Human Growth Hormone

A Preliminary Report

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INTRODUCTION

The clinical picture of acromegaly has intrigued physicians since the first description by Vicenzo Brigidi in 1877,[1] and the better known description by Pierre Marie in 1891.[2] This natural experiment in hyperpituitarism demonstrated that the human pituitary produced a growth factor effective in adults as well as in children. Despite the striking clinical alterations in acromegalic patients the only major chemical abnormalities consistently recorded in these patients have been the elevated inorganic serum phosphorus and the impaired glucose tolerance with insulin resistance. A frequent paradoxical observation has been the hypercalciuria of acromegalic patients who would be expected to conserve calcium for their obvious new bone formation. A little-emphasized but intriguing question underlying this bizarre clinical picture concerns the physiological role of growth hormone in normal adults. This then is part of the background to which recent studies with human growth hormone (HGH) pertain.[3-9]

METHODS

The demonstration by Knobil in 1954[10a] and 1957[10b] that beef growth hormone was ineffective in monkeys whereas monkey growth hormone was effective indicated that species specificity was probably responsible for the previous failures to demonstrate activity of beef growth hormone in man. Accordingly, collections were begun of human pituitaries obtained at autopsies and from this material human growth hormone was extracted by Drs. Raben,[11] Li,[12] Wilhelmi,[13] and a group in England.[9] At least four different methods of chemical isolation have been devised. Our studies have utilized an acid-soluble extract prepared by Dr. Raben. This preparation is well-tolerated and appears to be free of significant contamination with other anterior pituitary hormones.

The data which follow concern balance studies and measurements of levels of intermediary carbohydrate and fat metabolites in the blood and urine of fourteen patients during both brief and prolonged administration of HGH. Studies have ranged in duration from six days to eighteen months and have been carried out* upon four children with hypopituitarism, one young adult with congenital hypopituitarism, one elderly individual with idiopathic acquired hypopituitarism, an infant with hyperinsulinism,† a markedly obese middle-aged woman, a cachectic man with malignant melanoma, a young woman with gonadal dysgenesis and short stature, a young woman with Addison's disease, and three adult patients with mild diabetes mellitus.‡ The analytical and balance study technics have been described previously.[14,15]

RESULTS: CLINICAL AND BALANCE DATA

1. *Growth.* Growth hormone, 1-2 mg. daily, has been administered to three children with hypopituitarism for nine to eighteen months each. Growth rates in these three cases increased from 0.20, 0.20, and 0.25 cm. per month to 0.35, 1.0, and 0.4 cm. per month respectively. Treatment has been withdrawn in two children for prolonged periods with return to previous growth rates. Growth appears to have been somewhat more rapid the first six months of treatment than the second six months of treatment in each patient. Growth appears to have been somewhat greater during a treatment schedule consisting of HGH 2 mg. daily for "two weeks on" and no treatment for "two weeks off" repeated in a cyclical fashion than the growth rate observed during treatment with HGH 2 mg. every other day without interruption.

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*Studies were carried out on the metabolic ward of the Massachusetts General Hospital.

†Studied in cooperation with Dr. John Crigler at the Childrens Medical Center, Boston.

‡Two of these patients were studied on the metabolic ward of the Royal Victoria Hospital, Montreal, in cooperation with Drs. J. C. Beck and E. McGarry.

2. *Balance Data.* The administration of 0.2-10 mg. human growth hormone daily regularly produced retention of nitrogen, phosphorus, sodium, and magnesium in the same proportion as these substances occur in general protoplasm.[14] Nitrogen retention was absent to slight the first day of treatment, reached a maximum the third to fifth days, continued at levels of 2-6 gm. per day for two to three weeks, and in the fourth and fifth weeks of therapy gradually waned so that in the sixth week of therapy nitrogen retention was barely evident in the balance data. In one patient HGH was omitted from the sixth to eighth weeks and then resumed with prompt return of sustained nitrogen retention. On discontinuing growth hormone there was slight negative nitrogen balance, but losses of nitrogen did not approach the quantities retained during HGH. The changes in nitrogen excretion were matched by a progressive fall in the blood urea nitrogen to approximately 50 per cent of control level. On the other hand, serum inorganic phosphorus gradually rose 1 to 2 mg. per 100 ml. during prolonged treatment.

Although potassium retention in general paralleled nitrogen retention, it is noteworthy that potassium retention greater than that predicted from the nitrogen balance occurred the first day on treatment, and disproportionate loss of potassium occurred the first day off therapy. This peculiar response recalls the disproportionate loss and retention of potassium the first day on and the first day off cortisone respectively.

Calcium metabolism deserves special description. During HGH the urinary calcium gradually rose to levels two to three times control. This unexpected increase in urinary calcium appeared to be accounted for by an almost identical simultaneous decrease in fecal calcium, and calcium balance was little altered. Whereas nitrogen retention waned on prolonged HGH administration, hypercalciuria and increased calcium absorption continued unabated. On discontinuing HGH the urinary calcium promptly fell to nearly zero and fecal calcium remained low for at least eighteen days. Thus, marked calcium retention occurred following HGH in contrast to the minimal retention observed on HGH. It should be noted that this pattern of calcium metabolism was observed not only in the hypopituitary children with open epiphyses, but in the adult hypopituitary patient with closed epiphyses and in the adult obese patient with closed epiphyses.

3. *Theoretical Weight Curves.* In careful balance studies on stable patients there is generally an excellent correlation between changes in the weight of the patient and changes in nitrogen, sodium, and potassium balance. During growth hormone administration the "theoretical weight"[14] predicted from the balance data and from the weight changes of the fore-control consistently diverged from the actual weight of the patient. In each case actual weight failed to increase as much as predicted from the balance data. This discrepancy appeared rapidly during the first week of therapy and changed very slowly thereafter.

INTERPRETATIONS OF BALANCE DATA

The observed increased growth rate and retention of nitrogen and other constituents of protoplasm indicate that human growth hormone is effective in man. Special features are retention of nitrogen at a rate far greater than the rate observed in normally growing children and waning of the nitrogen response on prolonged administration. The pattern of calcium metabolism suggests that growth hormone increases calcium absorption but, at least initially, does not promote calcium deposition in bone. This recalls the assay in rats in which growth hormone stimulates chondrogenesis with widening of the epiphyseal plate but, at least initially, produces little evidence of accelerated osteogenesis. It is noted that only after discontinuing HGH does one observe marked calcium retention consistent with active osteogenesis. The nitrogen and calcium findings suggest that intermittent withdrawal of treatment may be more effective than constant therapy and may even simulate the normal pattern of secretion. Finally, the discrepancy between actual and theoretical weight during growth hormone administration suggests either loss of water or loss of fat. That the weight loss in part represents increased fat mobilization and utilization is further suggested by the loss of appetite observed in most of the patients treated with HGH and the improvement in appetite on discontinuing HGH.

RESULTS: CARBOHYDRATE AND FAT METABOLISM

Beck and associates[3] and Ikkos, Luft, and Gemzell[7] demonstrated that human growth hormone in man produced a decreased tolerance to administered glucose. Luft et al.[7] and Pearson et al.[8] noted accelerated production of ketone bodies in diabetic and hypophysectomized diabetic subjects. The degree of ketosis appeared related in some manner to the degree of hypopituitarism as well as to the degree of diabetes.

A. *Effects of* HGH *in Patients with Hypopituitarism, Hyperinsulinism, and Obesity.* 1. HGH produced changes in blood and urinary levels of carbohydrate and fat metabolites which preceded major changes in nitrogen balance by approximately twenty-four hours. Elevations

in fasting levels of plasma NEFA, as previously reported by Raben,[17] were observed four hours after HGH in hypopituitarism in an obese subject on an 800-calorie diet and in an infant with hyperinsulinism. Fasting levels of NEFA and citrate determined twenty-four hours after HGH also increased as the rate of retention of protoplasmic constituents became maximal (third to fifteenth days of HGH).

In hypopituitarism urinary excretion of ketones and citrate increased within twenty-four hours. Increases in ketones were variable thereafter but generally remained above control during maximal nitrogen retention. Control levels of urinary citrate were below normal during the control period; these progressively increased to normal or above normal levels during marked nitrogen retention.

In the obese patient the reduced caloric intake prior to HGH produced increased levels of plasma NEFA, serum citrate, urinary ketones and urinary citrate. As HGH produced maximal retention of protoplasmic constituents, levels of urinary ketones decreased while those of serum and urinary citrate and plasma NEFA rose.

2. No significant changes were observed in levels of blood and urinary glucose, pyruvate, lactate, or alpha-ketoglutarate in the patients with hypopituitarism, or in the infant with hyperinsulinism during either the initial phase (four hours to three days) or the phase of maximal nitrogen retention (three to fifteen days). Fasting, twenty-four-hour post levels of blood glucose and alpha-ketoglutarate rose while those of pyruvate fell in the obese patient during the first two weeks of therapy.

3. After two weeks of HGH, waning retention of protoplasmic constituents was paralleled by a fall to control levels of plasma NEFA and serum and urinary citrate; urinary ketones fell to control in hypopituitarism and decreased further in the obese subject. Blood glucose decreased to control in the obese subject and remained the same or fell below control in the hypopituitary patients. Tolerance to administered glucose decreased in all three hypopituitary patients and was accompanied by an abnormal postglucose elevation in serum citrate in two patients.

B. *Effects of* HGH *in Mild Diabetes.* In mild diabetes, HGH produced marked alterations in levels of fat and carbohydrate metabolites despite normal or totally absent retention of protoplasmic constituents. The three patients studied had minimal diabetes of late onset and were uncomplicated. The degree of hyperglycemia and glycosuria either prior to or during HGH could not be correlated with the degree of nitrogen retention produced by HGH.

In E.L. (male, age sixty-three, controlled by diet alone, normal weight), HGH produced marked retention of protoplasmic constituents. This was accompanied by elevations in fasting plasma NEFA and fasting serum citrate, a normal *fall* in postprandial levels of ketones and citrate, and a decrease in fasting blood and urinary levels of ketones. A marked and progressive increase in both fasting and postprandial glucose and glucosuria accompanied these changes. When HGH was discontinued, levels of glucose, NEFA, and serum citrate returned to control. However, urinary ketones remained below control and urinary citrate levels remained twice normal. There were no significant alterations in pyruvate, lactate, or alpha-ketoglutarate levels. Pertinent data are shown in figures 1 and 2.

In I.P. (female, age forty-three, history of diabetes

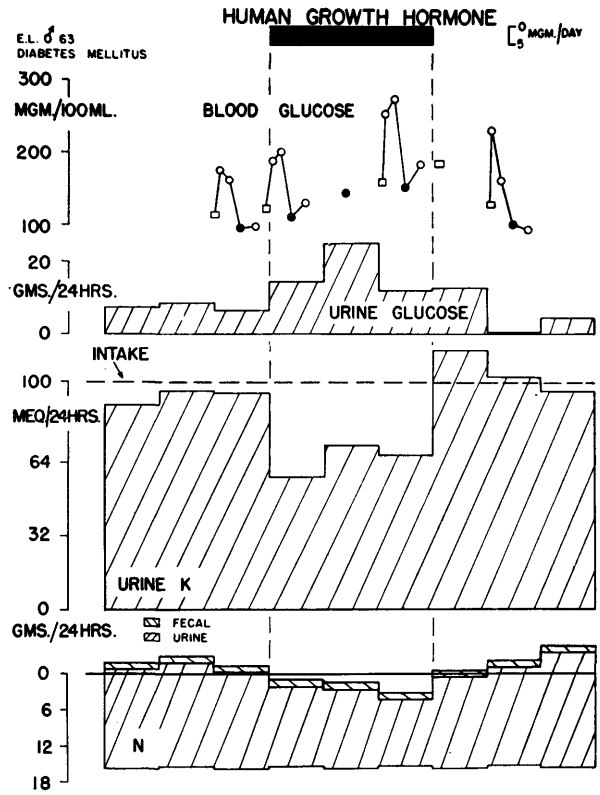

FIG. 1. Effects of HGH in a patient with mild diabetes. Blood samples were taken fasting at 8:00 a.m. (open square), after breakfast at 8:30 and 10:00 a.m. (open circle), at 12 noon (solid circle), and after lunch at 4:00 p.m. (open circle). HGH was injected immediately after the 8:00 a.m. venipuncture. Breakfast consisted of 100 gm. of carbohydrate. The study included three days each of forecontrol, HGH treatment, and postcontrol.

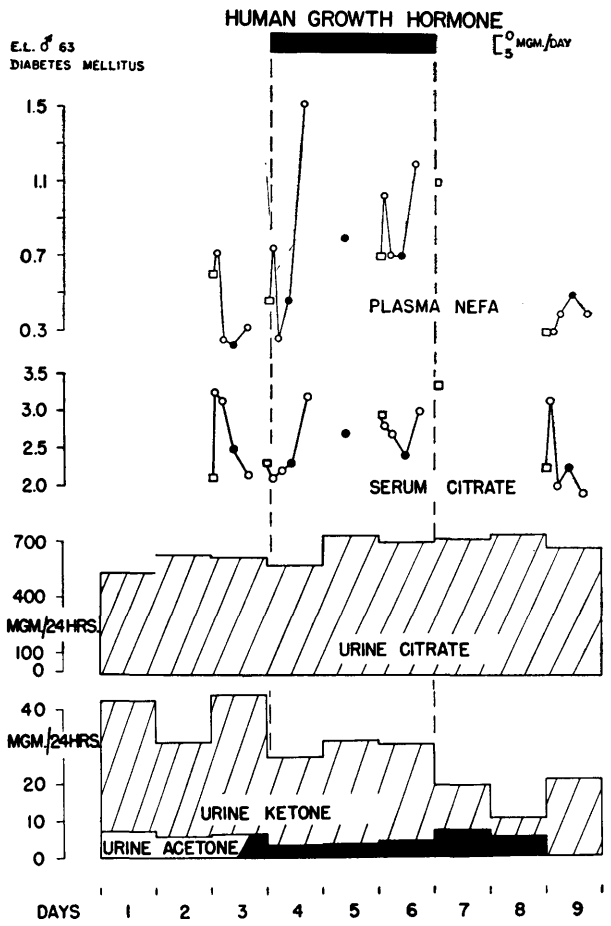

FIG. 2. Effect of HGH in a patient with mild diabetes. Scale for plasma NEFA (nonesterified fatty acids) is milliequivalents per liter and for serum citrate milligrams per 100 ml. Normal values for fasting NEFA are less than 0.7 mEq./L. and for serum citrate less than 2.6 mg. per 100 ml. Normal levels of urinary ketones are less than 20 mg. per twenty-four hours and for urinary citrate less than 480 mg. per twenty-four hours. For details of blood sampling see legend for figure 1.

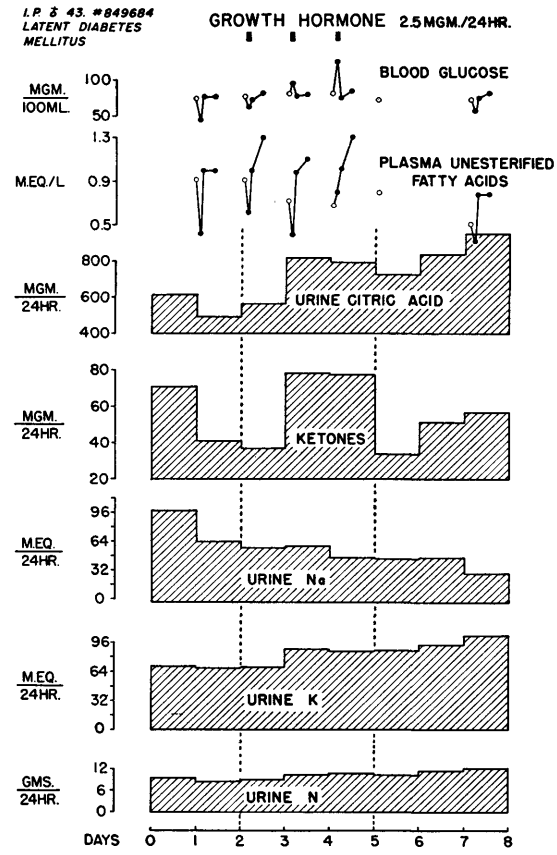

FIG. 3. Effects of HGH in a patient with mild diabetes. Blood values were obtained fasting at 8:00 a.m. (open circles), two and four hours after breakfast and four hours after lunch (solid circles). HGH was injected following the fasting venipuncture. Breakfast was eaten at 8:05 a.m. and lunch at 12:05 p.m.

prior to weight reduction, moderately obese at time of study), HGH produced no retention of protoplasmic constituents. No increase in fasting plasma NEFA or serum citrate was observed, but postprandial plasma NEFA progressively increased as did blood and urinary ketones. Fasting blood glucose showed no change but postprandial concentrations progressively increased. When HGH was discontinued, glucose, NEFA, and ketones returned to or below control values. Urinary citrate, however, continued to increase to twice normal levels during the postcontrol period. Pertinent data are shown in figure 3.

In the third diabetic patient, I.K. (female, age sixty-four, obese, controlled by diet and 8 units of Protamine Zinc Insulin), HGH produced changes which initially were comparable to those observed in E.L., but which thereafter were comparable to those seen in the second patient, I.P. Less than normal retention of nitrogen occurred on the first day accompanied by transient elevations in fasting plasma NEFA and serum citrate and transient decreases in blood and urinary ketones. Thereafter nitrogen retention waned, fasting and postprandial NEFA fell toward control, and urinary ketones gradually increased as did urinary citrate and alpha-ketoglutarate. Postprandial citrate rose in an abnormal fashion. Blood and urinary glucose increased with each dose of HGH and returned to less than control when HGH was discontinued. Urinary ketones, alpha-ketoglutarate, lactate and citrate continued to rise when HGH was discontinued.

INTERPRETATION OF CHANGES IN CARBOHYDRATE AND FAT METABOLITES LEVELS

The prompt and continued increase in plasma NEFA levels produced by HGH in hypopituitarism, hyperinsulinism, and simple obesity suggests that this hormone may accelerate the mobilization of fat. The ketonuria produced in hypopituitarism further suggests an increase in the availability of two-carbon fragments with the rate of production exceeding the rate of disposal of acetyl Co A. Increases in serum and urinary citrate suggest alterations in the tricarboxylic acid cycle though the specific mechanism remains obscure. The direction and sequence of changes in potassium, sodium or calcium balances during HGH do not explain the changes in citrate.[18]

The shift from very marked to slight nitrogen retention in the patients with hypopituitarism or in the patient with obesity does not appear related to insulin lack.

The changes in blood and urinary glucose, citrate, and ketone levels observed in these patients during the phase of waning retention were not comparable to those observed in the diabetic patients in whom minimal or absent nitrogen retention occurred.

Milman et al.[19] and Lukens[20] demonstrated in animals that insulin was essential for the anabolic effect of growth hormone. This appears to be true in man also in view of absent or minimal nitrogen retention in two of three mild diabetics. It is to be especially noted that although the anabolic effect was impaired by minimal insulin lack, a marked effect on blood and urinary levels of glucose, citrate, alpha-ketoglutarate, and ketones was still present.

This may reflect, in part, the increase in glucose production reported by Altszuler et al.[21] when growth hormone is administered to hypophysectomized dogs.

Under certain circumstances growth hormone appears to increase the secretion of endogenous insulin.[19,22] The evidence which suggests that HGH in man may produce a similar increase in insulin secretion is as follows: (1) Ketone levels decreased from above normal to normal in the obese subject on an 800-calorie diet and in the diabetic patient who showed normal nitrogen retention during HGH; (2) blood and urinary glucose fell below control in this same diabetic subject when growth hormone was discontinued; and (3) fasting levels of plasma NEFA, and serum citrate and urinary ketones and citrate fell below control during prolonged administration or following its discontinuation in the patients with hypopituitarism.

SUMMARY

Human growth hormone (HGH as prepared by Raben) is well tolerated and appears free of other hormones. Growth rate is stimulated in hypopituitarism and balance studies reveal retention of nitrogen, phosphorus, sodium, and magnesium. Potassium retention is disproportionately large the first day on HGH and loss is disproportionately large the first day off HGH. HGH produces hypercalciuria equal in degree to the observed increase in calcium absorption. Calcium retention is prominent only after HGH is stopped. Theoretical weight changes are consistent with fat mobilization.

Human growth hormone produced a prompt rise in plasma NEFA and serum and urinary citrate and ketones without significant changes in glucose, pyruvate, or lactate in patients with hypopituitarism. Levels of these constituents returned to control as nitrogen retention waned on prolonged HGH administration. Minimal or absent nitrogen retention in two of three patients with mild diabetes was accompanied by evidence of progressive impairment in glucose utilization and abnormal elevations in levels of citrate and alpha-ketoglutarate.

SUMMARIO IN INTERLINGUA

Hormon de Crescentia Human: Un Reporto Preliminar

Hormon de crescentia human (HGH, preparate secundo Raben) es ben tolerate e pare esser libere de altere hormones. Le crescentia es stimulate in hypopituitarismo, e studios de balancia revela retention de nitrogeno, phosphoro, natrium, e magnesium. Le retention de kalium es disproportionalmente grande le prime die del administration de HGH, e le perdita de kalium es disproportionalmente grande le prime die del re-suspension de HGH. HGH produce hypercalciuria equal in grado al observate augmento del absorption de calcium. Le retention de calcium es prominente solmente post le suspension de HGH. Le alterationes theoric del peso es in congruentia con le mobilisation de grassia.

HGH produceva un prompte augmento del non-esterificate acido grasse del plasma e del citrato e cetones del sero sin significative alterationes de glucosa, pyruvato, o lactato in patientes con hypopituitarismo. Le nivellos de iste constituentes retornava al valores de controlo in tanto que le retention de nitrogeno declinava con un prolongate administration de HGH. Grados minimal de retention de nitrogeno o le complete absentia de illo in duo inter tres patientes con leve diabete esseva accompaniate de evidentia de un progressive vitiation del utilisation de glucosa e de elevationes anormal del nivellos de citrato e de alpha-cetoglutarato.

ACKNOWLEDGMENT

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