THE INSULIN-LIKE ACTION OF GROWTH HORMONE*

BY

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It has been reported that pituitary growth hormone affects the rate of glucose uptake by isolated rat diaphragm (Ottaway, 1951). Two types of effect were observed. When the diaphragm was given a preliminary soaking for 20 minutes in a growth-hormone solution without glucose, and then incubated in a second solution containing glucose but no growth hormone, the glucose uptake was found to be increased. When the diaphragm was incubated straightforwardly in one solution growth hormone depressed the glucose uptake. The concentration of growth hormone in both types of experiment was 100 $\mu\text{g. per ml.}$

The effect of growth hormone *in vitro* in a straightforward incubation of rat diaphragm has now been investigated further. The inhibitory effect of growth hormone at a concentration of 100 $\mu\text{g. per ml.}$ was confirmed, but at lower concentrations, especially at 1–5 $\mu\text{g. per ml.}$, the results were conflicting. Some series of experiments showed an inhibition, and others an activation. The mean of all the observed glucose uptakes with growth hormone present at concentrations of 5 $\mu\text{g. per ml.}$ or below did not differ from the mean control uptake, but the variance was significantly greater than that of the controls. 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 uptake, and it was first necessary to find some means of making the muscle metabolize more consistently. Perlmutter *et al.* (1952) and Krahl and Krahl (1951) have both recently drawn attention to this problem of variance in work with rat diaphragm, and Krahl (1951) has evolved a technique which greatly reduces the variance of the control glucose uptake. The diaphragm is allowed to remain, after excision from the rat, for 5 to 15 minutes in ice-cold bicarbonate buffer. The glucose uptake subsequently is found to be at a higher rate than usual, but there is much less variation between pieces of muscle from one diaphragm. This method, which has apparently been in use for some years in Krahl's laboratory, has been tried by us and its effectiveness confirmed. Unfortunately, as Krahl himself found, after this treatment the diaphragm of the normal unoperated rat is quite insensitive to growth hormone added *in vitro*.

Experimental Technique

Another technique, described below, has been developed in this laboratory. Hitherto experiments on rat diaphragm have been carried out by a single operator, using four pieces of muscle from one diaphragm; in these circumstances it is inevitable that one hemi-diaphragm will be dealt with at a later time than the other. Study of results suggested that the later, or second, hemi-diaphragm often had a higher glucose uptake than the former. At first it was thought that the difference might in some way be connected with a

leakage of potassium from what is to some extent a damaged tissue. However, this explanation was found not to be the correct one, which agrees with the findings of Perlmutter *et al.* Another possible cause was thought to be the fact that one half of the diaphragm remained in more or less anaerobic conditions for a longer time than the other, and a technique was developed in which both hemi-diaphragms are treated in an identical way (see also Stadie *et al.*, 1949).

For the experiments reported in this paper the medium devised by Stadie and Zapp (1947) was used. It was modified by raising the proportion of dibasic sodium phosphate to bring the pH up to 7.2. This was done to ensure that the growth hormone (I.E.P. 6.75) remained in solution. The diaphragm was excised whole 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 at a steady rate through the sintered glass bottom of the crucible. This precaution was taken to ensure that the muscle had the same $p\text{O}_2$ in all the experiments. It remained in this solution for two minutes, during which time it was divided into two along the central tendon, without removing it from the liquid. The addition of ascorbic acid (0.005M) 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 hemi-diaphragm was taken simultaneously by one operator, blotted, and divided into two, and the four pieces were placed into four Warburg flasks. The flasks were being gassed in the bath (with oxygen) three and a half to four minutes after excision.

Results

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, which was 25 $\mu\text{g. per ml.}$ in all the experiments reported here. A stimulation was also observed at 10 $\mu\text{g. per ml.}$, but at 40 $\mu\text{g. per ml.}$ the hormone was apparently without effect. The hormone was dissolved in a small quantity of medium with the aid of a drop of N/10 NaOH, and this solution was diluted as required. The insulin-like activity disappears quite rapidly from the solution, and it is necessary to use it not more than 20 minutes after making. The results were very consistent: a stimulation was found in 26 out of 30 pairs of observations. The results are summarized in Table I.

TABLE I.—Effect of Growth Hormone on Glucose Uptake in mg./g. of Tissue/Hour, or Normal Rat Diaphragm After Two Minutes in Anaerobic Conditions. Growth Hormone Added *in vitro*, Concentration 25 $\mu\text{g./ml.}$ Glucose Concentration, 2 mg./ml. Incubated for 1–2 Hours

No. of Observations	Control $\pm$ S.E.	Growth Hormone $\pm$ S.E.
30	3.44 $\pm$ 0.16	4.06 $\pm$ 0.16

Difference, +0.62 $\pm$ 0.13; $t = 2.70$, $P = 0.01$.

It seemed desirable to show whether insulin was in some way responsible for this increase in glucose uptake before postulating a new stimulating principle. It seems unlikely that, prepared by the usual methods, growth hormone itself contains insulin (see, in particular, the work of Krahl and his colleagues. 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 of the diaphragm after excision.

The experiments were therefore repeated, using alloxan-diabetic rats. The rats were not used until at least ten days after subcutaneous injection of 25 mg. of alloxan per 100 g. of rat, after the method of Kass and Waisbren (1945). Table II shows that growth hormone had no effect on the glucose uptake from diabetic rat diaphragm—that is, from animals with a fasting blood sugar greater than 150 mg. per 100 ml.

*The tables included in this paper have already been reported in *Biochimica et biophysica acta*, 1953, 11, 443.

TABLE II.—Effect of Growth Hormone on Glucose Uptake in mg./g./hr., of Diabetic Rat Diaphragm, After Two Minutes in Anaerobic Conditions. Growth Hormone Added *in vitro*, Concentration 25 µg./ml. Glucose Concentration, 2 mg./ml. Incubated for 1–2 Hours

Fasting Blood Sugar	No. of Observations	Control $\pm$ S.E.	Growth Hormone $\pm$ S.E.
150 mg. per 100 ml.	11	3.50	3.59
		Difference, $+0.09 \pm 0.27$	
150 „ „ „ „	28	3.68	3.57
		Difference, -0.11 ± 0.16	
(Significantly different from the normal difference) $t=2.65$, $P=0.01$.			

This work was done before the results of Groen *et al.* (1952) became available. These workers found that it may be as long as three weeks before the last trace of insulin disappears from the blood of a diabetic animal. The present work would thus have been more conclusive if the animals had been kept for a longer time before use. However, the Chart shows that the stimulation induced by growth hormone added *in vitro* varies inversely with the fasting blood sugar: with the most severely diabetic animals there was indeed a slight inhibition of glucose uptake. This relationship between stimulation and severity of diabetes may be regarded as further evidence that it is insulin which is directly responsible for the stimulation.

As a corollary to these experiments it is desirable to show that the stimulation is increased when the concentration of insulin in the tissue is raised above normal—for example,

Chart showing effect of growth hormone on glucose uptake.

by injection of insulin into the intact animal. Injection of insulin into normal animals, with the consequent fall in blood-sugar concentration, might, however, have led to secretion of the anti-insulin factor, which has been shown by Somogyi (1948) to play a large part in the regulation of the blood-sugar level in human beings. A preliminary experiment showed that injection of 1–2 units of insulin subcutaneously

TABLE III.—Effect of Growth Hormone on Glucose Uptake in mg./g./hr. of Diaphragms from Fasting Diabetic Rats. The Rats had been Injected 30 Minutes Previously with 1–2 Units of Insulin. Other conditions as in Tables I and II

Mean Fasting Blood	Mean Blood Sugar at Death	No. of Observations	Glucose Uptake	
			Control S.E.	Growth Hormone S.E.
160 mg./100 ml.	60 mg./100 ml.	20	4.59	5.04

Difference, +0.45 ± 0.15 (significantly greater than zero); $t=3.00$, $P=0.01$

This dose of insulin was, however, too large, since many of the animals went into coma; and another experiment was performed with unfasted diabetic rats. Injection of 0.2 unit of insulin two hours before death was found to bring the blood-sugar level down in most cases to about 100 mg.

per 100 ml. Some animals were given a supplementary dose of about 0.1 unit about 30 minutes before death. Table IV 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 from fed normal animals, and incubated at the same time.

TABLE IV.—Effect of Growth Hormone on the Glucose Uptake in mg./g./hr. of Fed Diabetic Rats Injected Two Hours Previously with 0.2 Unit of Insulin. Results from Uninjected Diabetic Rats and From Normal Animals are Included for Comparison. Conditions as in Table I

	Mean Blood Sugar Before Injection	Mean Blood Sugar at Death	No. of Observations	Glucose Uptake	
				Control S.E.	Growth Hormone S.E.
Diabetic rats injected with insulin ..	430	255	16	1.97	2.28
Difference, +0.31 ± 0.11 (significantly greater than zero); $t=2.8$, $P=0.02$.					
Diabetic rats not injected ..	380	415	12	2.23	2.07
Difference, -0.16 ± 0.15; $t=1.07$, $P=0.10$					
Normal rats ..	130	6	6	3.11	3.58
Difference, +0.47 ± 0.17 (significantly greater than zero); $t=2.75$, $P=0.05$.					

Conclusions

These experiments show that growth hormone can have, in certain conditions, an insulin-like effect on muscle from normal rats, but that it does not have this effect 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; the existence of a compound in human serum which reduces the activity of insulin has been inferred by Marsh and Haugaard (1952). I prefer the second hypothesis as being more fruitful, especially if it is considered that endogenous growth hormone may also liberate insulin in anaerobic conditions. This concept applied to *in vitro* experiments suggests that the great variation in rate of glucose uptake observed in diaphragm experiments is due to the fact that insulin is set free to an extent depending on the oxygen tension of the muscle during the preparation of any experiment. 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” may have been complete before incubation of the tissue was begun; only with muscle from hypophysectomized animals, lacking any endogenous growth hormone, would it then be possible to demonstrate an *in vitro* effect.

The evidence that this reaction occurs *in vivo* is not at present conclusive. It has been shown that an injection of growth hormone into rats raises the glucose uptake of diaphragm excised at a later time (Park, 1953), and also that more massive doses cause a fall of blood sugar in all but diabetic rats (Milman and Russell, 1950). Both these are insulin-like effects. If the reaction can be shown to occur in physiological conditions it might be regarded as a device to ensure a greater supply of glucose for muscle rendered temporarily anaerobic by a sudden muscular effort.

Two problems arise from this work. One is the nature of the compound which makes insulin in the blood, and the mechanism by which it is liberated; some progress has been made in this direction. The other problem is the characterization of the entity which is responsible for the liberation, and which has been referred to in this paper as “growth hormone.” No serious attempt has yet been made in this laboratory to relate the effect to growth potency, or to attempt the preparation of an active submolecule from crystalline growth hormone.

Summary

Conditions are described in which pituitary growth hormone consistently produces an "insulin-like" effect on the glucose uptake of diaphragm from normal rats.

The effect is shown to be absent when the rats have been made diabetic, and to return when diabetic rats are injected with insulin. It is postulated that the effect is due to the liberation by growth hormone of insulin held in an inactive state in the blood or tissue.

REFERENCES

- Groen, J., Kamminga, C. E., Willebrands, A. F., and Blickman, J. R. (1952). *J. clin. Invest.*, **31**, 97.
 Kass, E. H., and Waisbren, B. A. (1945). *Proc. Soc. exp. Biol., N.Y.*, **60**, 303.
 Krah, M. E. (1951). *Ann. N.Y. Acad. Sci.*, **54**, 649.
 Marsh, J. B., and Haugaard, N. (1952). *J. clin. Invest.*, **31**, 107.
 Milman, A. E., and Russell, J. A. (1950). *Endocrinology*, **47**, 114.
 Ottaway, J. H. (1951). *Nature, Lond.*, **167**, 1064.
 Park, C. R. (1953). *Phosphorus Metabolism*, vol. 2. Johns Hopkins Press.
 Perlmutter, M., Weisenfeld, S., and Mufson, M. (1952). *Endocrinology*, **50**, 442.
 Somogyi, M. (1948). *J. biol. Chem.*, **174**, 597.
 Stadie, W. C., Haugaard, N., Marsh, J. B., and Hills, A. G. (1949). *Amer. J. med. Sci.*, **218**, 265.
 — and Zapp, J. A. (1947). *J. biol. Chem.*, **170**, 55.

STRUCTURAL CHANGES IN PROSTATIC CANCER AFTER BILATERAL ADRENALECTOMY REPORT OF A CASE

BY

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[WITH SPECIAL PLATE]

Bilateral adrenalectomy and orchidectomy is claimed by Huggins (Huggins and Scott, 1945; Huggins and Bergenstal, 1952; West *et al.*, 1952) to have induced substantial clinical improvement in some cases of advanced prostatic carcinoma. It is also claimed that post-operative substitution therapy enables the patient to enjoy a fair measure of health and activity. The operation is still in the stage of therapeutic trial, but is being carried out on an increasing number of patients in this country. Most of the published evidence in its support is clinical in character, and remarkably little has been written on the effects of deprivation of adrenal cortical hormones on the structure of the growth and its metastases.

This report concerns a man of 63 with an oestrogen-resistant prostatic carcinoma, who was subjected to orchidectomy with no clinical improvement and finally to bilateral adrenalectomy. He died from an acute pulmonary infection 40 days after this operation. Massive necrosis was found in the primary growth and in its metastases in the regional lymph nodes, liver, and lung. The changes in all these situations had occurred simultaneously a few weeks before death, and, although incapable of rigid scientific proof, it is reasonable to suppose that this widely scattered necrosis was the direct consequence of total deprivation of adrenal hormones.

Case Report

A man aged 63 was admitted to hospital on August 14, 1952, complaining of rectal pain and urinary frequency for six weeks. The prostate was hard, craggy, and fixed. Serum acid phosphatase was 3.3 units (King-Armstrong).

An x-ray film of the spine and pelvis showed osteoarthritis; no metastases. An endoscopic resection on August 19 relieved the urinary symptoms, but the rectal pain persisted, and on September 16 a bilateral subcapsular orchidectomy was done. Severe rectal pain and dysuria increased in spite of this, and was not relieved by large doses of stilboestrol (30–60 mg. daily). He was then transferred to St. Philip's Hospital, where an enlarged liver and palpable enlarged lymph nodes in the groin were found. Bilateral adrenalectomy was advised, and he was transferred to the Central Middlesex Hospital for operation. A pre-operative blood-sugar curve revealed moderately severe diabetes mellitus, but the patient's general condition remained fair.

Substitution therapy with cortisone acetate (200 mg. decreasing to 66.5 mg. daily), deoxycortone (5 mg. daily, decreasing to 5 mg. on alternate days), and sodium chloride (5 g. decreasing to 3 g. daily), as described by Huggins and Bergenstal (1951), was begun on December 4 and continued post-operatively. Both adrenals were removed on December 5 by Mr. J. D. Fergusson. The left kidney was absent. There was no immediate clinical improvement, and stilboestrol, 100 mg. daily, was given from December 22 onwards. The diabetes mellitus remained unchanged and was controlled by diet and insulin, but in spite of this there were a number of hypoglycaemic episodes. On January 14, 1953, he collapsed, complaining of dyspnoea, and died the next day, apparently from a pulmonary embolus.

Post-mortem Examination

Necropsy was performed eight and half hours after death. The lungs showed basal congestion, scattered bronchopneumonic areas, many small tumour nodules, and a large wedge-shaped area at the periphery of the right upper lobe showing central degeneration and haemorrhage—apparently an infarct, but the pulmonary arteries, which showed nodular atheroma in the large branches, contained no embolus. There were many large and small tumour deposits in the liver, some with a zone of congestion around them. The right kidney was large (weight 13½ oz.; 383 g.). The left kidney and ureter were absent, and the renal vessels on this side were of normal size but ended blindly in a dense mass of fibrous tissue. The adrenals were absent. The bladder was dilated and there were several small diverticula. The left ureteric orifice could not be found. The prostate was largely replaced by a firm nodular tumour mass spreading into the seminal vesicles, periprostatic tissues, and the base of the bladder. The rectum was compressed but not obviously infiltrated. There were large tumour deposits in left and right internal, external, and common iliac, left inguinal, para-aortic, and mediastinal lymph nodes, some of which showed central degeneration. The breasts were normal. Bones (8–12 thoracic, 1–5 lumbar vertebrae, and sacrum) were normal. The pituitary was small. All other organs were normal. The immediate cause of death was thought to be the lesions in the lung.

Histology

Tissues were examined by a "big section" technique which has been in routine use in these laboratories for the past two years. By this method histological sections (5–8 μ) of whole organs are cut and examined microscopically. The pathological changes in this case might have been overlooked had smaller sections been cut, because the extent and distribution of the degenerative changes was much greater than was apparent to the naked eye. In all sections from the primary tumour and metastatic deposits in lymph nodes, liver, and lung there was a massive central necrosis involving both neoplastic tissue and stroma. The necrotic central mass was surrounded by a narrow zone of granulation tissue and, beyond this, a rim of surviving tumour cells (Plate, Figs. 1, 2, and 3). The change involved both large and small deposits, and the zone of granulation tissue was apparently about the same age (2–3 weeks) in all. The ratio between the amount of necrotic and surviving tumour tissue was approximately the same in the primary growth as in its