have been manipulated to give values for the fixed time points as well as for each 24 h period. Thus growth rates at the fixed times when protein synthesis was measured were assumed to be the mean values of the rates observed over the 24 h periods before and after each measurement. Rates of protein synthesis over the 24 h periods of the treated rats were assumed to be the mean of the values measured at the start and finish of each 24 h period. Values for body-weight growth are mean values ($\pm$ 1 S.E.M.) for all treated and control groups described in Table 1. Since muscle protein growth rates were calculated from mean values for whole-body growth and gastrocnemius protein content, and daily rates of protein synthesis were calculated from the mean values of the measured rate in each group, variability of the rates of protein degradation cannot be precisely calculated.

Treatment duration (days)	Body weight growth (%/day)	Muscle protein growth (%/day)	Synthesis rate (%/day)	Degradation rate (%/day)
Control				
0	10.26 $\pm$ 0.22	11.55	18.5	6.95
5	4.8 $\pm$ 0.86	6.09	13.5	7.41
12	3.3 $\pm$ 1.3	4.59	10.9	6.31
Corticosterone-treated (10 mg)				
0	10.26 $\pm$ 0.22	11.55	18.5	6.95
0-1	4.28 $\pm$ 0.72	11.2	15.9	4.7
1		2.9	13.3	10.4
1-2	-3.48 $\pm$ 0.68	-5.7	12.7	18.4
2		-4.7	10.1	14.8
2-3	-0.64 $\pm$ 0.28	-3.7	9.75	13.45
3		-4.6	9.4	14.0
3-4	-2.56 $\pm$ 0.49	-5.5	8.65	14.15
4		-1.8	7.9	9.7
4-5	-0.85 $\pm$ 0.43	2.0	7.25	5.25
5		0.9	6.6	5.7
5-6	-1.88 $\pm$ 1.40	-0.2		
11-12	1.2 $\pm$ 1.22	+0.02		
12			6.3	6.3

the body weight, since gastrocnemius-muscle protein as a fraction of body weight was increasing with age (Table 1). Over the first 24 h of treatment with corticosterone the control growth rate was maintained, but during day 2 there was a sharp fall and protein was lost. This negative balance continued until after the end of day 4 there was a cessation of the wasting, and balance was more or less maintained after that time.

The synthesis rates were measured at one time point, whereas growth rates were measured over 24 h periods before and after the measurements. This means that degradation rates can be calculated in two ways. Thus a continuous change in synthesis rates can be assumed between individual measurements, and the average rate for each 24 h period can be calculated to compare with the measured growth rate. Alternatively growth rates at the time of measurement can be calculated as the mean of the rates measured before and after, and this rate can be compared with the measured synthesis rate. In Table 6 we have made both sets of calculations, since there is no reason to prefer one method over the other. In any case the values are only likely to be approxi-

mate, since there are almost certainly some diurnal changes in synthesis, particularly in the control rats. In the treated rats, however, with protein synthesis suppressed, the single measurements of synthesis may be closer to the average rate for the day. This is supported by our observations that in malnourished rats diurnal variations in protein synthesis are not noticeable (M. Cox & D. J. Millward, unpublished work), as reported in obese patients on a low-energy diet (Clugston & Garlick, 1981).

It is clear that by the end of the first 24 h and during day 2 of treatment the growth suppression and net catabolism involved a marked increase in protein degradation, which appears to be maintained until the end of day 3. After this time the degradation rate returned to normal values. Thus in our previous reports (Odedra & Millward, 1982) our inability to observe increased degradation was a result of limiting our measurements to 5-6 days of treatment. The increase in degradation as measured by this method appears therefore to be transient.

The rates of excretion of 3-methylhistidine are shown in Table 7 together with changes in the concentration in the free pool in the muscle and

Table 7. *Effect of corticosterone on free 3-methylhistidine concentration in muscle and plasma, and daily urinary excretion*

Concentration of free 3-methylhistidine in gastrocnemius muscle and plasma was determined in the groups of rats treated as described in Table 1. Urinary 3-methylhistidine excretion was measured on 24 h collections in two groups of rats treated with corticosterone or vehicle alone, which were eventually killed after 12 days. The urinary excretion is expressed as the total amount per g of gastrocnemius muscle by dividing the excretion by the weight of one gastrocnemius muscle. Values are expressed as means $\pm$ 1 S.E.M.

Treatment duration (days)	Muscle (nmol/g)	Plasma (nmol/ml)	Urine (μ mol/g of gastrocnemius muscle per day)
Control			
0	6.17 $\pm$ 1.34	3.65 $\pm$ 1.10	2.43 $\pm$ 0.28
5	7.03 $\pm$ 0.35	3.78 $\pm$ 0.98	2.15 $\pm$ 0.40
12	6.98 $\pm$ 0.57	4.12 $\pm$ 0.09	2.08 $\pm$ 0.36
Corticosterone-treated			
1	5.75 $\pm$ 1.03	2.64 $\pm$ 0.30	3.40 $\pm$ 0.44
2	6.65 $\pm$ 1.30	3.29 $\pm$ 0.48	4.53 $\pm$ 0.58
3	4.43 $\pm$ 0.45	3.27 $\pm$ 0.80	4.71 $\pm$ 0.74
4	4.85 $\pm$ 0.30	3.04 $\pm$ 0.54	3.97 $\pm$ 0.80
5	3.77 $\pm$ 0.58	2.65 $\pm$ 0.40	3.18 $\pm$ 0.29
6	5.54 $\pm$ 1.20	3.79 $\pm$ 0.79	2.54 $\pm$ 0.12
12	5.06 $\pm$ 0.65	3.17 $\pm$ 0.53	2.56 $\pm$ 0.30

plasma. We have expressed the output in terms of muscle mass as judged by the gastrocnemius weight. The excretion was increased during day 1 of treatment and reached a maximum on day 3–4. By day 6 the values had returned to normal. These changes are similar to those reported by Tomas *et al.* (1979) and particularly by Santidrian *et al.* (1981b). Thus the changes in urinary 3-methylhistidine excretion fit reasonably well with the change in rate of muscle protein degradation. Clearly this is one occasion when the changes in urinary 3-methylhistidine excretion do reflect the changes in muscle protein degradation, possibly because of similar changes in muscle and non-muscle 3-methylhistidine pools, in contrast with conditions such as Duchenne muscular dystrophy (Rennie *et al.*, 1982) and several other clinical conditions (Rennie & Millward, 1983), where 3-methylhistidine excretion and muscle degradation do not correlate.

In our previous paper (Oedra & Millward, 1982) we reported that in adrenalectomized rats treated with catabolic doses of corticosterone we observed a marked increase in the concentration of free 3-methylhistidine in muscle but not in plasma. In the present experiment these changes were not observed.

No substantial changes in free concentration of 3-methylhistidine in either muscle or plasma were observed, neither was the muscle/plasma concentration gradient changed. This was surprising, since we had thought that the increase in free 3-methylhistidine concentration in muscle was a sensitive index of muscle protein degradation (Rennie *et al.*, 1980), and in the present experiments increases in urinary 3-methylhistidine excretion and muscle protein degradation did occur. Clearly this indicates a very rapid clearance of free 3-methylhistidine by the kidney as well as no block to 3-methylhistidine transport out of muscle. We can only surmise that in our previous experiments (Oedra & Millward, 1982) the fact that the rats were adrenalectomized, or insulin-maintained diabetic animals, in some way decreased rates of 3-methylhistidine efflux out of muscle. What is clear, however, is that the measurement of free 3-methylhistidine concentration in muscle or the concentration gradient is not a good index of muscle protein degradation in this case.

Although the present results do reconcile the previous discrepant findings of the effects of glucocorticoid treatment *in vivo*, the physiological relevance of these findings is far from clear. Firstly, in incubated muscle from animals treated with glucocorticoids, degradation is suppressed (Shoji & Pennington, 1977), and this effect can be achieved in a few hours when glucocorticoids are added to the incubated muscles (McGrath & Goldspink, 1982). However, these changes are probably best seen as a partial suppression of the enhanced rate of degradation which normally occurs in incubated muscle (see, e.g., McGrath & Goldspink, 1982). There is no direct evidence that we know of that glucocorticoid treatment actually lowers the rate of degradation *in vivo*.

Secondly, it is not clear how the response to exogenous corticosterone observed here relates to the response to physiological changes in the hormone. The fact that the increase is only transient, whereas the suppression of protein synthesis is maintained at least over 12 days, would indicate that the effects of the hormone on the two processes are not directly linked. Furthermore, the response to treatment with exogenous glucocorticoids and to physiologically elevated concentrations of corticosterone that occurs during starvation would appear to be quite different. In response to starvation in rats, increases in corticosterone occur promptly (e.g. 24 h; Malatava & Ahlers, 1977), as does the decrease in insulin (Millward *et al.*, 1974). In contrast, the increase in muscle protein degradation is not an early response to starvation, only occurring after 3–4 days according to measurements *in vivo* (Millward & Waterlow, 1978; Cotellesa *et al.*, 1983) or in the perfused hemi-corpus (Goodman *et al.*, 1981). Since insulin lack is supposed to be as-

Table 8. *Effect of corticosterone treatment on protein synthesis in liver*

The synthesis rate measured over the 10 min after the flooding dose of phenylalanine will be the rate of both fixed and export protein synthesis. Synthesis rates were calculated by assuming the value for the labelling of free phenylalanine in liver measured after 10 min was similar to the mean precursor labelling over the 10 min injection period. Values for synthesis per rat are calculated from the fractional synthesis rate and liver protein content determined as the product of liver size (Table 1) and protein content. Values are shown as means $\pm$ 1 S.E.M. of the groups treated as described in Table 1.

Treatment duration (days)	Liver protein (mg/100 g body wt.)	RNA protein (μ g/mg)	Synthesis rate		RNA activity (g of protein/day per g of RNA)
			(%/day)	(mg/100 g body wt. per day)	
Control					
0	78 $\pm$ 3	65 $\pm$ 2	89 $\pm$ 3	68 $\pm$ 4	13.5 $\pm$ 0.9
5	73 $\pm$ 4	62 $\pm$ 3	86 $\pm$ 1	64 $\pm$ 3	13.9 $\pm$ 1.0
12	74 $\pm$ 5	64 $\pm$ 4	86 $\pm$ 3	63 $\pm$ 4	13.4 $\pm$ 0.9
Corticosterone-treated					
1	94 $\pm$ 5	61 $\pm$ 4	102 $\pm$ 3	95 $\pm$ 5	16.4 $\pm$ 1.1
2	97 $\pm$ 4	61 $\pm$ 3	91 $\pm$ 2	89 $\pm$ 3	14.8 $\pm$ 1.0
3	111 $\pm$ 7	59 $\pm$ 4	86 $\pm$ 5	94 $\pm$ 6	14.7 $\pm$ 1.2
4	100 $\pm$ 4	62 $\pm$ 4	86 $\pm$ 2	85 $\pm$ 4	13.9 $\pm$ 0.9
5	124 $\pm$ 8	55 $\pm$ 7	89 $\pm$ 4	111 $\pm$ 7	16.4 $\pm$ 1.1
12	122 $\pm$ 4	40 $\pm$ 3	70 $\pm$ 2	86 $\pm$ 5	17.8 $\pm$ 1.3

sociated with increased degradation (Jefferson *et al.*, 1977), the increased protein breakdown after exogenous corticosterone treatment is all the more surprising given the marked hyperinsulinaemia that occurs as a result of such treatment.

Effects of corticosterone treatment on hepatic protein synthesis

The changes in hepatic protein metabolism are shown in Table 8. Rates of protein synthesis were similar in the three control groups, the higher rate compared with muscle reflecting the higher RNA concentration, since RNA activities were similar to those in muscle. Hormone treatment did not change the liver composition, so that the increased mass (Table 1) was associated with increased protein and RNA, judging by the constant RNA/protein ratio, at least over the first 4 days. After this the RNA/protein ratio fell. In the treated groups the synthesis rates were well maintained throughout the first 5 days of treatment. Indeed on day 1 the mean value was 11% higher in the treated group, reflecting the higher RNA activity, and this difference was significant from the overall mean value for the three control groups ($P < 0.01$). However, after 12 days of treatment the rate was suppressed below the rate observed in any of the control groups. This was a result of the lower RNA concentration, since the RNA activity was elevated above control values at this time. Clearly the response to glucocorticoid treatment by the liver is quite different from that of muscle.

Although the effects of glucocorticoid treatment on hepatic protein mass and composition have been

extensively studied (e.g. Munro, 1964), few investigations of the mechanism of the change in liver protein mass have been reported (see Waterlow *et al.*, 1978). Kim & Kim (1975) reported a transient suppression (after 2 h), followed by an increase (after 8 h) of the overall rate of synthesis in response to prednisolone. Similarly Jeejeebhoy *et al.* (1973) reported a transient suppression of albumin synthesis (after 3 h) followed by a substantial increase after 24 h of treatment. We have previously made measurements of the rate of fixed liver protein synthesis in both well-fed and malnourished rats treated with glucocorticoids (Millward *et al.*, 1976a), which indicated increases in the rate, especially in the malnourished rat. The reliability of these results is questionable, however, since the actual labelling of the precursor pool was not measured. Quartey-Papafio (1982) reported that treatment with cortisone for 8 days suppressed the overall rate of hepatic protein synthesis, whereas dexamethasone had little effect on the rate. In each case the synthesis rate of albumin as a proportion of the overall rate was maintained at the control value. Thus all these results are reasonably consistent with the present finding, a transient increase in the fractional rate followed after prolonged treatment by a fall. However, the persistent enlargement of the liver throughout the treatment period means that the absolute rate of liver protein synthesis (fractional rate $\times$ liver mass) was markedly increased and remained elevated throughout the period.

The present measurements of protein synthesis include both fixed liver proteins and those exported. This latter fraction amounts to 25–40% of the total

(Scornik & Botbol, 1976; Pain *et al.*, 1978). Given this fact and given the report, by Jeejeebhoy *et al.* (1973) and John & Miller (1969), that the synthesis rate of albumin and other exported proteins is increased in response to glucocorticoids, it is quite possible that all of the initial increase in the fractional rate of protein synthesis could have been accounted for by changes in export-protein synthesis alone, the fractional synthesis rate of the fixed liver proteins remaining unchanged. It is certainly the case that the 25% increase in hepatic protein could not have been achieved by an increase in protein synthesis alone, since the overall rate increased by only 12.7%/day and the component of this increase associated with increased plasma synthesis would have no effect on hepatic mass. It would appear highly likely, therefore, that the rate of hepatic protein degradation was decreased by the treatment.

The difficulty of accounting for the action of glucocorticoid on the liver is to separate possible direct effects of the hormone on hepatic protein metabolism from secondary effects induced by the hormone's effect on hepatic amino acid supply and concentrations. Hepatic free amino acid concentrations are increased by glucocorticoid treatment in fed rats (Munro, 1964; Jeejeebhoy *et al.*, 1973) and the concentrations of alanine and several non-essential amino acids are markedly increased in the liver of treated malnourished rats (Lunn *et al.*, 1976). One well-known consequence of increased amino acid supply to the liver is markedly to suppress protein degradation (Mortimore & Schworer, 1977; Millward, 1980). Thus a decrease in hepatic protein degradation would be an expected response to glucocorticoid treatment.

In the present experiments, after the growth of muscle protein has stopped (after 24 h of treatment), since dietary amino acid supply was maintained, increased amounts of amino acids must have been available for oxidation, and this would explain elevated concentrations of amino acids in the liver. However, according to our current measurements during the first 24 h, dietary protein was deposited as muscle, and since dietary intake was only increased by 6% (Table 2) the extent of the increased amino acid availability for oxidation must have been small. Whether this small increase was sufficient to trigger the hepatic anabolic response without involving direct action of corticosterone remains to be determined.

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