SUMMARY

1. A method for the colorimetric estimation of arginine has been described.

2. The applicability of the method for the estimation of other guanido compounds, including streptomycin, has been demonstrated.

3. The method was shown to be suitable for the estimation of the arginine content of proteins.

4. The advantages of the method, as compared with that of Sakaguchi, have been discussed.

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The Short-Term Effect of Pituitary Growth Hormone on the Catabolism of Fat in the Liver

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Rats treated with anterior-pituitary growth hormone undergo a change in metabolic pattern in favour of a retention of nitrogen and water and a loss of fat (Lee & Schaffer, 1934; Young, 1945; Greenbaum, 1953). In a previous communication it was shown that injections of growth hormone caused profound changes in the catabolism of fat, particularly during the first 3 days of treatment (Greenbaum & McLean, 1953). At short time intervals after treatment (6 hr.) there was an inhibition of fat oxidation and an increase in the endogenous respiration in liver-tissue suspensions from treated rats as compared with similar preparations from control rats; while at 12 hr. this pattern was reversed, i.e. an inhibition of the endogenous respiration and increase in fat catabolism was found. At periods of 24 hr. or longer after the initiation of treatment there appeared to be no difference between control and experimental animals, although there is little doubt that the energy metabolism of the treated animals was based largely on fat and that of the controls on

the usual mixture of carbohydrate, protein and fat (Greenbaum, 1953). It seemed possible from these results that the liver responded rapidly to growth hormone but that the effect was essentially short-term, and that the longer-term effect of growth hormone in stimulating fat catabolism was possibly effected in the extrahepatic tissues. It was also suggested that the two-phase picture of fat metabolism obtained in the livers of treated rats in the first 24 hr. of growth-hormone treatment might be explained on the basis of a pancretotropic effect of growth hormone. This would result in a release of insulin over the first few hours, and this insulin would then produce its characteristic effect, an inhibition of fat oxidation. At the longer time interval the secretion of insulin would have declined and the growth hormone effect would then become obvious.

The present communication describes experiments which tend to show that some of the short-term effects of growth hormone are indeed due to insulin, and that the full effect of growth hormone

on fat metabolism in the intact animal does not become manifest until some 12 hr. after the injection.

EXPERIMENTAL

Animals. Virgin female hooded Norway rats, of the Medical Research Council strain, aged 4 months and with initial body weights from 170 to 210 g., were used. They were allowed free access to food. The rats which were to be made diabetic were injected subcutaneously with alloxan monohydrate dissolved in citrate-phosphate buffer, pH 4.0, at a dose level of 180 mg. of the monohydrate/kg. of body weight. These rats were kept for 21 days after the induction of diabetes before use to ensure that they were free of endogenous insulin. Their blood sugar levels (non-fasting) varied between 400 and 600 mg./100 ml.

Both the normal and the diabetic animals were divided into three groups: one group of controls and two groups injected intraperitoneally with 0.5 mg. of growth hormone (fraction A) prepared by the procedure of Wilhelm, Fishman & Russell (1948). One of the injected groups was killed 6 hr. after injection and the other group 12 hr. after.

Methods. The rats were killed by dislocation of the cervical vertebrae and the livers were rapidly excised. The fatty acid oxidase activity and the acetoacetate production were determined, and the data so obtained were used to calculate the rate of formation and rate of oxidation of the two-carbon fragments. The methods used have been described previously (Greenbaum & McLean, 1953). The substrate used throughout was octanoate prepared from the redistilled acid (b.p. 237°).

RESULTS

The results of this experiment are summarized in Table 1 and Figs. 1 and 2. All the results given are expressed as the activity of the entire liver. This form of expressing the results is used in preference to the more conventional activity/g. of tissue (*a*) because in *in vivo* experiments such as that described here the entire liver is the physiologically functional unit, and (*b*) because the livers of growth-hormone-treated rats become infiltrated to a variable degree with large amounts of fat, and this renders the expressing of results on the basis of activity/g. of tissue somewhat misleading.

The diabetic rats lost some 15% of their body weight during the 21 days of their diabetic state, but there does not appear to have been any parallel loss in liver weight.

As can be seen from Table 1 the endogenous oxygen uptake of the liver of the normal animals is not affected by the injection of growth hormone, but that of the diabetic rats is markedly stimulated, the level of oxygen uptake being significantly greater 12 hr. after the injection than that found either in the controls or in the rats treated for the shorter time interval.

The effect of the growth-hormone injection on the uptake of oxygen due to the oxidation of

Table 1. *Changes in the oxidation of fat in the livers of growth-hormone-treated normal and diabetic rats*

Treatment	No. of animals	Body wt. (g.)	Liver wt. (g.)	Total endogenous respiration (μ moles of O_2 /hr.)	Total fat oxidation (μ moles of O_2 /hr.)	2-C unit formation (μ moles of O_2 /hr.)	2-C unit oxidation (μ moles of O_2 /hr.)	Acetoacetate formed (μ moles/hr.)
Normal								
Control	6	189 $\pm$ 6.5	7.04 $\pm$ 0.37	1476 $\pm$ 162	951 $\pm$ 42	599 $\pm$ 42	352 $\pm$ 10	314 $\pm$ 28
Growth hormone for 6 hr.	6	193 $\pm$ 6.4	7.61 $\pm$ 0.43	1708 $\pm$ 88	706 $\pm$ 60	574 $\pm$ 60	132 $\pm$ 13	346 $\pm$ 40
Growth hormone for 12 hr.	6	189 $\pm$ 12.8	7.91 $\pm$ 0.27	1671 $\pm$ 78	1383 $\pm$ 52	808 $\pm$ 44	575 $\pm$ 50	392 $\pm$ 43
Diabetic								
Control	6	164 $\pm$ 11.6	7.61 $\pm$ 0.50	1647 $\pm$ 149	1165 $\pm$ 156	674 $\pm$ 96	491 $\pm$ 46	324 $\pm$ 55
Growth hormone for 6 hr.	6	154 $\pm$ 12.9	7.16 $\pm$ 0.18	1812 $\pm$ 97	1143 $\pm$ 69	646 $\pm$ 12	497 $\pm$ 35	304 $\pm$ 18
Growth hormone for 12 hr.	6	169 $\pm$ 6.2	8.21 $\pm$ 0.37	2167 $\pm$ 92	1923 $\pm$ 154	1556 $\pm$ 130	367 $\pm$ 27	943 $\pm$ 81
Normal				Statistical analysis of differences (values of <i>P</i>)*				
Control v. 6 hr. treated			0.489	0.240	0.014	0.742	<0.001	0.537
Control v. 12 hr. treated			0.975	0.336	<0.001	0.009	0.002	0.135
Control v. 12 hr. treated 6 hr. treated v. 12 hr. treated			0.276	0.762	<0.001	0.002	<0.001	0.439
Diabetic								
Control v. 6 hr. treated			0.374	0.513	0.893	0.595	0.923	0.722
Control v. 12 hr. treated			0.320	<0.001	0.006	0.001	0.050	<0.001
Control v. 12 hr. treated 6 hr. treated v. 12 hr. treated			0.024	<0.001	0.002	<0.001	0.018	<0.001

* *P*: probability that difference is due to chance alone.

octanoate is shown in column 6 of the table. Injection of the hormone into normal rats causes first a decreased rate of fatty acid oxidation and later a marked increase. Both the inhibition and stimulation of fatty acid oxidation are statistically significant. The inhibitory effect of growth hormone on fatty acid catabolism 6 hr. after injection into a normal animal is not found when the hormone is injected into diabetic rats, the values for the control group and 6 hr.-injected rats in this latter group being virtually identical. The stimu-

latory effect 12 hr. after injection is, however, found both in normal and diabetic animals.

When the oxygen uptake attributable to the oxidation of octanoate is partitioned into the two components, namely the oxygen used in the formation of the 2-C fragments and the oxygen used in the oxidation of the 2-C fragments to CO_2 and water, some differences between the behaviour of the normal and the diabetic rats become manifest. From column 7 of the table it is clear that the rate of formation of the 2-C fragment in normal rats is not increased 6 hr. after the injection, but a significant increase is found 12 hr. after the injection. The same is essentially true of the diabetic rats, except that the increased rate of 2-C fragment formation found at 12 hr. is much more striking, the new rate being over twice the control level.

When the oxygen used in the oxidation of the 2-C fragments to CO_2 and water is considered (column 8), two different patterns emerge for the normal and diabetic rats. In normal rats the rate of oxidation is cut to roughly one-third 6 hr. after the injection, but by 12 hr. the rate has been so increased that it is now significantly greater than the control level. In diabetic rats there is neither an inhibition of oxidation 6 hr. after growth-hormone treatment nor a stimulation 12 hr. after. On the contrary, the rate of oxidation of the 2-C fragment is reduced after 12 hr., although this reduction is only just statistically significant ($P=0.05$). This inhibition at 12 hr. is in marked contrast to the increased rate of 2-C fragment formation at the same time and is clearly reflected in the very high rate of acetoacetate formation in the diabetic rats at this time interval.

The relative rates of 2-C fragment formation and the rate of 2-C oxidation show contrasts (Fig. 1). In normal animals the ratio fatty acid oxidase activity:2-C oxidation is increased from 1.71 in control animals to 4.35 in animals treated for 6 hr., but by 12 hr. this had returned again to a ratio of 1.40. In diabetic animals the ratio 6 hr. after growth-hormone treatment is the same as that in controls, but by 12 hr. it has increased from 1.37 to 4.23.

In normal animals the increased ratio is not due to any change in the rate of formation of the 2-C fragments but to a decreased ability to oxidize these fragments through the Krebs cycle. In diabetic animals, on the other hand, the high ratio is not due to any marked decrease in the ability to oxidize the fragments through the cycle but rather to the large increase in the rate of formation of the 2-C units.

A similar contrast in pattern between normal and diabetic rats is found when the ratio between acetoacetate production and oxygen used in fatty acid oxidation is examined (Fig. 2).

Fig. 1. Comparison of the rate of formation of two-carbon fragments from octanoate to the rate of oxidation of the two carbon-fragments. The height of the columns represents the ratio two-carbon formation:two-carbon oxidation.

Fig. 2. Comparison of rate of acetoacetate production and rate of fatty acid oxidation. The height of the column represents the ratio μmoles of acetoacetate formed: μmoles octanoate oxidized.

DISCUSSION

The results of the experiments described here on the effect of the *in vivo* treatment of normal intact rats with growth hormone on the *in vitro* oxidation of octanoate confirm, in general, the results previously obtained by Greenbaum & McLean (1953). Treatment of the intact rat with growth hormone leads to a two-phase response: a decreased rate of fat oxidation 6 hr. after treatment, and an increased rate of fat oxidation 12 hr. after treatment. The decreased rate of oxidation appears to be due to a slowing down of the rate of oxidation of the two-carbon fragments to CO_2 and water rather than to a diminution in the rate of the two-carbon-fragment formation. The increased oxidation rate 12 hr. after treatment with growth hormone appears to be due to both an increased rate of two-carbon-fragment formation and an increased rate of two-carbon oxidation.

Inactivation of the pancreas abolishes the two-phase response of rats to growth hormone by the elimination of the first effect at 6 hr. The injection of growth hormone into a diabetic rat produces no discernible effect on fat catabolism 6 hr. after the injection. In contrast to the findings in the intact animal, there is no diminution in the total oxygen used for fatty acid oxidation, nor is there any reduction in the rate of oxidation of the two-carbon fragments formed by the fatty acid oxidase system. The second phase of the response remains. The increase in total oxygen used for fatty acid oxidation and the increased rate of the formation of the two-carbon fragments is found in both normal and diabetic rats, and indeed the increase seems even more marked with the diabetic than with the normal animals.

There is, however, this difference between the response of normal and diabetic rats. At 12 hr. normal rats are oxidizing an increasing proportion of the fatty acid to CO_2 and water, whereas the diabetic animals have a decreased rate of oxidation of octanoate to CO_2 and water.

The results presented here indicate that the response of fat catabolism to growth hormone at the shorter time interval is to a large degree dependent on a functional pancreas. The second phase, at the longer time interval, does not appear to require the presence of the pancreas. It would appear possible therefore that the primary response is mediated via the pancreas. There is little doubt that pituitary growth hormone exerts a powerful pancreatropic effect, although it is by no means certain that it causes primarily a secretion of insulin. Although there is a great deal of indirect evidence that growth hormone causes a secretion of insulin, the only direct work so far available (Anderson & Long, 1947) seems to indicate that

growth hormone actually causes an inhibition of insulin secretion. Too much emphasis, however, should not be placed on the results of Anderson & Long, in view of the criticism by Young (1953) of their assay procedure.

The indirect evidence for the pancreatropic effect of growth hormone has been reviewed by Young (1953) and by Campbell (1955). Especially relevant to the present work is the paper by Milman & Russell (1950), who found a depression in the blood sugar of normal rats treated with growth hormone for periods up to 8 hr. after the injection. No similar depression was found when growth hormone was injected into diabetic animals, and they therefore ascribed the lowered blood sugar to a stimulation of insulin secretion by the injected growth hormone. In the light of the results presented here it may be suggested that the primary response of fat metabolism to growth hormone is not due to growth hormone itself but to the secretion of insulin which it stimulates. In this context the exact mechanism of the release of insulin, whether it is a direct stimulation of the β -cells by growth hormone as suggested by Milman & Russell (1950), whether it is an indirect stimulation of the β -cells by the glucagon released by growth hormone as suggested by Young (1953), or whether the insulin is released from the tissues as suggested by Ottaway (1953), is not important. The effect of any of these mechanisms would mean that for a period of about 6–8 hr. after the injection of growth hormone the animal is predominantly under the influence of insulin. This would account for the increased endogenous respiration, and the decrease in the rate of fatty acid oxidation to CO_2 and water would also be consistent with this suggestion. After 8 hr. or so, the secretion of insulin diminishes and the true growth-hormone pattern, which has so far been masked, is revealed.

Thus the pattern of fat catabolism obtained 12 hr. after the injection of growth hormone is the characteristic picture, showing an increased rate of degradation of the fatty acids to the two-carbon fragment and an increased ability to oxidize these fragments through the Krebs cycle.

SUMMARY

1. The *in vitro* oxidation of octanoate has been studied in the livers of normal and diabetic rats injected 6 and 12 hr. previously with growth hormone.

2. In normal rats there is an inhibition of fatty acid oxidation to CO_2 and water 6 hr. after the injection of the hormone, but in diabetic animals this inhibition is not obtained.

3. In normal rats there is a stimulation of fatty acid oxidation, both as far as the two-carbon

fragment and to CO_2 and water, 12 hr. after the injection of the hormone. In diabetic rats there is also a stimulation of the rate of two-carbon-fragment formation at this time interval, but no increase in the rate of oxidation of the two-carbon units.

4. It is suggested that the inhibition of fatty acid oxidation found 6 hr. after the injection is due to a release of insulin caused by the pancreatropic effect of the growth hormone, and not by the growth hormone itself. The effect of growth hormone is to produce an increased rate of fat degradation.

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The Effect of Pituitary Growth Hormone and of Insulin on the Level of Oxidized and Reduced Coenzyme I in the Livers of Normal and Diabetic Rats

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The last few years have seen considerable advances in our knowledge of the mechanism of fat oxidation and synthesis. Reports from Green's laboratory (see Green, 1954), and from Lynen & Ochoa (1953), have contributed to the building-up of a composite picture in which acyl fatty acids are either shortened by 2-carbon atoms or lengthened by 2-carbon atoms as a result of four consecutive reactions, which together constitute the 'fatty acid cycle'. This cycle is envisaged as being completely reversible. Two of the reactions in the cycle involve hydrogen transfers, in one case by the flavin-adenine dinucleotide-linked enzyme, ethylene reductase, and in the other by the coenzyme I-linked enzyme, β -keto reductase. Lynen (1952-53) has suggested that the direction in which the cycle turns, i.e. whether it is a synthetic or a degradative cycle, depends on the availability of reduced coenzyme I (DPNH) as a source of hydrogen or of oxidized coenzyme I (DPN) to accept hydrogen. Indeed, he states: 'It would appear that in the living cell also the DPNH:DPN ratio may determine whether synthesis or degradation of the carbon chain will occur'. This suggestion has been verified by *in vitro* experiments (Hele & Popják, 1955), but not by *in vivo* experiments, where the experimental conditions are not so readily controlled. It seemed to us that the necessary *in vivo* conditions could best be obtained in animals

exposed to various hormonal influences which have previously been shown to favour either fat synthesis or breakdown. We have accordingly studied the DPN:DPNH ratio in insulin-treated rats, which are actively synthesizing fat, and in rats treated with pituitary growth hormone in which fat oxidation predominates. The results below indicate that although the DPN:DPNH ratios in diabetic and insulin-treated rats follow the predictions of Lynen, the ratio in growth-hormone-treated rats does not. In fact there appears to be little correlation between the cellular level of DPN or DPNH and the direction of the fatty acid cycle.

METHODS

Animals. The animals used were female hooded Norway rats aged 3-4 months, weighing 170-200 g. and maintained on stock diet (diet 41, Bruce & Parkes, 1946). In animals treated with growth hormone, the diet used was that described by Greenbaum (1953), which was supplied *ad lib.* before the commencement of treatment and at a daily level of 22 g. thereafter. Control rats for this group also received 22 g. of the special diet/day.

Diabetic animals were obtained by injecting 180 mg./kg. of body wt. of alloxan monohydrate dissolved as a 2% solution in a citrate-phosphate buffer, pH 4.0. The mortality rate was high, only about one-third of the rats surviving until the 21st day. The diabetic rats were kept for 3 weeks to ensure that there was no residual endogenous insulin. The surviving diabetic rats used in these experiments had