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Absence of serum growth hormone binding protein in patients with growth hormone receptor deficiency (Laron dwarfism)

(growth hormone/serum binding proteins)

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ABSTRACT **It has recently been recognized that human serum contains a protein that specifically binds human growth hormone (hGH). This protein has the same restricted specificity for hGH as the membrane-bound GH receptor. To determine whether the GH-binding protein is a derivative of, or otherwise related to, the GH receptor, we have examined the serum of three patients with Laron-type dwarfism, a condition in which GH refractoriness has been attributed to a defect in the GH receptor. The binding of ^{125}I-labeled hGH incubated with serum has been measured after gel filtration of the serum through an Ultrogel AcA 44 minicolumn. Nonspecific binding was determined when ^{125}I-hGH was incubated with serum in the presence of an excess of GH. Results are expressed as percent of specifically bound ^{125}I-hGH and as specific binding relative to that of a reference serum after correction is made for endogenous GH. The mean ± SEM of specific binding of sera from eight normal adults (26–46 years of age) was 21.6 ± 0.45%, and the relative specific binding was 101.1 ± 8.6%. Sera from 11 normal children had lower specific binding of 12.5 ± 1.95% and relative specific binding of 56.6 ± 9.1%. Sera from three children with Laron-type dwarfism lacked any demonstrable GH binding, whereas sera from 10 other children with other types of nonpituitary short stature had normal relative specific binding. We suggest that the serum GH-binding protein is a soluble derivative of the GH receptor. Measurement of the serum GH-binding protein may permit recognition of other abnormalities of the GH receptor.**

When normal human serum is subjected to gel filtration in neutral buffers, higher-molecular-weight forms of growth hormone (GH) have been frequently reported (1, 2). While these big forms of GH have been attributed to incompletely processed precursor forms of the hormone or aggregated GH molecules, there is now evidence that complexes of GH with a specific serum binding protein exist in human serum and the serum of a number of mammals.

The presence of a specific GH-binding protein (GH-BP) was first convincingly established in rabbit serum by Ymer and Herington (3). They added ^{125}I-labeled human GH (hGH) or ^{125}I-labeled bovine GH to normal rabbit serum, and after 2 hr of incubation, the reaction mixture was filtered through Ultrogel. As much as 45% of the added ^{125}I-labeled hGH (^{125}I-hGH) was bound to a component with a M_r of >100,000. Rabbit GH-BP had little affinity for either prolactin or human placental lactogen. The serum GH-BP, like a cytosolic GH-BP found in liver, exhibited 100% cross-reactivity with a monoclonal antireceptor antibody raised by Simpson *et al.* (4, 5) against GH receptors isolated from rabbit liver membranes. These observations led Ymer and Herington to suggest that the serum GH-BP arises from the liver and might be structurally related to the membrane-bound GH receptor.

Herington *et al.* (6) then demonstrated a similar GH-BP in human serum by gel filtration. The techniques of charcoal adsorption and PEG precipitation, which are often used to separate hormones from their binding proteins, were not effective. Human serum GH-BP is specific for human GH (hGH) and is unreactive with nonprimate GHs, other pituitary hormones, placental lactogen, and prolactin from a number of species. The authors found GH-BP in all 39 sera that they had studied. This included patients with hypopituitarism and acromegaly, as well as normal subjects. A weak negative correlation between GH binding and GH content was observed, but this can be attributed to occupancy of binding sites of GH-BP by the GH present in the serum. Specific binding of ^{125}I-hGH by human serum was independently reported by Baumann *et al.* (7).

In this paper we describe a method for measuring the binding of ^{125}I-hGH by human serum that corrects for differences in endogenous GH. We have applied this method to sera from normal children and adults and also to sera of children with nonpituitary forms of short stature. This group included sera from three patients with Laron-type dwarfism because there is evidence that this condition is due to a marked deficiency of growth hormone receptors (8).

HUMAN SUBJECTS

Sera were obtained from 12 children aged 2 to 15.5 yr of normal height and without pituitary disease. There were nine boys and three girls in the group. Sera were also obtained from five men and three women of normal stature whose ages ranged from 23 to 46 yr. We also studied sera from 13 children whose height was more than 2 SD below the normal mean (Table 1). All had serum GH concentrations >10 ng/ml in response to clonidine or other GH provocative testing. Three children were diagnosed as having Laron-type dwarfism on the basis of serum GH responses to testing of 42 to 248 ng/ml, low serum insulin-like growth factor I (IGF-I), and unsatisfactory responses to GH treatment. A brief description of these three boys follows:

Patient 1 was a Caucasian boy born after 34 weeks of gestation with a birthweight of 2.12 kg; his parents are not consanguineous, and there are no siblings. When first examined at Saint Louis Children's Hospital at 3.5 years of age, his height was 79.1 cm (height age of 14 mo), and his bone age was 18 mo. Correction of a mild renal tubular acidosis did not improve his growth velocity. At 4.1 yr of age, his baseline serum hGH concentrations were 37 and 42 ng/ml. Serum insulin-like growth factor I was 0.18 unit/ml. At age 6, he failed to have a normal rise of serum IGF-I after 4 doses of GH, 0.05 unit/kg, subcutaneously every 12 hr. Serum IGF-I levels at 0, 24, 48, and 54 hr were 0.1, 0.2, 0.25, and 0.21 unit/ml.

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Abbreviations: GH, growth hormone; hGH, human GH; GH-BP, GH-binding protein; IGF-I, insulin-like growth factor I.

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Table 1. Non-GH-deficient short stature

	Sex	Age, yr	Height, cm	Ht. age, yr	IGF-I*, units/ml	GH peak†, ng/ml
			Laron type			
1	M	6	94.9	3	<0.1	42
2	M	16.8	109	5.1	<0.2	248
3	M	14.8	142.8	10.7	<0.1	68
			Unclassified			
4	M	8.6	120	6.7	<0.1	14
5	F	8.6	108.6	5.1	0.2	18
6	M	14.9	141	10.6	0.3	13
7	M	6.5	106	4.2	0.3	20
8	M	11.9	52	8.8	0.4	16
9	F	5.0	94.6	3.0	0.7	42
10	M	14.8	139.5	10	0.6	14
11	M	8.8	115	5.8	0.8	13
12	F	3.7	88.8	2.2	0.9	44
13	M	12.5	126.9	7.9	1.1	20

Ht. age, height age.
†Peak value after clonidine or other provocative tests of GH secretion >10 ng/ml is considered a normal response.
*IGF-I concentrations vary as a function of age and pubertal status. For this study, concentrations ≤0.4 unit/ml are considered low.

Patient 2 was a black boy born at term weighing 2.7 kg and 43.2 cm in length. His father was 170 cm, and his mother was 124.5 cm; there was no known consanguinity. One sister has grown normally. His early growth was slow and complicated by symptomatic hypoglycemia. When he was examined by Dr. Paul V. DeLamater of Toledo, Ohio, at the age of 5.6 yr, his height was 71.1 cm (height age 6 mo). Baseline hGH levels were 45 and 164 ng/ml and rose to 152 ng/ml after levodopa and 248 ng/ml after oral glucose. The concentration of IGF-I (radioreceptor assay) was <0.2 unit/ml in four separate sera. He was treated with 2 units of hGH three times weekly for 1.75 yr without significant acceleration of growth. When last seen at 16.2 yr, his height was 108.9 cm (height age, 5.1 yr), and he was beginning puberty (Tanner II). Tests of thyroid and adrenal function were normal.

Patient 3 was a Caucasian boy born at term weighing 2.77 kg; his parents are of normal height and are not consanguineous. Two male and one female siblings are of normal height. When first examined at Saint Louis Children's Hospital at 3.9 yr, his height was 77.7 cm (height age 15 mo). Basal serum hGH concentrations were 27 and 32 ng/ml and rose as high as 68 ng/ml on provocative testing. Serum somatomedin concentrations were below 0.1 unit/ml before and after administration of hGH, 0.05 unit/kg body weight twice a day for 4 doses. Measurements of thyroid and adrenal function were normal. He was treated with hGH (7 units per week and then 12 units per week for 13 mo) without a significant change in his growth velocity. When seen at age 14.8 yr, he was 142.8 cm in height (height age of 10.5 yr).

MATERIALS

Recombinant human [Thr^{59}]IGF-I was purchased from Amgen (Thousand Oaks, CA). Highly purified hGH, hGH antiserum, somatomedin C antiserum (donated by L. V. Underwood and J. J. Van Wyk of Chapel Hill, NC) were provided by the National Pituitary Program, National Institute of Diabetes and Digestive and Kidney Diseases, Baltimore, MD. Ultrogel AcA 34 and 44 were purchased from LKB. Bovine serum albumin RIA grade, tris(hydroxymethyl)aminomethane hydrochloride (Trizma hydrochloride), and sodium azide were purchased from Sigma. ^{125}I-hGH purified by HPLC, specific activity 100–150 μCi/μg (1 Ci = 37 GBq), was purchased from Radioassay Systems Laboratories (Carson, CA). It was repurified weekly by filtration through an Ultrogel AcA 34, 0.9 × 23 cm column, in binding buffer (25 mM Tris·HCl/10 mM $CaCl_2$/0.02% sodium azide/0.1% bovine serum albumin, pH 7.5).

Binding Studies. These were conducted according to the minicolumn method of Herington *et al.* (5). The reaction mixture contained 100 μl of human serum with and without 500 or 1000 ng of hGH, ^{125}I-hGH (≈20,000 cpm), made up to 250 μl with binding buffer. After 2 hr at 20°C, 200 μl of the reaction mixture was added to Ultrogel AcA 44 minicolumns, 0.9 × 15 cm, and eluted at a rate of 0.12 ml/min. Ten-drop fractions were collected. The same column was used for the gel filtration of an individual serum without and with excess hGH. To minimize day-to-day variation in the binding properties of the ^{125}I-hGH, a normal reference serum obtained from a 23-year-old normal woman was similarly treated daily. The hGH content of this serum was 2.8 ng/ml.

With normal serum ^{125}I-hGH eluted from the column as two distinct peaks (Fig. 1). The first peak represents ^{125}I-hGH bound to GH-BP, and the second peak is unbound ^{125}I-hGH. Excess hGH virtually completely displaced the radioactivity from peak one to peak two. The cpm in peak one divided by the total cpm in peaks one and two was calculated and expressed as a percentage. This represented total binding, and specific binding was obtained by subtracting the percent of nonspecific binding in the presence of 500 ng of hGH.

To calculate relative specific binding, the specific binding of the reference serum was determined with various additions of hGH. This allowed us to calculate what the specific binding of the reference serum would be if it had contained the same hGH as the unknown serum. Relative specific binding is defined as the specific binding of the unknown serum divided by the calculated specific binding of the reference serum with a hGH content of the unknown serum expressed as a percentage. The GH-BP in serum is stable at −17°C for at least 6 mo. The percent specific binding of the reference serum in seven separate assays over a 3-month period was 21.6 ± 2.1 SD.

Hormone Measurements. Serum GH was measured by RIA with separation of bound from free ^{125}I-hGH by the double antibody method. Serum IGF-I was measured after acid ethanol extraction with ^{125}I-labeled recombinant human [Thr^{59}]IGF-I and somatomedin C antisera as previously

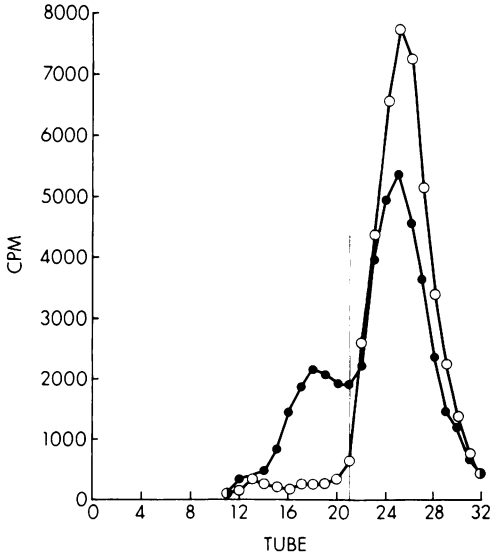

FIG. 1. ^{125}I-hGH was incubated with serum and then passed through an Ultrogel AcA 44 minicolumn. Cpm eluted in the absence (●) and presence (○) of an excess of hGH are shown. The vertical line indicates the boundary used to calculate the percentage of ^{125}I-hGH bound to GH-BP.

described (9). Results are expressed as units/ml. Our reference serum contains 280 ng of IGF-I per ml.

RESULTS

Characterization of GH-BP. The binding of ^{125}I-hGH by normal reference serum is shown in Fig. 1. A definite biphasic pattern is observed. The first component represents ^{125}I-hGH bound to the serum GH-BP, and the second represents free ^{125}I-hGH. With high quality ^{125}I-hGH tracer virtually all the ^{125}I-hGH associated with GH-BP was displaced with excess unlabeled GH.

Fig. 2 is a plot of the cpm with serum alone minus the cpm with serum plus an excess of hGH. This type of plot more clearly indicates the presence of a symmetrical peak of ^{125}I-hGH bound to GH-BP without evidence of a significant larger-molecular-weight GH-BP.

^{125}I-hGH was displaced from GH-BP of the normal reference serum by unlabeled GH with the expected logarithmic dose relationship of competitive inhibition for the binding site (Fig. 3). Fifty percent displacement occurred with 4.8 ng per tube. This would represent the displacement of binding anticipated with 100 μl of serum containing 48 ng of hGH per ml.

GH-BP in Normal Serum. The mean percent specific binding of sera from 8 normal individuals, aged 25 to 46 years, was 21.6 ± 0.45 (SEM). The mean binding of adult normal sera expressed relative to the reference serum corrected for GH content was 101.1 ± 8.6%.

The specific GH binding of sera from 11 normal children with ages of 2.7–13.7 years was 12.5 ± 1.95%, and the mean binding expressed relative to the reference serum corrected for GH content was 56.6 ± 9.1%. Both measures of GH binding of children are significantly different than that for the adult subjects when analyzed by Student's t test ($P < 0.005$). Relative binding as a function of age is shown in Fig. 4.

GH-BP in Sera from Laron-Type Dwarfs. The sera from the three boys with Laron-type dwarfism had no demonstrable GH-BP (Fig. 4). This abnormality could not be attributed to occupancy of GH-BP by GH because the GH concentrations in the sera examined were 42, 4.0, and 27 ng/ml for patients

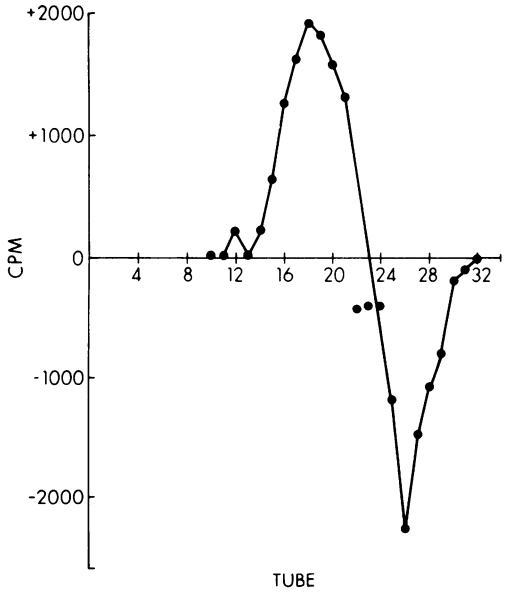

FIG. 2. The specific binding of ^{125}I-hGH (total minus nonspecific binding) of each tube is plotted from a different run than shown in Fig. 1. Positive numbers show binding to GH-BP as a single peak. Negative numbers indicate ^{125}I-hGH displaced from GH-BP and appearing in the unbound GH peak.

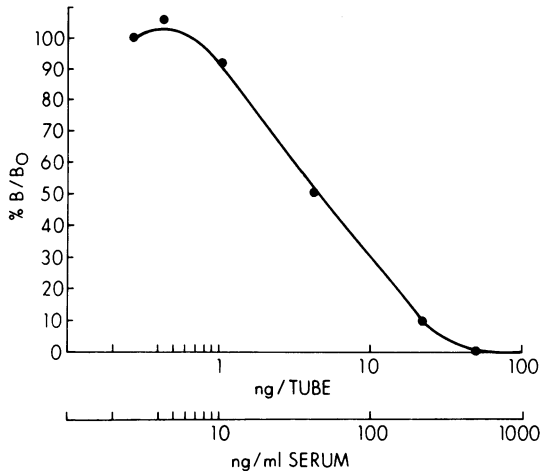

FIG. 3. Different amounts of unlabeled GH were added to the reference serum, and the percent specific binding was determined. Specific binding without added GH was considered 100% (B_0). The displacement curve was used to determine the decrease of specific binding by the reference serum if it had contained the same GH content of an unknown serum.

1, 2, and 3. These concentrations of GH are insufficient to abolish GH binding by normal sera.

Other Nonpituitary Forms of Dwarfism. Sera from 10 patients with unclassified short stature were examined for GH-BP. The percent specific binding of the group with low IGF-I was 6.2 ± 0.78 and with normal IGF-I was 6.9 ± 1.6. It should be noted that, inadvertently, sera selected for study were obtained during GH provocative testing. When the percent relative binding was calculated after correction for endogenous GH, the group with low initial IGF-I was 51.9 ± 10% and the group with high initial IGF-I was 50.0 ± 10.5%. These results were not significantly different from those obtained with sera from normal-statured children.

DISCUSSION

We have confirmed the finding of Herington *et al.* (6) that specific binding of ^{125}I-hGH can be easily and reproducibly measured in human serum by a gel filtration method. The interassay coefficient of variation of our reference serum was 10%. Interference with endogenous GH in the estimation of specific binding presented a problem. We elected not to

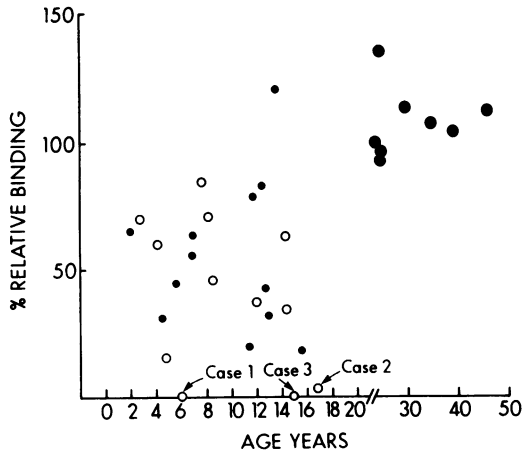

FIG. 4. The ^{125}I-hGH binding of sera relative to that of the reference serum after correction for endogenous GH is plotted as a function of age. Normal adults (●), normal-statured children (●), short-statured children with normal GH secretion (○). The three cases of Laron-type dwarfism are indicated.

remove endogenous GH by methods such as charcoal adsorption, affinity chromatography, or extraction. We considered that these procedures would be time consuming and when applied to small volumes of serum, might result in unpredictable losses of GH-BP. We, therefore, measured the serum GH in our unknown sera and calculated the extent to which that amount of GH would lower the binding of our reference standard serum. For serum GH concentrations of <10 ng/ml, the calculated reduction of binding of the reference serum is small (Fig. 3).

The plot of specific binding illustrated in Fig. 2 provides evidence of only a single significant component of GH-BP and argues against the presence of two separate binding proteins of molecular mass 76 and 124 kDa as suggested by Baumann and Shaw (10). When we have used older, unrepurified ^{125}I-hGH, the larger component is evident but is poorly displaceable with excess unlabeled GH. Our experience leads us to suspect that this large component may be an artifact resulting from partially denatured ^{125}I-hGH.

The percent of specific binding of ^{125}I-hGH by the sera of eight normal adults showed little variance about the mean of 21.6 (SD 1.27). These results correspond to those of Herington *et al.* (6) but are considerably higher than reported by Baumann *et al.* (7) who reported that 5 to 10% of ^{125}I-hGH added to plasma was bound. It is likely that the larger column used by these authors (1.5 × 100 cm) allowed elution of ^{125}I-hGH from GH-BP during the course of gel filtration. GH-BP was significantly lower in children than in adults. The size of the sample tested was insufficient to recognize possible regulatory factors. There did not seem to be a difference between sexes and in the 11–13 age range there were no obvious differences between prepubertal and pubertal children in respect to their GH-BP.

Except for the patients suspected clinically of having Laron-type dwarfism, the serum GH-BP of the ten children with short stature was not significantly different from that in the sera of normal-statured children. The sera of the three cases of Laron-type dwarfism had no demonstrable GH-BP as illustrated in Fig. 5. The absence of GH-BP in the sera of these patients cannot be attributed to occupancy of binding sites by the unlabeled GH in their sera or as a consequence of their functional hyposomatotropism, because as shown by Herington *et al.* (6) GH-BP was always demonstrable in sera of patients with growth hormone deficiency and growth hormone excess.

Laron-type dwarfism is transmitted as a simple autosomal recessive trait and is characterized by refractoriness to endogenous and exogenous GH suggestive of a defect in the GH receptor. Direct support for such a defect was provided by Eshet *et al.* (8), who showed that membranes prepared from liver tissue of two of these patients did not bind ^{125}I-hGH, whereas membranes prepared from liver of normal individuals had readily demonstrable ^{125}I-hGH binding. A similar lack of binding of ^{125}I-hGH by sera of such patients provides the most suggestive evidence thus far brought forward that the GH-BP is directly related to the GH receptor.

The other evidence that suggests a linkage of the serum GH-BP with the membrane-bound GH receptor is that in the rabbit, which has a serum GH-BP comparable to that of man, a monoclonal antibody raised against the rabbit liver membrane GH receptor cross-reacts with the serum GH-BP (3). In addition, human GH-BP has the same limited specificity for human (and presumably primate) GH as possessed by the IM-9 lymphoid cell GH receptor (11) and the human liver GH receptor (12). It would seem unlikely that an unrelated serum carrier protein for GH would acquire exactly the same specificity as the membrane receptor. It should be recalled that the hormonal specificities of the corticosteroid-binding globulin (13), the sex steroid-binding globulin (14), the thyroid-binding globulin (15), and the somatomedin-binding proteins (16) differ substantially from those of the receptors for their respective hormones. We doubt that the GH-BP is an important transport protein because only about 20–25% of plasma GH is complexed. This is to be compared to the nearly complete binding of the thyroid hormones, the steroid hormones, and the somatomedins to their respective binding proteins. Also, the association constant of the GH-BP is sufficiently low that the GH-BP would readily give up GH to tissue receptors.

Our results do not provide any insights into the mechanism by which GH-BP is released from cells into the plasma. According to Herington *et al.* (17), the serum GH-BP has an

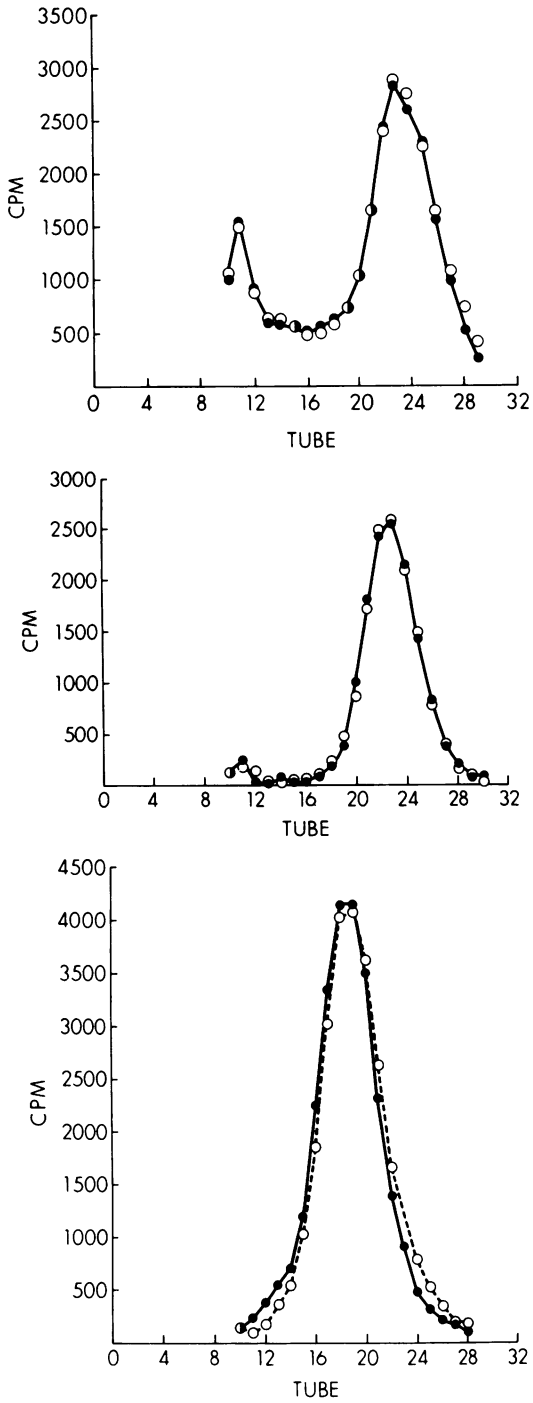

FIG. 5. The elution pattern from Ultrogel minicolumns of ^{125}I-hGH incubated with sera from three patients with Laron-type dwarfism. (*Top*) Case 1; (*Middle*) case 2; (*Bottom*) case 3. No evidence of specific binding of ^{125}I-hGH was found.

estimated M_r of 63,000 and 73,000. This is smaller than the estimated size of 109,000 for the IM-9 lymphoid cell GH receptor (18). A truncated receptor might arise from abnormal processing of the GH receptor mRNA or post-translational enzymatic cleavage. Such a defective receptor would not be retained by the plasma membrane, as is the case of the low-density lipoprotein receptors of fibroblasts with a deletion in the portion of the gene that codes for the transmembrane portion of the receptor (19). Certain cultured lymphocyte lines rapidly (over 1–2 hr) lose their plasma membrane GH and insulin receptors when incubated in a serum-free medium, and soluble receptors appear in the medium (20, 21). It is not known whether or not the soluble receptors recovered in the conditioned medium retain the hydrophobic transmembrane domain of these receptors.

Whatever the mechanism of GH-BP release from cells is, it is a process that occurs widely in mammalian species. In addition to its presence in human and rabbit serum, Ymer and Herington (3) found it in sheep serum but not in adult rat serum. We have demonstrated GH-BP also to be present in dog, pig, and pregnant rat serum (B.T., unpublished). In view of the high concentration of GH membrane receptors in rabbit and human liver and the presence of soluble GH-BP in rabbit liver cytosol, this organ might be suspected to be the source of serum GH-BP. The lack of GH-BP in liver of normal male rats and its presence in the serum of pregnant rats is consistent with the marked increase in the concentration of GH receptors in liver membranes during pregnancy (22).

Clinical studies of GH receptors in physiologic and pathologic conditions have been greatly hampered by the lack of an ethically accessible cell type with reproducibly demonstrable GH receptors. While GH receptors have been reported on human peripheral mononuclear cells (23), fibroblasts (24), and adipose cells (25), measurement of these receptors has been inconstant and unreliable in our hands. Our studies indicate that measurement of GH-BP will permit the recognition of patients with Laron dwarfism without resorting to detailed metabolic study before and after hGH administration. The present method may be clinically useful in providing information about other types of abnormal GH receptors.

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