sists in continuously extracting urine acidified to pH 1 for 48–72 hr. with ether. Recently Burstein & Lieberman (1958) have described a solvolysis procedure for splitting these conjugates. Although these procedures hydrolyse the sulphates of the 3-hydroxy-5 α - and -5 β -steroids in addition to the 3 β -hydroxy- Δ^5 -steroids, the former method has the disadvantage that the hydrolysis is slow and, more important, that more labile steroids, e.g. 3 β :16 α -dihydroxyandrost-5-en-17-one, are destroyed by these conditions. Whether or not these labile steroids are destroyed by the method of Burstein & Lieberman remains to be determined. The hydrolysis procedure used in the present method is comparatively quick and the more labile steroids appear not to be destroyed, so that the method should be capable of extension to include the other 3 β -hydroxy- Δ^5 -steroids that have been shown to occur in the urine of normal males (Fotherby, 1958, 1959).

SUMMARY

1. A method for the estimation of dehydroepiandrosterone in urine is described in which the sulphate of this steroid is hydrolysed by heating urine without acidification at 100° for 6 hr.

2. The criteria of reliability of the method are discussed.

I wish to thank Professor G. F. Marrian, F.R.S., for his interest in the work and Miss N. Balloch and Miss A. Fleming for technical assistance.

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Diet and the Action of Cortisone on Protein Metabolism

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(Received 11 December 1958)

The general action of administered cortisone is to cause a loss of protein from the body. Some organs, however, do not share in this catabolic

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effect. In the liver and other viscera, there is an accumulation of protein in response to cortisone administration (see review by Trémolières, Derache & Lowy, 1955). In the experiments to be described below, the influence of diet on these actions of cortisone has been examined. Diets

varying in both protein and energy content were given to rats and it was found that neither dietary factor affected the general loss of nitrogen from the body which results from cortisone administration. The increase in liver-protein content induced by cortisone was also uninfluenced by the protein content of the diet, but it was found to depend to a considerable degree on an adequate energy intake. This latter finding has been related to the levels of energy-yielding metabolites in the liver on the various diets.

A preliminary report of this work has already appeared (Goodlad & Munro, 1958).

EXPERIMENTAL

Animals. Female albino rats weighing 180–200 g. were housed under thermostatic conditions (23–25°) in individual cages fitted for collection of urine and faeces (Thomson & Munro, 1955). Their body surface areas were computed from the formula of Lee (1929).

Diets and treatment with cortisone. The general dietary arrangements were modelled on those used in previous experiments to provide different levels of protein and of energy intake (Munro & Naismith, 1953). At 9 a.m., 1 g. of a vitamin-mineral-roughage mixture (Munro, 1949) was given to all animals. At 5 p.m. they ate a meal which provided all the protein of the diet. With rats receiving an adequate protein intake, the evening meal contained 2.4 g. of casein (unextracted grade, Glaxo Laboratories Ltd., Greenford, Middlesex), 1.2 g. of carbohydrate (equal parts of glucose and potato starch) and 0.4 g. of fat (margarine). The corresponding evening meal for animals on the lower level of protein intake contained 1.2 g. of casein, the caloric content of the meal being kept constant by increasing the carbohydrate to 2.5 g. For rats receiving the protein-free diet, all the casein of the meal was replaced by an isocaloric amount of carbohydrate, bringing the total carbohydrate content of the meal to 3.8 g.

At each of these three levels of protein intake, the basic diet provided 21 kcal./rat/day and carbohydrate was added to the morning meal to bring the energy intake up to any desired level. At first, all rats were given a total energy intake of 33 kcal./day (about 1200 kcal./sq.m. body surface area) for a period of 5 days, in order that they might become accustomed to the diets. This energy intake was obtained by adding 2.5 g. of starch and 0.5 g. of glucose to the morning meal. After this preliminary period of adjustment, the animals at each level of dietary protein were divided into a group receiving a high-energy intake and a group receiving a low-energy intake. Those on the high-energy intake were given 5 g. of starch and 1 g. of glucose in the morning meal, the total daily energy content of the diet being 45 kcal. (about 1800 kcal./sq.m.). With animals receiving the low-energy intake, the supplementary carbohydrate was withdrawn from the morning meal and they were thus reduced to the 21 kcal. provided by the basic diets (about 850 kcal./sq.m.). At each of these two levels of energy intake, half of the rats were injected subcutaneously with 2.5 mg. of cortisone acetate (Upjohn of England Ltd., London, W. 1) suspended in 0.1 ml. of aq. 0.9% NaCl soln., 1 hr. after each of the two daily meals. The other animals (control series) were injected with 0.1 ml. of aq. 0.9%

NaCl soln. at these times. In this way, the animals were divided into 12 groups, comprising cortisone-treated and control animals receiving either a high or a low level of energy intake at each of three levels of dietary protein.

Administration of the various diets and cortisone was continued for 4 days, during which urine and faeces were collected individually from the rats by the method of Thomson & Munro (1955). At the end of the period, the rats were killed in the fasting state by stunning and exsanguination, either at 8 p.m. on the last day of feeding (in which case the meal at 5 p.m. was withheld), or at 10 a.m. on the morning after the end of the 4-day period. The liver was removed for analysis.

Analysis of excreta. The total output of N in the urine and faeces over the period of collection was estimated by the micro-Kjeldahl procedure (Munro & Naismith, 1953).

Analysis of liver. The livers were analysed for protein N, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) by procedures described previously (Munro & Naismith, 1953). The total amounts of these liver constituents have been expressed as mg./100 g. of initial body wt.

The adenine nucleotide content and glycogen content of the livers were estimated in some experiments. Adenosine mono-, di- and tri-phosphate (AMP, ADP and ATP respectively) were measured by the spectrophotometric enzymic assay of Kalckar (1947). Preliminary freezing of the liver was not carried out because Rowles (1952) and Dianzani (1957) obtained higher values for the ATP content of unfrozen tissues. A sample of liver of about 2 g. was rapidly weighed and disintegrated in a homogenizer (Measuring and Scientific Equipment Ltd., London) for 2 min. in 20 ml. of ice-cold 1.5% (w/v) perchloric acid. After removal of the precipitate by centrifuging and washing with 1.5% perchloric acid, a sample (10 ml.) was added to 10 ml. of succinate buffer (Kalckar, 1947) and the pH adjusted to 6.1 by addition of NaOH. A further 15 ml. of buffer was added to the sample and the total volume made up to 50 ml. with water. The three adenosine phosphates were then estimated enzymically on 2.5 ml. samples of this solution, with adenylic deaminase prepared from rabbit muscle (Kalckar, 1947), commercial myokinase (Boehringer und Soehne, Mannheim) and apyrase prepared from potatoes (Krishnan, 1949).

RESULTS

Our results represent the action of cortisone over a 4-day period at different levels of protein intake, on each of which energy intake was also varied.

Effect of cortisone on body weight and liver weight. A number of investigators have demonstrated in short-term experiments that cortisone administration causes loss of body weight accompanied by enlargement of the liver (Kochakian & Robertson, 1951; Silber & Porter, 1953; Bodansky & Money, 1954; Trémolières, Derache & Griffaton, 1955; Clark & Pesch, 1956). Most of this information was obtained under conditions of unrestricted feeding. Our data (Table 1) confirm these actions of cortisone under conditions of controlled food intake and show that they are independent of diet. The body weights and liver weights in the control series of

Table 1. *Effect of cortisone administration over a 4-day period on the body weight and liver weight of rats receiving diets varying in protein and energy content*

After a preliminary period on the diet fed at an energy level of 33 kcal./day, the rats at each protein level were divided into a group on a low-energy intake (21 kcal./day) and a group on a high-energy intake (45 kcal./day). Rats in each group were injected with 2.5 mg. of cortisone acetate twice daily for 4 days; control animals received an injection of aq. 0.9% NaCl soln. They were killed on the morning after the end of the 4-day period. Results are the means of seven experiments $\pm$ s.e. Statistical analysis shows that the change in body weight over the 4-day period and the final liver weight were affected significantly ($P < 0.01$) by dietary protein level, by dietary energy level and by cortisone administration; the magnitude of the change produced by cortisone was not significantly affected by diet in either case ($P > 0.05$ for interaction of cortisone with dietary protein and energy intakes).

Dietary protein	Dietary energy	Initial body wt.		Change in body wt.		Liver wt./100 g. of initial body wt.	
		Control series (g.)	Cortisone series (g.)	Control series (g.)	Cortisone series (g.)	Control series (g.)	Cortisone series (g.)
None	Low	175	173	-14 $\pm$ 5	-16 $\pm$ 3	2.27 $\pm$ 0.23	2.98 $\pm$ 0.13
	High	181	182	-3 $\pm$ 2	-17 $\pm$ 3	2.65 $\pm$ 0.10	3.26 $\pm$ 0.13
Low	Low	173	173	-12 $\pm$ 3	-21 $\pm$ 3	2.54 $\pm$ 0.08	2.84 $\pm$ 0.07
	High	176	181	+4 $\pm$ 1	-7 $\pm$ 2	2.67 $\pm$ 0.27	3.70 $\pm$ 0.22
Adequate	Low	174	175	-10 $\pm$ 2	-15 $\pm$ 2	2.78 $\pm$ 0.14	3.40 $\pm$ 0.20
	High	169	164	+3 $\pm$ 3	-5 $\pm$ 3	2.98 $\pm$ 0.11	3.58 $\pm$ 0.17

Fig. 1. *Effect of cortisone administration over a 4-day period on the nitrogen balance of rats receiving diets varying in protein and in energy content. Experimental conditions were similar to those described in Table 1. Each graph shows the influence of energy intake on nitrogen balance at an adequate level of dietary protein (upper line), at a low level of dietary protein (middle line) and on a protein-free diet (lower line). Results are the means of five experiments. Statistical analysis shows that nitrogen balance was affected significantly ($P < 0.01$) by protein intake, by energy intake and by cortisone administration. The action of cortisone on nitrogen balance was not significantly altered by diet ($P > 0.05$ for the interaction of cortisone with dietary energy and protein intakes).*

rats were both considerably affected by the protein and energy content of the diet fed during the 4-day period, but the loss of body weight and gain in liver weight resulting from cortisone administration were not significantly different on the various diets.

Effect of cortisone on nitrogen balance. In agreement with previous evidence (Munro, 1951), N balance was affected by both the protein content of the diet and its energy content (Fig. 1). Comparison of the N balances of the cortisone-treated groups with those of the control groups receiving corresponding levels of protein and energy in the diet shows that cortisone administration caused an increased loss of N from the body on all diets. This action of cortisone was of similar magnitude at all levels of protein and energy intake. In consequence, the improvement in N balance after an increase in protein intake or in energy intake was the same in the cortisone-treated and control series of animals (Fig. 1). Thus cortisone does not interfere with the capacity of the body as a whole to utilize dietary protein or energy.

Effect of cortisone on the protein and nucleic acid content of the liver. The total protein and RNA content of the liver is influenced by the protein and energy content of the diet (Munro & Naismith, 1953; Munro, Naismith & Wikramanayake, 1953). Our present series of experiments confirm these effects of diet on liver composition (Table 2). Cortisone administration increased the amount of protein and of RNA. This increment was independent of the dietary protein level, but it was influenced by energy intake to a significant degree with liver-protein deposition and probably also with RNA accumulation. At each dietary protein level, the rats receiving the higher-energy intake responded to cortisone with a greater deposition of protein and of RNA in the liver, the average deposition over the three protein levels being about twice that of animals fed on diets of low-energy content. Thus the catabolic action of cortisone on the body as a whole is independent of diet, whereas

Table 2. *Effect of cortisone administration over a 4-day period on the protein and ribonucleic acid content of the livers of rats receiving diets varying in protein and in energy content*

Experimental conditions were similar to those described in Table 1. Results are the means of seven experiments $\pm$ s.e. Analysis of variance shows that cortisone administration caused a significant deposition of protein in the liver at both levels of energy intake ($P < 0.01$), but the action of cortisone was significantly greater at the higher-energy level ($P < 0.05$ for interaction between cortisone and energy); the action of cortisone was not significantly modified by dietary protein level ($P > 0.05$ for this interaction). The action of cortisone on liver RNA content was only significant at the higher level of energy intake ($P < 0.01$). This influence of dietary energy level on the RNA deposition caused by cortisone administration is also suggested by a tendency for the interaction between energy intake and cortisone to be positive (P almost 0.05).

Dietary protein	Dietary energy	Liver protein N (mg./100 g. of initial body wt.)			Liver RNA P (mg./100 g. of initial body wt.)		
		Control series	Cortisone series	Difference	Control series	Cortisone series	Difference
None	Low	52.3 $\pm$ 3.3	62.1 $\pm$ 2.5	+ 9.7	2.26 $\pm$ 0.12	2.44 $\pm$ 0.08	+ 0.18
	High	49.9 $\pm$ 2.1	67.0 $\pm$ 4.2	+ 17.1	2.43 $\pm$ 0.16	2.75 $\pm$ 0.16	+ 0.32
Low	Low	57.8 $\pm$ 1.7	58.7 $\pm$ 2.0	+ 0.9	2.32 $\pm$ 0.17	2.33 $\pm$ 0.09	+ 0.01
	High	63.7 $\pm$ 2.2	75.7 $\pm$ 4.1	+ 12.0	2.65 $\pm$ 0.15	3.10 $\pm$ 0.18	+ 0.45
Adequate	Low	64.2 $\pm$ 2.1	73.7 $\pm$ 3.5	+ 9.5	2.59 $\pm$ 0.16	2.82 $\pm$ 0.17	+ 0.23
	High	71.3 $\pm$ 3.1	84.3 $\pm$ 4.4	+ 13.0	2.75 $\pm$ 0.15	3.37 $\pm$ 0.15	+ 0.62

Table 3. *Influence of diet and of cortisone on the content of adenosine phosphates in rat liver*

Diets were given as two meals, at 9 a.m. and 5 p.m., the energy content of the diet being varied by addition of carbohydrate to the morning meal and the protein content by changes in the evening meal. After 5 days at a constant energy intake, rats at each protein level were divided into groups on low and on high intakes of energy and were injected with cortisone or aq. 0.9% NaCl soln. (control group) 1 hr. after each meal. They were killed either at 8 p.m. on the fourth day of cortisone treatment (the evening meal being omitted on that day), or at 10 a.m. on the morning of the next day (the morning meal being omitted). The results given are the means of three experiments at each time of killing $\pm$ s.e.; the factors affecting them have been evaluated statistically by analysis of variance as ++ ($P < 0.01$), + ($P < 0.05$) or - ($P > 0.05$). There were no significant interactions, which indicates that the concentrations of adenosine phosphates in the liver were influenced by diet to the same extent in the cortisone-treated and control groups.

Treatment	Dietary protein	Dietary energy	AMP (μ mole/g.)	ADP (μ mole/g.)	ATP (μ mole/g.)
(a) Animals killed in the evening					
None	None	Low	1.72 $\pm$ 0.19	0.92 $\pm$ 0.23	0.43 $\pm$ 0.17
		High	1.41 $\pm$ 0.06	1.05 $\pm$ 0.07	1.28 $\pm$ 0.14
	Adequate	Low	1.70 $\pm$ 0.22	1.41 $\pm$ 0.04	0.61 $\pm$ 0.11
		High	1.20 $\pm$ 0.18	0.83 $\pm$ 0.04	1.50 $\pm$ 0.33
Cortisone	None	Low	1.28 $\pm$ 0.08	1.40 $\pm$ 0.05	1.47 $\pm$ 0.32
		High	1.09 $\pm$ 0.12	1.14 $\pm$ 0.06	1.97 $\pm$ 0.32
	Adequate	Low	1.34 $\pm$ 0.19	1.38 $\pm$ 0.14	1.45 $\pm$ 0.22
		High	1.18 $\pm$ 0.13	1.23 $\pm$ 0.08	1.54 $\pm$ 0.28
Statistical analysis:					
Protein			-	-	-
Energy			+	+	+
Cortisone			+	+	+
(b) Animals killed in the morning					
None	None	Low	1.38 $\pm$ 0.10	1.23 $\pm$ 0.05	0.65 $\pm$ 0.17
		High	1.13 $\pm$ 0.23	1.02 $\pm$ 0.17	1.02 $\pm$ 0.04
	Adequate	Low	1.61 $\pm$ 0.14	1.16 $\pm$ 0.02	0.90 $\pm$ 0.05
		High	1.75 $\pm$ 0.07	1.20 $\pm$ 0.17	0.60 $\pm$ 0.10
Cortisone	None	Low	0.91 $\pm$ 0.05	1.06 $\pm$ 0.13	1.15 $\pm$ 0.07
		High	1.19 $\pm$ 0.06	1.06 $\pm$ 0.15	1.19 $\pm$ 0.12
	Adequate	Low	1.21 $\pm$ 0.09	1.44 $\pm$ 0.20	1.33 $\pm$ 0.02
		High	1.09 $\pm$ 0.16	1.06 $\pm$ 0.33	1.39 $\pm$ 0.23
Statistical analysis:					
Protein			+	-	-
Energy			-	-	-
Cortisone			+	-	+

the anabolic effect of cortisone on the liver is partly dependent on energy intake.

Measurement of the total DNA content of the liver (not recorded in Table 2) showed it to be uninfluenced by any of the dietary conditions or by cortisone administration. Silber & Porter (1953) also found no effect of cortisone on the DNA content of liver.

Action of cortisone in relation to the content of adenosine phosphates in the liver. Since our results show that the amount of protein deposited in the liver after cortisone administration depends on energy intake, it was of interest to know whether this finding could be related to the concentrations of adenosine phosphates in the liver at different levels of energy intake. The diets fed to our animals were given in the form of two meals, differences in energy intake being obtained by changing the amount of carbohydrate in the morning meal and differences in protein intake by varying the composition of the evening meal. In order to assess the effect of diet and of cortisone on the adenosine phosphate content of the liver throughout the day, it was therefore necessary to kill animals at definite times after each of these meals. One series of rats was killed at 8 p.m. on the fourth day of cortisone treatment, i.e. 11 hr. after the morning meal; a second series was killed the following morning at 10 a.m., 17 hr. after the evening meal. At both of these times of killing, the cortisone-treated animals differed from the corresponding animals of the control series by having a lower concentration of AMP and a higher concentration of ATP in their livers (Table 3). In the rats killed in the evening, the concentrations of these metabolites were influenced in addition by the energy content of the morning meal, animals on the higher level of energy intake having lower concentrations of AMP and ADP, and higher concentrations of ATP. This effect of level of energy intake was not significantly reduced by cortisone treatment. In the animals killed in the morning, however, the energy content of the diet no longer had a significant effect on the adenosine phosphate content of the liver.

Our data thus show that, for part of each day, animals on the higher level of energy intake have more ATP in their livers, and that cortisone administration does not obliterate this effect of dietary energy level.

DISCUSSION

In a preceding series of experiments (Munro, Black & Thomson, 1959) evidence was obtained of a reciprocal relationship between protein metabolism in muscle and in the viscera. After administration of glucose to fasting rats, there was an increased

uptake of labelled amino acids by muscle, and this was accompanied by a fall in the urinary excretion of nitrogen and in the protein content of the liver. It was concluded that the primary action of glucose on protein metabolism is to cause deposition of amino acids in muscle. This curtails the supply of amino acids to other tissues, and in consequence there is a reduction in protein synthesis by these organs and in urea formation. The action of cortisone on protein metabolism is the converse of this picture. Administration of cortisone over short periods results in an increased excretion of nitrogen due to loss of protein from the carcass (Kochakian & Robertson, 1951; Clark, 1953; Silber & Porter, 1953). This is accompanied by an increase in the protein content of the liver and other viscera (Kochakian & Robertson, 1951; Clark, 1953; Silber & Porter, 1953; Bodansky & Money, 1954; Trémolières, Derache & Griffaton, 1955; Clark & Pesch, 1956; Fritz, 1956). As with administered glucose, these metabolic actions of cortisone can be interpreted in terms of a reciprocal relationship between protein metabolism in the carcass and in the viscera. The wasting of carcass protein caused by cortisone provides a greater supply of amino acids for other organs and in consequence protein synthesis in these organs and urea formation are increased. This interpretation gains strength from the fact that other agents causing loss of carcass protein also increase the protein content of the viscera. Deposition of protein in the liver has been observed after administration of thyroid preparations to rats (Sternheimer, 1939; Addis, Karnofsky, Lew & Poo, 1938; Guggenheim, Halevy, Singer & Usieli, 1958), and it also accompanies wasting of the carcass in tumour-bearing rats (Stewart & Begg, 1953), although in the latter case the changes in the liver differ in several respects from those produced by cortisone administration (Goodlad & Munro, unpublished data).

The present experiment were undertaken to determine whether the actions of cortisone on protein metabolism are influenced by diet. Silber & Porter (1953) noted that administration of cortisone increased the urinary output of nitrogen and the protein content of the liver independently of dietary protein intake. Our data show that the catabolic action of cortisone on the body as a whole is independent of the protein and energy content of the diet (Fig. 1), whereas the anabolic action of cortisone on the liver is partly dependent on energy intake (Table 2). The latter finding suggests that the capacity of the liver to utilize amino acids coming from carcass breakdown may be influenced by the energy available in the liver. Direct examination of energy-yielding compounds in the livers of our animals (Table 3) showed that the energy

content of the diet had a considerable effect on the concentrations of AMP and of ATP during part of the day. Although cortisone administration also produced changes in the concentrations of AMP and of ATP, the influence of dietary energy intake was not significantly diminished among such animals. This shows that, in spite of the considerable deposition of glycogen in the liver which is known to result from cortisone administration, the energy content of the diet continued to influence energy metabolism in the livers of the hormone-treated group, and may thus have affected utilization of amino acids arising from carcass breakdown.

Changes in the RNA content of the liver after cortisone administration are consistent with an increased supply of amino acids coming to the liver. A number of authors (e.g. Munro *et al.* 1953) have shown that, when the protein content of the diet is raised, there is an increase in the amount of RNA as well as of protein in the liver. If cortisone administration increases the protein content of the liver because it augments the supply of amino acids coming from the carcass, the RNA content of the liver would be expected to respond in the same way as it does to an increased supply of amino acids from the diet. Silber & Porter (1953) recorded a substantial increase in the liver RNA of cortisone-treated animals, but Lowe & Rand (1956), using much larger doses of the hormone, did not obtain any appreciable increment. In our own experiments, cortisone administration increased the RNA content of the liver (Table 2). As with protein deposition, there was a tendency for the increase in RNA to be greater when the diet provided an abundant supply of energy.

SUMMARY

1. The effect of cortisone on nitrogen balance and on liver protein and ribonucleic acid content has been studied on rats receiving diets varying in protein and in energy content.

2. The catabolic action of cortisone on the body as a whole was independent of the protein and energy content of the diet, whereas the anabolic action of cortisone on the liver was greater when the dietary energy intake was high.

3. The adenosine phosphate content of the liver was affected by the level of energy in the diet, even in the cortisone-treated series of animals.

4. It has been concluded that loss of protein from the carcass after cortisone administration results in a greater supply of amino acids for other organs and in consequence protein synthesis in these organs and urea formation are increased. The capacity of the liver to utilize amino acids arising from the carcass is probably dependent on the supply of energy available in the liver cell.

We wish to acknowledge a grant for materials from the Medical Research Council to one of us (H.N.M.). Skilled technical assistance was provided by Miss R. Pevie and Miss M. A. Mathieson.

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