The Effect of Treatment of Rats with Pituitary Growth Hormone on the Activities of some Enzymes Involved in Fatty Acid Degradation and Synthesis

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1. Measurements have been made of the activities of acyl-CoA dehydrogenase, enoyl-CoA hydratase, β -hydroxyacyl-CoA dehydrogenase and ketothiolase in the livers of rats treated for either 12 hr. or 3 days with pituitary growth hormone. 2. There was a significant increase in the activity of acyl-CoA dehydrogenase in rats treated with the hormone for 3 days. 3. Measurements were also made of the lipogenic enzymes acetyl-CoA carboxylase and palmitate synthase in the livers of similarly treated animals. 4. There was a depression of the activity of both enzymes after 12 hr. treatment and a further decline after 3 days. 5. The results are discussed in relation to the known increase in the rate of fatty acid oxidation and inhibition of fatty acid synthesis in rats treated with growth hormone.

Previous studies on the influence of pituitary growth hormone on lipid metabolism have revealed that the hormone exerts a profound effect on both fatty acid synthesis and degradation. Administration of the hormone causes an acceleration of the rate of breakdown of octanoate or oleate accompanied by a lowering of the respiratory quotient to about 0.7 and the production of ketone bodies (Greenbaum & McLean, 1953b), all indicative of an increased rate of fatty acid oxidation. At the same time a decreased rate of incorporation of labelled substrates into triglyceride fatty acids (Welt & Wilhelmi, 1950-51; Brady, Lukens & Gurin, 1951; Greenbaum & Glascock, 1957) and phospholipids (Leal & Greenbaum, 1961) has been observed.

There have been no studies on the enzymic mechanisms by which these effects are achieved. For this reason, measurements have been made of several enzymes involved in the oxidation of fatty acids [acyl-CoA dehydrogenase (acyl-CoA-cytochrome *c* oxidoreductase, EC 1.3.2.2); enoyl-CoA hydratase (α -3-hydroxyacyl-CoA hydro-lyase, EC 4.2.1.17); β -hydroxyacyl-CoA dehydrogenase (α -3-hydroxyacyl-CoA-NAD oxidoreductase, EC 1.1.1.35); ketothiolase (acyl-CoA-acetyl-CoA acyl-transferase, EC 2.3.1.16)] and of lipogenesis [acetyl-CoA-CO₂ ligase (ADP), EC 6.4.1.2, and palmitate synthase].

Since both of the enzyme systems measured (the Lynen-Green cycle and the malonyl-CoA pathway)

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are capable of synthesizing fatty acids and only one of them (the Lynen-Green cycle) is implicated in fatty acid oxidation, it was thought that the effects of the hormone could most readily be ascertained if two periods of treatment were studied. At the shorter time-interval, 12 hr., both the increase of oxidation and the decrease of synthesis were maximal, whereas at the longer time-interval, 72 hr., the oxidation rate is more or less normal although lipogenesis is still strongly inhibited (Greenbaum & McLean, 1953b; Greenbaum & Glascock, 1957). The use of two lengths of treatment might also prove useful in differentiating between the type of control effected by variations in cofactor or product concentrations and that achieved by variations in the enzyme concentration.

The results described below show that the activities of all four enzymes of the degradative cycle are relatively unchanged 12 hr. after treatment with growth hormone and only the activity of the acyl-CoA dehydrogenase is decreased after 72 hr. On the other hand, the activities of both acetyl-CoA carboxylase and of palmitate synthase are markedly depressed at the two time-intervals studied.

METHODS

Animals. Adult hooded Norway female rats of body wt. 180-200 g. were used. For the shorter treatment with growth hormone they were injected intraperitoneally with 1 mg. of the hormone in 0.9% NaCl 12 hr. before being killed. For the longer treatment each rat received 1 mg. of the hormone subcutaneously on each of 3 days and was killed on the fourth

day. Control animals were given injections of the same volume of 0.9% NaCl.

Preparation of tissue extracts. The rats were killed by cervical dislocation and the livers rapidly exposed and excised. Thereafter, one of the two types of tissue homogenates was prepared.

(a) Phosphate buffer homogenate. The entire liver was washed in approx. 60 ml. of ice-cold 66 mM-KH₂PO₄ buffer, pH 7.0, and then homogenized in fresh 66 mM-KH₂PO₄ buffer, pH 7.0, to give a tissue suspension such that 1 g. was equivalent to 3.3 ml. of homogenate. The suspension was then frozen and thawed five times in rapid succession to liberate the mitochondrial enzymes. The suspension was centrifuged at 105 000g for 1 hr. and the supernatant taken.

(b) Sucrose homogenates. The entire liver was washed in approx. 60 ml. of ice-cold 0.25 M-sucrose-1 mM-EDTA and then homogenized in fresh 0.25 M-sucrose-1 mM-EDTA to give a tissue suspension such that 1 g. was equivalent to 3.3 ml. of homogenate. This homogenate was then centrifuged at 105 000g for 30 min. and the supernatant taken. The protein content of each of these preparations was measured by the method of Warburg & Christian (1941).

Enzyme estimations. The activities of acyl-CoA dehydrogenase, β -hydroxyacyl-CoA dehydrogenase and of ketothiolase were measured essentially as described by Wieland, Reinwein & Lynen (1956) except for the minor modifications described below.

Assay of acyl-CoA dehydrogenase. A 3 ml. portion of the phosphate buffer homogenate was dialysed against 300 ml. of a medium containing (final concentrations): KCl (50 mM), cysteine (1 mM), disodium EDTA (1 mM) and KH₂PO₄ buffer, pH 7.5 (50 mM). The final volume was measured and its protein content determined, and this extract was then used for the assay of acyl-CoA dehydrogenase. In our hands some additional precautions were necessary. The whole reaction was carried out in cuvettes that had been adapted to carry vaccine sealing caps and to which a stainless-steel ball for stirring had been added. The cuvettes were set up as described by Wieland *et al.* (1956) and the enzyme added (0.3 or 0.6 ml.) before sealing with the vaccine caps. A small amount of freshly prepared Na₂S₂O₄ solution in buffer was then injected to reduce the dye until the solution was a very faint red colour. Mixing was achieved by means of the steel ball and a magnet. An excess pressure of nitrogen was maintained above the mixture by means of two syringe needles inserted through the cap. When the colour had stabilized (about 8–10 min.), the reaction was started by the injection of 0.1 ml. of *S*-crotonyl-*N*-acetylthioethanolamine (50 μ moles/ml.) and the development of colour at 545 m μ followed over the next 8 min. The increase in extinction from the third to the seventh minute was taken as being proportional to the activity of the enzyme. It was found essential to have a strong current of nitrogen passing through the cell throughout the procedure. Under the conditions of the assay the reaction was linear with time up to 10 min. and with enzyme concentration at least up to twice that used here. There appeared to be very little loss of activity of the enzyme during the dialysis procedure. The results are expressed as μ moles of leuco-safranin oxidized/hr.

Assay of enoyl-CoA hydratase. The estimation was carried out as described by Stern (1955). For this determination the undialysed supernatant from the phosphate buffer homogenate was diluted 1:100 with 20 mM-KH₂PO₄ buffer, pH 7.4, and then, shortly before use, it was further diluted:

0.1 ml. of the diluted homogenate was added to 2.4 ml. of a mixture containing (final concentrations) KH₂PO₄ buffer, pH 7.4 (20 mM), potassium EDTA (1 mM) and bovine plasma albumin (0.1%), and the reaction started by the addition of 0.1, 0.2 or 0.3 ml. of the diluted enzyme. This reaction was also linear with respect to time and enzyme concentration.

Assay of β -hydroxyacyl-CoA dehydrogenase. In this estimation the undialysed homogenate in phosphate buffer was used. This enzyme preparation was further diluted 1:20 with 50 mM-sodium pyrophosphate buffer, pH 7.4, and 0.1, 0.2 or 0.3 ml. of this was used for the estimation. The reaction was started by the addition of acetoacetylthioethanolamine. The reaction was linear with respect to time and enzyme concentration under the conditions used. The results are expressed as μ moles of NADH oxidized/hr.

Assay of ketothiolase. For this estimation the undialysed supernatant fraction from the phosphate buffer homogenate was diluted 1:60 with 0.2 M-tris-HCl buffer, pH 7.5. Then 0.5 ml. (or 1.0 ml.) of this diluted enzyme was added to 1.0 ml. of M-MgCl₂ in 0.2 M-tris-HCl buffer, pH 7.5, and the volume made up to 7 ml. with tris-HCl buffer; 0.75 ml. of a fresh solution of ice-cold acetoacetylthioethanolamine (2.25 μ moles/ml.) in tris-HCl buffer was added and 3 ml. of this mixture used in the blank cuvette and 2.8 ml. in the reaction cuvette. The reaction was started by the addition of 0.2 ml. of ice-cold pantotheine (100 μ moles/ml.) to the reaction cuvette and the change in extinction at 313 m μ measured, with the stock solution as blank, for 8 min. The activity of this enzyme, also, was linear with respect to time and enzyme concentration under the conditions of this assay.

Assay of acetyl-CoA carboxylase. This enzyme was measured by the method described by Levy (1963), involving a preincubation of the enzyme with citrate to achieve activation. The enzyme preparation used was the supernatant from the sucrose homogenate that had been dialysed for 2 hr. against 100 vol. of 7 mM-thioethanol in 50 mM-KH₂PO₄ buffer, pH 6.5. The removal of unchanged bicarbonate after acidification with Dowex 50 (H⁺ form) proved troublesome and the method was modified as follows. After acidification the reaction tubes were exposed to a vacuum for 30 sec., during which they were constantly vibrated. Then 0.5 ml. of a solution of cold bicarbonate (containing 5 μ moles of bicarbonate) was added and the vacuum treatment repeated. After standing for 30 min. the vacuum treatment was once again applied and then the samples of the solution were removed for counting on a Nuclear-Chicago scintillation counter. The results are expressed as μ moles of CO₂ incorporated/hr.

Assay of palmitate synthase. The activity of this enzyme system was measured on the supernatant fraction, prepared from sucrose homogenates, by the method of Gibson & Hubbard (1960). Since dialysis of the enzyme preparation against KH₂PO₄-thioethanol made no difference either to the stability of the enzyme or its activity, this step was omitted. The reaction, started by the addition of 0.05, 0.10 or 0.15 ml. of the enzyme preparation, was measured in a Carey spectrophotometer at 365 m μ by using the 0.0–1 slide wire. The reaction, which was linear with time over at least 5 min., was followed for 3 min. The results are expressed as μ moles of NADPH oxidized/hr.

Calculation of the enzyme results. The activities of the enzymes are expressed as μ moles of substrate changed/hr.

Table 1. *Effect of acute (12 hr.) and long-term (3-day) treatment of rats with growth hormone on the activities of the enzymes of the fatty acid cycle*

Enzyme activities are expressed as follows: (a) acyl-CoA dehydrogenase as μ moles of leuco-safranin oxidized/hr.; (b) enoyl-CoA hydratase as m-moles hydrated/hr.; (c) β -hydroxyacyl-CoA dehydrogenase as μ moles of NADH formed/hr.; (d) ketothiolase as μ moles of acetoacetyl-pantotheine deacetylated/hr. Assay procedures are given in the text. The results are given as means $\pm$ s.e.m. Fisher's *P* represents the probability of difference between the means; N.S. signifies 'not statistically significant'.

Treatment	No. of animals	Body wt. (g.)		Acyl-CoA dehydrogenase			Enoyl-CoA hydratase		
		Initial	Final	(units/g. of liver)	(units/whole liver)	(units/100 g. body wt.)	(units/g. of liver)	(units/whole liver)	(units/100 g. body wt.)
Control	6	—	178.7	2.09	16.97	9.48	23.57	185.6	104.2
Growth hormone (12 hr.)	6	—	± 2.6 195.5	± 0.12 2.16	± 1.31 16.18	± 0.66 8.35	± 3.98 23.57	± 25.9 175.1	± 14.49 91.0
Fisher's <i>P</i>			± 12.1	± 0.17 N.S.	± 0.92 N.S.	± 0.51 N.S.	± 3.62 N.S.	± 21.1 N.S.	± 11.3 N.S.
Control		197.0	191.0	2.34	16.59	8.69	29.37	206.4	108.6
Growth hormone (3 days)	6	± 10.9 188.0	± 5.4 183.2	± 0.17 2.53	± 0.29 20.83	± 0.14 11.32	± 4.85 20.60	± 26.0 166.3	± 15.0 89.6
Fisher's <i>P</i>		± 6.7	± 7.3	± 0.24 N.S.	± 0.51 <0.001	± 0.1 <0.001	± 2.46 N.S.	± 23.3 N.S.	± 9.1 N.S.

Treatment	No. of animals	Body wt. (g.)		β -Hydroxyacyl-CoA dehydrogenase			Ketothiolase		
		Initial	Final	(units/g. of liver)	(units/whole liver)	(units/100 g. body wt.)	(units/g. of liver)	(units/whole liver)	(units/100 g. body wt.)
Control	6	—	178.7	57.7	447.1	249.3	391	3115	1731
Growth hormone (12 hr.)	6	—	± 2.6 195.5	± 11.1 45.6	± 55.0 348.8	± 27.5 177.5	± 66 308	± 464 3053	± 243 1591
Fisher's <i>P</i>			± 12.1	± 3.2 N.S.	± 36.4 N.S.	± 11.7 0.037	± 35 N.S.	± 185 N.S.	± 133 N.S.
Control	6	197.0	191.0	65.0	448.0	237.0	417	2929	1538
Growth hormone (3 days)	6	± 10.9 188.0	± 5.4 183.2	± 13.7 53.5	± 76.0 435.0	± 43.0 237.0	± 52 428	± 229 3569	± 124 1934
Fisher's <i>P</i>		± 6.7	± 7.3	± 8.8 N.S.	± 54.0 N.S.	± 28.0 N.S.	± 37 N.S.	± 292 N.S.	± 83 0.024

either per g. wet wt. of liver, per whole liver or per 100g. body wt.

Chemical methods. *N*-Acetylthioethanolamine, b.p. 85–86°/0.15 mm. Hg, was prepared by the method of Kuhn & Quadbeck (1951). *N*-Acetyl-*S*-crotonylthioethanolamine, m.p. 60–61°, b.p. 128°/0.1 mm. Hg, which was prepared by the method of Seubert & Lynen (1953), was purified by distillation, chromatography of a carbon tetrachloride solution on alumina (suitable for chromatography; E. Merck A.-G., Darmstadt, Germany), and recrystallization from ether–light petroleum (b.p. 40–60°). *S*-Acetoacetyl-*N*-acetylthioethanolamine, m.p. 61–62°, was prepared by the method of Lynen & Wieland (1955) and recrystallized from ether after treatment with charcoal. Pantotheine was prepared by the condensation of β -alathoine (King, Stewart & Cheldelin, 1953) with pantolactone (L. Light and Co. Ltd., Colnbrook, Bucks.), as described by Baddiley & Thain (1952). It was stable for several months when stored at –20° under nitrogen as an approx. 50 mm solution in butan-1-ol. Butan-1-ol was removed under vacuum immediately before use, and any subsequent manipulations were carried out under nitrogen. *S*-Acetoacetyl-pantotheine was prepared by the reaction of equimolar amounts of pantotheine and diketene (British Celanese Ltd., 345 Folds-hill Road, Coventry) at room temperature in 0.05 M-potassium phosphate buffer, pH 5.4, and was stable for a month when stored as this solution at –20°. CoA (Sigma Chemical Co.) was acetylated according to the method described by Stadtman (1957). Malonyl-CoA was prepared by the reaction of CoA and *S*-malonyl-*N*-octanoylthioethanolamine as described by Lynen (1962). Acetyl-CoA and malonyl-CoA were stable in solution at pH 5.5 and –20° for months.

RESULTS

The effect of acute (12 hr.) and longer-term (3-day) treatment of rats with pituitary growth hormone on the enzymes of the Lynen–Green fatty acid cycle is shown in Table 1.

At the shorter time-interval there is no change in the activity of acyl-CoA dehydrogenase, enoyl-CoA hydratase or ketothiolase. Only the activity of β -hydroxyacyl-CoA dehydrogenase is changed and this only when calculated as hepatic activity/100 g. body wt. The activity of this enzyme is diminished by the treatment at a time when fatty acid oxidation is proceeding at virtually the maximum rate. After treatment of rats with growth hormone for 3 days the activity of β -hydroxyacyl-CoA dehydrogenase returns to control levels and the activities of both acyl-CoA dehydrogenase and of ketothiolase increase above control levels. As at the shorter time-interval, the activity of enoyl-CoA hydratase remains unchanged. The enzyme changes are not apparent when the results are calculated as activity/g. wet wt. of liver but become so when the total weight of liver is considered. In hormonally treated animals calculation of results on the basis of activity/g. wet wt. may not be very meaningful because of the changes of water and fat content that often accompany such treatment. The values found for the

Table 2. Effect of acute (12 hr.) and long-term (3-day) treatment of rats with growth hormone on the activities of acetyl-CoA carboxylase and palmitate synthase

Enzyme activities are expressed as follows: (a) acetyl-CoA carboxylase as μ moles of CO ₂ incorporated/hr.; (b) palmitate synthase as μ moles of NADPH oxidized/hr. Assay procedures are given in the text. The results are given as means $\pm$ s.e.m. Fisher's <i>P</i> represents the probability of difference between the means; N.S. signifies 'not statistically significant'.				Acetyl-CoA carboxylase			Palmitate synthase		
Treatment	No. of animals	Body wt. (g.)		(units/g. of liver)	(units/whole liver)	(units/100 g. body wt.)	(units/g. of liver)	(units/whole liver)	(units/100 g. body wt.)
		Initial	Final						
Control	7	—	197.3 $\pm$ 4.71	264 $\pm$ 33	2174 $\pm$ 293	1100 $\pm$ 144	24.2 $\pm$ 1.5	199.8 $\pm$ 11.6	101.1 $\pm$ 5.2
Growth hormone (12 hr.) Fisher's <i>P</i>	8	—	206.5 $\pm$ 2.73	191 $\pm$ 31	1358 $\pm$ 197	659 $\pm$ 92	22.3 $\pm$ 1.5	165.7 $\pm$ 12.0	80.3 $\pm$ 5.6
				0.072	0.037	< 0.001	N.S.	0.049	0.012
Growth hormone (3 days) Fisher's <i>P</i>	6	213.6 $\pm$ 8.1	215.5 $\pm$ 5.57	146 $\pm$ 36	1103 $\pm$ 121	515 $\pm$ 59	12.4 $\pm$ 1.2	94.2 $\pm$ 9.2	43.9 $\pm$ 4.4
				0.035	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

activities of the different enzymes are in agreement with those in the literature [acyl-CoA dehydrogenase, β -hydroxyacyl-CoA dehydrogenase and ketothiolase (Wieland *et al.* 1956); enoyl-CoA hydratase (Stern, 1955)].

The effect of treating animals for either 12 hr. or 3 days with growth hormone on the activities of acetyl-CoA carboxylase and palmitate synthase is shown in Table 2.

The activity of acetyl-CoA carboxylase is decreased 12 hr. after the administration of a single dose of 1 mg. of growth hormone and declines even further if the treatment is extended up to 3 days. Palmitate synthase is also decreased in activity after the shorter-term treatment with growth hormone although it is less markedly affected than that of acetyl-CoA carboxylase. After 3 days of treatment with growth hormone the palmitate-synthase activity is severely depressed (expressed by any of the three methods employed) and is even more depleted than that of acetyl-CoA carboxylase. The value of 1.6 μ moles/mg. of protein/hr. found for the activity of acetyl-CoA carboxylase compares well with the value of 3.9 μ moles/mg. of protein/hr. reported by Numa, Matsubashi & Lynen (1961). However, we have found the activity of the palmitate synthase to be higher than their reported value, i.e. 180 compared with 79 μ moles/mg. of protein/hr.

DISCUSSION

The depletion of body fat in rats treated with growth hormone may be ascribed both to an increased rate of fatty acid catabolism and to an inhibition of lipogenesis. In previous studies of fat oxidation in such hormone-treated animals (Greenbaum, 1953; Greenbaum & McLean, 1953b) a discrepancy emerged between results *in vivo* and *in vitro* in that, on the basis of measurements of respiratory quotient on intact animals or liver slices, there was an accelerated rate of lipid oxidation for up to 50 days or more of treatment, whereas measurements of the rate of lipid oxidation by homogenates of liver from similarly treated animals only showed an increased rate of fat oxidation for some 18 hr. (maximum at 12 hr. after injection of the hormone) thereafter returning to normal levels in spite of continued hormone treatment. Although the methods available at that time for the estimation of 'fatty acid-oxidase' activity were not entirely satisfactory the results may be interpreted to indicate that the adaptation to a higher rate of fat oxidation in animals treated with growth hormone was achieved not so much by an increase in the amounts of enzyme available as by the changes in the factors that regulate the activities of these enzymes. There was no evidence to show which of these two methods mediated the decline in the rate

of fatty acid synthesis. The present measurements *in vitro* show that after 12 hr. of treatment with growth hormone fatty acid oxidation is at its maximum and lipogenesis severely inhibited, and that after 3 days of treatment the rate of oxidation is back to normal, whereas measurements *in vivo* show that oxidation is still above normal.

Fatty acid oxidation. At the time of maximum stimulation of fatty acid oxidation, 12 hr. after the administration of growth hormone, only one of the four enzymes of the fatty acid cycle shows a change in activity (Table 1). The activity of β -hydroxyacyl-CoA dehydrogenase is decreased by almost 30% when calculated as total hepatic activity/100 g. body wt., although calculation of the change by either of the other two methods gives less significant differences. It is rather surprising to find that this is the only enzyme change found in view of the known increase in fat catabolism occurring at this time and because it is not the rate-limiting enzyme. β -Hydroxyacyl-CoA dehydrogenase is present in some 20-fold excess over the rate-limiting enzyme (acyl-CoA dehydrogenase), and it is difficult to understand how a 30% change of the hydroxyacyl-CoA dehydrogenase can produce much effect. Treatment of the rats for 3 days with growth hormone produces changes in hepatic enzyme activity that are both striking and more consistent with the results *in vivo*. The activities of acyl-CoA dehydrogenase (both as activity/whole liver and as activity/100 g. body wt.) and ketothiolase (as activity/100 g. body wt.) are both increased.

The maximum rate of fatty acid oxidation from the activity of the rate-limiting member of the fatty acid cycle as measured here was compared with the rate of oleate oxidation as measured in the experiments of Greenbaum & McLean (1953b), where a more intact tissue preparation was used. The activity of the total hepatic acyl-CoA dehydrogenase is sufficient to account for the oxidation of 20.8 μ moles of oleate/hr., and Greenbaum & McLean (1953b) found that the rate of oxidation of this substance in homogenates of liver from similarly treated rats was 17.9 μ moles of oleate/hr. for the entire liver. The other enzymes of the cycle are in considerable excess. Thus it might be that the activity of acyl-CoA dehydrogenase sets an upper limit on the rate of fatty acid degradation and that the relative stability of the enzyme activity under conditions of increased fat oxidation might imply that the physiological activity of the system is normally governed by the availability of substrate. The increased rate of fatty acid oxidation in intact rats treated with growth hormone may thus be explained in part by the small increase in the activity of the rate-limiting enzyme, acyl-CoA dehydrogenase, and, in part, by the known activity of the hormone as a fat-mobilizing factor (Szego & White,

1949; Campbell & Lucas, 1951; Greenbaum & McLean, 1953a), together with its ability to cause a release from the fat depots of free fatty acids (Engel, Hallman, Siegel & Bergenstal, 1958; Raben & Hollenberg, 1959; Buckle, 1962) that are ultimately oxidized in the liver.

Fatty acid synthesis. Fatty acid synthesis is sharply inhibited in the livers of rats treated with growth hormone for 6 hr. After 12 hr. of treatment hepatic lipogenesis in the treated animals may be as low as 10% of the control rate (Greenbaum & Glascock, 1957). This inhibition persists in rats treated with the hormone for at least up to 5 days (R. S. Leal & A. L. Greenbaum, unpublished work). Investigation of the activities of the two enzymic stages of fatty acid synthesis via the malonyl-CoA pathway, i.e. acetyl-CoA carboxylase and palmitate synthase, shows that growth hormone affects the activities of both enzymes.

At the shorter time-interval (12 hr.) both the carboxylase and the synthase activities are significantly lowered, calculated either as activity/whole liver or as activity/100 g. body wt. (carboxylase, 40%; synthase, 20%) (Table 2). The inhibition is even greater after 72 hr. of treatment and is statistically significant by all three modes of calculation (carboxylase, 53%; synthase, 57%; percentage inhibitions based on activities/100 g. body wt.) (Table 2). Previous studies of these two enzymes in conditions of depressed lipogenesis have shown that both enzymes are affected. Wieland, Neufeldt, Numa & Lynen (1962) found the specific activities of both enzymes diminished by diabetes, whereas Gibson & Hubbard (1960) found the synthase activity decreased but not that of the carboxylase in this condition. In starvation Numa *et al.* (1961) again found diminished activities of the acetyl-CoA carboxylase and palmitate synthase, whereas Gibson & Hubbard (1960) found a lower activity of palmitate synthase and no change in that of carboxylase. The pattern of enzyme change after treatment with growth hormone therefore resembles that found in other conditions of decreased fatty acid synthesis. The decreased activity of the palmitate synthase is particularly noteworthy in view of the many reports that the carboxylase is the rate-limiting enzyme (Ganguly, 1960; Numa *et al.* 1961; Wieland *et al.* 1962) and the observation that the rate of lipogenesis could be restored to normal in preparations of liver from starved rats by the addition of purified acetyl-CoA carboxylase in spite of the decreased palmitate-synthase content of these extracts (Numa *et al.* 1961).

The changes in the activity of acetyl-CoA carboxylase are more readily understood. Not only is this enzyme the rate-limiting enzyme of fatty acid synthesis but it is also subject to either activation or inhibition by a number of naturally occurring

compounds. The enzyme is activated by citrate (Martin & Vagelos, 1962; Waite & Wakil, 1963) and inhibited by palmitoyl-CoA (Bortz & Lynen, 1963). Long-chain acyl-CoA compounds may also decrease the activity of the enzyme indirectly by their inhibitory action on citrate synthase, which occurs at concentrations comparable with those found in the liver (Tubbs, 1963; Wieland & Weiss, 1963), thus depriving the carboxylase of its activator. The present work confirms that acetyl-CoA carboxylase is the rate-limiting enzyme in lipogenesis and that it is subject to hormonal control. The lowered activity of this enzyme recorded here for hormone-treated rats cannot be due to the lack of citrate since in the assay procedure the enzyme is fully activated by incubation in the presence of citrate before estimation of its activity. Nor is it likely that there was sufficient endogenous long-chain acyl-CoA carried over in the enzyme preparation to inhibit it in the assay *in vitro*, since this involved a 33-fold dilution. It would seem more probable that growth hormone causes a depletion of enzyme protein.

However, the 53% decrease in the activity of the rate-limiting enzyme in animals treated with growth hormone is insufficient to account for the profound depression of lipogenesis actually found, particularly when this is considered in the light of the close correspondence found by Numa *et al.* (1961) between the activity of the enzyme and the actual rate of fatty acid synthesis. An analogous situation has been reported by Korchak & Masoro (1962) in the livers of rats starved for 24 hr. In such animals the activity of the carboxylase system has decreased by some 50%, but lipogenesis had declined to less than 5% of the control. Wieland *et al.* (1962) found a similar discrepancy in the livers of alloxan-diabetic rats deprived of insulin for 1 day. In these experiments lipogenesis was powerfully depressed in liver slices but not in extracts from the same livers, and measurements of the activity of acetyl-CoA carboxylase showed this to be also but little changed. It therefore seems likely that in rats treated with growth hormone, as in starving and diabetic rats, factors other than the concentration of the enzyme may also be involved.

A feature that starvation, diabetes and treatment with growth hormone all have in common is that all three lead to the liberation of free fatty acids from the depots, accumulation of triglyceride in the liver (at least transiently) and an increased rate of fatty acid oxidation. All three conditions also affect carbohydrate metabolism and lead to a diminished rate of glycolysis and, presumably, a lower rate of production of α -glycerophosphate. Vaughan (1961), Tzur, Tal & Shapiro (1964) and Howard & Lowenstein (1964) have all pointed out that the availability of α -glycerophosphate is a rate-determinant

in fatty acid synthesis. Thus it may be suggested that the depressed rate of glycolysis after treatment with growth hormone would lead to a deficiency of α -glycerophosphate and hence to an accumulation of long-chain acyl-CoA compounds. These, in turn, would inhibit citrate formation and the activity of acetyl-CoA carboxylase as described above and lead to the severe depression of lipogenesis actually found in these animals, a greater inhibition of fatty acid synthesis than would be anticipated from the smaller amount of carboxylase actually present. The depressed formation of citrate would also account, in part, for the ketosis found in these three types of animal, and the ketone bodies so produced would compound the trend of events by inhibiting the phosphofructokinase reaction through their action on citrate formation (Garland, Randle & Newsholme, 1963) and so decrease even further the flow of glycolytic material. It is not clear that the initial inhibition of lipogenesis by long-chain acyl-CoA molecules in rats treated with growth hormone is a feed-back in the classical sense. These compounds are not necessarily 'end products' of the synthetic system. It is, in fact, difficult to visualize fatty acid synthesis as a self-regulating process operating through a feed-back mechanism if, as has been proposed by Bressler & Wakil (1962), the end product of the palmitate-synthase reaction is free palmitate and not palmitoyl-CoA and if, until this point is reached, the fatty acid intermediate is either enzyme-bound or exists bound to acyl-(carrier protein) (Majerus, Alberts & Vagelos, 1964; Wakil, Pugh & Sauer, 1964) unless the fatty acid-acyl-(carrier protein) compound is also inhibitory or unless the free fatty acids themselves are inhibitory, as Levy (1963) has shown to be the case in mammary gland. An inhibition of fatty acid synthesis by the mechanism proposed by Lynen, Matsushashi, Numa & Schweitzer (1963) can be expected to occur whenever there is a mobilization of fat or fatty acid from the extrahepatic tissue to the liver for oxidation.

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