

PHYSIOLOGICAL AND PATHOLOGICAL ROLES OF GROWTH HORMONE

M. HARTOG, B.A., B.M., B.CH., M.R.C.P.

Department of Medicine, Hammersmith Hospital, London, W.12.

It is now more than 40 years since Evans and Long first demonstrated the growth-promoting activity of crude extracts prepared from the anterior pituitary. Since then the hormone responsible has been extracted from the pituitary glands and purified. It has been shown to be a polypeptide and important differences have been demonstrated between growth hormones from different species. Bovine growth hormone, in particular, has been studied intensively, and its physico-chemical properties (Ketterer, Randle and Young, 1957) and amino-acid composition (Franklin, Li and Dunn, 1947) have been determined. Modern methods of cyto-chemistry have supported previous circumstantial evidence that, in normal glands, the eosinophil cells of the anterior pituitary are the site of origin of the hormone (Pearse, 1952); and, more recently, the fluorescence of these cells when exposed to an antiserum to growth hormone conjugated with fluorescein, has again confirmed them to be the source of the hormone (Leznoff, Fishman, Goodfriend, McGarry, Beck and Rose, 1960). Russfield, Reiner and Klaus (1956), however, have reported that the predominant cells of the pituitaries from seven acromegalic patients were not eosinophils, but were cells which were only sparsely granular and stained weakly with periodic-acid Schiff (amphophil cells); these cells were presumably secreting growth hormone.

A variety of techniques have been described for extracting the hormone from the pituitary, and as much as 2.7 mg. to 5.6 mg. has been estimated to be obtained from a single human pituitary (Gemzell and Li, 1959). The hormone is fortunately relatively stable, and can be extracted from pituitary glands which have been stored for months either in acetone or frozen. It has been stated that as much growth hormone can be obtained from adult pituitaries as from those of the young (Gershberg, 1957).

The hormone has widespread metabolic effects, and has an important part to play in the homeostasis of the body's internal environ-

ment. It is the purpose of this article to describe briefly the actions of growth hormone on the metabolism of protein, fat and carbohydrate, and then to describe its effects in man and its possible pathological roles. For more complete descriptions of its metabolic effects the reader is referred to reviews by Knobil and Hotchkiss (1964), Korner (1961a) and Ketterer and others (1957), and for its action in man to the article by Raben (1962).

Effect on Protein Metabolism

Lee and Schaffer (1934) performed carcass analyses on rats injected with anterior pituitary extract, and showed them to have higher protein and lower fat contents than did the control animals; conversely Lee and Ayres (1936) showed that hypophysectomised rats on a limited food intake lost more protein and less fat than did control animals on the same diet. It thus appeared that secretions from the pituitary had a "protein sparing" action, and when pure growth hormone became available it was soon shown that it produced nitrogen retention with a marked reduction in urine nitrogen (Bennett, Li and Laundrie, 1948). An important feature of this effect of growth hormone is that it appears to be dependent upon the presence of insulin. Milman, DeMoor and Lukens (1951) showed that growth hormone had no effect on the nitrogen balance of cats which had been pancreatectomised, a small effect if the cats were kept on maintenance doses of insulin, and a normal effect if the dose of insulin was increased during the period of growth hormone administration. Variation of insulin dosage by itself did not alter the nitrogen excretion.

The exact method whereby growth hormone induces nitrogen retention remains uncertain; there is strong evidence for an effect in stimulating protein synthesis, and it may also have an effect in inhibiting protein catabolism. Manchester and Young (1959) have shown that growth hormone added *in vitro* will stimulate the uptake of radioactive glycine by isolated diaphragm muscle from hypophysectomised rats, and that this is unaffected by the addition

of anti-insulin in serum, which will inhibit stimulation produced by insulin. Korner (1961b) in some interesting experiments with cell-free systems, has shown that growth hormone will increase the incorporation of radioactive amino-acids into the ribonucleoprotein microsomal particles of rat liver; these microsomal particles are a site of protein synthesis within the cell.

Effect on Fat Metabolism

The results of the studies on carcass analyses already mentioned indicated that growth hormone encourages the breakdown of fats within the body, and subsequent work has confirmed this view. Growth hormone will produce a rise of the blood ketone bodies, an increase in the liver fat content, and a fall in the respiratory quotient of the whole animal and of isolated tissues (Greenbaum and McLean, 1953a; Greenbaum, 1953). These, and other experiments, suggested that growth hormone acts by stimulating the utilisation of fats by the liver and peripheral tissues (Greenbaum and McLean, 1953b). However, some subsequent work has failed to support this view. Thus, Franklin and Knobil (1961) studied the oxidation of ^{14}C -labelled fatty acids in the rat, and were unable to detect any acceleration after the administration of growth hormone. There is considerable evidence that growth hormone will depress the formation of fats. Welt and Wilhelmi (1950) found that treatment with growth hormone depressed the incorporation of deuterium-labelled water into carcass and liver fats of rats. Goodman (1963) found that the fat content of omental and epididymal fat of hypophysectomised rats was decreased by treatment with growth hormone, and the *in vitro* synthesis of fat by adipose tissue was diminished. He suggested that the effects of growth hormone on the respiratory quotient and blood ketone bodies mentioned above, could be the result of depressed fat synthesis rather than increased fat oxidation.

Raben and Hollenberg (1959) have shown that growth hormone produces a marked increase in the level of non-esterified fatty acids (NEFA) in the plasma. The NEFAs are liberated from the fat depots and are metabolised further both by the liver and the peripheral tissues; an indication of their rate of utilisation can be obtained from the fact that the half-life of their rate of disappearance from plasma has been estimated as two minutes (Havel and Fredrickson, 1956). Some aspects of their metabolism are reviewed by Fredrickson and Gordon (1958). Raben (1959) has commented

upon the very small dose of growth hormone which is required to produce a rise in the plasma NEFA level, suggesting the physiological importance of this action of growth hormone.

Effect on Carbohydrate Metabolism

Houssay showed that the pituitary has an effect on carbohydrate metabolism when he showed that hypophysectomy would ameliorate diabetes in the dog; in 1949, Cotes, Reid and Young showed that purified bovine growth hormone was diabetogenic in intact adult cats. Hypophysectomised animals are unduly sensitive to insulin, and de Bodo, Kurtz, Ancowitz and Kiang (1950) were able to restore the insulin sensitivity of hypophysectomised dogs to normal with growth hormone. With more prolonged therapy the dogs became insulin-resistant and ultimately diabetic.

Young (1945) has emphasised the importance of the response of the beta cells of the islets of Langerhans in determining the effect of injections of growth hormone. It has already been mentioned that the simultaneous secretion of insulin seems essential for the nitrogen-retaining action of growth hormone. There is considerable evidence that growth hormone stimulates the secretion of insulin. Kinash, MacDougall, Evans, Bryans and Haist (1953) found an increase in islet weight after the administration of growth hormone, and rises of serum insulin-like activity have been demonstrated (Randle, 1956; Engel, Albertson, Fredericks and Lopez, 1958). In puppies and kittens growth hormone administration will stimulate growth, and the animals do not develop diabetes; Young has suggested that these young animals show this effect because they are able to increase their insulin secretion sufficiently. In adult dogs and cats growth hormone will produce a diabetic state during the period of administration (idihypophyseal diabetes), which may, if the hormone is given long enough, persist even after the injections have stopped (metahypophyseal diabetes). These animals may be unable to increase their insulin secretion adequately, and therefore become diabetic; if irreversible changes are produced in the islets the diabetes will be permanent. By avoiding hyperglycaemia during injections of pituitary extracts, Lukens and Dohen (1942) were able to prevent permanent diabetes; it would therefore appear that it is the persistent hyperglycaemia which is responsible for permanent damage to the islets. The mechanism whereby growth hormone stimulates insulin secretion is uncertain; it may be by

means of hyperglycæmia or it may represent a direct effect on the islets; the recent demonstration of a rise in pancreatic vein insulin after the infusion of ketone bodies into the pancreatic artery (Madison, Mebane, Ungar and Lochner, 1964) may also be important in this respect.

Administration of growth hormone has been shown by Park, Brown, Cornblath, Daughaday and Krahl (1952) to have a biphasic effect on the glucose uptake of isolated rat diaphragm. Initially, the uptake is stimulated and subsequently it becomes depressed. The initial stimulation may be partly due to the increased insulin secretion, but also is due to a direct effect of growth hormone. Thus, Manchester and Young (1959) showed that growth hormone added *in vitro*, increased the glucose uptake by isolated rat diaphragm from hypophysectomised animals, and this effect was not inhibited by the addition of an antiserum to insulin. The subsequent period of depression of glucose uptake was not produced by growth hormone *in vitro*, and so presumably depended upon some transformation or metabolic effect of growth hormone in the body.

The way in which growth hormone depresses glucose uptake has been extensively studied. Colowick, Cori and Slein (1947) found that anterior pituitary extracts produced an inhibition of the enzyme hexokinase, which is concerned with the immediate phosphorylation of glucose, once it has passed the cell membrane, to glucose-6-phosphate, and that insulin reversed this inhibition. More recently Park, Morgan, Henderson, Regen, Cadenas and Post (1961) have studied the regulation of glucose intake in perfused rat heart preparations. The major rate-limiting steps for glucose uptake by muscle are its transport across the cell membrane and its subsequent phosphorylation. The major effect of insulin is the stimulation of transport across the membrane, and growth hormone was shown to have an effect in reducing the sensitivity of this transport system to insulin; growth hormone also caused depression of phosphorylation. In the normal animal, regulation of glucose uptake is provided by the balance between these and other hormones. The insulin sensitivity of the hypophysectomised animal would be understandable on the basis of the effect of insulin in stimulating glucose transport, in the absence of the normal restraining effect of growth hormone on transport and phosphorylation. Another mechanism for the effect of growth hormone on the insulin hypersensitivity of the hypophysectomised animal, was indicated by Alts-

zuler, Steele, Dunn, Wall and de Bodo (1959), who used a ^{14}C -labelled-glucose dilution technique, and found that growth hormone increased the rate of glucose flow into the plasma, presumably from the liver, in hypophysectomised dogs.

Williamson and Krebs (1961) found that acetoacetate decreased the oxidation of glucose by the isolated rat heart, both in the presence and absence of added insulin. Randle, Garland, Hales and Newsholme (1963), in a stimulating paper have shown that ketone bodies and fatty acids will depress the glucose uptake by isolated rat diaphragm and rat heart. They have suggested that this might provide the mechanism of some of the abnormalities of carbohydrate metabolism found not only after the administration of growth hormone, but also in diabetes, starvation and after the administration of glucocorticoids. Such a mechanism would explain the depression of glucose uptake by growth hormone when administered *in vivo* but not *in vitro*.

The Effect on Growth

Provision of an adequate diet is clearly essential for normal growth to occur. Greenbaum (1953) has shown that rats on a limited food intake, when given growth hormone, will show an initial growth spurt similar to rats on an unlimited diet also receiving growth hormone. The increased growth rate in the rats on a restricted diet, however, tailed off at a time which he found to coincide with the depletion of their fat stores by about 50 per cent. Protein is laid down in muscle with a considerable amount of water, and its calorific value has been estimated as 1 Cal./g. (Young, 1945); fat depots, on the other hand, are composed of almost pure fat with a calorific value of 9 Cal./g. Thus, it can be seen how an animal can gain weight even on a restricted food intake if fat is oxidised in preference to protein.

As was discussed in the section on protein metabolism, the simultaneous secretion of insulin seems essential for the nitrogen-retaining action of growth hormone; it is, therefore, important to consider whether insulin itself is responsible for the growth stimulation of growth hormone. Manchester, Randle and Young (1959) showed that growth hormone has the effect of reducing the stimulation of glucose uptake by insulin in the rat diaphragm, insulin thereby perhaps becoming more effective as regards nitrogen retention. Salter and Best (1953) succeeded in maintaining hypophysectomised rats on a long-acting insulin, thereby avoiding the effects of hypoglycæmia. They

found that the insulin-treated animals gained weight; the majority of this weight gain being due to an increased fat content, but there was also a definite rise of body protein. Wagner and Scow (1959) have done similar experiments, and found that force-feeding of hypophysectomised rats produced very similar effects on the weight and body composition as did injections of insulin. In marked contrast was another group of rats treated with growth hormone in whom the excess tissue consisted largely of protein. They concluded that insulin has no specific effect upon growth, other than its effect in stimulating appetite.

The Extraction of Human Growth Hormone

Once growth hormone from animal sources had been identified and purified it was natural to investigate its activity in man. Though some metabolic changes with such preparations were described, the results were, on the whole, disappointing. Wilhelmi (1955) reported that growth hormone extracted from fish pituitaries was inactive in rats, though it was active in fish, and furthermore, bovine growth hormone was also active in fish. This drew attention to the possibility of the species specificity of human growth hormone (HGH) being responsible for the failure to obtain consistent effects with animal growth hormones in man. Li and Papkoff (1956) extracted and purified growth hormone from human pituitaries, and showed that it differed in many of its characteristics from bovine growth hormone. Thus, the molecular weight of HGH was estimated as 25,400 and that of bovine growth hormone as 46,000.

Much work has been done in establishing the composition of HGH, the amino-acid structure of which is described by Li and Liu (1964). Despite fulfilling many of the physicochemical criteria of a pure substance, HGH when submitted to starch gel electrophoresis gave rise to four distinct lines (Ferguson and Wallace, 1961; Barrett, Friesen and Astwood, 1962). After elution, the material composing these fractions was shown to have the usual metabolic effects of growth hormone when tested in the rat (Ferguson and Wallace, 1961) and in the hypopituitary subject (Laron, Assa and Menache, 1963). Starch-gel electrophoresis has also demonstrated the close similarity in the lines obtained from HGH, and those obtained from preparations of prolactin extracted from sheep and human pituitaries (Ferguson and Wallace, 1961; Barrett and others, 1962). It is, therefore, of particular interest that the purest preparations of HGH have been shown to have lactogenic activity when assayed bio-

logically (Chadwick, Folley and Gemzell, 1961), and conversely sheep prolactin has been shown to have growth-hormone-like activity in rats (Reisfeld, Tong, Rickes and Brink, 1961) and to cause nitrogen retention in man (Bergental and Lipsett, 1958).

The Effects of Human Growth Hormone in Man

In 1956, Knobil, Wolf and Green showed that monkey growth hormone was effective in monkeys, and the following year Beck, McGarry, Dyrenfurth and Venning (1957) demonstrated that preparations of monkey and HGH were effective in man. Since then these observations have been repeatedly confirmed, and a number of papers have described the effects of HGH in man (Beck, McGarry, Dyrenfurth and Venning, 1958; Ikkos, Luft and Gemzell, 1959; Clinical Endocrinology Committee of the MRC, 1959; Henneman, Forbes, Moldawer, Dempsey and Carroll, 1960).

The majority of the reports have stated that hypopituitary subjects are more sensitive to the action of HGH than are subjects with a normal endogenous HGH production. A rise of plasma NEFA levels is among the earliest changes after an injection of HGH (Raben and Hollenberg, 1959), the levels rising two- to three-fold above the control levels; a rise of plasma and urine ketones also occurs soon after the administration of HGH. Nitrogen retention is a prominent effect, and the blood urea and urine nitrogen are lowered within 24 hours, the effect of a single injection on urinary nitrogen lasting for approximately ten days. There is a considerable retention of sodium with expansion of the extracellular space; an increased urinary excretion of aldosterone was found by Beck and others (1958) which might have been responsible for this sodium retention. However, others have not found a consistent rise in urinary aldosterone, and the sodium retention occurred in an adrenalectomised patient (Biglieri, Watlington and Forsham, 1961), where it must have been independent of aldosterone secretion. The potassium balance is also positive, even when allowance is made for the retention associated with the laying down of tissue. A surprising feature of the effect of HGH is the production of a marked hypercalcuria; the intestinal absorption of calcium is usually increased and the overall calcium balance is variable, depending on which of these two effects is the greater. The Clinical Endocrinology Committee of the MRC (1959) have suggested that the increase in urinary calcium may be an over-dosage

effect of HGH, as doses which produce no further increase in nitrogen retention, showed increased hypercalcuria.

The effects of HGH on carbohydrate metabolism in man have also been closely studied. The early reports of Beck and others (1958) and Ikkos and others (1959) showed that the administration of HGH to hypopituitary subjects can produce diminished carbohydrate tolerance, as measured by oral or intravenous glucose tolerance tests. When large doses of HGH were given to two non-diabetic women who had been hypophysectomised for disseminated breast cancer, fasting hyperglycaemia and glycosuria occurred (Ikkos and Luft, 1960). When the HGH injections finished, the carbohydrate tolerance returned to normal. It thus appeared that a state of "idiopathous diabetes" comparable with that found in animals had been produced, and it was interesting that, as was found in animals, a marked positive nitrogen balance occurred despite the development of the diabetic state. Diabetic patients are more sensitive to the "diabetogenic" effect of HGH. Three insulin-requiring diabetics who had been hypophysectomised for the treatment of diabetic retinopathy, were given HGH by Luft, Ikkos, Gemzell and Olivecrona (1959); they all showed a pronounced deterioration in their diabetic control with hyperglycaemia, ketonuria and acidosis. In two, the HGH injections had to be stopped after 36 hours. The third patient continued with HGH for 8 days, and, during this time, no appreciable nitrogen retention was noted, again providing a comparison with the animal studies already mentioned, which indicated that increased insulin secretion was necessary for the nitrogen-retaining effect of growth hormone. Administration of HGH has also been found to be accompanied by an immediate and short-lived period of hypoglycaemia (Zahnd, Steinke and Renold, 1960), during which no rise of insulin-like activity in the blood was found, and must have presumably reflected a direct action of HGH. Rabinowitz and Zierler (1962, 1963) have done some interesting experiments in which the metabolism of the forearm was studied by cannulating the brachial artery and the superficial and deep veins of the forearm, (venous samples from the deep vein drain predominantly muscle tissue, and those from the superficial vein mainly adipose tissue). They found that intra-arterial insulin stimulated the uptake of glucose by muscle and adipose tissue and inhibited the release of NEFA from adipose tissue. HGH

stimulated the release of NEFA from adipose tissue and stimulated their uptake by muscle tissue; it also inhibited glucose uptake. When both hormones were given together or when insulin was given to acromegalic subjects, the effect of insulin on glucose uptake was no longer present, though its effect in inhibiting the release of NEFA was unchanged. This evidence, together with evidence obtained from assays of insulin and assays of HGH in plasma (Roth, Glick, Yalow and Berson, 1963a), led them to suggest three phases of hormonal release action after the intake of food. The first one, immediately after the meal, is dominated by the effect of insulin and encourages the storage of carbohydrate and fat, the next phase in which both insulin and HGH are effective encourages protein synthesis, and the third "remote post-absorptive" phase in which HGH is dominant, results in fat mobilisation.

The Assay of Growth Hormone in Human Serum

Early methods for the assay of growth hormone have depended upon its biological effects. Thus, satisfactory assays have been performed measuring the weight gain of intact or hypophysectomised rats, and the width of the tibial epiphyseal cartilage of hypophysectomised rats (Geschwind and Li, 1955). When applied to the measurement of HGH in serum these methods have proved inadequate owing to their lack of sensitivity. Gemzell (1959), however, developed a technique for the extraction of growth hormone from plasma, and, applying this to the tibial epiphysis method, he was able to detect growth hormone-like activity in acromegalic subjects, extracts from normal subjects being inactive. Salmon and Daughaday (1957) reported a method for the assay of a "sulphation factor" which stimulated the uptake of radioactive sulphate by costal cartilage from hypophysectomised rats. This "sulphation factor" paralleled the expected levels of growth hormone in blood, but could not have been growth hormone itself as this was found to be inactive *in vitro*. The assay of the "sulphation factor" has been developed, in particular, by Almqvist, who has used it widely in studying hypopituitary subjects and acromegalic subjects before and after treatment (Almqvist, 1961, Almqvist, Ikkos and Luft, 1961).

Read (1960) developed an immuno-assay for HGH which depended on the "hæmagglutination-inhibition" technique, in which prepared red blood cells were coated with HGH and agglutinated by an antibody prepared to HGH

in a rabbit; this agglutination could then be inhibited by solutions containing HGH. The assay is a sensitive one, but unfortunately is affected by factors in serum, probably plasma protein, which interfere with the hæmagglutination (Fraser and Hartog, 1962). The technique has been further elaborated by Dominguez and Pearson (1962) who have used an extraction procedure to remove these interfering substances, and find it to be sensitive and reliable. The methods which are likely to become the most widely adopted are immuno-assays depending on the use of radioactive-labelled HGH. When labelled HGH is exposed to an antibody to HGH some of it becomes bound to the antibody, and the amount of this binding is affected by the amount of unlabelled HGH in the system. Hunter and Greenwood (1962) have devised a method for the production of ^{131}I -labelled HGH of high specific activity. The different methods which have been developed depend upon different ways of separating the free and the bound HGH (Hunter and Greenwood, 1964a; Glick, Roth, Yalow and Berson, 1963; Utiger, Parker and Daughaday, 1962).

Using these methods most interesting results have been obtained, which have already clarified some of the factors which affect growth hormone secretion. Roth, Glick, Yalow and Berson (1963 a and b) found that the level of serum HGH was raised by fasting, hypoglycæmia and exercise. It would seem that the serum HGH rises whenever the supply of glucose to the cells is diminished, and perhaps when their glucose requirement increases. The existing methods of assay are not sensitive enough to permit the detection of subnormal values of HGH, but the absence of a rise of serum HGH following the production of hypoglycæmia by insulin or tolbutamide, is likely to be useful in detecting deficient secretion of HGH (Hunter and Greenwood, 1964b).

Pathological Rôles of HGH

The most obvious rôles of HGH in disease in man are in states of dwarfism and overgrowth. Dwarfism is a marked feature of hypopituitarism, though such dwarfism does not usually manifest itself until 1-4 years of age (Martin and Wilkins, 1958). The extent to which growth hormone deficiency is responsible for other conditions of dwarfism, in which pituitary function appears otherwise normal, remains uncertain, though it is hoped that the new methods of assay of HGH will help to clarify them. The so-called primordial or constitutional dwarf would seem unlikely to

be the result of growth-hormone deficiency. These children characteristically show retarded growth from birth onwards, and they have failed to show any nitrogen retention following injections of HGH (Lipsett, Bergenstal and Dhyse, 1961), which raised the possibility of their dwarfism being due to a lack of responsiveness to a normal endogenous production of HGH. Nadler, Neumann and Gershberg (1963) have reported the case of an infant who was severely dwarfed, and who was persistently hypoglycæmic. Tests of thyroid and adrenal function were normal, but the "sulphation factor" in his serum was low. He was given HGH to which he made a dramatic response both in terms of his growth and his carbohydrate metabolism. Nadler and others have suggested that this infant was suffering from an isolated growth hormone deficiency. With regard to conditions of acromegaly and gigantism, it is to be hoped that the methods of assay of HGH now available should make the assessment of activity of these conditions simpler than it has been to date.

Other pathological conditions in which HGH might be speculated to play some part are the hormone-dependent cancers and diabetes mellitus. Atkins, Falconer, Hayward, MacLean, Schurr and Armitage (1960) showed that hypophysectomy had a slight but significant advantage over adrenalectomy in the treatment of breast carcinomatosis, and it is possible that the factor, the removal of which makes this difference, is HGH. Direct evidence that HGH plays a part in hormone-dependent cancers is difficult to acquire. Pearson and Ray (1959) gave HGH to five women suffering from disseminated cancer of the breast, two of whom complained of bone pain after the injections. However, using the urinary excretion of calcium as a criterion, Lipsett and Bergenstal (1960) were unable to find any effect of HGH.

The occurrence of permanent diabetes in experimental animals following treatment with growth hormone has raised the possibility that HGH may be implicated in the aetiology of diabetes mellitus in man. The incidence of diabetes in acromegaly is known to be raised, and was 17 per cent in Coggeshall and Root's series (1940); this is presumably due to the chronic overproduction of HGH. Some degree of hereditary predisposition seems also to be necessary, as the acromegalics with diabetes have been shown to have a stronger family history of diabetes than do those without diabetes (Coggeshall and Root, 1940). The incidence of big babies among diabetic and pre-

diabetic mothers has been thought to be possibly due to overproduction of HGH. However Jackson (1954) studied 33 babies from 11 acromegalic mothers and found that their birth weights were not as high as those of pre-diabetic mothers. Barns and Swyer (1952) injected pregnant rats with anterior pituitary extracts and growth hormone, and were unable to find any unequivocal evidence of an increase in foetal weight under the influence of growth hormone. White (1959) has measured diabetic children within three months of the onset of their symptoms, and found that they exceeded the expected height for their age by about one year. This again invites speculation as to whether growth hormone may be involved.

Luft, Olivecrona, Sjogren, Ikkos and Ljunggren (1954) reported encouraging results after hypophysectomy for the treatment of severe diabetic complications, and Poulsen (1953) noticed the regression of diabetic retinopathy in a patient who spontaneously developed Simmond's disease. Since these reports, the destruction or removal of the pituitary has been performed in several centres as a treatment of diabetic retinopathy (e.g., Hernberg, Bjorkestén and Vannas, 1959; Field, Hall, Contreras and Sweet, 1961; Joplin, Hill, Scott and Fraser, 1962), with improvement in some of the features of the retinopathy. Whether the removal of the source of HGH is the mechanism of this effect is entirely unknown.

REFERENCES

- ALMQVIST, S. (1961): Studies on Sulphation Factor Activity of Human Serum, *Acta endocr. (Kbh.)*, **36**, 31.
- ALMQVIST, S., IKKOS, D., and LUFT, R. (1961): Serum Sulphation Factor in Hypopituitarism and Acromegaly, *Acta endocr. (Kbh.)*, **36**, 577.
- ALTSZULER, N., STEELE, R., DUNN, A., WALL, J. S., and DE BODO, R. C. (1959): Diminution of Insulin Effect by Growth Hormone in Hypophysectomised Dogs; Studies with C-14 Glucose, *Amer. J. Physiol.*, **196**, 231.
- ATKINS, H. J. B., FALCONER, M. A., HAYWARD, J. L., MACLEAN, K. S., SCHURR, P. H., and ARMITAGE, P. (1960): Adrenalectomy and Hypophysectomy for Advanced Cancer of the Breast, *Lancet*, **i**, 1148.
- BARNs, H. H. F. and SWYER, G. I. M. (1952): Effects on Foetal Weights of Growth Hormone-containing Anterior Pituitary Extracts Given to Pregnant Rats, *Brit. med. J.*, **ii**, 914.
- BARRETT, R. J., FRIESEN, H., and ASTWOOD, E. B. (1962): Characterisation of Pituitary and Peptide Hormones by Electrophoresis in Starch Gel, *J. biol. Chem.*, **237**, 432.
- BECK, J. C., MCGARRY, E. E., DYRENFURTH, I., and VENNING, E. H. (1957): Metabolic Effects of Human and Monkey Growth Hormone in Man, *Science*, **125**, 884.
- BECK, J. C., MCGARRY, E. E., DYRENFURTH, I., and VENNING, E. H. (1958): The Metabolic Effects of Human and Monkey Growth Hormone in Man, *Ann. intern. Med.*, **49**, 1090.
- BENNETT, L. L., LI, C. H., and LAUNDRIE, B. (1948): Production of Nitrogen Retention in Hypophysectomised Rats by Small Doses of Hypophysected Growth Hormone, *Proc. Soc. exp. Biol. (N.Y.)*, **68**, 94.
- BERGENSTAL, D. M., and LIPSETT, M. B. (1958): The Anabolic Effect of Sheep Prolactin in Man, *J. clin. Invest.*, **37**, 877.
- BIGLIERI, E. G., WATLINGTON, C. O., and FORSHAM, P. H. (1961): Sodium Retention with Human Growth Hormone and its Subfractions, *J. clin. Endocr.*, **21**, 361.
- CHADWICK, A., FOLLEY, S. J., and GEMZELL, C. A. (1961): Lactogenic Activity of Human Pituitary Growth Hormone; *Lancet*, **ii**, 241.
- CLINICAL ENDOCRINOLOGY COMMITTEE OF THE MEDICAL RESEARCH COUNCIL (1959): The Effectiveness in Man of Human Growth Hormone. *Lancet*, **i**, 7.
- COGGESHALL, C., and ROOT, H. F. (1940): Acromegaly with Diabetes Mellitus, *Endocrinology*, **26**, 1.
- COLOWICK, S. P., CORI, G. T., and SLEIN, M. W. (1947): The Effect of Adrenal Cortex and Anterior Pituitary Extracts and Insulin on the Hexokinase Reaction, *J. biol. Chem.*, **168**, 583.
- COTES, P. M., REID, E., and YOUNG, F. G. (1949): Diabetogenic Action of Pure Anterior Pituitary Growth Hormone, *Nature (Lond.)*, **164**, 209.
- DE BODO, R. C., KURTZ, M., ANCOWITZ, A., and KIANG, S. P. (1950): Anti-Insulin and Diabetogenic Actions of Purified Anterior Pituitary Growth Hormone, *Amer. J. Physiol.*, **163**, 310.
- DOMINGUEZ, J. M., and PEARSON, O. H. (1962): Immunological Measurement of Growth Hormone in Human Sera, *J. clin. Endocr.*, **22**, 865.
- ENGEL, F. L., ALBERTSON, T., FREDERICKS, J., and LOPEZ, E. (1958): Evidence for Stimulation of Insulin Secretion by Growth Hormone in the Rat, *Endocrinology*, **63**, 99.
- FERGUSON, K. A., and WALLACE, A. L. C. (1961): Prolactin Activity of Human Growth Hormone, *Nature (Lond.)*, **190**, 632.
- FIELD, R. A., HALL, W. A., CONTRERAS, J. S., and SWEET, W. H. (1961). Hypophyseal Stalk Section in the Treatment of Advancing Diabetic Retinopathy, *New Engl. J. Med.*, **264**, 689.
- FRANKLIN, A. L., LI, C. H., and DUNN, M. S. (1947): The Amino-Acid Composition of Anterior Hypophyseal Growth Hormone, *J. biol. Chem.*, **169**, 515.
- FRANKLIN, M. J., and KNOBIL, E. (1961): The Influence of Hypophysectomy and Growth Hormone Administration on the Oxidation of Palmitate-I-C-14 by the Unanæsthetised Rat, *Endocrinology*, **68**, 867.
- FRASER, R., and HARTOG, M. (1962): Immunological Studies with Human Growth Hormone, *Ciba Found. Coll. Endocrinology*, **14**, 105.

- FREDRICKSON, D. S., and GORDON, R. S. (1958): Transport of Fatty Acids, *Physiol. Rev.*, **38**, 585.
- GEMZELL, C. A. (1959): Demonstration of Growth Hormone in Human Plasma, *J. clin. Endocr.*, **19**, 1049.
- GEMZELL, C. A., and LI, C. H. (1959): Estimation of Growth Hormone Content in a Single Human Pituitary, *J. clin. Endocr.* **18**, 149.
- GERSHBERG, H. (1957): Growth Hormone Content and Metabolic Actions of Human Pituitary Glands, *Endocrinology*, **61**, 160.
- GESCHWIND, I. I., and LI, C. H. (1955): The Tibia Test for Growth Hormone, in 'Hypophyseal Growth Hormone: Nature and Actions', pp. 28-51. New York: McGraw-Hill.
- GLICK, S. M., ROTH, J., YALOW, R. S., and BERSON, S. A. (1963): Immuno-assay of Human Growth Hormone in Plasma, *Nature (Lond.)*, **199**, 784.
- GOODMAN, H. M. (1963): Effect of Chronic Growth Hormone Treatment on Lipogenesis by Rat Adipose Tissue, *Endocrinology*, **72**, 95.
- GREENBAUM, A. L. (1953): Changes in Body Composition and Respiratory Quotient of Adult Female Rats Treated with Purified Growth Hormone, *Biochem. J.*, **54**, 400.
- GREENBAUM, A. L., and MCLEAN, P. (1953a): Mobilisation of Lipid by Anterior Pituitary Growth Hormone, *Biochem. J.*, **54**, 407.
- GREENBAUM, A. L., and MCLEAN, P. (1953b): The Influence of Pituitary Growth Hormone on the Catabolism of Fat, *Biochem. J.*, **54**, 413.
- HAVEL, R. J., and FREDRICKSON, D. S. (1956): The Metabolism of Chylomicra: I. The Removal of Palmitic Acid-I-C-14 Labelled Chylomicra from Dog Plasma, *J. clin. Invest.* **35**, 1025.
- HENNEMAN, P. H., FORBES, A. P., MOLDAWER, M., DEMPSEY, E. F., and CARROLL, E. L. (1960): Effects of Human Growth Hormone in Man, *J. clin. Invest.*, **39**, 1223.
- HERNBERG, C. A., BJÖRKESTEN, G., and VANNAS, S. (1959): Hypophysectomy in the Treatment of Retinopathy and Nephropathy in Severe Juvenile Diabetics, *Acta Endocr. (Kbh.)*, **31**, 241.
- HUNTER, W. M., and GREENWOOD, F. C. (1962): Preparation of Iodine-I-131 Labelled Human Growth Hormone of High Specific Activity, *Nature (Lond.)*, **194**, 495.
- HUNTER, W. M., and GREENWOOD, F. C. (1964a): A Radio-immunoelectrophoretic Assay for Human Growth Hormone, *Biochem. J.*, **91**, 43.
- HUNTER, W. M., and GREENWOOD, F. C. (1964b): Studies on the Secretion of Human Pituitary Growth Hormone, *Brit. med. J.*, **1**, 804.
- IKKOS, D., LUFT, R., and GEMZELL, C. A. (1959): The Effect of Human Growth Hormone in Man, *Acta Endocr. (Kbh.)*, **32**, 341.
- IKKOS, D., and LUFT, R. (1960): 'Idiophyphyseal' Diabetes Mellitus in Two Hypophysectomised Women, *Lancet*, **ii**, 897.
- JACKSON, W. P. U. (1954): The Prediabetic Syndrome. Large Babies and the (pre)Diabetic Father, *J. clin. Endocr.* **14**, 177.
- JOPLIN, G. F., HILL, D. W., SCOTT, D. J., and FRASER, R. (1962): Pituitary Ablation in the Treatment of Diabetic Retinopathy, in 'Disorders of Carbohydrate Metabolism', pp. 207-217. London: Pitman.
- KETTERER, B., RANDLE, P. J., and YOUNG, F. G. (1957): The Pituitary Growth Hormone and Metabolic Processes, *Ergebn. Physiol.*, **49**, 127.
- KINASH, B., MACDOUGALL, I., EVANS, M. A., BRYANS, F. E., and HAIST, R. E. (1953): Effects of Anterior Pituitary Extracts and of Growth Hormone Preparations on the Islets of Langerhans and the Pancreas, *Diabetes*, **2**, 112.
- KNOBIL, E., WOLF, R. C. and GREEP, R. O. (1956): Some Physiological Effects of Primate Pituitary Growth Hormone Preparations in the Hypophysectomised Rhesus Monkey, *J. clin. Endocr.*, **16**, 916.
- KNOBIL, E., and HOTCHKISS, J. (1964): Growth Hormone, *Ann. Rev. Physiol.*, **26**, 47.
- KORNER, A. (1961a): Growth Hormone, in 'Modern Trends in Endocrinology', pp. 19-43. London: Butterworths.
- KORNER, A. (1961b): The Effect of Hypophysectomy and Growth Hormone Treatment of the Rat on the Incorporation of Amino-Acids into Isolated Liver Ribosomes, *Biochem. J.*, **81**, 292.
- LARON, Z., ASSA, S., and MENACHE, R. (1963): Metabolic Activity of Individual Electrophoretic Fractions of Human Growth Hormone, *J. clin. Endocr.*, **23**, 315.
- LEE, M. O., and SCHAFFER, N. K. (1934): Anterior Pituitary Growth Hormone and the Composition of Growth, *J. Nutr.*, **7**, 337.
- LEE, M. O., and AYRES, G. B. (1936): The Composition of Weight Lost and the Nitrogen Partition of Tissues after Hypophysectomy, *Endocrinology*, **20**, 489.
- LEZNOFF, A., FISHMAN, J., GOODFRIEND, L., MCGARRY, E., BECK, J., and ROSE, B. (1960): Localisation of Fluorescent Antibodies to Human Growth Hormone in Human Anterior Pituitary Glands, *Proc. Soc. exp. Biol. (N.Y.)*, **104**, 232.
- LI, C. H., and PAPKOFF, H. (1956): Preparation and Properties of Growth Hormone from Human and Monkey Pituitary Glands, *Science*, **124**, 1293.
- LI, C. H., and LIU, W. K. (1964): Human Pituitary Growth Hormone, *Experientia*, **20**, 169.
- LIPSETT, M. B., and BERGENSTAL, D. M. (1960): Lack of Effect of Human Growth Hormone and Ovine Prolactin in Carcinoma in Man, *Cancer Res.*, **20**, 1172.
- LIPSETT, M. B., BERGENSTAL, D. M., and DHYSE, F. G. (1961): Metabolic Studies with Human Growth Hormone in Dwarfism and Acromegaly, *J. clin. Endocr.*, **21**, 119.
- LUFT, R., IKKOS, D., GEMZELL, C. A., and OLIVECRONA, H. (1959): The Effect of Human Growth Hormone in Hypophysectomised Human Diabetic Subjects, *Acta Endocr. (Kbh.)*, **32**, 330.
- LUFT, R., OLIVECRONA, H., SIOGREN, B., IKKOS, D., and LJUNGGREN, H. (1954): Therapeutic Results of Hypophysectomy in Metastatic Cancer of the Breast and in Severe Diabetes Mellitus: Adrenocortical Function after Hypophysectomy, *Ciba, Found. Coll., Endocrinology*, **8**, 438.
- LUKENS, F. D. W., and DOHEN, F. C. (1942). Pituitary Diabetes in the Cat; Recovery Following Insulin or Dietary Treatment, *Endocrinology*, **30**, 175.

- MADISON, L. L., MEBANE, D., UNGAR, R. H., and LOCHNER, A. (1964): Evidence of Stimulatory Feedback of Ketones on Pancreatic Beta Cells, *J. clin. Invest.*, **43**, 3.
- MANCHESTER, K. L., and YOUNG, F. G. (1959): Hormones and Protein Biosynthesis in Isolated Rat Diaphragm, *J. Endocr.*, **18**, 381.
- MANCHESTER, K. L., RANDLE, P. J., and YOUNG, F. G. (1959): The Effect of Growth Hormone and of Cortisol on the Response of Isolated Rat Diaphragm to the Stimulating Effect of Insulin on Glucose Uptake and on Incorporation of Amino-Acids into Protein, *J. Endocr.*, **18**, 395.
- MARTIN, M. M., and WILKINS, L. (1958): Pituitary Dwarfism: Diagnosis and Treatment, *J. clin. Endocr.*, **18**, 679.
- MILMAN, A. E., DEMOOR, P., and LUKENS, F. D. W. (1951): Relation of Purified Pituitary Growth Hormone and Insulin in Regulation of Nitrogen Balance, *Amer. J. Physiol.*, **166**, 354.
- NADLER, H. L., NEUMANN, L. L., and GERSHBERG, H. (1963): Hypoglycaemia, Growth Retardation and Probable Isolated Growth Hormone Deficiency in a One-Year-Old Child, *J. Paediat.*, **63**, 977.
- PARK, C. R., BROWN, D. H., CORNBLATH, M., DAUGHADAY, W. H., and KRAHL, M. E. (1952): The Effect of Growth Hormone on Glucose Uptake by the Isolated Rat Diaphragm, *J. biol. Chem.*, **197**, 151.
- PARK, C. R., MORGAN, H. E., HENDERSON, M. J., REGEN, D. M., CADENAS, E., and POST, R. L. (1961): The Regulation of Glucose Uptake in Muscle as Studied in the Perfused Rat Heart, *Recent Progr. Hormone Res.*, **17**, 493.
- PEARSE, A. G. E. (1952): Cytochemical Localisation of the Protein Hormones of the Anterior Hypophysis, *Ciba Found. Coll. Endocrinology*, **4**, 1.
- PEARSON, O. H., and RAY, B. S. (1959): Results of Hypophysectomy in the Treatment of Metastatic Mammary Carcinoma, *Cancer (Philad.)*, **12**, 85.
- POULSEN, J. E. (1953): Houssay Phenomenon in Man: Recovery from Retinopathy in a Case of Diabetes with Simmond's Disease, *Diabetes*, **2**, 7.
- RABEN, M. S., and HOLLENBERG, C. H. (1959): Effect of Growth Hormone on Plasma Fatty Acids, *J. clin. Invest.*, **38**, 484.
- RABEN, M. S. (1959): Human Growth Hormone, *Recent Progr. Hormone Res.*, **15**, 71.
- RABEN, M. C. (1962): Growth Hormone, *New Engl. J. Med.*, **226**, 31, 82.
- RABINOWITZ, D., and ZIERLER, K. L. (1962): Acute Effects of Human Growth Hormone on Forearm Metabolism in Man, *Clin. Res.*, **10**, 402.
- RABINOWITZ, D., and ZIERLER, K. L. (1963): A Metabolic Regulating Device Based on the Actions of Human Growth Hormone and of Insulin, Singly and Together, on the Human Forearm, *Nature (Lond.)*, **199**, 913.
- RANDLE, P. J. (1953): The Influence of Growth Hormone on Blood Insulin and Glycogen Activity, in 'Hypophyseal Growth Hormone: Nature and Actions', pp. 413-437. New York: McGraw-Hill.
- RANDLE, P. J., GARLAND, P. B., HALES, C. N., and NEWSHOLME, E. A. (1963): The Glucose Fatty-Acid Cycle, *Lancet*, **i**, 785.
- READ, C. H. (1960): The Immunologic Assay of Human Growth Hormone, *Clinical Endocrinology*, **I**, pp. 598-605. New York: Grune and Stratton.
- REISFELD, R. A., TONG, G. L., RICKES, E. L., and BRINK, N. G. (1961): Purification and Characterisation of Sheep Prolactin, *J. Amer. chem. Soc.*, **83**, 3717.
- ROTH, J., GLICK, S. M., YALOW, R. S., and BERSON, S. A. (1963a): Secretion of Human Growth Hormone: Physiologic and Experimental Modification, *Metabolism*, **12**, 577.
- ROTH, J., GLICK, S. M., YALOW, R. S., and BERSON, S. A. (1963b): Hypoglycaemia: a Potent Stimulus to Secretion of Growth Hormone, *Science*, **140**, 987.
- RUSSFIELD, A. B., REINER, L., and KLAUS, H. (1956): The Endocrine Significance of Hypophyseal Tumours in Man, *Amer. J. Path.*, **32**, 1055.
- SALMON, W. D., and DAUGHADAY, W. H. (1957): A Hormonally Controlled Serum Factor which Stimulates Sulphate Incorporation by Cartilage *in vitro*, *J. Lab. clin. Med.*, **49**, 825.
- SALTER, J., and BEST, C. H. (1953): Insulin as a Growth Hormone, *Brit. med. J.*, **ii**, 353.
- UTIGER, R. D., PARKER, M. L., and DAUGHADAY, W. H. (1962): Studies on Human Growth Hormone, I. A Radio-Immuno-Assay for Human Growth Hormone, *J. clin. Invest.*, **41**, 254.
- WAGNER, E. M., and SCOW, R. O. (1957): Effect of Insulin on Growth in Force-fed Hypophysectomised Rats, *Endocrinology*, **61**, 419.
- WELT, I. D., and WILHELMI, A. E. (1950): Effect of Adrenalectomy and of Adrenocorticotrophic and Growth Hormones on the Synthesis of Fatty Acids, *Yale J. Biol. Med.*, **23**, 99.
- WHITE, P. (1959): Diabetic Children and their Later Lives, in 'Treatment of Diabetes Mellitus', pp. 655-690. London: Henry Kimpton.
- WILHELMI, A. E. (1955): Comparative Biochemistry of Growth Hormone from Ox, Pig, Horse and Fish Pituitaries, in 'The Hypophyseal Growth Hormone: Nature and Actions', pp. 59-69. New York: McGraw-Hill.
- WILLIAMSON, J. R., and KREBS, H. A. (1961): Aceto-Acetate as Fuel of Respiration in the Perfused Rat Heart, *Biochem. J.*, **80**, 540.
- YOUNG, F. G. (1945): Growth and Diabetes in Normal Animals Treated with Pituitary (Anterior Lobe) Diabetogenic Extract, *Biochem. J.*, **39**, 515.
- ZAHND, G. R., STEINKE, J., and RENOLD, A. E. (1960): Early Metabolic Effects of Human Growth Hormone, *Proc. Soc. exp. Biol. (N.Y.)*, **105**, 455.