# THE INSULIN-LIKE EFFECT OF GROWTH HORMONE

by

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Pituitary growth hormone has been reported by OTTAWAY[1] to stimulate (the "insulin-like" effect) and to depress the glucose uptake of rat diaphragm. KRAHL[2] and PARK[3] have been unable to show any effect of growth hormone on diaphragm from normal rats, although KRAHL has reported an "insulin-like" effect on diaphragm from hypophysectomised rats.

The effect of growth hormone *in vitro* in a straightforward incubation of rat diaphragm has been further investigated. The inhibitory effect of growth hormone at a concentration of 100 $\mu$g/m was confirmed, but at lower concentrations, especially at 1–5 $\mu$g ml, the results were conflicting. Some series of experiments showed an inhibition, and others an activation. It was felt that the growth hormone could indeed produce two quite different effects, but their induction depended on unidentified changes in experimental conditions.

The elucidation of these changes proved difficult because of the wide scatter of the control rates of glucose uptake, and a technique to be described here has been developed in this laboratory for making the muscle metabolise more consistently. The technique described by KRAHL has been found by us to reduce variance of glucose uptake, but also to render the muscle completely insensitive to growth hormone.

A possible cause of the scatter was that during preparation one half of the diaphragm remained in more or less anaerobic conditions for a longer time than the other. In the experiments below both hemidiaphragms are treated in an identical way.

The medium devised by STADIE AND ZAPP[4] was used, modified by raising the pH to 7.2, to ensure that the growth hormone (I.E.P. 6.75) remained in solution. The whole diaphragm was excised from a fasted male albino rat, and washed for a moment in medium to remove adhering blood. It was then placed in a Gooch crucible containing 10–15 ml of medium with glucose; nitrogen was passed through the sintered glass bottom of the crucible, so that the muscle had the same $pO_2$ in all the experiments. It remained in this solution for 2 minutes, during which time it was divided into two along the central tendon, without removing it from the liquid. The addition of ascorbic acid (0.005 *M*) to the medium used in this preliminary gassing was found to reduce the variation in control uptakes; its use did not affect the type of result obtained. Each hemidiaphragm was taken simultaneously by one operator, blotted, and divided into two, and the four pieces placed into four Warburg flasks. The flasks were being gassed in the bath (with oxygen) 3½–4 minutes after excision.

The rigid use of this routine led to the finding that growth hormone stimulated the glucose uptake of normal rat diaphragm at the concentration used (25 $\mu$g ml in all the experiments reported here). A stimulation was also observed at 10 $\mu$g/ml, but at 40 $\mu$g/ml the hormone was apparently without effect. It was dissolved in a small quantity of medium with the aid of a drop of *N*/10 NaOH, and this solution diluted as required. The insulin-like activity disappears quite rapidly from the solution and it is necessary to use it not more than 20′ after making. The results were very consistent; a stimulation was found in 26 out of 29 pairs of observations (Table I).

TABLE I

EFFECT OF GROWTH HORMONE ON GLUCOSE UPTAKE IN mg/g OF TISSUE/h, OR NORMAL RAT DIAPHRAGM AFTER 2 MINUTES IN ANAEROBIC CONDITIONS. GROWTH HORMONE ADDED *in vitro*, CONCENTRATION 25 $\mu$g/ml. GLUCOSE CONCENTRATION 2 mg ml

Incubated for 1–2 hours

| *Number of observations* | *Control* ± *S.E.* | *Growth hormone* ± *S.E.* |
|---|---|---|
| 30 | 3.44 ± 0.16 | 4.06 ± 0.16 |
| | Difference + 0.62 ± 0.13 | |
| | $t = 2.70$, $p = 0.01$ | |

It seems unlikely that growth hormone prepared by the usual methods itself contains insulin (PARK[5] *et al.*). There remained the possibility that insulin was in some way liberated by growth hormone after the preliminary anaerobic treatment of the diaphragm, either in the muscle cells themselves or in the blood remaining in the blood vessels after excision.

The experiments were repeated, using alloxan-diabetic rats. The rats were not used until at least 10 days after subcutaneous injection of 25 mg of alloxan per 100 g of rat, after the method of KASS AND WAISBREN[6]. Table II shows that growth hormone had no effect on the glucose uptake from diabetic rat diaphragm, *i.e.* that from animals with a fasting blood sugar greater than 150 mg%. A graph showed that the stimulation induced by growth hormone added *in vitro* varies inversely with the fasting blood sugar (correlation coefficient 0.62), which is further evidence that it is insulin which is directly responsible for the stimulation.

TABLE II

EFFECT OF GROWTH HORMONE ON GLUCOSE UPTAKE OF DIABETIC RAT DIAPHRAGM. ALL VALUES IN mg/g/h. CONDITIONS AS IN PREVIOUS TABLE

| *Blood sugar at death* | *Number of observations* | *Control ± S.E.* | *Difference* | *Growth Hormone ± S.E.* |
|---|---|---|---|---|
| 1. Fasted rats < 150 mg% | 11 | 3.50 ± 0.12 | + 0.09 ± 0.27 | 3.59 ± 0.43 (*a*) |
| 2. Fasted rats > 150 mg% | 28 | 3.68 ± 0.23 | — 0.11 ± 0.16 | 3.57 ± 0.21 (*a, c*) |
| 3. Fasted rats injected with insulin 60 mg% | 20 | 4.59 ± 0.25 | + 0.45 ± 0.15 | 5.04 ± 0.27 (*b*) |
| 4. Fed rats; mean b.s. 380 mg% 380 mg% | 12 | 2.23 ± 0.19 | — 0.16 ± 0.15 | 2.07 ± 0.27 (*a*) |
| 5. Fed rats; mean b.s. 430 mg%, injected with insulin 255 mg% | 16 | 1.97 ± 0.13 | + 0.31 ± 0.11 | 2.28 ± 0.09 (*b*) |

*a* Not significantly greater than zero
*b* Significantly greater than zero
*c* Significantly different from response of normal rats (Table I)

It was desirable to show that the stimulation was increased when the concentration of insulin in the tissue is raised above normal. Injection of insulin into normal animals would complicate interpretation because of the secretion of insulin antagonists (SOMOGYI[7]).

Injection of 1–2 units of insulin subcutaneously into fasting mildly diabetic rats 30–45 minutes before death caused the insulin-like effect to rise to a significant level (Table II, section 3); all resuts from animals in which the blood sugar at death was greatly below normal have been excluded.

Many of the animals went into coma, and another experiment was performed using unfasted diabetic rats. Injection of 0.2 unit of insulin 2 hours before death brought the blood sugar level down in most cases to about 100 mg%. Some animals were given a supplementary dose of about 0.1 unit about 30 minutes before death. Table II, section 5, shows that this treatment effectively restored the "insulin-like" response to growth hormone. As a control, tissue was taken from uninjected diabetic animals from the same batch and incubated at the same time.

These experiments show that growth hormone can have, in certain conditions, an insulin-like effect on muscle from normal rats, but not on muscle from diabetic rats. Two explanations suggest themselves: that insulin in some way "potentiates" the tissue so that it is able to respond to the growth hormone; or that growth hormone is able to liberate insulin from a complex in which it is inactive. This hypothesis is more fruitful especially if it is considered that endogenous growth hormone may also liberate insulin in anaerobic conditions. This concept suggests that the great variation in rate of glucose uptake observed in diaphragm experiments is partly due to insulin being set free to an extent depending on the $pO_2$ of the muscle during preparation. Again, the fact that KRAHL was unable to show an insulin-like effect upon muscle from normal animals may have been because

in his conditions the "liberation" was complete before incubation of the tissue was begun; only with muscle from hypophysectomised animals, lacking any endogenous growth hormone, would it then be possible to demonstrate an *in vitro* effect.

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# FACTORS INFLUENCING THE FORMATION OF CEROID IN THE LIVERS OF CHOLINE-DEFICIENT RATS*

## I. DIETARY FATS

by


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The golden yellow, lipid pigment which may occur in the cirrhotic livers of choline-deficient rats was named *ceroid* by LILLIE and his associates[1]. Studies on the *in vitro* preparation and histochemical properties of substances resembling it support the suggestion that this material might be formed by the autoxidation of pathologically accumulated, unsaturated fats[2]. Through their influence upon tissue lipids, therefore, dietary fats might be expected to affect the formation of ceroid in the livers of choline-deficient rats. Cod liver oil favours this[3,4,5], while replacing the lard in the diet by butter decreases it and by hydrogenated cottonseed oil prevents it[6]. Even reduction in the amount of fat has an effect[7]. This preliminary note concerns a systematic study of the influence of the degree of unsaturation of the dietary fat upon the amount of ceroid formed in the livers of choline deficient rats.

Seven similar groups of male, Wistar rats, each consisting of 10 animals initially weighing 58–65 g, were fed choline-deficient diets***. The group receiving the basal, fat-free diet was given methyl linoleate, 50 mg per 100 g body weight, orally twice weekly. The other 6 groups were fed diets in which 10% of the sucrose in the basal mixture was replaced by 10% of a fat: hydrogenated cottonseed oils (Iodine Number = 12 or 36), cocoa butter (I. No. = 36), beef tallow (I. No. = 40), cottonseed oil (I. No. = 111), or a mixture of 4 parts cod liver oil (I. No. = 151) and 6 parts corn oil (I. No. = 125). To reduce the incidence of fatal renal lesions, the diets were supplemented with choline chloride, 0.08% for the first 10 days, and 0.03% for the next 14 days, but not any thereafter. Individual food consumptions were determined daily, body weights twice weekly. Rats from each group were sacrificed at random between the 55th and 126th days. Sections from 4 regions of each of their livers were graded for the amount of ceroid present (paraffin sections, Oil Red O method).

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*** Basal choline-deficient diet contained: extracted peanut meal, 30%; vitamin and fat-free casein, 6%; powdered sucrose, 49.90–49.98%; salt mixture[11], 3%; vitamin mixture[11], 1%; choline chloride (see text), 0.00–0.08%; cod liver oil concentrate (200,000 I.U. vitamin A and 50,000 I.U. vitamin D per g), 0.01%, and α-tocopherol acetate, 0.01%. When fat was added, this replaced 10% of the sucrose.