The Effect of Cortisol on the Synthesis of Rat Plasma Albumin, Fibrinogen and Transferrin

By K. N. JEEJEEBHOY, A. BRUCE-ROBERTSON, J. HO and U. SODTKE
Department of Medicine, University of Toronto, Toronto 181, Ont., Canada

(Received 26 June 1972)

A decrease of absolute synthesis of albumin, no change in that of fibrinogen and an increased fractional synthesis of transferrin were observed 3 h after intraperitoneal administration of a pharmacological dose of 5 mg of cortisol to 220 g rats in the post-absorptive state and previously kept on a diet with 40% protein. The concentration in liver of total free amino acids was practically unchanged at this time. Intraperitoneal administration of a mixture of amino acids with the cortisol raised this concentration and was accompanied by an almost complete de-repression of the synthesis of albumin, with no real effect on that of fibrinogen. In considerable contrast, in rats studied at 24 h after intraperitoneal administration of cortisol, and who had been fed once in the interim (but who had received no amino acids intraperitoneally), there was a marked increase in the absolute synthesis of albumin and fibrinogen, with an increase in fractional synthesis that was less proportionately but still very significant and which included transferrin. The amino acid concentrations had risen above the supplemented values at 3 h but not as much proportionately as the fractional synthesis rates, and of course not as much as the absolute synthesis rates, of albumin and fibrinogen. These time-dependent effects of cortisol suggest to us that our studies resolve the apparently conflicting results of the effect of cortisol on the synthesis of albumin reported by others.

Cortisone is known to stimulate the synthesis of hepatic RNA and proteins (Feigelson *et al.*, 1962). On chronic administration of adrenal cortical hormones, the synthesis of plasma albumin, a liver-made protein, is enhanced in animals, including man (Rothschild *et al.*, 1958, 1961; Grossman *et al.*, 1960). Further, John & Miller (1969) have shown that supplementation of amino acids with hormones, including cortisol, is required for maximal plasma protein synthesis in the isolated perfused liver. In contrast, in short-term studies, cortisol decreases albumin synthesis in the isolated perfused liver (Sellers *et al.*, 1969; Gordon, 1964) and *in vivo* [A. Koj (unpublished work) quoted by McFarlane (1969)]. These conflicting results of the effect of glucocorticoids on albumin synthesis have not been resolved. Further, there is little information on the effect of glucocorticoids on the synthesis of other liver-made plasma proteins *in vivo*.

The studies reported in the present paper were designed to observe and compare the immediate and delayed effects of cortisol on the synthesis of rat albumin, fibrinogen and transferrin in the same animal, by using the [^{14}C]carbonate method (McFarlane, 1963; Reeve *et al.*, 1963; McFarlane *et al.*, 1965). The results showed that within 3 h of cortisol treatment the fractional and absolute syntheses of albumin were decreased, those of fibrinogen were unchanged and the fractional synthesis of trans-

ferrin was increased, when compared with control values. In contrast, 24 h after the administration of cortisol, the fractional synthesis of all three proteins was increased and the absolute synthesis of albumin and fibrinogen increased even more. The concentration of total free amino acids in the liver was unchanged at 3 h. The increases in synthesis at 24 h were greater proportionately than corresponding increases in concentration of these free amino acids in the liver.

Materials and Methods

Animals and cortisol

Male Wistar rats (190–250 g) were used for these experiments. Cortisol was obtained as the sodium succinate (Solu-Cortef; Upjohn Co. of Canada, Don Mills, Ont., Canada) and dissolved in water to a concentration of 10 mg/ml; a 0.5 ml dose, equivalent to 5 mg, was injected intraperitoneally into each rat. It should be noted that this commonly used experimental dose (23 mg/kg) is a high pharmacological one, and not physiological.

Plan of experiments

Eight batches of 8–20 Wistar rats were used for the studies with cortisol. The results were compared with control values already published (Jeejeebhoy *et al.*, 1970; 1972) combined with further control values for

one batch for albumin and transferrin. Control data were obtained over a period of 27 months and experimental results over the last 20 months of this span of time. As in our earlier studies, the animals were kept on a diet containing 40% protein (as casein) and providing $7100 \text{ kJ} \cdot \text{m}^{-2}$ ($1700 \text{ kcal} \cdot \text{cm}^{-2}$) surface area for 4–6 days before the study. Only animals eating the diet and gaining weight steadily were used for experiments. The last feed was given to the rats at about 16:00h and the study begun the next day about 08:00h. At 3 or 24 h before injection of the $\text{Na}_2^{14}\text{CO}_3$ each rat was injected intraperitoneally with 0.5 ml of the cortisol solution. Plasma protein synthesis in the 3 h experiment was performed on animals in the post-absorptive state, whereas synthesis studied at 24 h was in rats that had been fed once after receiving cortisol. In one batch synthesis was measured 3 h after giving both cortisol and an amino acid mixture (4 ml of Amigen, a protein hydrolysate; Baxter Laboratories Ltd., Alliston, Ont., Canada). Under light ether anaesthesia the animals received $25 \mu\text{Ci}$ of $\text{Na}_2^{14}\text{CO}_3$ intraperitoneally followed by $5 \mu\text{Ci}$ of either ^{125}I -labelled rat albumin, ^{125}I -labelled rat fibrinogen or ^{125}I -labelled rat transferrin via the dorsal vein of the penis. After injection of ^{14}C - and ^{125}I -labelled compounds, blood was withdrawn (under ether anaesthesia) from the retro-orbital plexus into heparin at 10 min after injection and then hourly for 3 h after the administration of $\text{Na}_2^{14}\text{CO}_3$. Where necessary, blood from two or three rats was pooled to obtain sufficient plasma for the determinations. At the fifth hour the rats were anaesthetized and a large sample of blood was withdrawn from the inferior vena cava under direct vision. The samples were centrifuged and the plasma was separated.

Separate batches of rats were given $5 \mu\text{Ci}$ of [^{14}C]-urea via the dorsal vein of the penis to determine the distribution and turnover of [^{14}C]urea (McKinley *et al.*, 1970).

Labelled compounds, experimental procedures (including determination of radioactivity), protein separations and calculations

The details have been published previously (Jeejeebhoy *et al.*, 1972), including the calculation of fractional synthesis rates and the method of correcting for losses from the circulation of ^{14}C -labelled proteins during the 5 h period over which synthesis was measured (Norwich, 1972). The magnitude of this correction for losses was not significantly different for rats in the control and cortisol-treated groups.

The term fractional, as applied to synthesis, and as used by us, means that fraction (or percentage) of the intravascular (i.e. freely circulating) pool of the plasma protein concerned, which is synthesized per day. This use of fractional has been customary for some years in dynamic studies of plasma protein

metabolism in the intact mammal (McFarlane, 1963). A main reason for use of the term fractional synthesis rate (F.S.R.) as applied to a plasma protein, and for relating it to total circulating mass of the protein was the early finding that catabolism of the major plasma proteins occurs in closer association with this circulating mass of the protein (and therefore in or very near to the intravascular compartment) than with any other parameter so far considered (McFarlane, 1957; Gitlin, 1957). Another reason for this use of F.S.R. is that it allows some measure of synthesis to be made even when the concentration of the plasma protein is not known (as is the case with transferrin in our experiments). However, because F.S.R. is related to total circulating mass of the given protein (i.e. plasma volume $\times$ plasma concentration of the protein), a parameter which does not necessarily parallel body weight and which may fluctuate rather more, and also more rapidly, than body weight does, there need not be a strict parallel between fractional and absolute rates. Absolute synthesis refers to this phenomenon in terms of mass synthesized/day per unit of body or liver weight, and when so expressed takes into account any alteration in the mass of total circulating plasma protein.

The test of significance used when comparing experimental results with control data was Student's *t* test (Gosset, 1925) with Welch's (1947) modification for possibly different population variances and unequal sample sizes as summarized in *Documenta Geigy* (1962).

Determination of free amino acid concentrations in liver

A 1 g sample of liver was homogenized in 3 ml of 20% (w/v) trichloroacetic acid and the homogenate centrifuged. The precipitate was washed twice with 10% trichloroacetic acid and the supernatants were combined and made up to 10 ml for ^{14}C counting and for amino acid analysis by using a Beckman amino acid analyser (physiological column). The essential amino acids measured were histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine and valine. The non-essential ones measured were alanine, aspartic acid, glycine and serine.

Results

Plasma volume, plasma albumin and fibrinogen, total circulating albumin and fibrinogen

The results are given in Table 1. Plasma volume was increased, but not significantly by 3 h, but the increase continued and was significant by 24 h, being 4.52 ± 0.436 compared with the control value of $3.88 \pm 0.348 \text{ ml}/100 \text{ g}$ body wt. The plasma albumin

Table 1. Comparison of plasma volume and of plasma albumin and fibrinogen concentrations in control and cortisol-treated rats

Results are given as means $\pm$ 1 s.d., with numbers of determinations in parentheses. For experimental details see the text. Values of *P* are denoted by asterisks: * <0.05 , ** <0.01 (values compared with corresponding control value) and *** <0.001 (values compared with cortisol-treated result after 3 h, i.e. without amino acid supplementation).

	Cortisol-treated rats		
	Control rats	After 3 h	After 24 h
Plasma volume (ml/100 g body wt.)	3.88 $\pm$ 0.348 (31)	4.13 $\pm$ 0.475 (16)	4.52 $\pm$ 0.436 (17)**
Plasma albumin (g/100 ml) at 5 h	2.90 $\pm$ 0.30 (21)	2.41 $\pm$ 0.40 (20)**	3.57 $\pm$ 0.68 (15)**
Total circulating albumin (mg/100 g body wt.)	111.0 $\pm$ 12.5 (19)	111.4 $\pm$ 12.8 (10)	162.4 $\pm$ 29.1 (14)**
Plasma fibrinogen (mg/100 g body wt.)	247.0 $\pm$ 43.8 (28)	270.1 $\pm$ 82.8 (59)	335.9 $\pm$ 75.3 (14)**
Total circulating fibrinogen (mg/100 g body wt.)	10.21 $\pm$ 2.07 (26)	12.48 $\pm$ 3.16 (21)**	18.45 $\pm$ 5.15 (10)**

concentrations (mean $\pm$ s.d.) were 2.90 ± 0.30 in controls and 2.41 ± 0.40 g/100 ml 3 h after cortisol treatment. This decrease was significant ($P < 0.001$). At 24 h after cortisol treatment the plasma albumin concentration showed the opposite effect and had increased significantly ($P < 0.005$) to 3.57 ± 0.68 g/100 ml. Because of the slight increase in plasma volume, however, the total circulating albumin at 3 h after cortisol treatment was not significantly altered from the control value, being 111.4 ± 12.8 and 111.0 ± 12.5 mg/100 g body wt. respectively, but after cortisol treatment for 24 h it very definitely ($P < 0.001$) had risen above the control value, to 162.4 ± 29.1 mg/100 g body wt.

The plasma fibrinogen concentration behaved differently with the same cortisol treatment. Within 3 h there was a tendency ($0.05 < P < 0.1$) to rise from the control value of 247.0 ± 43.8 to 270.1 ± 82.8 , and by 24 h after cortisol treatment there occurred a further, significant, rise to 335.9 ± 75.3 mg/100 ml ($P < 0.001$). Correspondingly the total circulating fibrinogen tended to rise from 10.21 ± 2.07 to 12.48 ± 3.16 mg/100 g body wt. at 3 h after cortisol treatment ($0.05 < P < 0.1$) and the further increase to 18.45 ± 5.15 by 24 h after cortisol treatment ($P < 0.001$) was very definite.

Fractional albumin synthesis

The results are set out in Table 2. At 3 h after cortisol treatment the mean value ($\pm$ s.d.) of fractional albumin synthesis had decreased from control values of 75.23 ± 11.17 to $46.25 \pm 19.00\%$ of the intravenous pool per day ($P < 0.001$). Since the total circulating albumin remained constant during this period (Table 1) the absolute synthesis (fractional synthesis $\times$ total circulating albumin) had also fallen. In strong contrast, by 24 h after cortisol treatment the fractional synthesis had risen significantly ($P < 0.005$) to $118.9 \pm 18.1\%$ of the intravascular pool per day. The elevation in absolute synthesis is even more impressive when it is recognized that the total circulating albumin had also increased 24 h after cortisol treatment.

Fractional fibrinogen synthesis

The results are shown in Table 2. At 3 h after cortisol treatment the fractional synthesis, 73.5 ± 25.7 , was unchanged ($0.4 < P < 0.5$) from the control value of $66.2 \pm 13.5\%$ of the intravascular pool per day. Nevertheless the total circulating fibrinogen had tended to increase during this period, so that there was a similar trend of increment in the absolute synthesis rate (fractional synthesis rate $\times$ total circulating fibrinogen), in contrast to the decrease for albumin. However, 24 h after cortisol treatment, the fractional synthesis rate of fibrinogen, as of albumin, had much increased ($P < 0.001$), to $106.6 \pm 28.8\%$ of the intra-

Table 2. Fractional synthesis of plasma proteins in control and cortisol-treated rats

Results (expressed as % of intravascular pool per day) are given as means $\pm$ 1 s.d. with numbers of determinations in parentheses. For experimental details see the text. Values of *P* are denoted by asterisks: * <0.02 , ** <0.005 (values compared with corresponding control value) and *** <0.001 (value compared with cortisol-treated result after 3 h, i.e. without amino acid supplementation).

	Cortisol-treated rats		
	Control rats	After 3 h	After 3 h, + amino acids
Albumin	75.23 $\pm$ 11.17 (18)	46.25 $\pm$ 19.00 (14)**	118.92 $\pm$ 18.14 (6)**
Fibrinogen	66.2 $\pm$ 13.5 (14)	73.5 $\pm$ 25.7 (18)	106.6 $\pm$ 28.8 (7)*
Transferrin	85.8 $\pm$ 23.4 (12)	148.4 $\pm$ 13.5 (5)**	184.6 $\pm$ 44.9 (7)**

vascular pool per day. Because of the further, and this time significant, increase in total circulating fibrinogen at this point, absolute synthesis had increased much more proportionately.

Fractional transferrin synthesis

The results are shown in Table 2. The means ($\pm$ s.d.) of the fractional synthesis rate of transferrin were 85.8 ± 23.4 and $148.4 \pm 13.5\%$ of the intravascular pool per day in control animals and 3 h after cortisol treatment respectively. The increase in synthesis with this cortisol treatment was significant ($P < 0.005$) and is a departure from the observations on the synthesis of albumin and fibrinogen.

By 24 h after cortisol treatment the transferrin synthesis, in common with that of albumin and fibrinogen, was elevated significantly ($P < 0.005$), to $184.6 \pm 44.9\%$ of the intravascular pool per day.

Total free amino acid concentrations in liver

The results are given in Table 3. There was no significant difference between the free amino acid concentration of control animals and those studied 3 h after being given cortisol alone. On the other hand, in rats given Amigen and cortisol, there was at 3 h a significant rise in the concentration of all amino acids measured, except valine. This rise (again excepting valine) was progressive and even more marked by 24 h after cortisol treatment (which was without amino acid supplementation but with one feeding, as noted in the Materials and Methods section).

Correlation of total free amino acid concentration with fractional protein synthesis

From Tables 2 and 3 in conjunction, it is obvious that although there was no change in the concentration of total free amino acids in the liver within 3 h of giving cortisol, there appeared to be a significant fall in albumin synthesis and a significant elevation in transferrin synthesis. Fibrinogen synthesis was not really altered.

Giving amino acids with the cortisol resulted in an increase at 3 h in free amino acids, to about $1\frac{1}{2}$ times the control value ($P < 0.02$). This rise in amino acid concentration was associated with a significant reversal of the repression of albumin synthesis found in those animals given cortisol alone, and was associated with still no real change in fibrinogen synthesis compared with controls or at 3 h in rats given cortisol alone.

In contrast, although the further increase in free amino acid concentration by 24 h was only about 20% above that observed at 3 h in animals given amino acids, the synthesis of all three proteins at this time had increased disproportionately, so that

Table 3. Concentrations of total free amino acids in liver of control and cortisol-treated rats

The essential amino acids measured were histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine and valine. The non-essential ones measured were aspartic acid, serine, glycine and alanine. Results are given as means $\pm$ 1 s.d., with numbers of determinations in parentheses. Values of *P* are from comparison with the respective control value: * <0.02; ** <0.005.

	Concn. of amino acids (μ mol/g wet wt.)	
	Essential amino acids	Non-essential amino acids
Control	36.00 $\pm$ 12.81 (6)	32.17 $\pm$ 5.26 (6)
Cortisol-treated		
After 3h	31.26 $\pm$ 9.32 (14)	31.05 $\pm$ 5.60 (14)
After 3h, + amino acids	54.17 $\pm$ 8.90 (12)*	46.37 $\pm$ 5.50 (12)**
After 24h	67.79 $\pm$ 9.03 (16)**	52.86 $\pm$ 5.76 (16)**

while there was a relationship of the albumin and fibrinogen synthesis rates to the concentration of total free essential amino acids the association appeared to be non-linear and non-logarithmic.

Discussion

The results show that initially cortisol primarily depresses albumin synthesis when compared with control animals with a similar hepatic free amino acid concentration. In strong contrast, synthesis of fibrinogen was unaffected and that of transferrin was stimulated, showing that initially cortisol has opposite effects on albumin and on so-called 'acute phase' proteins, namely fibrinogen and transferrin.

However, 24h after cortisol was given, the synthesis of all three plasma proteins was stimulated greatly. Since at this time there was an increased concentration of free amino acids in the liver, an elevation of synthesis could have been due to both a direct effect, as for example through stimulation of hepatic nuclear RNA [Kenney & Kull (1963), and reviewed by Kenney (1970)], and to an increase in the availability of amino acids to the liver. This increased availability could have been due to a shift of amino acids from muscle to liver, as reported by others (Munro, 1964, p. 442). To clarify this aspect, some rats were given amino acids together with cortisol, and synthesis was measured 3h after the administration of both these agents. It is clear from the results that despite a definite increase in the free amino acid pool, the change in albumin synthesis at 3h, although an almost complete reversal of the depression noted with cortisol alone, was not equivalent to that observed 24h after giving cortisol alone. Further, 24h after cortisol was given fibrinogen synthesis also was substantially increased, but in this case increase of the amino acid concentration (at 3h) had not increased or

altered fibrinogen synthesis. These findings suggest that a shift of amino acids can only partially account for the rise in synthesis observed 24h after giving cortisol. Hence cortisol appears to influence the synthesis of proteins both directly (reviewed by Kenney, 1970) and through a shift of amino acids. The direct effect which is observed immediately (within 3h of giving cortisol) is clearly different from the direct effect which is observed later on (at 24h) and also from the indirect effect of amino acid shift at this later time.

Our findings resolve the seemingly conflicting results of cortisol on albumin synthesis reported by Rothschild *et al.* (1958, 1961) and Grossman *et al.* (1960) on the one hand and by Sellers *et al.* (1969), Gordon (1964) and A. Koj [unpublished work, quoted by McFarlane (1969)] on the other hand. The last three authors measured synthesis with a short period of cortisol influence, in the perfused liver and *in vivo*, and noted a fall in albumin synthesis during cortisol administration. The first two authors, however, noted a rise in albumin synthesis, but this was during the chronic administration of cortisol.

A possible hypothesis for the opposing effects of cortisol on different plasma proteins, observed within 3h of its administration, lies in different turnover times of mRNA for specific plasma proteins (John & Miller, 1966, 1968) and a time-dependent effect of cortisol on the availability of ribosomes. Normal albumin synthesis is likely to involve a major part of the hepatocyte's ribosomal population and utilize much of the cell's amino acid substrate because of the relatively large amount of this protein that is synthesized. Stimulation of ribosomal RNA formation (Jacob *et al.*, 1969) and of mRNA for other, more rapidly turning-over, proteins would decrease the availability of ribosomes (reviewed by Munro, 1970, p. 74) and amino acid substrate for this synthesis of

albumin. Increasing hepatocytic amino acid concentrations by administration of Amigen may have prevented a decrease in such synthesis by not only increasing available substrate but also by decreasing RNA catabolism (Munro, 1964, p. 398). In studies where synthesis was measured 24h after cortisol administration, and in chronic experiments, an elevation in liver RNA (Feigelson *et al.*, 1962) would make more ribosomes available for protein synthesis, resulting in the generalized increases in albumin, fibrinogen and transferrin synthesis. Further, a shift of amino acids to the liver would reinforce the increase in RNA (Munro, 1964, p. 447).

We continue to be grateful to Dr. A. S. McFarlane for helpful comments on many aspects. A. B.-R. thanks Schering Corp. Ltd., Pointe Claire, Quebec, Canada, for its Travel Award, which enabled him to visit Dr. McFarlane. We thank Mrs. Janet Chrupala for much and excellent typing. This work was given generous financial support by our own Department of Medicine, by the Medical Research Council (Canada) Grants MA. 3204 and MA.785 and by a capital grant from the Bickell Foundation.

References

- Documenta Geigy* (1962) *Scientific Tables* (Diem, K., ed.), 6th. edn., p. 172, J. R. Geigy S.A., Basle
- Feigelson, M., Gross, P. R. & Feigelson, P. (1962) *Biochim. Biophys. Acta* **55**, 495
- Gitlin, D. (1957) *Ann. N.Y. Acad. Sci.* **70**, 122
- Gordon, A. H. (1964) *Biochem. J.* **90**, 18P
- Gosset, W. D. (1925) *Metron* **5**, 105
- Grossman, J., Yalow, A. A. & Weston, R. E. (1960) *Metab. Clin. Exp.* **9**, 528
- Jacob, S. T., Sadjel, E. M. & Munro, H. N. (1969) *Eur. J. Biochem.* **7**, 449
- Jeejeebhoy, K. N., Bruce-Robertson, A., Sodtke, U. & Foley, M. (1970) *Biochem. J.* **119**, 243
- Jeejeebhoy, K. N., Phillips, M. J., Bruce-Robertson, A., Ho, J. & Sodtke, U. (1972) *Biochem. J.* **126**, 1111
- John, D. W. & Miller, L. L. (1966) *J. Biol. Chem.* **241**, 4817
- John, D. W. & Miller, L. L. (1968) *J. Biol. Chem.* **243**, 268
- John, D. W. & Miller, L. L. (1969) *J. Biol. Chem.* **244**, 6134
- Kenney, F. T. (1970) *Mammalian Protein Metab.* **4**, 148
- Kenney, F. T. & Kull, F. J. (1963) *Proc. Nat. Acad. Sci. U.S.* **50**, 493
- McFarlane, A. S. (1957) *Ann. N.Y. Acad. Sci.* **70**, 19
- McFarlane, A. S. (1963) *Biochem. J.* **89**, 277
- McFarlane, A. S. (1969) *Physiol. Pathophysiol. Plasma Protein Metab., Proc. Int. Symp. 4th. 1967*, p. 98 (in discussion)
- McFarlane, A. S., Irons, L., Koj, A. & Regoeczi, E. (1965) *Biochem. J.* **95**, 536
- McKinley, J. E., Gilbert, D. B., Chao, P.-Y. & Reeve, E. B. (1970) *Amer. J. Physiol.* **218**, 491
- Munro, H. N. (1964) *Mammalian Protein Metab.* **1**, 381
- Munro, H. N. (1970) *Mammalian Protein Metab.* **4**, 74
- Norwich, K. N. (1972) *Biochem. J.* **126**, 1124
- Reeve, E. B., Pearson, J. E. & Martz, D. C. (1963) *Science* **139**, 914
- Rothschild, M. A., Schreiber, S. S., Oratz, M. & McGee, H. L. (1958) *J. Clin. Invest.* **37**, 1229
- Rothschild, M. A., Wimer, E. & Schreiber, S. S. (1961) *J. Clin. Invest.* **40**, 545
- Sellers, A. L., Bonorris, G. & Katz, J. (1969) *Physiol. Pathophysiol. Plasma Protein Metab., Proc. Int. Symp. 4th. 1967*, p. 99
- Welch, B. L. (1947) *Biometrika* **34**, 28