GROWTH HORMONE[1]

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Pituitary growth hormone, alternatively known as somatotropin, is an anabolic agent which promotes the growth of virtually all tissues except the brain and other nervous tissue and the eyeballs; it is, in addition, a prominent factor in the regulation of the metabolism of fat and carbohydrate. The relation of the metabolic to the anabolic action of growth hormone is not as yet understood; but, in each of these areas, as well as in regard to the regulation of secretion, the species specificity, and the chemistry of the hormone, much new information has accrued in recent years.

Physicochemical and immunological properties.—The species-specific nature of growth hormone is suggested by the findings that fish pituitary growth hormone is inactive in rats [Wilhelmi (1)], and that the monkey does not respond to bovine growth hormone [Knobil & Greep (2)]. The concept of species-specificity has been well supported by the evidence of differences in physicochemical and immunological properties of growth-hormone preparations from various species and by the demonstration of effects in man with primate growth hormone which had been unobtainable with animal preparations [Beck et al. (3); Raben (4, 5)].

The differences in the chemistry of the growth hormones from various species are such that the hormones differ in their antigenicities and in the range of animals in which they can produce biological responses. They differ also in isoelectric points, N-terminal and C-terminal amino acid residues, and amino acid composition. Their molecular weights range from 21,500 for human (6) to 47,400 for ovine (7) growth hormone. According to the delay on gel filtration, the molecular weights of bovine, ovine, porcine, and human growth hormone preparations would be estimated to be considerably less than the values indicated by ultracentrifugation (8, 9). The precise structure of the protein has not yet been determined for any species.

Despite the marked differences in physicochemical properties, the rat responds to many growth hormones, including human, simian, porcine, bovine, ovine, and cetaceous, indicating that the hormones must have properties in common. Essentially the same extraction methods have been applied to obtain growth hormones from many species, a fact which also indicates the basic similarity of the growth hormones. Recently, it was claimed that pepsin digestion of bovine growth hormone gave a component with similar mobility to that of undigested human growth hormone on agar gel electrophoresis and that this component gave a positive hemagglutination reaction with antiserum to human growth hormone (10). This would suggest that a section of

[1] The survey of literature pertaining to this review was concluded on July 15, 1964.

the molecule was common to both species. That primate growth hormone is both biologically active and antigenic in the rat suggests that different parts of the protein are responsible for biological and antigenic determinants.

When purified growth-hormone preparations were submitted to electrophoresis in starch gel, they were separated into several bands (11). Five human growth-hormone preparations, extracted by different methods, contained four identical major bands in common. The components of two human growth-hormone preparations, separated on starch gel, were tested by tibia assay, and all of the components tested were active (12). It was also shown that the two bands of a bovine growth-hormone preparation were equally active.

The separable components of the various growth hormones seem to be slightly different in physicochemical properties, but they still have the same biological activities and immunochemical properties. When antiserum to human growth hormone was tested against two human growth-hormone preparations made by different methods and against freshly prepared saline homogenates of human pituitary glands, the lines of precipitation formed during immunodiffusion completely fused without any spur formation, and only a single precipitin arc was demonstrated by immunoelectrophoresis (13). By comparing the freshly prepared saline extracts of human pituitary glands with a preparation of purified human growth hormone, however, it was observed that the arc of precipitation on immunoelectrophoresis was shorter with the saline extracts and lacked the more anodal components of the arc to purified growth hormone (13). Heterogeneity was also shown by the detection of at least five precipitin lines by immunodiffusion on cellulose acetate (14).

The source of this heterogeneity has been the subject of much discussion. It could be due to genetic heterogeneity, to polymorphism of native hormone, or to denaturation or other chemical alteration either after death, during storage, or during the purification procedure. Recently, the detection, in the serum of patients, of antibody to exogenously administered human growth hormone (15), raised the same problem as well as the question of whether there is a difference between the hormone in the pituitary and circulating human growth hormone. The difference between fresh extracts of human pituitary glands and purified growth-hormone preparations by electrophoresis and the formation of additional bands during storage of growth hormone solutions, indicate a chemical change in human growth-hormone preparations during storage and purification, but the presence of multiple components in simple buffer extracts of fresh pituitaries (12) suggests that some heterogeneity is intrinsic in the hormone. All the components so far separated fall within the same family of peptides sharing the same biological activity and common antigenic determinants and may conceivably vary by no more than a difference in number of amide groups.

Growth hormone, prolactin, and the "placental lactogen."—The existence of a pituitary growth hormone was not readily believed by all, and Riddle,

in particular, resisted its acceptance. He and his associates worked with pigeons and found that, in this species, prolactin supported the growth of body and viscera. Several more recent reports have claimed stimulation of growth in the rat with prolactin (16); and effects in man, similar to those obtained with human growth hormone and unobtainable with animal growth hormone, including positive nitrogen balance and hypercalciuria, have been seen with ovine prolactin [Bergenstal & Lipsett (17); Beck (18)]. Certain actions seen *in vitro* with growth-hormone preparations, such as increased uptake of amino acids by diaphragm and glucose by fat pads, have also been reproduced with prolactin preparations. In each case in which prolactin simulated growth hormone, the dose required for prolactin was much larger.

Although growth hormone from animal pituitaries has been prepared free of prolactin, human and simian growth-hormone preparations have been found to be about 10 percent as potent in prolactin-like activity as purified ovine prolactin. Ferguson & Wallace recovered the protein from each of three separate bands yielded by a human growth-hormone preparation on starch gel electrophoresis and found growth-promoting and crop sac-stimulating activities in each fraction, in similar although not identical proportions (12). Chen & Wilhelmi prepared fractions from human pituitaries with different ratios of growth-promoting to prolactin-like activities, but no preparation of human growth hormone has been obtained free of prolactin activity, and no prolactin preparation has been made which is not a fairly potent growth hormone. It is of interest, in regard to these unresolved problems regarding the relationship of growth hormone to prolactin, that both hormones are believed to be produced by the eosinophilic cells of the pituitary, and that there is a striking similarity of electrophoretic mobility on starch gel of ovine prolactin and human growth hormone, but there is no immunological cross-reaction between the two substances. It has been suggested that bovine growth hormone may be enzymatically converted to prolactin (19), but definitive proof of this is lacking.

In 1961, four papers appeared in Japanese journals declaring that placental extracts produced somatotropic and prolactin-like effects (20–23). Shortly thereafter, Josimovich & McLaren (24) reported that extracts of human placenta reacted with antiserum to human growth hormone and Showed evidence of antigenic partial identity with the growth hormone on Ouchterlony plates. The affinity for the antibody has since been shown to be only about 1/10,000 that of the growth hormone. The placental substance was termed "placental lactogen." It differed from human chorionic gonadotropin, which also cross-reacts with human pituitary hormone, luteinizing hormone, but appeared, according to Sciarra, Kaplan & Grumbach (25), to be synthesized in the same cells, the syncytiotrophoblasts. The substance is present in the placenta as early as the twelfth week of pregnancy, and it has also been detected in pregnancy serum and in retroplacental blood. The concentration in pregnancy serum would appear to reach concentrations as high as 5000 mμg per ml, measured by complement fixation.

Partial purification by Josimovich yielded material with both growth-promoting and crop sac-stimulating activity; but further purified antigenically cross-reacting material did not stimulate growth itself but enhanced the potency of human growth hormone in hypophysectomized rats. The prolactin-like activity of the purified material was about half that of ovine prolactin. Friesen (26) purified human placental extracts by gel filtration, and his purified product was shown to have a mobility on starch gel electrophoresis different from that of human growth hormone, but the molecular weights, judged from gel filtration and the amino acid compositions, were very similar. Growth-promoting activity was found in the product of some batches but not in others. An "insulin-like" action, also seen with growth hormone on isolated adipose tissue, promoting glucose oxidation and incorporation into triglyceride, was obtained even with batches which were not growth-promoting. It has been speculated that the placental factor may be an anabolic agent of pregnancy and that it may also be responsible for the elevated free fatty acids and impaired glucose tolerance found prepartum.

Amino acid transport.—The regulation of active transport of amino acids through the cell membrane has attracted attention as a possible initial step in the stimulation of protein synthesis by growth hormone. Growth-hormone treatment resulted in increased concentration of injected AIB (α-amino-isobutyric acid) in intracellular fluid, as compared to that of plasma in various tissues of adult female rats [Noall, Riggs & Walker (27)]. Hypophysectomy reduced the intracellular accumulation of AIB, while growth-hormone administration restored it toward normal within 1 hr (28). The *in vitro* uptake of AIB by isolated intact diaphragm was also reduced in tissues from hypophysectomized rats; treatment or *in vitro* addition of growth hormone restored the greatly reduced uptake of AIB (29, 30). Cut diaphragm also responded to growth hormone *in vitro*, but the response was less than with intact tissue. A concentration of bovine growth hormone as low as 1 μg per ml in the incubation medium caused unequivocal stimulation with intact diaphragm (31). The action of growth hormone on the amino acid transport system was very rapid and persistent, since only a brief dipping in a dilute growth-hormone solution was necessary, and prolonged washing after exposure to growth hormone did not eliminate the effect (32).

Both growth hormone and insulin increase uptake of amino acids by diaphragm, but differ in the amino acids which they influence. Insulin increases the uptake of glycine, serine, threonine, methionine, proline, and hydroxyproline in addition to AIB (33), whereas growth hormone increases the intracellular accumulation of glycine, alanine, serine, threonine, proline, histidine, tryptophan, glutamine, and asparagine, but not that of methionine, valine, tyrosine, phenylalanine, leucine, lysine, arginine, or the dicarboxylic amino acids (34).

Growth hormone increases the incorporation of certain amino acids into protein without measurable change in the size or specific activity of their intracellular pools. This may be attributable to the presence of an intra-

cellular pool which turns over rapidly and which may well be different from the total or expandable pool of the amino acid. An amino acid, upon entering the cell, may be rapidly activated to form a complex with soluble RNA without being mixed with the entire measurable pool.

Thus, it seems now clear that growth hormone increases the intracellular accumulation of certain amino acids against a concentration gradient. Although a parallelism between the level of intracellular amino acid and protein synthesis was demonstrated in the studies of Riggs & Walker (35), the relationship of amino acid transport to protein synthesis is not well defined. Evidence of a functional dissociation between the two steps has been reported. The blocking of protein synthesis by puromycin did not interfere with the AIB transport by diaphragm (36). Conversely, sodium-free medium inhibited the *in vitro* accumulation of AIB or glycine and yet growth hormone, added *in vitro*, stimulated the incorporation of glycine into protein in hypophysectomized rat diaphragm (37). Since there is some evidence that the intracellular amino acids are not all equally available for protein synthesis, the physiological meaning of increased total amino acid pool cannot be easily evaluated. Whether the regulation of protein synthesis by the hormone is primarily related to increased availability of amino acids remains to be elucidated.

Protein synthesis.—It has, naturally, been of major interest to explore the action of the hormone on the processes intimately concerned with the synthesis of protein. In the same experimental design as used for amino acid uptake, growth hormone was administered along with isotopically-labeled amino acids to hypophysectomized rats, and increased incorporation into protein was demonstrated (38, 38a). *In vitro* incorporation of amino acids into protein of diaphragm (39, 39a) and levator ani (40) was also shown to be augmented by chronic treatment with pituitary growth hormone. A single injection of the hormone 3 hr before sacrifice failed to cause an effect (40). In an attempt to observe a direct action on protein synthesis, growth hormone was added to isolated diaphragms from hypophysectomized rats, resulting in increased incorporation of labeled amino acids into protein (40–42). Levator ani muscle responded in a similar way (40). Kostyo & Knobil demonstrated that simian growth hormone, added *in vitro*, consistently increased the incorporation of leucine into the protein of diaphragms from hypophysectomized rats (41). The same amount of porcine and bovine growth hormone produced inconsistent effects. Growth hormone, added *in vitro*, increased the incorporation of C^{14} from palmitate into protein but not from acetate (43).

The extensive studies of Korner have attempted to define the mechanism of the growth hormone effect on protein synthesis. He demonstrated that a cell-free system, prepared from hypophysectomized rat liver, was less active than normal in incorporating leucine-C^{14} into protein, and treatment with growth hormone restored it toward normal (44). It was shown that the liver microsomes, and not the amino acid activating enzymes or the sRNA of the soluble fraction (44), were responsible for the altered ability to incorporate

protein. The control by the hormone could be explained by an effect on the ability of ribosomes to assemble activated amino acids into polypeptide chains (45). Korner also presented evidence that growth hormone treatment stimulated orotic acid incorporation into nuclear and ribosomal RNA, including rapidly labeled mRNA; he concludes that "the effects of growth hormone on protein synthesis and growth can be explained in terms of its control of m-RNA synthesis" (46). This conclusion is supported by Talwar, Gupta & Gras (47).

Observations, showing that hypophysectomy and growth-hormone treatment alter sulfate incorporation into cartilage, led to the detection in normal serum of a nondializable substance which stimulates the incorporation of sulfate into the mucopolysaccharides of hypophysectomized rat cartilage. The substance has been termed the "sulfation factor" (48). The factor is low in the serum of hypophysectomized rats and appears after treatment with growth hormone. Sera from hypopituitary patients contain very little, although it is increased in acromegalics (48). Growth hormone also stimulates in the collagen of hypophysectomized rat cartilage, the conversion of proline to hydroxyproline (49). The similarities of the sulfation and hydroxyproline factors are notable, since direct addition of growth hormone to the incubation medium has little effect in either case, and both factors appear in the serum after growth-hormone treatment. The sulfation factor reappears in the serum of hypophysectomized rats in 2 to 6 hr after treatment with growth hormone and continues to increase in amount for 24 hr.

In studies on protein synthesis, growth hormone was given to the animals several hours to several days before sacrifice. Stahelin, upon injection of a single dose of growth hormone 3 hr prior to sacrifice, found only an extremely small stimulation of protein synthesis by levator ani muscle, as compared to a marked effect after 5 days of treatment (40). In view of the time lag in stimulating protein synthesis *in vivo* and the example provided by the delayed and indirect action on sulfate and proline incorporation, the possibility exists that the effect of growth hormone on protein synthesis might not be a direct one but rather mediated through some active intermediate(s).

However, direct actions of growth hormone on amino acid uptake and incorporation into protein have been shown, so that the hormone is at least capable of stimulating protein synthesis directly in an isolated tissue. The effects on isolated diaphragm were of a lesser magnitude compared with *in vivo* administration of the hormone. It is noteworthy that, in *in vitro* experiments, the stimulus to protein synthesis was evident in periods of less than 90 min, which would not be sufficient time for an effect after administration *in vivo*.

A speculative explanation of the discrepancy between the *in vivo* and *in vitro* stimulation of protein synthesis by growth hormone is suggested by the recent work of Hamilton on estradiol-induced RNA and protein synthesis in ovariectomized rat uterus (50). A small rise was observed in protein synthesis between 30 min and 1 hr after the injection of estradiol, and then marked

stimulation occurred reaching a peak 3 to 4 hr after the injection. The first small rise was not inhibited by actinomycin D. The time sequence and the magnitude of the biphasic response could well be correlated with the *in vivo* and *in vitro* effects of growth hormone. The *in vitro* effect, being of a lesser degree and observed within 90 min, coincides with the first increase; and the *in vivo* effect, being markedly greater and requiring some latent period, coincides with the second peak.

Fat mobilization.—The relation between fat metabolism and growth hormone is seen most clearly in the fat-mobilizing action of the hormone. Small amounts of growth hormone were found to elevate the fasting-free-fatty acid level in the plasma of man, dog (4, 51), and monkey (2) and, to a lesser extent, in the rat (52). This evidence of relatively rapid fatty acid mobilizing activity supported the inference from less direct observations of fat mobilization, such as reduction in fat stores due to growth hormone, increased fat deposition in liver, promotion of ketosis, and a depression of respiratory quotient. An increased output of free fatty acid *in vitro* by adipose tissue was found, when the hypophysectomized rat was pretreated with growth hormone (53, 54).

Despite the prominent fatty acid mobilizing activity *in vivo*, the effect of growth hormone added *in vitro* is by no means clear. The large amount of growth-hormone preparations required *in vitro* makes it uncertain as to whether lipolytic activity is stimulated by growth hormone or by contaminating substances, particularly in view of the extreme sensitivity of rat adipose tissue to the direct addition of ACTH (55) or TSH (56). Moreover, the lipolytic potency varies with different preparations of growth hormone of similar anabolic activity. However, the *in vitro* behavior of the adipose tissue probably does not simulate precisely that of the tissue *in vivo*. If the appropriate *in vitro* conditions were provided, perhaps requiring addition of a suitable combination of insulin, sympathomimetic substance, and others to mimic the more elaborate *in vivo* conditions, it might be possible to reproduce the fat mobilizing characteristics of the hormone. Alternatively, growth hormone may exert its adipokinetic effect through an intermediary substance, comparable to its effect on sulfate uptake via the "sulfation factor."

Pituitary peptides other than growth hormone also possess fat mobilizing activity, particularly ACTH, alpha and beta melanocyte stimulating hormone, TSH, peptides I and II, or Fraction H (55–59). These substances, like catecholamines, very rapidly elevate plasma-free fatty acid in responsive species and are very potent in stimulating its release from adipose tissue *in vitro*, and at least ACTH enhances the activity of a lipase in adipose tissue upon brief *in vitro* exposure (55). The pattern of growth-hormone effect is unique: the increase in plasma-free fatty acid begins only after about 2 hr, and the maximal concentration is not reached until at least 4 hr. Its increase after growth hormone is preceded by a slight fall, at 30 min, with a concomitant fall in blood sugar.

Long-term treatment with growth hormone results in a reduction in total

carcass fat (60, 61). Reduced lipogenesis, as well as increased mobilization of fat, may contribute to this result. Inhibition of lipogenesis in the liver has been demonstrated in some experiments (62–64) but not in others (65, 66). The effect on lipogenesis has been shown to be dependent on the nutritional condition of the experimental animal (67). Using hypophysectomized rats which had been on high carbohydrate and low fat diet, Goodman reported that bovine growth hormone administered for a period of 4 days reduced the epididymal and omental fat content as well as lipogenesis *in vitro* from glucose or leucine (68). On the other hand, growth hormone, added *in vitro* (69, 70), had an "insulin-like" effect, increasing fat synthesis from glucose and leucine.

It may be reasonable to subordinate some of the metabolic effects of growth hormone to its fat mobilizing action including increased liver fat, ketosis, increased content of glycogen in heart and muscle, impaired glucose tolerance and insulin responsiveness, and diabetes. This interpretation, in the case of the effects on carbohydrate, is based on the finding, in perfused rat heart (71–73), that long chain fatty acids and ketone bodies are preferred to glucose as substrates. An increased supply of acetyl CoA from fatty acids may inhibit decarboxylation of pyruvate, thus diminishing the oxidative metabolism of carbohydrate and favoring accumulation of glycogen and eventually of glucose. Since insulin does not correct the impairment of pyruvate oxidation, insulin-resistance would be encountered. The abundant supply of fatty acid to the liver may be sufficient explanation for the increased ketone formation. The metabolic changes brought about by excessive fat mobilization can be shifted toward normal by insulin by its effect of reducing fatty acid release from adipose tissue. The extreme sensitivity of the partially pancreatectomized dog and of the hypophysectomized severely diabetic human to the metabolic effects of growth hormone attest to the importance of this action of insulin in modulating the response to growth hormone. Indeed, the interplay and proper balance of insulin and growth hormone seem to be most important for the normal regulation of fat and carbohydrate metabolism.

The importance of fat mobilization by growth hormone for its anabolic action is suggested by the fact that growth hormone-treated rats, on a calorically inadequate diet, can grow at the expense of the oxidation of their own fat, and growth ceases when the adipose tissue stores are exhausted (60). A parallelism between protein anabolism and fat catabolism is also indicated by the increase in protein and decrease in fat content in the carcass, with growth-hormone treatment. Hypophysectomized rats lose less fat and more protein when fed inadequately than do pair-fed normal rats. With fasting or when caloric intake is low, fat mobilization is fundamental in protein sparing and, thus, is anabolic of itself. By possessing the biphasic properties of mobilizing fat and stimulating protein synthesis, growth hormone is indeed an ideal anabolic agent.

Interrelationship of growth hormone and insulin.—In some respects, growth hormone is insulin-like and in others, it is insulin-antagonistic. At times,

insulin-like and antagonistic actions can be shown in regard to the same process, for example, the stimulation of fat synthesis with growth hormone *in vitro* and inhibition by chronic treatment of the animal. When both types of action are demonstrable, insulin-antagonism appears to be the more physiologically important. Indeed, there has been a lingering suspicion, with some experimental support, that some insulin-like actions are caused by impurities in growth-hormone preparations, but until proven, such effects must be attributed to growth hormone.

Growth hormone *in vitro*, like insulin, stimulates the uptake, oxidation, and conversion to fatty acids of glucose and leucine by adipose tissue from hypophysectomized rats (69, 70). Insulin has been shown to be anabolic, as has been reviewed by several authors [see Randle (74)]. Both growth hormone and insulin stimulate amino acid transport and also accelerate its incorporation into protein *in vitro*. Insulin also shares, with growth hormone, the activity of stimulating protein synthesis by rat liver microsomes (75). It has even been claimed that the anabolic action of growth hormone may be mediated by insulin (76). However, enough evidence exists to favor an anabolic action of growth hormone independent of insulin.

The independence of the action of growth hormone is indicated by a variety of observations. Growth hormone stimulated amino acid uptake (77) and the appearance of sulfation factor (78) in alloxan diabetic hypophysectomized rats. Antiserum to insulin abolished the stimulatory effect of insulin on amino acid incorporation *in vitro*, but not that of growth hormone (42). Growth hormone stimulated protein synthesis and weight gain in hypophysectomized pancreatectomized rats maintained on constant amounts of insulin (79). Growth hormone differed from insulin in its *in vitro* effect on carbohydrate metabolism in adipose tissue (70); glycogen synthesis and mannose utilization were stimulated by insulin but not by growth hormone, and the nonutilizable glucose analogue α-methyl glucoside inhibited the effect of insulin but not that of growth hormone.

A striking difference exists in the action of insulin and growth hormone on fatty acids in adipose tissue: insulin inhibits fatty acid release by promoting re-esterification, when glucose is present, and by a direct hormonal inhibition of the activation of lipase by other agents; on the other hand, growth hormone stimulates fatty acid mobilization. Insulin is a potent stimulus to fat formation and storage, and growth hormone acts to release fat stores and perhaps to diminish their formation.

Growth of hypophysectomized rats can be stimulated by insulin (80), but only with increased food intake and an excessive amount of fat formation. The effect of insulin might be attributable entirely to increased food intake, since forced feeding can produce the same pattern of weight gain and composition of gain as insulin (81).

Thus, a clear qualitative difference exists between the effect of growth hormone and that of insulin or forced feeding, in that growth hormone can exert its anabolic effect without a high caloric intake or even with an inade-

quate caloric supply as long as depot fat is present, and unlike insulin it reduces fat stores. Despite its flaws, the attractiveness of the hypothesis, that insulin rather than growth hormone exerts the anabolic effect, is in the not unreasonable supposition that if growth hormone did nothing but prevent the accumulation of fat and promote its discharge as fatty acids, insulin could exert an anabolic action, without the caloric drain of enlarging fat stores.

Clinical use of growth hormone.—Growth in man is influenced by several hormones and by nutrition, but, as in other species, there is a specific requirement for growth hormone to obtain normal growth. Hypopituitary children may grow at nearly normal rates for the first year or two, but from then on grow at markedly subnormal rates. At age 17, a pituitary dwarf may be 50 in tall, but since such persons also lack gonadotropins and remain sexually immature, a slow rate of growth may continue for several more decades. Eventually, a normal height may even be attained; however, the limbs will be disproportionately long for the size of the torso. In the rat, growth virtually ceases if hypophysectomized at 6 wk of age and resumes if growth hormone is administered.

Human pituitary dwarfs can be made to grow at a normal rate, and at times faster than normal, with injections of human growth hormones (5) but not with animal growth hormone. In one instance, a male hypopituitary subject who measured 51 in at age 17 attained a height of 66 in after 5½ years of treatment with 2 to 5 mg of human growth hormone injected intramuscularly three times a wk. This patient, as have others treated with the hormone, developed antibodies to it (15), but this did not seem to interfere with the growth response. Patients receiving insulin typically develop antibodies which do not prevent their responsiveness to the hormone, and perhaps the pattern with growth hormone will prove similar. However, some pituitary dwarfs have responded less well than others, and, in one instance, a diminishing response to human growth hormone was associated with a high titer of antibody (82). The appearance of antibodies is of more concern in relation to attempts to treat short normal children with growth hormone. Such children often grow more rapidly when treated with the hormone, but it is still questionable whether substantial increases in adult height can be achieved in short individuals who are not hypopituitary.

A child who suffered from hypoglycemia and short stature and was thought to have an isolated deficiency of growth hormone was reported to show improvement, both of the hypoglycemia and of growth rate, with human growth hormone. It was also shown to improve hypoglycemia in other children and in adults (83). One report indicated an improvement in nitrogen balance in burned patients after receipt of the hormone, but there has been little investigation, as yet, of the use of human growth hormone as an anabolic agent.

The effects which have been produced with the hormone in man include

in addition to stimulation of growth, evidence of anabolism from balance studies as indicated by storage of nitrogen, phosphorus, potassium, and, less regularly, of calcium and sodium; decrease in blood urea nitrogen; a slow increase in serum phosphorus and alkaline phosphatase; increase in calcium in the urine; increase in hydroxyproline in the urine; increase in plasma free fatty acids (after a transient fall) and evidence of ketogenesis; diminution in insulin sensitivity and either no change or decrease in glucose tolerance; transient fall in blood sugar; increase in urinary citrate; enlargement of chloride space; increase in renal clearance of inulin, creatinine, and para-aminohippurate; increase in Tm_{PAH}; and increase in serum "sulfation factor."

Regulation of growth hormone secretion.—The development of methods for the immunoassay of growth hormone has made it possible to study the control of growth-hormone secretion. The growth-hormone content of human serum has been measured by hemagglutination inhibition, by complement fixation, and by use of radioiodinated antigen with separation of free and bound hormone by chromatoelectrophoresis or precipitation. The original estimates of Read (84) have been revised downward by Hunter & Greenwood (85), Berson et al. (15), and Pearson (86), and others. It now appears that the serum content after an overnight fast is of the order of 1 to 2 mμg per ml, and that children and adults have similar amounts of circulating growth hormone. Acromegalic patients have greater amounts, commonly about 10 times the normal value.

The immuno-assay has yielded some quite unanticipated information about growth-hormone secretion, particularly the indications that secretion fluctuates rapidly with changes in metabolic state. Roth et al. (87) found that hypoglycemia is a potent stimulus to growth-hormone secretion. After intravenous insulin, causing a minimal blood sugar value at 30 min, plasma growth hormone increased rapidly to typical values of 25 to 50 mμg per ml 30 min later. Hypoglycemia induced without insulin, and interference with glucose utilization without hypoglycemia, through the use of deoxyglucose, also increased growth hormone. Exercise was another rapid stimulus to growth-hormone secretion, not attributable to hypoglycemia, and feeding glucose rapidly decreased the blood value. The response to hypoglycemia was diminished by pretreatment with corticosteroids (88). Plasma growth hormone rose progressively with fasting; after 60 hr, the concentration was 8 mμg per ml, falling to 1 mμg 1 hr after refeeding. Obese subjects responded normally to hypoglycemia, but did not respond to fasting or to exercise with an increase of growth hormone.

After stalk section of the pituitary, both the stimulating effect of hypoglycemia and the suppressive effect of hyperglycemia were absent (89). Electrolytic lesions in the anterior median eminence, but not posteriorly placed lesions, prevented the response to hypoglycemia in monkeys (90). These suggestions, that the control of growth-hormone secretion operates

through a suprahypophyseal center, are supported by evidence that hypothalamic tissue contains a factor which stimulates the release of growth hormone from the pituitary (91).

The stimuli to growth-hormone secretion, which have been reported, seem logical in respect to the fat-mobilizing action of the hormone, but less intelligible in respect to its function of regulating the chronic process of growth. The conditions which stimulate growth-hormone secretion are those in which the release of fatty acids is desirable and in which an increase of plasma fatty acids normally occurs. The rapid stimuli to growth-hormone secretion are related to a metabolic rather than anabolic need for growth hormone. The slower stimulus of fasting may serve both needs, since adequate provision of calories to tissues through fatty acid mobilization would help keep protein loss to a minimum.

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