Biochem. J. (1979) **178**, 139–146
Printed in Great Britain

Effect of Glucocorticoid Administration on the Rate of Muscle Protein Breakdown *in vivo* in Rats, as Measured by Urinary Excretion of N^{τ}-Methylhistidine

By FRANK M. TOMAS, HAMISH N. MUNRO and VERNON R. YOUNG
Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, MA 02139, *U.S.A.*

(*Received* 26 *July* 1978)

The role of glucocorticoids in regulating the rate of muscle protein breakdown was evaluated by measuring excretion of N^{τ}-methylhistidine during administration of various doses of corticosterone to adrenalectomized rats. Groups of rats received daily subcutaneous injections of 0, 0.2, 0.5, 1.0, 5.0 or 10.0mg of corticosterone/day per 100g body wt. for 7 days, followed by 3 days without hormone treatment, after which they were killed. A group with intact adrenal glands served as an additional control. All animals were pair-fed with the untreated adrenalectomized group. No significant differences were noted in growth rate or N^{τ}-methylhistidine excretion between the intact or adrenalectomized control groups, or those given 0.2, 0.5 and 1.0mg of corticosterone, whereas growth ceased and N^{τ}-methylhistidine excretion rose markedly in the groups receiving 5 and 10mg of corticosterone. After these two high doses of corticosterone, but not after lower doses, there was a loss of weight of the gastrocnemius muscle per 100g of final body wt., but not of the soleus and extensor digitorum longus muscles. The two highest doses of corticosterone also resulted in an increase in liver weight per 100g of final body wt. Lower doses of corticosterone did not cause these changes. Plasma corticosterone concentrations, measured on the final day of injection and again at the time of killing, were decreased to near zero by adrenalectomy and were little raised by doses of 0.2 and 0.5mg daily, but were increased to within the normal range by the 1mg dose. At 5 and 10mg doses, plasma corticosterone concentrations were sustained at 2–3 times those of intact rats, and thus in the range reported for rats exposed to severe stress. Rats given 5 and 10mg doses of corticosterone had glycosuria, and showed considerably elevated concentrations of insulin in the plasma. It is concluded that plasma concentrations of glucocorticoids within the normal range do not regulate the rate of muscle protein breakdown, whereas excessive plasma concentrations of corticosteroids, equivalent to those observed in severe stress, can accelerate muscle protein breakdown.

The net catabolic effects of glucocorticoid hormones from endogenous or exogenous sources on skeletal muscle are widely recognized and well documented (Young, 1970). Net loss of muscle protein occurs whenever breakdown rate exceeds synthesis rate. A decrease in muscle protein-synthesis rate after glucocorticoid administration has been established (Wool & Weinshelbaum, 1960; Young, 1970; Hanoune *et al.*, 1972), but a specific increase in the breakdown rate of muscle proteins has not yet been unequivocally demonstrated, although it has been assumed to occur on the basis of increased proteolytic activity in the muscles of corticosteroid-treated animals (Mayer *et al.*, 1976; Mayer & Rosen, 1977). An increase in the breakdown rate of muscle protein after treatment of rats with high doses of glucocorticoids has been reported (Goldberg, 1969, 1975). More recently, however, Shoji & Pennington (1977), using similar techniques *in vitro*, could not detect a change in muscle protein breakdown in corticosteroid-treated animals, a result that agrees with measurements *in vivo* by Millward *et al.* (1976). These various studies are difficult to interpret, since they involve different muscles and differing types and doses of administered glucocorticoid. In addition, in some of these studies, the problem of reutilization of the labelled amino acid introduces methodological complications that cannot be readily minimized.

We have used the urinary excretion of N^{τ}-methylhistidine, an amino acid present in myofibrillar protein, as an index *in vivo* of muscle protein breakdown. We have previously shown that this amino acid is released on breakdown of the myofibrillar proteins myosin and actin, and excreted quantitatively (Young *et al.*, 1972; Haverberg *et al.*, 1975*a*,*b*; Young & Munro, 1978). This approach thus provides an inte-

grated assessment of protein breakdown rates in the total skeletal musculature and is an elegant means of measuring changes in the rate of protein breakdown *in vivo* in muscle itself under the influence of various treatments. In the present paper, we report the effects of corticosterone administration to rats on the rate of muscle protein breakdown, as determined by urinary N^{τ}-methylhistidine excretion; the results are correlated with plasma corticosterone concentrations. It is concluded that glucocorticoids do not influence the rate of muscle protein breakdown until plasma concentrations of the hormone rise to values observed in states of severe stress.

Experimental

Animals and treatments

Adrenalectomized and intact male Sprague–Dawley rats, about 120g body wt. (Charles River Breeding Laboratories, Wilmington, MA, U.S.A.), were housed in individual metabolic cages. For 1 week after surgery, they were fed *ad libitum* on a purified diet containing 18% (w/v) lactalbumin (Haverberg *et al.*, 1975*a*), and the adrenalectomized rats were given 1% NaCl to drink. The adrenalectomized rats were then randomly assigned to six treatment groups of five rats each. Treatments consisted of a daily subcutaneous injection of corticosterone (purchased from Calbiochem, San Diego, CA, U.S.A.) for a 7-day period; each group received one of the following doses: 0.0, 0.2, 0.5, 1.0, 5.0 or 10.0mg of corticosterone/day per 100g body wt. A group of intact rats served as an additional control group. The steroid was injected in 0.5ml of vehicle, consisting of NaCl (0.8%), polysorbate 80 (Fisher Scientific, Pittsburgh, PA, U.S.A.) (0.4%), sodium CM-cellulose (0.5%) and benzyl alcohol (0.9%), administered between 11:00 and 12:00h each day, after the rats had been weighed. Both intact and adrenalectomized control groups received injections of the vehicle only. Intact controls and hormone-treated rats were pair-fed with the adrenalectomized control group; the daily ration was offered after completion of other experimental procedures, at about 12:00h.

Complete 24h urine collections were obtained from each rat, beginning 3 days before and ending 3 days after the 7-day hormone-treatment period; thymol was added as a preservative. On the final day of hormone treatment, blood samples were obtained from the tail of each rat immediately before (T_0) and 5h after (T_5) administration of the hormone. Then 3 days later, on completion of the urine collections, each rat was decapitated. Immediately, a blood sample was taken, the liver was excised and weighed, and the soleus, extensor digitorum longus and gastrocnemius muscles from the left rear leg were removed by careful dissection and individually weighed.

Determination of urine constituents

Samples of the daily urine collections were pooled within each group for N^{τ}-methylhistidine and creatinine analysis. Concentrations of N^{τ}-methylhistidine were determined individually on a Beckman 121 amino acid analyser, as previously described (Haverberg *et al.*, 1975*b*), after hydrolysis of the *N*-acetyl derivative with 2M-HCl in a boiling-water bath for 1–2h, and subsequent desalting on a cation-exchange column (Dowex AG50-X8; 6cm × 1cm), followed by stepwise elution with HCl of the acidic, neutral (2.0–2.5M-HCl) and basic (4.0–5.0M-HCl) amino acids. The acid eluate containing the basic amino acids was dried in a rotary evaporator, and the sample was reconstituted with citrate buffer (0.2M adjusted to pH2.2 with HCl) before application to the amino acid analyser.

Urinary creatinine was determined by the method of Hare (1950). Urinary glucose was monitored qualitatively with an enzymic test strip (Tes-Tape; Eli Lilly and Co., Indianapolis, IN, U.S.A.).

Plasma hormone assays

Plasma corticosterone was determined by the direct radioimmunoassay method described by Gomez-Sanchez *et al.* (1975). {The antibody was kindly provided by Dr. Gordon Williams, Peter Bent Brigham Hospital, Boston, MA, U.S.A. [1,2,6,7-^{3}H]-Corticosterone (sp. radioactivity 92Ci/mmol) was obtained from New England Nuclear, Boston, MA, U.S.A.} Substantial blank values were obtained with this method, owing to non-specific binding to the antibody by materials that could not be removed by charcoal treatment. A similar problem has been noted by others with this method (G. Williams, personal communication). Thus it is likely that the values obtained for the adrenalectomized rats are in fact the true blank values.

Insulin assays on pooled plasma samples were kindly carried out by radioimmunoassay by Dr. John Allsop, Shriner's Burns Institute, Boston, MA, U.S.A.

Statistical procedures

Statistical evaluations were carried out by conventional one- and two-way analysis of variance. Least significant differences were calculated and are shown in Table 1.

Results

Effect of corticosterone on body weight and organ weights

Fig. 1 shows changes in body weight of the groups of rats during the experiments. The animals were given about 10g of diet daily, which was essentially fully

Table 1. *Summary of statistical analyses of body weight gain, N^{τ}-methylhistidine and creatinine excretion, liver and muscle weights, and plasma corticosterone concentrations in intact and adrenalectomized rats receiving various doses of corticosterone*

For details see the text. Abbreviations: LSD, least significant difference between group means for $P<0.01$; NS, not significant ($P>0.05$).

Table or figure	Significance (P) of differences with respect to: Corticosterone treatment	Time	LSD
Fig. 1. Body weight gain during treatment (g/day)	<0.01		1.56
Table 2. Organ weights			
Liver (g)	<0.01		0.86
(g/100g body wt.)	<0.01		0.58
Soleus (mg)	<0.05		17.2
(mg/100g body wt.)	NS		
Gastrocnemius (mg)	<0.01		216.0
(mg/100g body wt.)	<0.01		59.1
Extensor digitorum longus (mg)	<0.01		14.5
(mg/100g body wt.)	<0.05		7.75
Urinary excretion during treatment			
Fig. 2. N^{τ}-Methylhistidine			
(μmol/day)	<0.01	NS	0.37
(μmol/100g body wt.)	<0.01	0.05>P<0.1	0.26
(μmol/g of creatinine)	<0.01	<0.01	320
Fig. 3. Creatinine (mg/day)	<0.01	<0.01	0.55
(mg/100g body wt.)	<0.01	<0.025	0.39
Fig. 4. Plasma corticosterone (μg/100ml)			
at 0h	<0.01		190
at 5h	<0.01		12.4
at 100h	<0.01		9.0

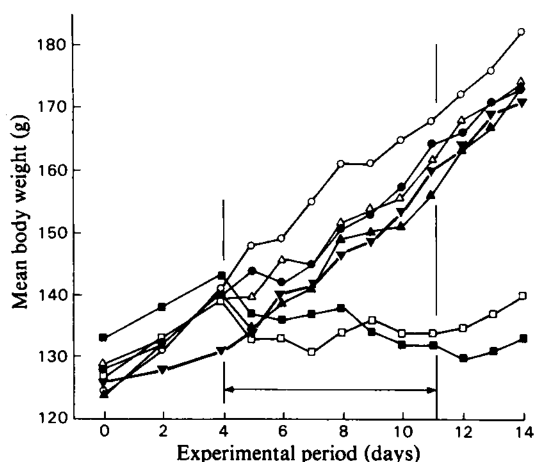

Fig. 1. *Daily body-weight changes of rats before, during and after corticosterone treatment*

Values are means for five rats in each of the following treatment groups: intact+vehicle (▼); adrenalectomized+vehicle (○); +0.2 (mg of corticosterone/day per 100g body wt.) (●); +0.5 (△); +1.0 (▲); +5.0(□); +10.0 (■). Injections were given subcutaneously for 7 days (period indicated by horizontal arrow). All rats were pair-fed with the average intake of adrenalectomized controls.

consumed, even by the rats receiving the high corticosterone dosages. Since all rats were pair-fed with the adrenalectomized control group, the intact controls did not achieve the normal rate of weight gain for males of the Sprague–Dawley strain receiving an 18% lactalbumin diet *ad libitum* (about 6–7g/day; Haverberg *et al.*, 1975*a*).

During the 7-day period of hormone treatment, the intact controls and the adrenalectomized groups given 0.0, 0.2, 0.5 and 1mg of corticosterone/day per 100g body wt. gained about 25g; the rates of weight gain did not differ among these groups (Table 1 and Fig. 1). Rats receiving 5 and 10mg of corticosterone/day per 100g body wt. either maintained weight or lost 5–10g, with the major weight loss occurring on the first 1 or 2 days of treatment. Although a similar initial weight loss was observed in rats of the group receiving 1mg of corticosterone, this group resumed a normal growth pattern from the second day of steroid treatment.

The weights of the livers and of the three skeletal muscles removed at the end of the experiment are shown in Table 2. Livers of animals receiving 5 and 10mg of corticosterone showed a considerable ($P<0.01$) increase in both absolute weight and weight/100g final body wt., in agreement with liver

hypertrophy known to be induced by corticosteroid treatment (Goodlad & Munro, 1959). The three muscles, representing predominantly red (soleus), white (extensor digitorum longus) and mixed (gastrocnemius) fibres, showed differing responses. Relative to final body weight, the gastrocnemius weighed significantly ($P < 0.01$) less in rats given the two highest steroid doses, in comparison with the adrenalectomized controls (Table 2). However, the soleus and extensor digitorum longus muscles were not significantly decreased by corticosteroid treatment below those in the adrenalectomized controls when the weights of these muscles were expressed per 100 g final body wt. (Table 2).

Excretion of N^{τ}-methylhistidine and creatinine

Fig. 2 shows the daily output of N^{τ}-methylhistidine/100 g body wt. for groups of rats. Intact and adrenalectomized controls and rats given 0.2, 0.5 and 1 mg of corticosterone/day showed similar output patterns during the collection period. The initial output of about 1.3 μmol/100 g body wt. is similar to that observed by us previously (Haverberg *et al.*, 1975*a*) for 100 g rats, and, as the rats gained weight, the progressive decrease of about 25% in N^{τ}-methylhistidine output/100 g (Fig. 2) also agrees with our earlier observations on growing rats (Haverberg *et al.*, 1975*a*). Output of N^{τ}-methylhistidine per unit body weight or per unit of creatinine excretion did not differ significantly between intact rats and adrenalectomized rats receiving up to 1 mg of corticosterone. In contrast, administration of 5 mg of corticosterone/day per 100 g body wt. rapidly increased daily N^{τ}-methylhistidine output over 3 days to 2.3 μmol/100 g body wt., followed by a decrease to about 1.3 μmol/100 g, whereas administration of 10 mg of steroid caused a persistent increase at about 1.9 μmol of N^{τ}-methylhistidine/100 g body wt. Output of N^{τ}-methylhistidine by both of these groups declined to values below those of the other groups after steroid treatment was stopped (Fig. 2).

To provide an index of muscle mass, and to minimize variability from daily urine excretion, creatinine output was measured. Fig. 3 shows the ratio of N^{τ}-methylhistidine output to creatinine excretion, expressed as a percentage change from the mean ratio of each group during the 3-day period before hormone treatment. The ratio increased sharply throughout treatment for animals receiving 10 mg, and during the first half of treatment for those given 5 mg of corticosterone; these elevated ratios persisted until after treatment was stopped. At the 1 mg dosage, there was a tendency for output of N^{τ}-methylhistidine/mg of creatinine to increase on the first 3 days of dosage, whereas groups on lower doses showed no change in ratio as a result of treatment (Fig. 3).

Table 2. *Liver and muscle weights of intact and adrenalectomized rats killed 3 days after the final day of treatment with graded doses of corticosterone for 7 days*

Values are means ± S.E.M. for four or five rats. For details see the text.

Treatment (mg of corticosterone/day per 100 g body wt.)	Liver		Gastrocnemius		Soleus		Extensor digitorum longus	
	(g/100 g body wt.)	(% of adrenalectomized control)	(mg/100 g body wt.)	(% of adrenalectomized control)	(mg/100 g body wt.)	(% of adrenalectomized control)	(mg/100 g body wt.)	(% of adrenalectomized control)
Intact (0)	3.50±0.12	98	585±20	105	37.4±1.6	91	49.6±2.6	107
Adrenalectomized (0)	3.57±0.10	100	559±16	100	40.9±1.5	100	46.2±1.6	100
Adrenalectomized (0.2)	2.89±0.11	81	585±15	105	37.4±2.3	91	53.0±2.2	115
Adrenalectomized (0.5)	3.20±0.06	90	580±8	104	33.4±4.0	82	55.4±2.4	120
Adrenalectomized (1.0)	3.28±0.19	92	553±6	99	38.4±2.4	94	49.8±1.5	108
Adrenalectomized (5.0)	5.07±0.27	142	442±25	79	38.3±1.3	94	49.0±2.2	106
Adrenalectomized (10.0)	5.96±0.23	167	400±11	72	38.8±1.2	95	45.2±0.4	98

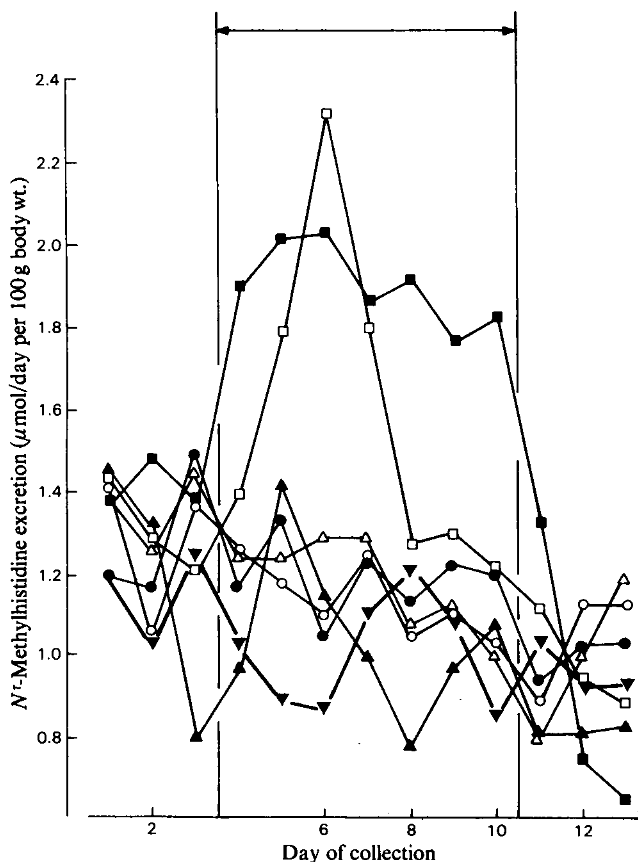

Fig. 2. *Daily output of N^{τ}-methylhistidine/100g body wt. before, during and after corticosterone treatment*
Each point is a pooled sample from five rats. Explanation of the symbols and other details are as described in the legend to Fig. 1.

Plasma hormone concentrations

Fig. 4 shows corticosterone concentrations in plasma drawn from the tail just before and 5h after the last injection of corticosterone, and at the time of killing 3 days later. A certain amount of non-specific displacement of antibody-bound radioactivity by compounds in the plasma other than corticosterone was demonstrated after removal of authentic corticosterone by charcoal treatment. The data have therefore been interpreted in relation to this blank value. The values obtained for intact controls are within the normal range reported by others (Gomez-Sanchez *et al.*, 1975; Pollard *et al.*, 1976). The untreated adrenalectomized control group still showed small amounts of apparent corticosterone binding compared with charcoal-treated blank values (Fig. 4); this also is probably non-specific, and the values obtained for this group presumably represent the true blank values for the assay. Rats receiving 0.2 and 0.5mg of corticosterone showed no apparent increase in plasma concentrations of the steroid, whereas rats injected with 1, 5 or 10mg of corticosterone had increasingly elevated plasma concentrations 5h after treatment, which, at the two higher doses, persisted into the post-injection period, in comparison with values observed for the untreated adrenalectomized animals (Fig. 4).

Insulin content was measured on pooled plasma samples for each group. The values within all groups except for the adrenalectomized controls were highest at 5h after corticosterone administration (Fig. 5). Corticosterone doses above 0.5mg raised plasma insulin to concentrations exceeding those of control rats. At the 5 and 10mg doses, insulin values were still elevated 24h after corticosterone injection, and these groups also showed gross glycosuria.

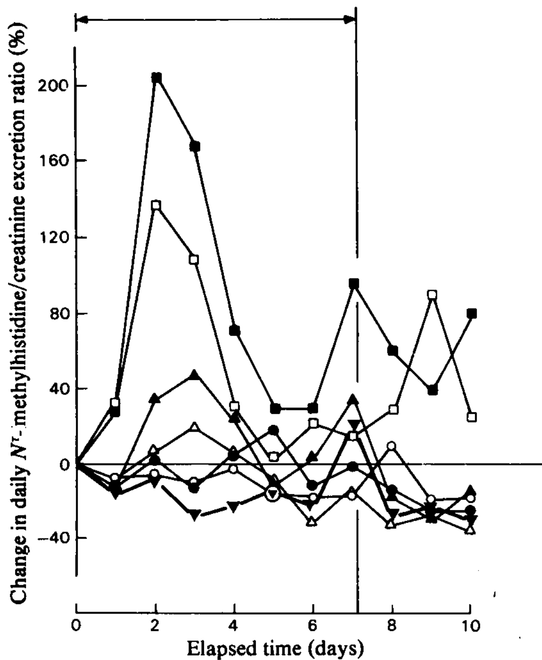

Fig. 3. *Daily N^{τ}-methylhistidine/creatinine ratios for each treatment group from commencement of corticosterone treatment, expressed as the percentage change from the mean ratio of the preceding* 3 *days*

Each point is a pooled sample from five rats. Explanation of the symbols and other details are as described in the legend to Fig. 1.

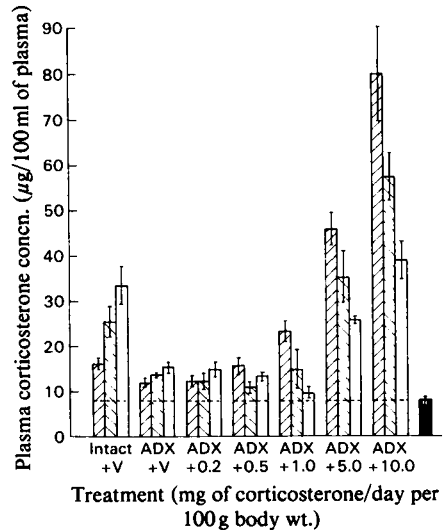

Fig. 4. *Mean concentrations of corticosterone in plasma of rats*

Plasma was sampled from the tail just before the 7th injection (24h after the 6th injection) (T_0, ■), 5h after the 7th daily injection (T_5, ■), and at time of killing, about 100h after the 7th injection (T_{100}, □). Values are means±S.E.M. for four or five animals. The charcoal-treated plasma (■) value is the mean± S.E.M. for 12 samples within the range 11.5–71.0µg/100ml before charcoal treatment. Abbreviations used: V, vehicle; ADX, adrenalectomized.

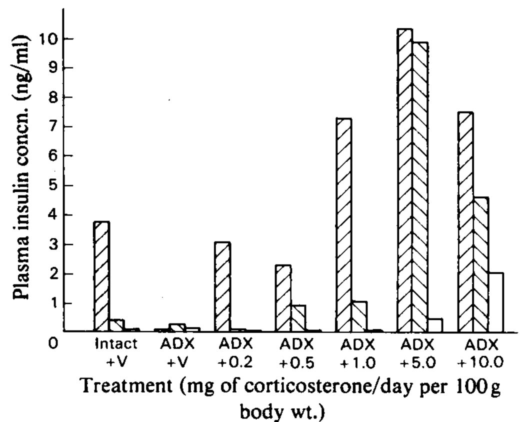

Fig. 5. *Concentrations of insulin in plasma of rats*

Plasma was sampled at 0, 5, and 100h after corticosterone injection. Each value is a pooled sample from four or five rats. For further details see the legend to Fig. 4.

Discussion

As judged by changes in growth rate and urinary excretion of N^{τ}-methylhistidine, neither adrenalectomy nor replacement treatment with 0.2 or 0.5mg of corticosterone/day per 100g body wt. had any effect when these groups were pair-fed with the adrenalectomized group receiving the vehicle only. These two doses of corticosterone are similar to those considered by Steele (1975), on the basis of various criteria, to be optimal for replacement therapy of adrenalectomized rats. Our data confirm this by showing that the plasma corticosterone concentration 5h after administration of 0.5mg of corticosterone was within the normal range in intact rats (Fig. 4). In contrast, growth ceased and N^{τ}-methylhistidine output rose sharply when rats received 5 or 10mg of corticosterone/day per 100g body wt. These doses caused persistent increases in plasma corticosterone above the values observed in intact controls, indicating that corticosterone increases the breakdown rate of myofibrillar protein only when excessive concentrations are present in the plasma. A critical dose for catabolism is confirmed by the loss of weight of the gastrocnemius muscle only at the two higher doses (Table 2). Our data do not allow us to be precise about the minimum effective

dose required to induce the catabolic response. At the 1 mg dose of the steroid, non-significant minor effects on growth rate and N^{τ}-methylhistidine output were observed. These findings emphasize the need to establish the critical catabolic dose of corticosterone, preferably under conditions of administration reflecting the pattern of normal adrenal secretory activity.

The literature is contradictory about the action of corticosteroids on muscle protein breakdown. Millward *et al.* (1976) treated rats for 2 days with triamcinolone (0.5 mg/100 g body wt.) and used the technique of continuous infusion of [^{14}C]tyrosine to measure protein synthesis in individual muscles, from which they computed breakdown as the difference between the estimated rate of synthesis and the net change in muscle protein content. Although they found that synthesis was inhibited, they did not observe a significant increase in protein-breakdown rate in rat gastrocnemius and quadriceps muscles. Shoji & Pennington (1977) reported that release of tyrosine *in vitro* (taken to be an index of protein breakdown) from the extensor digitorum longus muscle of rats did not increase after pretreatment with 10 mg of cortisone acetate/100 g body wt. for 3 days. On the other hand, Goldberg (1969) injected [^{14}C]leucine into rats and measured its release *in vivo* from plantaris and soleus muscles after administration of 10 mg of cortisone acetate/day per 100 g body wt. for 7–10 days, and observed an increased rate of muscle protein breakdown in plantaris but not in soleus. Karl *et al.* (1976) administered 20 mg of cortisone acetate on three successive days to 120 g rats; 24 h after the last dose, release of alanine by the epitrochlaris muscle *in vitro* was enhanced, implying a net protein loss from this muscle as a result of steroid treatment.

These studies, carried out on single muscles, are contradictory and difficult to resolve owing to the differences in the type of steroid administered, the amount and period of dosage, the age and sex of the rats, and the hormone-responsiveness of the individual muscles. Our data (Table 2) and those of others (Smith, 1964; Goldberg, 1969; Goldberg & Goodman, 1969) confirm that changes caused by corticosteroid administration differ among muscle types. Measurement of urinary N^{τ}-methylhistidine excretion, as reported in the present paper, offers the advantage of an integrated estimate of average protein breakdown for the entire skeletal musculature at various dosages of steroid.

The persistence of the catabolic response to corticosterone is also reflected in our studies. Since plasma corticosterone concentrations were assayed after 7 days of injections, it is possible that, owing to adaptive enhancement of corticosterone removal, the concentrations induced by the two highest doses declined, after having been more elevated at the start of treatment. Nevertheless, Fig. 4 indicates that the elevated values persisted 4 days after the last injection of the hormone. Alternatively, the target organs may have become to some degree refractory to the hormone, as indicated by the decline in N^{τ}-methylhistidine output after 3–4 days of dosage with 5 mg/day per 100 g body wt. Thus Goldman & Frohman (1974) noted that the body protein content of rats declined on the first day of glucocorticoid treatment. Results reported by other workers also indicate an adaptive decline in such parameters as nitrogen excretion and plasma glucose and insulin concentrations in response to continued hormone therapy (Steele, 1975).

The doses of corticosterone that caused increased output of N^{τ}-methylhistidine in our studies also produced liver enlargement (Table 2). The latter response to a high dose of glucocorticoid agrees with other data in the literature (Goodlad & Munro, 1959) and is in accord with observations that steroid treatment can enhance the synthesis of RNA and protein by perfused liver (Munro, 1964). In intact rats, the critical dose needed to produce this hypertrophy appears to be similar to that for skeletal-muscle catabolism (Table 2). Although Loeb (1976) found a decrease in DNA content of rat liver after low doses of cortisol, we saw no evidence of changes in liver weight or body weight when our doses were less than the high values that we used to produce a catabolic response.

The low concentrations of insulin in the untreated adrenalectomized control rats agree with the finding by Sutter (1968) of decreased pancreatic insulin secretion and lack of responsiveness to elevated plasma glucose values in adrenalectomized rats. This attests to the completeness of adrenalectomy in our rats. With higher corticosterone doses, the concomitant elevation of insulin concentrations (Fig. 4) is consistent with the findings of others (Steele, 1975). The reason for the relatively low plasma insulin concentrations in the group receiving 10 mg, compared with that receiving 5 mg, of corticosterone/day per 100 g body wt. is unclear. Both groups showed extensive glycosuria, indicative of corticosterone-related insulin resistance and consequent diabetes. Thus our data are in accord with the view that corticosteroids inhibit entry of glucose into peripheral tissues, with resulting elevation of plasma glucose concentrations and increased secretion of insulin caused by hyperglycaemia. In addition, it is well known that corticosteroids increase gluconeogenesis, which would accentuate the hyperglycaemia.

Our data do not permit any conclusions about the mechanism of the observed increase in the rate of muscle protein breakdown. Glucocorticoid treatment does not appear to activate or release muscle lysosomal hydrolases (Buchanan & Schwartz, 1967), but does lead to increased activity of several non-

lysosomal proteinases (Mayer *et al.*, 1976; Mayer & Rosen, 1977). Mayer *et al.* (1976) have reported a 2-fold increase in activity of a myofibrillar proteinase after glucocorticoid treatment, consistent with our finding of increased myofibrillar degradation, as indicated by N^{τ}-methylhistidine excretion, after administration of corticosterone to rats in amounts sufficient to maintain elevated plasma corticoid concentrations.

Finally, it should be noted that the concentration of plasma corticosteroids is elevated by a number of pathological stress conditions. For example, rats with extensive burns can maintain corticosterone concentrations higher (50–55μg/100 ml) than those noted by us in rats receiving 5mg of corticosterone/day per 100g body wt. for several days (J. Allsop, unpublished work). Similar elevations in corticosterone concentrations in response to physical stress have been reported by others (Dallman & Jones, 1973; Szarfarczyk *et al.*, 1974). Psychological stress superimposed on physical stress can elevate plasma corticosterone concentrations in rats to 90μg/100ml for several days (Pollard *et al.*, 1976). On the basis of these data, our results suggest that an increased rate of muscle protein breakdown caused by sustained increased steroid secretion is likely to be a feature of the response to severe stress, such as in burns with infection. Corticosterone does not have a regulatory effect on myofibrillar protein breakdown until concentrations well above the normal range are present in the plasma.

This study was supported by a National Institutes of Health grant AM 16654 and National Aeronautics and Space Administration grant NAS9-15194. The assistance given by Miss Robin Randall in developing the corticosterone assay is acknowledged. The preparatory procedures for rat urine before N^{τ}-methylhistidine analysis were largely devised by Dr. John Ballard of the CSIRO Division of Human Nutrition, Adelaide, South Australia, with the assistance of Miss Lorraine Pope. Ms. Christine Bilmazes kindly performed the N^{τ}-methylhistidine determinations. Mrs. Loreen Tomas assisted with the experimental procedures and rat care. This study was carried out during F. M. T.'s leave of absence from the CSIRO, Division of Human Nutrition, Adelaide 5000, South Australia, Australia.

References

Buchanan, W. E. & Schwartz, T. B. (1967) *Am. J. Physiol.* **212**, 732–736

Dallman, J. F. & Jones, M. T. (1973) *Endocrinology* **92**, 1367–1375

Goldberg, A. L. (1969) *J. Biol. Chem.* **244**, 3223–3229

Goldberg, A. L. (1975) in *Intracellular Protein Catabolism* (Hanson, H. & Bohley, P., eds.), pp. 548–555, Johann Ambrosius Barth Verlag, Leipzig

Goldberg, A. L. & Goodman, H. M. (1969) *J. Physiol.* (*London*) **200**, 667–675

Goldman, J. K. & Frohman, L. A. (1974) *J. Pharmacol. Exp. Ther.* **188**, 310–317

Gomez-Sanchez, C., Murry, B. A., Kein, D. C. & Kaplan, N. M. (1975) *Endocrinology* **92**, 796–798

Goodlad, G. A. & Munro, H. N. (1959) *Biochem. J.* **73**, 343–348

Hanoune, J., Chambaut, A. M. & Josiposicz, A. (1972) *Arch. Biochem. Biophys.* **148**, 180–184

Hare, R. S. (1950) *Proc. Soc. Exp. Biol. Med.* **74**, 148–151

Haverberg, L. N., Deckelbaum, L., Bilmazes, C., Munro, H. N. & Young, V. R. (1975*a*) *Biochem. J.* **152**, 503–510

Haverberg, L. N., Omstedt, P. I., Munro, H. N. & Young, V. R. (1975*b*) *Biochim. Biophys. Acta* **405**, 67–71

Karl, I. E., Garber, A. J. & Kipnis, D. M. (1976) *J. Biol. Chem.* **251**, 844–850

Loeb, J. N. (1976) *N. Engl. J. Med.* **295**, 547–560

Mayer, M. & Rosen, F. (1977) *Metabolism* **26**, 937–962

Mayer, M., Amin, R., Milholland, R. J. & Rosen, F. (1976) *Exp. Mol. Pathol.* **25**, 9–19

Millward, D. J., Garlick, P. J., Nnanyelugo, D. O. & Waterlow, J. C. (1976) *Biochem. J.* **156**, 185–188

Munro, H. N. (1964) in *Mammalian Protein Metabolism* (Munro, H. N. & Allison, J. B., eds.), vol. 1, pp. 381–481, Academic Press, New York

Pollard, I., Bassett, J. R. & Carcineross, K. D. (1976) *Neuroendocrinology* **21**, 312–330

Shoji, S. & Pennington, R. J. T. (1977) *Mol. Cell. Endocrinol.* **6**, 159–169

Smith, B. (1964) *Neurology* **14**, 857–863

Steele, R. (1975) *Handb. Physiol. Sect. 7* **6**, 135–167

Sutter, B. C. J. (1968) *Diabetologia* **4**, 295–304

Szarfarczyk, A., Moretti, J. M., Boissin, J. & Assenmacher, J. (1974) *Endocrinology* **94**, 284–287

Wool, I. G. & Weinshelbaum, E. I. (1960) *Am. J. Physiol.* **198**, 360–362

Young, V. R. (1970) in *Mammalian Protein Metabolism* (Munro, H. N., ed.), vol. 4, pp. 585–674, Academic Press, New York

Young, V. R. & Munro, H. N. (1978) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 77–86

Young, V. R., Alexis, S. C., Baliga, B. S., Munro, H. N. & Muecke, W. (1972) *J. Biol. Chem.* **247**, 3592–3600