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HUMAN GROWTH HORMONE*

Zvi Laron, M.D.**

For years human growth hormone (HGH) or somatotropin has been considered the prototype of an anabolic agent promoting normal growth. Recently, however, investigators have been puzzled by its isolation from the pituitaries of adults. The situation became even more confused when highly purified preparations became available about ten years ago,[1, 2] and it was found that HGH possesses protein anabolic and growth promoting activities as well as an adipokinetic or lipolytic property (both in vivo[3] and in vitro[4]) and a prolactin activity.[5] The question of whether the pituitary preparations used comprise only one hormone with several properties, or whether they actually contain two or three separate hormones has become an international controversy.[6, 7]

The development of a very sensitive radioimmunoassay[8, 9] has made it possible to measure the plasma GH concentration, thus adding to our knowledge of the physiological role of this hormone.

It is beyond the purpose of this paper to review all the aspects of the research concerning the growth hormone, I shall thus restrict myself to our own research in this field.

We prepare human growth hormone from pituitary glands obtained at autopsy and stored in acetone. The method of extraction is that described by Raben.[1] Our facilities permit studies *in vitro* and *in vivo* both in animals and in man.[10, 11]

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** Department of Pediatrics, Tel Aviv University Medical School and Rogoff-Wellcome Trust Medical Research Laboratories, Beilinson Hospital, Israel.

Immunochemical and Biological Studies

On agar gel electrophoresis, the human growth hormone is seen as two or three individual components migrating towards the anode.[12, 13] In most preparations the anodal component is contaminated with human albumin and antiserum. Such a preparation also contains antibodies against albumin.

Pure antiserum to HGH can be prepared by either adsorption with albumin or immunization with components 1 and 2 alone. The qualities of HGH remain unchanged as shown by the fact that all components were found to be immunologically similar to each other and to the whole hormone. Each component was found to be metabolically active in children[14] and to have an effect similar to that of the whole human growth hormone[15] i.e. a resulting retention of nitrogen, water and phosphorus. Whole HGH however always caused increased calcium excretion in the urine, whereas electrophoretic components 1 and 3 induced some reduction of urinary calcium.

Treatment with heat or pepsin showed that the immunological properties decreased progressively more slowly[16, 17] than the growth promoting activity.[18] Further evidence of lack of correlation between molecular integrity and various inherent properties of GH was demonstrated by finding that digestion, up to 64 per cent, did not reduce the ability to stimulate lipolysis.[19] These findings were also considered to be evidence that the stimulation of lipolytic activity is an inherent property of the HGH molecule. During these studies the interesting observation was made that digestion of bovine growth hormone (BGH) released a fraction that exhibited electrophoretic behavior similar to that of human GH and reacted immunologically with antiserum to the latter.[17] This was interpreted as proof that BGH and HGH possess a common core. Metabolic studies in man[11] did indeed show that digested BGH showed some nitrogen retention activity in man, whereas untreated BGH displayed no activity; however, these preparations were markedly antigenic and therefore unsuitable for prolonged clinical use.

Whether human prolactin exists as a separate hormone, as it does in sheep or cattle, or whether it is an inherent property of the human growth hormone molecule is as yet an unanswered question. The finding of Pasteels et al.[20] that tissue cultures of human pituitary cells rapidly lost GH activity but increased their prolactin activity favors the existence of two hormones.

In association with Apostolakis we have investigated the immunological properties of a highly active pituitary prolactin preparation.[21] We found that this preparation contained three antigens. One of these fused with HGH, a second was found to be albumin and the third remained unidentified.

The Physiological Aspects Studied by Means of Radiolabeled Growth Hormone

The recently introduced technique of radioimmunoassay[5] has made possible the accurate measurement of the minute amounts of growth hormone present in biological fluids. The principle of the radioimmunoassay is based on the competition between non-labeled and I^{131} -HGH to bind with a specific antiserum. The more there is of non-labeled HGH in the incubation mixture, the less I^{131} -HGH will be bound. The remaining free I^{131} -HGH is separated and a standard curve is constructed using the ratio of bound to free I^{131} -HGH.

Using a method employing flow chromatography on 3MC Whatman filter paper[9] for the separation of bound and free HGH-I^{131}, we have performed several thousand determinations of plasma or serum growth hormone in children and adults during the last two years.

It was found that in healthy children, over three months of age up to adulthood, fasting levels were low, ranging from between non-measurable quantities to 3-6 mμg/ml, so that no differentiation could be made between a normal state and pituitary insufficiency. The following conditions were found to have elevated fasting plasma growth hormone: newborn, acromegaly, cerebral gigantism and a form of genetic, familial pituitary dwarfism.

Newborns have high serum GH concentrations.[22, 23] The mean serum GH in the fetal umbilicus at delivery was found to be 23 mμg/ml as compared to 10 mμg/ml in the maternal cord and 15 mμg/ml in the mother at delivery. Within 24 hours the mean serum GH concentration rose to 44 mμg/ml and during the next day to 75 mμg/ml. This very active secretion of GH occurs concomitantly with a marked fall in blood sugar and we assume that it is the cause of the postnatal rise in plasma free fatty acids. We found that smaller babies had the highest GH levels. Big babies, including those of diabetic mothers had the lowest GH levels, in spite of marked hypoglycemia in the latter (as illustrated in Table 1). We consider these findings to be a direct proof that growth hormone is not the

TABLE 1

MEAN CONCENTRATIONS OF GROWTH HORMONE, GLUCOSE AND FREE FATTY ACIDS IN THE BLOOD OF MOTHER, CORD AND INFANT IN NORMAL, DIABETIC AND TOXEMIC PREGNANCIES

	HGH $m_{\mu}g$/ml			Glucose mg/100 ml		
	Mother	Cord	Infant	Mother	Cord	Infant
Group	N Mean ± S.E.	N Mean ± S.E.	N Mean ± S.E.	N Mean ± S.E.	N Mean ± S.E.	N Mean ± S.E.
Normal	9 15 ± 1.8	9 34 ± 9.7	9 69 ± 14	9 90 ± 8.6	9 81 ± 12	9 47 ± 4
Diabetic	9 21 ± 5.2	6 26 ± 8.7	7 40 ± 7.7*	9 114 ± 24	6 86 ± 7.7	6 37 ± 10
Toxemic	7 24 ± 3*	6 93 ± 15*	7 99 ± 42	8 69 ± 3*	6 94 ± 8.1	3 36 ± 10

N = number of determinations

* Statistically significant difference compared to values in normal group.
Student test.

etiologic factor responsible for the large babies of diabetic mothers. We also demonstrated the lack of placental transfer of GH from the mother by having injected unlabeled or I^{131} -labeled HGH into the mother before delivery.[21]

In acromegaly plasma GH concentrations ranged from 13 to 130 mμg/ml, depending upon the activity of the disease. The concentration dropped within several hours after hypophysectomy, so that this test is an index of the effectiveness of treatment, surgical or otherwise.

Elevated GH levels were also found in a few cases of gigantism accompanied by disease of the central nervous system.

A very interesting observation was made in a group of familial, genetic dwarfs who had clinical signs of growth hormone deficiency but who were found to have elevated fasting plasma concentrations of GH ranging from 40 to 100 mμg/ml.[25] We think that the pituitaries of the latter produce an abnormal growth hormone which is immunologically similar to GH but biologically inactive; i.e. we have before us a new inborn error of metabolism.

In order to differentiate between normal and reduced pituitary function, as must be done in many children with growth retardation, it was found necessary to examine growth hormone secretion during stimulation of its secretion. One of the most potent stimuli is known to be hypoglycemia.[26] Thus a standard test of measuring growth hormone reserve has been designed which measures the increase of plasma GH during the hypoglycemia induced by either insulin or Tolbutamide. Using the intravenous injection of insulin (0.1 unit regular insulin/kg. body weight) we measured the plasma insulin response in many conditions as well as in normal control in the pediatric age group. Normally the fasting plasma concentration of GH is either non-measurable or up to 3-5 mμg/ml, rising to 15-25 mμg/ml 30 minutes after the lowest blood glucose value, provided that the reduction was about 50 per cent of the fasting level. In children or adults with pituitary insufficiency of any origin the fasting plasma GH concentration is usually between 0-2 mμg/ml and there is no increase following hypoglycemia.

This test is also employed to determine the degree of effectiveness of the operation after hypophysectomies performed for therapeutic reasons in non-pituitary diseases (neoplastic disorders, diabetic retinopathy, etc.).

We also examined children with precocious puberty and/or increased growth for increased responses of GH secretion. In all cases, normal fasting plasma GH and reserve was found.

We have also used radio - labeled growth hormone for other purposes. After prior blocking of the thyroid with Lugol solution, we injected tracer amounts of I^{131}-HGH into several adolescent boys and girls and followed the disappearance of the TCA precipitable radioactivity in their plasma.[27] After a mixing phase of two to five minutes, two slopes were found. The first slope showed a half life ($T\frac{1}{2}$) of 20 to 30 minutes and the second slope a half life ($T\frac{1}{2}$) of 150 minutes. The first slope seems to represent the biological half life of metabolically active growth hormone. The meaning of the second slope is not fully understood. Similar studies in women before delivery revealed a faster than normal turnover rate of GH in this state.[24] The first slope showed a $T\frac{1}{2}$ of from 11 to 12 minutes, the second from 70 to 80 minutes.

These are the various aspects of research on growth hormone with which our group has been engaged during the last few years. We hope that our contribution, with those of other investigators, will help to further the understanding of the roles played by the so - called «growth hormone» in normal homeostasis and in pathological conditions.

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