Effect of Adrenalectomy and of Growth Hormone on Enzymes Concerned with Urea Synthesis in Rat Liver

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Although adrenalectomy and growth-hormone treatment both cause a decreased nitrogen excretion (Long, Katzin & Fry, 1940; Russell, 1955) and both decrease the arginase activity of rat liver (Fraenkel-Conrat, Simpson & Evans, 1943*a, b*; Folley & Greenbaum, 1948*b*), the changes in the general metabolic pattern produced by these two treatments are quite different, the common feature of decreased urea production being related in the adrenalectomized rats to decreased gluconeogenesis from protein and in growth-hormone-treated rats to an increased deposition of protein (Long *et al.* 1940; Young, 1945; Russell, 1955; Ketterer, Randle & Young, 1957).

With the recent development by Ratner (1955*a*) and Brown & Cohen (1959) of methods for the assay of the enzyme involved in the synthesis of urea from ammonia and carbon dioxide (Scheme 1), i.e. carbamoyl phosphate synthetase (adenosine triphosphate-carbamate phosphotransferase; EC 2.7.2.2), ornithine transcarbamoylase (carbamoyl phosphate-L-ornithine carbamoyltransferase, EC 2.1.3.3), both located in the mitochondrial fraction (Grisolia & Cohen 1953), argininosuccinate synthetase [L-citrulline-L-aspartate ligase (adenosine 5'-phosphate); EC 6.3.4.5], argininosuccinase (L-argininosuccinate arginine-lyase, EC 4.3.2.1), the last two together forming the arginine-synthetase system, and arginase (L-arginine ureohydrolase, EC 3.5.3.1), all in the soluble fraction (Ratner, 1955*a*; Rosenthal, Gottlieb, Gorry & Vars, 1956), the effects of these hormonal conditions on the individual enzyme reactions can be examined.

The rate-limiting reaction in the urea cycle in rat liver is the condensing enzyme of the arginine-synthetase system, argininosuccinate synthetase (Brown & Cohen, 1960); the activity of this enzyme is important, since it is at such rate-limiting reactions that metabolic control might be exerted (Krebs & Kornberg, 1957). McLean (1961) showed that the activity of this enzyme is markedly decreased in the livers of adrenalectomized rats, and Freedland & Sodikoff (1962) found that a striking increase occurs in hydrocortisone-treated rats and in diabetic or hyperthyroid rats.

In the present experiments measurements were made of all five enzymes of the urea cycle to determine whether the metabolic adaptation to decreased production of urea involves specifically the rate-limiting reaction, the most economical way of decreasing the production of urea or, if the activity of several enzymes changed, whether there was any difference in the response of enzymes located in the mitochondrial and soluble fractions of the cell.

METHODS

Materials. Commercial reagents were used, with the exception of argininosuccinic acid, α -isonitrosopropiophenone and growth hormone. Argininosuccinic acid was a gift from Dr R. G. Westall, University College Hospital, London. Two samples of growth hormone were used: one was prepared by Dr A. L. Greenbaum, University College London, and the other was kindly given by Dr A. E. Wilhelmi, Endocrinology Study Section, National Institutes of Health, U.S.A. α -Isonitrosopropiophenone was synthesized by the method of Claisen & Manasse (1889).

Scheme 1. Simplified urea cycle.

Purified arginase (Sigma) was used; this preparation had no argininosuccinate-synthetase or argininosuccinase activity.

Adrenalectomy and treatment with cortisone acetate. Adult male rats with initial body weights between 140 and 150 g. were used. The adrenalectomized rats were given 1% sodium chloride solution to drink. Paired feeding was begun immediately after the operation. Two weeks later the rats were killed by dislocation of the neck and liver extracts prepared for estimation of the activity of the urea-cycle enzymes. One group of rats, which had been adrenalectomized for 12 days, were treated with a saline suspension of cortisone acetate (Merck Inc., U.S.A.). Three intramuscular injections, each of 2.5 mg., were given at 24 hr. intervals. The rats were killed 1 hr. after the last injection. This short time of treatment was chosen to determine the order in which the enzymes were restored towards normal values (see Fig. 3).

In addition, a group of six normal adult male rats were treated intramuscularly with a saline suspension of cortisone acetate, 5 mg./day for 4 days. The rats were killed 1 hr. after the last injection (see Table 3). The food intake was controlled by paired feeding in all experiments.

Treatment with growth hormone. Adult female rats were used when their body weight had reached a plateau value of 180–190 g. A daily dose of 1.0 mg. of growth hormone was injected subcutaneously; the growth response was improved when the hormone was given in two divided doses. Treatment was continued for 9 days; the rats were pair-fed throughout the experimental period.

In the present experiments it was difficult to measure all five enzymes of the urea cycle in the liver homogenates of a large group of rats without undue delay and prolonged storage at 0° of the enzyme preparations. For this reason, the mitochondrial enzymes were measured in one group and the enzymes located in the soluble fraction of the cell in a second group of animals.

Urea-cycle enzymes. The activities of the urea-cycle enzymes were measured essentially according to the methods of Brown & Cohen (1959); the modifications made in the individual enzyme assays are given below.

Liver was homogenized in 0.1% (w/v) cetyltrimethylammonium bromide solution, in the ratio 1 g. of liver to 9.0 ml. of solution, the latter being added at room temperature, for 3 min. in a Potter homogenizer and then cooled in ice. The homogenate was centrifuged at 4000g for 15 min. at 5°. The supernatant fraction was used in the assay of all the urea-cycle enzymes except arginase. This treatment gave virtually complete extraction of these enzymes, as shown by further extraction of the residue with cetyltrimethylammonium bromide solution.

Carbamoyl phosphate synthetase and ornithine transcarbamoylase. The assay system of Brown & Cohen (1959) was used. Comparison of the enzymic activity of liver extracts prepared with 0.1% cetyltrimethylammonium bromide solution (used by Brown & Cohen, 1959) with 1 mM-GSH (used by Jones & Spector, 1960) showed that the latter gave slightly lower values for carbamoyl phosphate synthetase, and identical values for ornithine transcarbamoylase. Moreover, 1 mM-GSH extracts stored for 3 or 4 hr. in ice showed no loss of enzymic activity, a point of interest in relation to the unstable nature of rat-liver carbamoyl phosphate synthetase and the cold inactivation of carbamoyl phosphate-synthetase preparations from frog

liver (Rajman & Grisolia, 1961). The effect of Mn^{2+} and Cr^{3+} ions on the activity of carbamoyl phosphate-synthetase preparations was tested. These metals had no effect on the activity of the enzyme; with manganese sulphate as high as mM final concentration the enzyme activity (cetyltrimethylammonium bromide extract) was only 4% higher than in its absence. Ornithine transcarbamoylase is so active in rat liver (12 400 units/g.) that it proved unnecessary to supplement the carbamoyl phosphate-synthetase system (320 units/g.) with excess of ornithine transcarbamoylase.

Ornithine-transcarbamoylase activity was estimated by the method of Brown & Cohen (1959).

Citrulline was estimated by the method of Archibald (1944), diacetylmonoxime being used for colour development. It is clear from the non-linearity of the lower portion of the calibration curve (0–20 μ g. of citrulline, Fig. 1) that some fading had occurred despite precautions to exclude light (see Archibald, 1944). Glutathione interfered some-

Fig. 1. Effect of glutathione on the citrulline calibration curve. (a) Effect on colour development of addition of glutathione to different amounts of citrulline: ○, citrulline alone (20–80 μ g.); ●, citrulline + 30 μ g. of GSH; ◐, citrulline + 60 μ g. of GSH. (b) Percentage inhibition of the colour developed with 60 μ g. of citrulline by increasing concentrations of glutathione (15–90 μ g.). The colour reaction of Archibald (1944) was used, the time of heating was 10 min. and the colours were read 10 min. later at 490 $m\mu$.

what with the colour reaction and was therefore added to the citrulline standards in an amount equivalent to that in the incubation medium sample. The effect of increasing amounts of glutathione on the colour development is shown in Fig. 1.

Arginine synthetase and argininosuccinase. These enzymes were estimated according to the methods of Brown & Cohen (1959) by using the soluble fraction from homogenates in 0.1% cetyltrimethylammonium bromide solution. Extracts prepared in 1 mM-GSH were unsuitable for the assay of these enzymes since with these extracts the overall arginine-synthetase activity was low when the incubation mixture of Brown & Cohen (1959) was used.

Fig. 2. Enzyme-substrate concentration curves for argininosuccinase. Enzyme activity was measured according to the method of Brown & Cohen (1959) with a 15 min. incubation period. One part of liver was homogenized in 9 parts of 0.1% cetyltrimethylammonium bromide solution and the soluble fraction prepared by centrifuging at 4000g for 15 min. at 0° was used for the assay. (a) Effect of doubling the substrate concentration on the enzyme concentration-activity curve: ○, 2 μmoles of argininosuccinate; ●, 4 μmoles of argininosuccinate. (b) Substrate concentration-activity curve with a fixed amount of enzyme present (0.1 ml. of extract prepared as described above), the substrate concentration varying from 2 to 8 μmoles/ml. of medium.

In the assay of argininosuccinase Brown & Cohen (1959) used an amount of substrate giving only 68% V_{max} , and they suggest that higher concentrations of substrate should be employed where possible. In the present experiments twice the amount of substrate has been used (i.e. 4 μmoles). The effect of this alteration in substrate concentration on the enzyme activity is shown in Fig. 2.

Arginase activity. This was determined by the method of Folley & Greenbaum (1948a). The liver homogenate was prepared, with a top-drive homogenizer (Folley & Watson, 1948), in 'manganese saline' (containing 9.0 g. of sodium chloride and 8 g. of manganese sulphate tetrahydrate/l.) in the ratio 1 g. of liver to 19 ml. of manganese saline. This was further diluted to 1 part of liver in 200 parts of manganese saline for activation (30 min.) and assay of the enzyme activity.

The colour reaction with α -isonitrosopropiophenone (Archibald, 1945) was used for determining the urea formed.

The present values for the activities of the urea-cycle enzymes are very similar to those of Brown & Cohen (1960), with the exception of liver arginase, which is ten times as high. This might be explained by the different extraction procedure, the activation with Mn^{2+} ions, the higher substrate concentration and the shorter time of incubation used in the method of Folley & Greenbaum (1948a), which was used in the present experiments. The values for normal rat-liver arginase reported here agree with those of Folley & Greenbaum (1948b).

Enzyme units. The enzyme activities were all expressed in terms of a unit, defined as the amount of enzyme catalysing the formation of 1 μmole of urea/hr., under the conditions of the assay.

Validity of assay for rat liver. The overall arginine-synthetase system comprises a condensing enzyme, argininosuccinate synthetase, and a splitting enzyme, argininosuccinase. In rat liver, because of the relative activities of these enzymes and of arginase (78 units/g. overall arginine-synthetase system, 185 units/g. argininosuccinase and 128 000 units/g. arginase, normal male rat liver: average values for controls—Tables 2 and 3), each of these enzyme steps can be measured separately by using

Table 1. Activities of argininosuccinase and arginase measured under conditions used for the assay of arginine synthetase and argininosuccinase

A, Optimum conditions for arginine-synthetase system with respect to buffer and time of incubation (Brown & Cohen, 1959): (1) with substrates for arginine synthetase; (2) with 4 μmoles of argininosuccinate; (3) with 200 μmoles of arginine. B, Optimum conditions for argininosuccinase with respect to buffer and time of incubation (Brown & Cohen, 1959): (2) with 4 μmoles of argininosuccinate; (3) with 200 μmoles of arginine. C, Optimum conditions for arginase (Folley & Greenbaum, 1948a).

Conditions ...	Activity (μmoles of urea/hr. g. of liver)		
	A	B	C
Enzyme			
(1) Arginine synthetase	64	—	—
(2) Argininosuccinase	221	252	—
(3) Arginase	1060	3440	112 600

Table 2. *Effect of adrenalectomy on the activities of enzymes of the urea cycle in rat liver*

Results are expressed as means $\pm$ S.E.M. A unit of enzyme activity is defined as the amount catalysing the formation of 1 μ mole of urea/hr. under the conditions of assay. For details, see Methods section.

	No. of animals	Control group	Adrenalectomized group	P
Initial body wt. (g.)		144 $\pm$ 5	144 $\pm$ 5	—
Final body wt. (g.)	10	148 $\pm$ 4	149 $\pm$ 5	—
Liver wt. (g.)		7.53 $\pm$ 0.40	6.81 $\pm$ 0.28	—
Carbamoyl phosphate synthetase				
Units/g. of liver	9	318 $\pm$ 15	234 $\pm$ 22	0.003
Total units/100 g. body wt.		1 554 $\pm$ 85	1 064 $\pm$ 33	< 0.001
Ornithine transcarbamoylase				
Units/g. of liver	10	12 410 $\pm$ 650	9 550 $\pm$ 900	0.022
Total units/100 g. body wt.		62 100 $\pm$ 2 460	43 310 $\pm$ 4 860	0.003
Initial body wt. (g.)		150 $\pm$ 7	140 $\pm$ 6	—
Final body wt. (g.)	6	157 $\pm$ 12	150 $\pm$ 7	—
Liver wt. (g.)		7.37 $\pm$ 0.69	6.88 $\pm$ 0.35	—
Arginine-synthetase system				
Units/g. of liver	6	88 $\pm$ 3	38 $\pm$ 3	< 0.001
Total units/100 g. body wt.		413 $\pm$ 19	176 $\pm$ 13	< 0.001
Argininosuccinase				
Units/g. of liver	6	207 $\pm$ 16	142 $\pm$ 12	0.011
Total units/100 g. body wt.		965 $\pm$ 67	651 $\pm$ 58	0.006
Arginase				
Units/g. of liver	6	127 000 $\pm$ 4 000	46 500 $\pm$ 5 300	< 0.001
Total units/100 g. body wt.		594 000 $\pm$ 28 000	213 000 $\pm$ 23 000	0.002

appropriate substrates and estimating the urea formed without the necessity for supplementation with purified enzymes.

It is apparent from these figures that the overall arginine-synthetase system may be equated with the argininosuccinate synthetase, the condensing enzyme, which is rate-limiting.

Rat-liver arginase is present in great excess and it appears unnecessary to supplement either rat-liver argininosuccinate synthetase or argininosuccinase with excess of purified arginase (see Brown & Cohen, 1959). However, Folley & Greenbaum (1948*a*) showed that the activity of rat-liver arginase decreased in the absence of added Mn^{2+} ions and the arginase activity of the liver extract in cetyltrimethylammonium bromide was therefore measured with the same volume of extract, buffer, pH and time of incubation as that employed in the assay of arginine synthetase and argininosuccinase, but with 200 μ moles of arginine as substrate. Even with these conditions arginase is still present in ten-fold excess (Table 1). The addition of excess of purified arginase (Sigma) to the assay system did not alter the measured activity of either arginine synthetase or argininosuccinase. The activities of the condensing enzyme of the arginine-synthetase system and argininosuccinase are not widely separated and it was important to establish that the latter enzyme was not rate-limiting under the conditions of the arginine-synthetase assay, namely at pH 7.0 and with a 1 hr. incubation period. The results given in Table 1 show that argininosuccinase is more active than the overall arginine-synthetase system when measured under these conditions. The experiments of Schimke (1962) indicate that the excess of purified argininosuccinase is necessary to get full activity of argininosuccinate synthetase in water homogenates. In the present experiments the changes in the activity of arginine synthetase were often greater than

those of argininosuccinase (see Results section), and this, combined with the material in Table 1, makes it seem unlikely that argininosuccinase was rate-limiting; however, the present work is concerned with the relative changes in the activities of the urea-cycle enzymes produced by hormone treatment rather than with the absolute values.

Glutamic dehydrogenase. This was estimated by the method of Copenhaver, McShan & Meyer (1950) in aqueous homogenates, a treatment that activates the latent dehydrogenases in mitochondria (Bendall & de Duve, 1960).

Glutamic-oxaloacetic transaminase. This was estimated by the method of Tonhazy, White & Umbreit (1950).

The enzyme units for this and for glutamic dehydrogenase were defined as μ moles of substrate used or product formed/hr. at 37°.

Fisher's *t* test was used, a difference between two mean values being regarded as significant if *P* was no greater than 0.05.

RESULTS

Adrenalectomy reduced the activity of all five of the urea-cycle enzymes and of glutamic dehydrogenase. The most striking decrease was in the arginine-synthetase system and arginase; both enzymes were reduced to less than half the pair-fed control values (see Table 2 and Fig. 4). The changes in the activity of the urea-cycle enzymes located in the soluble fraction of the cell were rather more marked than those of the two mitochondrial enzymes. In contrast with this, glutamic-oxaloacetic transaminase remained completely unchanged (Fig. 4).

The effect of cortisone acetate on the rate of restoration of three of the urea-cycle enzymes in adrenalectomized rat liver, those located in the soluble fraction of the cell and most markedly affected by adrenalectomy, is shown in Fig. 3.

Fig. 3. Effect of cortisone acetate on the activities of enzymes of the urea cycle in livers of adrenalectomized rats. Results, as total enzyme units in the whole liver, are expressed as a percentage of the activity of the corresponding pair-fed control group. Vertical lines represent twice the S.E.M. The horizontal broken line is the arbitrarily fixed control value of 100%, and the area between the dotted lines on each side of this represents the S.E.M. value for control groups. Figures across adjacent columns are the values for Fisher's *P*, being a comparison of the adrenalectomized (open columns) and adrenalectomized treated with cortisone acetate (2.5 mg./day by intramuscular injection for 2 days) (cross-hatched columns). For details, see the Methods section.

A short period of treatment, only 2 days, was chosen in an attempt to determine the order in which these enzymes returned towards the control values. Arginase was most rapidly restored (see Fig. 3), reaching a value of 66% of the control in this time; argininosuccinase was raised but the difference was not significant; arginine synthetase was least affected.

Treatment of normal rats with cortisone acetate for 4 days resulted in a marked increase in the total activity of the three urea-cycle enzymes located in the soluble fraction of the cell (see Table 3). Here the metabolic adaptation was largely a result of the significant increase in the liver weight of the cortisone acetate-treated rats. The concentration (units/g. of liver) of arginine synthetase and argininosuccinase remained unchanged. With arginase there was a significant increase in both the concentration and the total activity of the enzyme.

The pattern of change of the enzymes concerned with urea synthesis in growth-hormone-treated rats is somewhat different from adrenalectomized rats (see Table 4 and Fig. 4). Carbamoyl phosphate synthetase and ornithine transcarbamoylase, the first two enzymes of the cycle located in the mitochondria, are scarcely altered by growth-hormone treatment. There is a small decrease in the concentration of ornithine transcarbamoylase; but when the results are expressed as total units (Fig. 4) or total units/100 g. body wt. (Table 4), there is no significant difference between treated and pair-fed control rats. The remaining three enzymes of the urea cycle, located in the soluble fraction of the cell, are all significantly decreased when expressed as units/g. of liver or as total units/100 g. body wt.

Since it was hoped to find a correlation between the activities of the urea-cycle enzymes and the known effects of the growth hormone on the nitrogen balance of the whole animal, it seemed that the

Table 3. *Effect of cortisone acetate on the activities of some enzymes of the urea cycle of normal rat liver*

Results are expressed as means $\pm$ S.E.M. A unit of enzyme activity is defined as the amount catalysing the formation of 1 μ mole of urea/hr. under the conditions of assay. For details, see Methods section.

	Control group	Cortisone acetate-treated group	<i>P</i>
No. of animals	6	6	—
Initial body wt. (g.)	151 $\pm$ 2	150 $\pm$ 2	—
Final body wt. (g.)	153 $\pm$ 2	143 $\pm$ 2	—
Liver wt. (g.)	7.13 $\pm$ 0.23	9.05 $\pm$ 0.22	< 0.001
Arginine synthetase			
Units/g. of liver	69 $\pm$ 8	84 $\pm$ 7	0.165
Total units/100 g. body wt.	319 $\pm$ 32	537 $\pm$ 57	0.008
Argininosuccinase			
Units/g. of liver	163 $\pm$ 12	173 $\pm$ 14	0.601
Total units/100 g. body wt.	756 $\pm$ 45	1 105 $\pm$ 122	0.022
Arginase			
Units/g. of liver	130 000 $\pm$ 11 000	161 000 $\pm$ 5 000	0.026
Total units/100 g. body wt.	606 000 $\pm$ 52 000	1 023 000 $\pm$ 58 000	< 0.001

Table 4. *Effect of growth hormone on the activities of enzymes of the urea cycle in rat liver*

Results are expressed as means $\pm$ S.E.M. A unit of enzyme activity is defined as the amount catalysing the formation of 1 μ mole of urea/hr. under the conditions of the assay. For details, see Methods section.

	No. of animals	Control group	Growth-hormone-treated group	P
Initial body wt. (g.)	6	194 $\pm$ 4	196 $\pm$ 6	—
Final body wt. (g.)		192 $\pm$ 5	218 $\pm$ 6	—
Liver wt. (g.)		7.36 $\pm$ 0.44	9.30 $\pm$ 0.41	—
Carbamoyl phosphate synthetase				
Units/g. of liver	6	202 $\pm$ 21	149 $\pm$ 27	0.153
Total units/100 g. body wt.		779 $\pm$ 68	618 $\pm$ 89	0.181
Ornithine transcarbamoylase				
Units/g. of liver	6	11 470 $\pm$ 1 140	8 200 $\pm$ 577	0.029
Total units/100 g. body wt.		43 270 $\pm$ 3 460	35 520 $\pm$ 3 600	0.159
Initial body wt. (g.)	9	181 $\pm$ 4	178 $\pm$ 5	—
Final body wt. (g.)		179 $\pm$ 4	193 $\pm$ 5	—
Liver wt. (g.)		7.60 $\pm$ 0.29	8.26 $\pm$ 0.30	—
Arginine-synthetase system				
Units/g. of liver	9	96 $\pm$ 4	67 $\pm$ 6	0.002
Total units/100 g. body wt.		409 $\pm$ 22	286 $\pm$ 26	0.002
Argininosuccinase				
Units/g. of liver	7	223 $\pm$ 13	177 $\pm$ 10	0.019
Total units/100 g. body wt.		969 $\pm$ 46	735 $\pm$ 47	0.004
Arginase				
Units/g. of liver	6	85 800 $\pm$ 2 400	68 700 $\pm$ 4 600	0.013
Total units/100 g. body wt.		376 000 $\pm$ 19 000	274 000 $\pm$ 18 000	0.005

total activity of the urea-cycle enzymes in the liver was of greater physiological significance than the concentration of the enzyme. The decreased enzyme activity/g. of liver in growth-hormone-treated rats is largely balanced by an increase in liver weight (Table 4). Fig. 4 shows that, with the total activity of the enzymes as the more important criterion, only the arginine-synthetase system was significantly reduced.

In these experiments the rats were pair-fed and the changes observed were not due to differences in food intake. The control of food intake is important, since Schimke (1962) has shown that the total liver content of enzyme activity of all the urea-cycle enzymes is directly proportional to the protein intake.

DISCUSSION

The common feature and, perhaps, the most striking change found in the present experiments is the decrease in the activity of arginine synthetase in both adrenalectomized and growth-hormone-treated rats. Under conditions of increased gluconeogenesis when normal or adrenalectomized rats were treated with cortisone acetate, a marked increase in the activity of this enzyme occurred. It is at rate-limiting reactions such as that catalysed by arginine synthetase that regulatory effects might be expected. The results of the present work, together with those of Freedland & Sodikoff (1962), showing that the activity of this enzyme increased under

conditions of excessive protein catabolism, fit smoothly together and may indicate a possible point of action of hormones in the control of urea production. The observation that the total liver content of arginine-synthetase activity, alone among the seven enzymes measured, was significantly altered in growth-hormone-treated rats indicates that there may be some degree of specificity in the response to the hormonal treatment. This change may account for the decreased production of urea by liver slices from growth-hormone-treated rats (Beaton, Ozawa, Beaton & McHenry, 1953).

There are at least three different physiological conditions in which several enzymes of the urea cycle change simultaneously: with changes in the protein content of the diet (Schimke, 1962), metamorphosis of tadpoles (Brown, Brown & Cohen, 1959) and, as shown in the present experiments, in adrenalectomy. This suggests that the mechanism controlling all these enzymes is similar and that the more economical change, that of the rate-limiting reaction alone, does not occur under these conditions. This may be another example of a 'constant-proportion group' of enzymes, in this case closely related in a metabolic chain, as recently discussed by Pette, Luh & Bücher (1962) and Pette, Klingenberg & Bücher (1962).

Carbamoyl phosphate synthetase is at a branch point in metabolism, since it supplies a precursor of both urea formation and pyrimidine synthesis. The

Fig. 4. Effect of (a) adrenalectomy and (b) growth-hormone treatment on the total activity of enzymes of the urea cycle, glutamic dehydrogenase and glutamic-oxaloacetic transaminase in rat liver. Results, as enzyme units in the whole liver, are expressed as a percentage of the activity in the corresponding pair-fed control groups. Vertical lines represent twice the S.E.M. The horizontal dashed line is the arbitrarily fixed control value of 100% and the area between the dotted lines on each side represents twice the S.E.M. value for the control groups. Fisher's *P* values are given at the foot of each column. Each group contains six or more animals; the numbers in each group correspond with those in Table 2 (adrenalectomized rats) and Table 4 (growth-hormone-treated rats).

activity of this enzyme is decreased in adrenalectomized rats, an observation in keeping with the demonstration by Reid (1962) of decreased aspartic-transcarbamoylase activity and decreased orotic acid formation 9 days after adrenalectomy. Reid (1962) has pointed out that carbamoyl phosphate synthetase is unlikely to be rate-limiting in the series of reactions leading to orotic acid; it is present at a concentration about 200-fold that of the rate-limiting reaction between carbamoyl aspartate and orotate.

There is an inverse relationship between the activity of ornithine transcarbamoylase and aspartic transcarbamoylase in certain conditions where rapid growth is taking place and there is a high requirement for pyrimidines for DNA and RNA synthesis (see Cohen & Sallach, 1961). In the present experiments the concentration of ornithine transcarbamoylase was decreased in growth-hormone-treated rats, although there was no significant difference when the results were expressed as total units in the liver. Since ornithine transcarbamoylase is an extremely active enzyme, this difference in enzyme concentration may be significant in making a greater amount of carbamoyl phosphate available for the carbamoyl aspartate-transcarbamoylase reaction. The effect of growth hormone on nucleic acid turnover has been studied by Reid, O'Neal, Stevens & Burnop (1956). These authors have shown that there is a decreased incorporation of orotic acid into the RNA of the mitochondrial and microsomal fraction of the cell in hypophysectomized rats; however, these effects were not reversed by growth-hormone treatment.

The mitochondrial enzymes measured in growth-hormone-treated rats, carbamoyl phosphate synthetase and glutamic dehydrogenase showed little change, although there is considerable evidence that growth hormone may profoundly alter both mitochondrial permeability (Melhuish & Greenbaum, 1961) and the total number of mitochondria in the cell (Greenbaum, Slater & Reid, 1955).

These results suggest that there is no general change in the activity of mitochondrial enzymes in growth-hormone-treated rats and perhaps support the view that there is regulation of metabolic processes by changes in permeability (Melhuish & Greenbaum, 1961) or that protein anabolic reactions are more immediately important (Russell, 1955; Korner, 1961).

The changes in rat-liver arginase are in accord with the previous demonstrations of decreased activity in adrenalectomized rats (Fraenkel-Conrat *et al.* 1943a; Folley & Greenbaum, 1946, 1948b) and growth-hormone-treated rats (Fraenkel-Conrat, Simpson & Evans, 1943b), although in the present experiments the total liver-arginase activity is not altered in growth-hormone-treated rats. These re-

sults are reported for comparison with other enzymes of the urea cycle with respect to magnitude of change and time of response. Although the fall in liver arginase of adrenalectomized rats is striking, the fact that under conditions of optimum activity there is approximately 1000-fold greater activity of this enzyme than the rate-limiting condensing reaction of the arginine-synthetase system raises again the question of the biochemical significance of changes in an enzyme present in such vast excess. However, the activity of this enzyme at pH 7.3 and in the absence of Mn^{2+} ions is more in keeping with those of the other urea-cycle enzymes (Table 1).

Measurements were made of the activity of glutamic-oxaloacetic transaminase and glutamic dehydrogenase for comparison with urea-cycle enzymes since, despite numerous studies in this field (see Ketterer *et al.* 1957; Knox, Auerbach & Lin, 1956), there is no clear agreement about the changes occurring in the activity of these enzymes; much seems to depend on the age, sex and dietary regimen of the rats (Harding, Rosen & Nichol, 1961).

The decreased activity of glutamic dehydrogenase in adrenalectomized rats is in contrast with the results of Umbreit & Tonhazy (1951*a, b*). A depression in the activity of this enzyme is of interest in view of the part it plays in deamination reactions and urea synthesis (see Ratner, 1955*b*). Moreover, glutamic dehydrogenase may more nearly fulfil the requirement for a primary action of a hormone on an enzyme reaction, since Engel & Scott (1960) found that corticosterone increased the activity of glutamic dehydrogenase *in vitro*. However, this finding was not confirmed by Yielding, Tompkins, Munday & Curran (1960).

Since glutamic dehydrogenase reacts with NAD and NADP at comparable rates (Olson & Anfinsen, 1953), the changes in the activity of this enzyme may have a marked effect on the ratio of oxidized to reduced nicotinamide-adenine dinucleotides. Reid (1961) has already shown that there is a reduction in the concentration of oxidized NADP in the livers of adrenalectomized rats.

In agreement with Harding *et al.* (1961), glutamic-oxaloacetic transaminase remained almost completely unaltered in adrenalectomized rats. These authors found that great changes in glutamic-pyruvic transaminase occurred in adrenalectomized rats and under conditions where there was an enhanced rate of gluconeogenesis (Harding *et al.* 1961; Rosen, Roberts & Nichol, 1959). This differential response might be explained by the different metabolic roles of alanine-pyruvic acid and aspartic acid-oxaloacetic acid transaminases. A steady supply of aspartic acid is necessary in many reactions, for purine and pyrimidine synthesis [the

formation of 2-(5-aminoimidazol-4-ylformamido)-succinate ribotide, adenylosuccinate and carbamoyl aspartate; see Buchanan & Hartman, 1959; Reichard, 1959], and in the formation of argininosuccinate in the urea cycle (see Ratner, 1955*b*). Each of the two transaminases clearly links with a different series of reactions and those of glutamic-pyruvic transaminase are much more closely related to gluconeogenesis.

Bush (1962) has pointed out the unrewarding results of attempts to find hormonal control of specific enzyme reactions. The present results suggest that the decreased activity of all five enzymes of the urea cycle in adrenalectomized rats was secondary to some more fundamental change. Further investigations of the effect of growth hormone on the activity of urea-cycle enzymes, and in particular arginine synthetase, will be necessary before this change can be properly assessed.

SUMMARY

1. The activities of enzymes of the urea cycle (carbamoyl phosphate synthetase, ornithine transcarbamoylase, arginine synthetase, argininosuccinase and arginase) have been measured in control, adrenalectomized, cortisone-treated and growth-hormone-treated rats. In addition measurements have been made of glutamic dehydrogenase and glutamic-oxaloacetic transaminase.

2. Adrenalectomy caused a reduction in the activity of all five enzymes of the urea cycle and of glutamic dehydrogenase. The most striking changes were in the activity of the rate-limiting enzyme of the urea cycle, the condensing enzyme of the arginine-synthetase system, and in liver arginase, both of which fell to about 40% of the pair-fed control values.

3. The effect of cortisone acetate on the activities of the three enzymes of the urea cycle located in the soluble fraction of the cell (arginine synthetase, argininosuccinase and arginase) was studied. In normal rats, treatment with this hormone raised the total liver-enzyme activities of all three enzymes well above control values. In adrenalectomized rats cortisone acetate restored the activities of these enzymes towards normal; arginase showed the most rapid response.

4. The most striking change in the growth-hormone-treated animals was the reduction in the activity of the arginine-synthetase system.

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The Production of Amino Acids by Cell Fractions, particularly Rat-Liver Mitochondria

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The uptake of radioactive amino acids into rat-liver-cell organelles has been widely documented (e.g. Roodyn, Reiss & Work, 1961), but there is little published work on protein breakdown.

Amino acid liberation from cell organelles has been observed (Ansell & Richter, 1954a, b; Dingle, 1961; Bartley, Sobrinho-Simões, Notton & Montesi, 1958) and Korner & Tarver (1957) have investi-