The Effect of Cortisol on the Activity of Glutamate–Pyruvate Transaminase and the Formation of Glycogen and Urea in Starved Rats

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(Received 24 January 1964)

The adrenocorticosteroids, cortisol, cortisone and corticosterone, may be regarded as catabolic hormones in that the long-term injection of these substances into rodents prevents growth and brings about a drop in body weight (Bodansky & Money, 1954; Cannon, Frazier & Hughes, 1956). The steroids differ widely in their potency (Noble, 1955), but the common action involves the breakdown of skeletal muscle (Engel, 1951) with an associated increase in the size of the liver (Silber & Porter, 1953; Bodansky & Money, 1954; Morita & Kamei, 1962). The latter effect may be a secondary response induced by an increased flow of amino acids to the liver for deamination. In this respect it is known that several hepatic processes concerned with amino acid metabolism are activated in response to a single injection of cortisol. For example, glycogen is deposited in the liver of the starved animal by gluconeogenesis (Long, Katzin & Fry, 1940) and there are corresponding increases in the activity of glutamate–pyruvate transaminase (L-alanine–2-oxoglutarate aminotransferase, EC 2.6.1.2) (Morita & Kamei, 1962; Morita & Kawada, 1962) and the rate of urea synthesis (Long et al. 1940; Engel, 1951). To investigate the possibility that all these responses were linked together, and dependent on a primary effect of cortisol on the release of amino acids from peripheral tissues, the rate of change of all three processes, and their dose–response relationships, were examined after single injections of cortisol into starved rats.

MATERIALS AND METHODS

Treatment of animals. Two-month-old male rats (about 150 g. body wt.) were taken from an inbred colony maintained in this Department. Cortisol (L. Light and Co. Ltd.) was prepared for injection by grinding in a mortar, with a solution of 0.9% NaCl containing 1% of sodium carboxymethylcellulose (L. Light and Co. Ltd.) and 1% of benzyl alcohol, until the suspension could be drawn easily into a no. 12 hypodermic needle. Animals were starved for 17 hr. before dorsal injections were made subcutaneously, just behind the head.

Glutamate–pyruvate transaminase. At various intervals after injection the starved animals were stunned, decapitated and the median lobe of the liver was removed and placed in crushed ice. Enzyme activity was measured by an adaptation of the method of Reitman & Frankel (1957). About 100 mg. of liver was homogenized in 10 ml. of water in a glass homogenizer (A. Gallenkamp Ltd.) by moving the pestle up and down 20 times. A sample of the homogenate (0.5 ml.) was added to 4.5 ml. of water and centrifuged at 25000g for 5 min. The tissue preparation was kept as close to 0° as possible during these operations. Exactly 45 min. after the death of the rat 0.2 ml. of the supernatant solution was added to 1.0 ml. of α-oxoglutarate–alanine substrate solution (Reitman & Frankel, 1957) at 40°. The mixture was kept at 40° for 30 min. then 1.0 ml. of 1 mx-dinitrophenylhydrazine–HCl was added. This mixture was left for 30 min. at room temperature before adding 10 ml. of 0.4x-NaOH. The extinction (at 505 μμ) of the resultant solution was measured 30 min. later against a tissue blank prepared by adding 0.2 ml. of tissue extract to 1.0 ml. of substrate and 1.0 ml. of dinitrophenylhydrazine hydrochloride and omitting the incubation at 40°. The calibration curve was linear.

Tyrosine–α-oxoglutarate transaminase (L-tyrosine–2-oxoglutarate aminotransferase, EC 2.6.1.5). The method of Canellakis & Cohen (1956) was used with modifications. The tyrosine–α-oxoglutarate substrate solution (8.0 ml.) was placed in a water bath at 38° for 10 min. A sample of liver homogenate (0.2 ml.), prepared by homogenizing 1 g. of liver in 10 ml. of water, was added and the suspension mixed thoroughly. A tissue blank was prepared by adding 3.0 ml. of mixture, as quickly as possible, to 0.1 ml. of 50% trichloroacetic acid. The remaining suspension was incubated for 90 min. and another 3.0 ml. sample treated in the same way. The samples were centrifuged, 2.5 ml. of the supernatant solution was added to 2.0 ml. of mixed colour reagent, and the extinction of the second sample was compared with the first at 850 μμ after standing at room temperature for 1 hr. The amount of p-hydroxyphenylpyruvic acid formed may be calculated by using E = 4100 (Canellakis & Cohen, 1956). Tissue blanks were found to give an extinction of about 0-05, and in practice one blank was used for a range of liver samples.

Estimation of liver glycogens. Freshly chilled liver (about 100 mg.) was homogenized in 10 ml. of 5% trichloroacetic acid. The homogenate was centrifuged at about 6000g for 10 min. and the glycogen content of the supernatant solution was measured by the method of van der Vies (1954). The calibration curve was linear.

Metabolites in urine. A 24 hr. sample of urine from each rat was dried on a rotary flash evaporator at 35° and dissolved in 2 ml. of water. Perchloric acid solution (8 ml. of 10%, v/v) was added and the mixture centrifuged at maximum speed in a MSE bench centrifuge for 10 min. Glucose (Nelson, 1944), amino nitrogen (Rosen, 1957) and urea (Archibald, 1946, as modified by Retner, 1955) were
determined by using 0-1 ml. samples of supernatant solution diluted 1/10, 1/20 and 1/50 respectively.

**Extraction and estimation of radioactivity.** Radioactive substances arising from injected tritiated cortisol were extracted and estimated as described previously (Bellamy, Phillips, Jones & Leonard, 1962; Bellamy, 1963a). Blood was collected in heparin-treated tubes, from decapitated animals. Plasma samples (0-1 ml.) were pipetted on to 3/4 in. diam. circles of filter paper, dried in air and shaken with 1 ml. of CHCl₃-methanol (2:1, v/v). The CHCl₃-soluble fraction of plasma and tissues was obtained by dissolving the material extracted with CHCl₃-methanol in CHCl₃ (100 ml/g. of original tissue) and washing this solution three times with an equal volume of water.

**RESULTS**

**Effect of cortisol on urea excretion.** A single subcutaneous injection of cortisol into starved rats was associated with an increased excretion of urea over a fourfold dose range (Table 1). At the highest dose (40 mg.) there was about a twofold stimulation of urea output, measured 24 hr. after injection.

<table>
<thead>
<tr>
<th>Cortisol injected (mg.)</th>
<th>Glucose (µmoles)</th>
<th>Amino N (µg. atoms)</th>
<th>Urea N (µg. atoms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>38.8±3.7</td>
<td>246±14</td>
<td>13.9±1.1</td>
</tr>
<tr>
<td>5</td>
<td>33.0±3.5</td>
<td>266±20</td>
<td>20.2±1.5</td>
</tr>
<tr>
<td>10</td>
<td>58.8±5.1</td>
<td>360±22</td>
<td>24.0±1.4</td>
</tr>
<tr>
<td>20</td>
<td>51.5±4.7</td>
<td>418±25</td>
<td>28.2±1.7</td>
</tr>
<tr>
<td>40</td>
<td>65.0±6.2</td>
<td>492±29</td>
<td>30.4±1.5</td>
</tr>
</tbody>
</table>

No changes were observed in the concentrations of tissue and plasma urea at this time, indicating that the increased urea excretion was due to increased urea synthesis. The amount of glucose and total free amino acids excreted also showed a tendency to rise with increasing dose (Table 1). This probably reflects a higher than normal concentration of these substances in the plasma (Ingle, 1949). An increased synthesis of urea is well established as a response to cortisol treatment and is thought to be related to a primary action of the steroid in changing the steady-state balance of protein metabolism in peripheral tissues so as to favour protein breakdown (Russell, 1951).

The amount of urinary amino nitrogen found as amino acids was equivalent to about 3 % of that excreted as urea. Calculations of the amount of protein carbon atoms released by the deamination reactions which were responsible for the increased urea synthesis (1 g. of nitrogen = 3-14 g. of carbon) showed that carbon atoms excreted in the form of glucose could account for less than 1 % of the protein metabolites.

**Effect of cortisol on liver glycogen and transaminases.** Cortisol injection brought about an increase in liver glycogen within 2 hr. at all doses (Table 2). The amount of liver glycogen found 24 hr. after injection at the higher doses could account for only about one-sixth of the protein carbon atoms metabolized to form urea. Glycogen was deposited at a linear rate for 24 hr. with doses of 10 and 20 mg. With doses smaller than 10 mg. the rate of increase of glycogen was the same for the first 6-12 hr. After this time the rate of glycogen deposition fell off and at the lowest dose of 2-5 mg. the amount of liver glycogen decreased between 12 and 24 hr. after injection.

In contrast with changes in glycogen, glutamate-pyruvate transaminase was not affected immedi-

**Table 1. Effect of cortisol on the urinary excretion of glucose, amino acids and urea in starved rats**

| Male rats (2 months old) starved for 17 hr. were injected subcutaneously with 0.5 ml. of 0.9 % NaCl containing various amounts of cortisol. The animals were kept starved in separate metabolism cages and urine was collected for 24 hr. after injection. Each figure is the mean ± s.e. obtained from five to eight animals. |

**Table 2. Effect of cortisol on liver glycogen in starved rats**

| Two-month-old litter mates (five to eight animals) were starved for 17 hr., and then injected subcutaneously with various amounts of cortisol suspended in 0.5 ml. of 0.9 % NaCl (containing 1 % of sodium carboxymethyl-cellulose). At intervals after injection each litter (maintained without food) was killed and the glycogen determined in a 5 % trichloroacetic acid extract of liver as described in the text. |

<table>
<thead>
<tr>
<th>Cortisol injected (mg.)</th>
<th>0</th>
<th>2.5</th>
<th>5.0</th>
<th>10.0</th>
<th>20.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time after injection (hr.)</td>
<td>0</td>
<td>2.5</td>
<td>5.0</td>
<td>10.0</td>
<td>20.0</td>
</tr>
<tr>
<td>0</td>
<td>0.210±0.008</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>—</td>
<td>0.516±0.030</td>
<td>—</td>
<td>0.345±0.023</td>
<td>0.442±0.015</td>
</tr>
<tr>
<td>4</td>
<td>—</td>
<td>1.560±0.075</td>
<td>0.930±0.038</td>
<td>1.238±0.068</td>
<td>1.703±0.098</td>
</tr>
<tr>
<td>6</td>
<td>—</td>
<td>2.190±0.090</td>
<td>1.533±0.090</td>
<td>1.965±0.105</td>
<td>2.093±0.165</td>
</tr>
<tr>
<td>12</td>
<td>—</td>
<td>2.303±0.120</td>
<td>2.445±0.105</td>
<td>2.828±0.143</td>
<td>3.188±0.165</td>
</tr>
<tr>
<td>24</td>
<td>0.143±0.008</td>
<td>1.245±0.075</td>
<td>3.570±0.195</td>
<td>6.068±0.353</td>
<td>5.288±0.270</td>
</tr>
</tbody>
</table>

* Means ± s.e. (comparing all figures with control, P = 0.01-0.001).
ately but began to rise only after a lag period of between 2 and 12 hr. (Table 3). The results show that an increase in the amount of cortisol injected brought about a decrease in the lag period. However, this was not apparently associated with any change in the rate of increase of the enzyme. For example, when the dose was lowered from 10 to 2.5 mg., the time at which the first significant rise in transaminase occurred was delayed for 12 hr., although the activity of transaminase 24 hr. after the lower dose was the same as that 12 hr. after the higher dose.

The results show that the mechanisms responsible for the change in glycogen and transaminase are not affected by cortisol in the same way. In particular, alterations in glycogen deposition precede changes in transaminase activity. Since the amount of urea excreted in 24 hr. indicated that protein catabolism was increased over the dose range 5–20 mg. (Table 1), and by contrasting this with the corresponding constant rate of glycogen deposition and the small amount of glucose in the urine, it may be concluded that gluconeogenesis was not limited by the availability of substrate. These results also raise the possibility that the change in the activity of glutamate–pyruvate transaminase is a secondary response in that it may be brought about when the products of a primary action of cortisol on peripheral tissues reach a critical concentration in liver.

Although several liver enzymes increase in amount after cortisol injection (Noble, 1955), they may differ considerably in the nature of their response to steroid treatment. Experiments on tyrosine–α-oxoglutarate transaminase showed that 0.5 mg. of cortisol increased enzymic activity 4 hr. after injection to the same degree as 10 mg. of cortisol (Table 4). This indicates that the enzyme was either much more sensitive to steroid treatment than glutamate–pyruvate transaminase or that the type of mechanism involved was somewhat different. Other work also suggests that these two enzymes differ in the rate of response to cortisol (cf. Rosen, Roberts & Nichol, 1959; Litwack, Sears & Diamondstone, 1963).

**Concentrations of injected steroid in plasma and liver.** To investigate the relationship between the intracellular steroid and the biochemical responses of liver, previous studies on the tissue distribution of intravenously injected cortisol (Bellamy et al. 1962; Bellamy, 1963a) were extended to include cortisol administered subcutaneously. Two hours after a subcutaneous injection of 10 mg. of [3H]cortisol (1.7 μg) the amount of radioactivity in plasma was equivalent to about 50 μg. of steroid/
ml. Thereafter the plasma radioactivity dropped exponentially and was reduced by about 75% after 24 hr. (Fig. 1). The initial radioactivity in liver was about twice that in plasma. Subsequently, however, the tissue radioactivity fell at a linear rate and 24 hr. after injection the concentration in liver was about four times that in plasma.

The chloroform-soluble radioactivity (cortisol and non-polar metabolites; Bellamy, 1963a) was higher in liver than in plasma (Fig. 1). The chloroform-soluble steroids fell at a slower rate than the total radioactivity so that the ratio of non-polar to polar steroids increased with time. This observation supports previous work which showed that the chloroform-soluble metabolites of cortisol were adsorbed more strongly to liver tissue than the water-soluble steroids (Bellamy, 1963a). The maximum concentration of unchanged radioactive cortisol in plasma, about 3 μg./ml., was reached 2 hr. after injection.

The observed time-course of the changes in total radioactivity would be expected if cortisol was removed from the site of injection over the whole 24 hr. period. From previous work it would appear that the bulk of the injected steroid was ultimately excreted into the intestine (Bellamy, 1963a), possibly by a route not involving the plasma (Bellamy, 1963b). Indeed, the present experiments showed that less than 10% of the injected dose was excreted in the urine in 24 hr.

The experiments on urea excretion (Table 1) suggested that an increased dose of cortisol resulted in an increased amount of biologically active steroid in the tissues. To examine this further, varying amounts of radioactive corticosterone in suspension were injected subcutaneously into normal rats. At the lowest dose the ratio of insoluble to soluble steroid in 0.5 ml. of injected suspension was of the order of 250:1. An increase in the amount of suspended steroid in the standard injection volume therefore would make no difference to the concentration gradient for the diffusion of corticosterone into the tissues, yet an increased dose, over a tenfold range, always brought about an increased concentration of radioactive steroids in the liver (Table 5). Thus the injected steroid was mobilized partly on a volume basis and the concentration of tissue steroids was to some considerable extent independent of the amount of soluble steroid per unit volume. That is to say, diffusion of the steroid into the tissues was not the predominant factor that limited uptake of injected corticosterone.

In agreement with previous work (Bellamy, 1963a) on intravenously injected corticosteroids, only about 5% of total radioactivity in liver and plasma was accounted for by unchanged hormone 2 hr. after administration. When related to liver volume, the maximum amount of unchanged corticosterone in liver at this time was equivalent to only about 0.05% of the injected dose.

The apparent half-life of the mobilized steroid in plasma, calculated from the fall in total radioactivity beginning 2 hr. after injection, was about 6 hr. This may be compared with a half-life of about 1 hr. for the intravenously injected steroid. The half-life of total liver steroid was about 24 hr. After intravenous injection of radioactive soluble cortisol in mice, previous workers (Berliner, Keller & Dougherty, 1961) have found the half-life of liver radioactivity to be about 70 min. Thus, with respect to the present work, there is a good deal of evidence in favour of both the long-term mobilization of subcutaneously injected cortisol suspension and the retention of liver steroids.

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**Figure 1.** Liver and plasma radioactivity after subcutaneous injections of [3H]cortisol. Normal 2-month-old male rats were injected subcutaneously as described in the text with 0.5 ml. of cortisol suspension (10 mg. of cortisol containing 1.67 μc of H/μg). Groups of animals (five to seven rats) were killed at intervals after injection and the total radioactivity (liver, ○; plasma, □) and chloroform-soluble radioactivity (liver, ■; plasma, ■) were determined as described in the text.

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**Table 5. Radioactivity in liver after subcutaneous injections of [3H]corticosterone**

<table>
<thead>
<tr>
<th>Cortisol Injected (mg.)</th>
<th>Expt. 1</th>
<th>Expt. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>0.187</td>
<td>0.167</td>
</tr>
<tr>
<td></td>
<td>0.172</td>
<td>0.174</td>
</tr>
<tr>
<td>5</td>
<td>0.376</td>
<td>0.204</td>
</tr>
<tr>
<td></td>
<td>0.256</td>
<td>0.206</td>
</tr>
<tr>
<td>10</td>
<td>0.333</td>
<td>0.346</td>
</tr>
<tr>
<td></td>
<td>0.357</td>
<td>0.301</td>
</tr>
<tr>
<td>20</td>
<td>0.414</td>
<td>0.666</td>
</tr>
<tr>
<td></td>
<td>0.495</td>
<td>0.702</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.637</td>
</tr>
</tbody>
</table>

Four mixed litters of normal rats were injected subcutaneously with various amounts of corticosterone containing [3H]corticosterone (8 μg/mg.). Two hours later the animals were killed and liver radioactivity was determined as described in the text. Figures refer to determinations on individual animals.
DISCUSSION

Bellamy (1963a) pointed out that strongly adsorbed cortisol in the cell, not in equilibrium with intracellular free cortisol, might be responsible for some of the biochemical results of cortisol administration. It can now be said that this type of hormone–receptor relationship is unlikely to account for glycogen deposition. The kinetics of glycogen formation and the distribution of injected steroid suggest that a relationship in which the hormone at the site of action is in free equilibrium with total cell cortisol is involved. In agreement with this, other experiments (D. Bellamy & R. Kilpatrick, unpublished work) show that liver glycogen in starved rats was not altered 24 hr. after the intravenous injection of 10 mg. of cortisol, although the initial amount of cortisol per liver was about 10 times higher than that observed when this quantity of steroid was injected subcutaneously (65 μg. compared with 5-6 μg.). These results also stress the importance of the long-term mobilization of subcutaneously administered cortisol in the present work.

The sites of action evidently have a great affinity for cortisol in that the maximum rate of glycogen deposition was obtained in the present work with doses as low as 0.5 mg. of steroid. As the dose was increased, the sites appeared to remain saturated for a longer time because of a higher initial amount of cellular hormone.

In contrast with the effect on glycogen formation, the changes in glutamate–pyruvate transaminase, which involve increased synthesis of enzyme protein (Segal, Beattie & Hopper, 1962; Segal, Rosso, Hopper & Weber, 1962), have not so far been demonstrated in vitro. However, the time-course of the alterations in glutamate–pyruvate transaminase suggest that the enzyme activity is not affected by cortisol directly. These conclusions are in agreement with other work which shows that glutamate–pyruvate transaminase increases in response to dietary changes in the absence of the adrenal glands (Harding, Rosen & Nichol, 1961). In addition there is evidence that alterations in plasma composition, due to a variety of extrahepatic processes not involving glucocorticoids, may produce marked alterations in liver-protein synthesis (Feigelson & Feigelson, 1963). The latter findings, when taken together with the major catabolic effect of injected cortisol on peripheral tissues, which results in the release of a large number of tissue components into the blood stream, make it extremely difficult to implicate this steroid directly in long-term changes in metabolism. This applies particularly to the increased respiration (Shida & Barker, 1962) and liver growth (Goodlad & Munro, 1959) observed by other workers.

From the changes in urea excretion, it is clear that the major effects of large single injections of cortisol were on protein metabolism. Calculations show that with the smallest dose (2.5 mg.) the initial rate of carbon deposition as liver glycogen was similar to the increased rate of release of amino acid carbon atoms from deamination reactions. However, unlike urea formation, the initial rate of glycogen deposition was not affected by the quantity of steroid injected. This resulted in only one-sixth of the amino acid residues being converted into liver glycogen at the highest steroid concentration. The fate of the remaining metabolites is not known. Since only about 0.5 g. of carbon was involved it would be difficult to detect this as a net change in body fat, muscle glycogen or expired carbon dioxide over a 24 hr. period.

The results show quite clearly that the rate of transamination does not limit the glycogen synthesis initiated by low doses of cortisol. However, it may be that glycogen in these experiments did not arise solely by gluconeogenesis since cortico-steroids may make blood sugar available for the formation of liver glycogen before the availability of amino acids is altered (Long, Smith & Fry, 1960; Ashmore, 1960).

Taking into account the concentrations of alanine and glutamic acid found in the liver of starved rats (Kaplan & Shimizu, 1962), it would appear that glutamate–pyruvate transaminase is not normally saturated with substrate and the conditions are favourable for transamination from alanine. Although there are indications that localized concentrations of amino acids may occur in specific regions of the cell (Bellamy, 1961), the available evidence suggests that an increased liver alanine concentration would bring about a faster rate of transamination. This conclusion is in agreement with other experiments which showed that the intravenous infusion of amino acids produced a large increase in urea formation (Engel, 1951) before any appreciable change in glutamate–pyruvate transaminase would be expected. Further, it may be calculated that the quantity of enzyme in the liver of normal rat would be more than sufficient to account for all deamination rates observed in the present work. In fact, since cortisol treatment has been found to double the concentration of plasma amino acids, without a corresponding change in liver amino acids (Kaplan & Shimizu, 1963), amino acid uptake by liver, rather than the rate of deamination, may limit amino acid metabolism.

These considerations raise the general question of the significance of changes in non-limiting enzymes by hormones. In a previous discussion of this topic the view was taken that variations in enzyme synthesis after hormone administration may often be an indirect homeostatic response of the tissues concerned.
to counteract undesirable changes in substrate concentration (Bellamy, 1964). Thus, although hormone action may alter the rate of formation of particular substances, the corresponding changes in metabolic rate occur with minimal alterations in the concentration of intracellular metabolites. As far as amino acids are concerned this concept is in agreement with extensive work of Roberts & Simonsen (1962), which shows a remarkable constancy of concentration of tissue amino acid in a variety of metabolic states.

Indeed, the present work supports their conclusions that extensive changes in amino acid turnover are not linked necessarily with abnormal steady-state concentrations.

SUMMARY

1. A single subcutaneous injection of cortisol suspension into starved rats increased the 24 hr. urea excretion over a wide dose range (2-5-40 mg./rat). At the highest dose there was a twofold rise in urea excretion with no change in plasma or tissue urea concentration.

2. At all doses cortisol increased liver glycogen within 2 hr. after injection. The rate of glycogen deposition was not affected by the amount of steroid injected but larger doses maintained the process for longer periods. With the higher doses liver glycogen amounted to about 6% of the wet weight of liver 24 hr. after injection.

3. Liver glutamate-pyruvate-transaminase activity was increased only after a lag period. An increased dose reduced the lag period without affecting the rate of increase of enzymic activity.

4. Two hours after a subcutaneous injection of $[^{3}H]$cortisol (10 mg.) the plasma radioactivity was equivalent to about 50 $\mu$g. of steroid/ml. Thereafter plasma radioactivity fell exponentially whereas liver radioactivity dropped at a linear rate. The apparent half-life of the injected steroid was approximately 6 hr. and 24 hr. for plasma and liver respectively.

The authors thank the Endocrinology Study Section, National Institutes of Health, Bethesda, Md., U.S.A., for the gift of radioactive steroids. The work was largely financed by grants from the Agricultural Research Council.

REFERENCES
