Observations on the Responsiveness of Human Subjects to Human Growth Hormone

EFFECTS OF ENDOGENOUS GROWTH HORMONE DEFICIENCY AND MYOTONIC DYSTROPHY

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ABSTRACT The effect of human growth hormone (HGH) on the N, P, Na, and K balance, and on the body weight (BW) of three groups of subjects was measured. In group I were nine cases (age 6–69) with HGH deficiency; in group II, eight cases (age 9–79) with normal endogenous HGH; in group III, four cases with myotonic dystrophy (age 45–51). After a 7 day control period, the hormone was administered for 7 days. Each subject was tested with three doses of HGH: dose *A*, 0.0168 U/kg $BW^{3/4}$ per day; dose *B*, 0.0532 U/kg $BW^{3/4}$ per day; dose *C*, 0.168 U/kg $BW^{3/4}$ per day.

In group I, the responsiveness to HGH declined with age. Two subjects aged 6 yr responded to all three doses of HGH with positive balances in N, P, Na, and K and increases in BW. At ages 15–17, responses were obtained only to doses *B* and *C* in three cases, and only to dose *C* in two cases. Two subjects, aged 42 and 69, responded only to dose *C*. Not only did the threshold dose increase with age in group I, but the magnitude of the responses declined with age as well.

Patients of group II were less responsive to all doses of HGH than were patients of group I at comparable age. None responded to dose *A* or dose *B*. All responded to dose *C*, but the increments in balances and in BW stimulated by this dose were only one-third to one-half as great as in HGH-deficient subjects of similar age.

Three patients of group III responded to all three doses of HGH, and one responded to doses *B* and *C*. The responses were similar in magnitude to those in the 6-yr old HGH–deficient children, and greater than those in all other subjects studied.

These data show that responsiveness to exogenous HGH rises with deficiency of endogenous HGH, and that individuals with myotonic dystrophy are hyperresponsive to exogenous HGH.

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INTRODUCTION

Some evidence suggests that age, endocrine status, and genetic factors may influence the responsiveness of animal and human subjects to growth hormone (GH):[1] (*a*) The pituitary glands of adult rats contain as much GH as those of young rats (1). Heins, Garland, and Daughaday (2) found that the in vitro responsiveness of the rat's costal cartilage to "sulfation factor" diminishes with age of the animal. Ray, Evans, and Becks (3) observed that the in vivo widening effect of bovine growth hormone (BGH), 0.8–8 mg daily for 10 days, on the proximal epiphysis of the hypophysectomized rat's tibia was only half as great in 150-day old rats as in 50-day old animals. Therefore, it is possible that cessation of growth in adult rat or man may result in part from a diminishing responsiveness of the peripheral tissues to GH with age. (*b*) Rimoin, Merimee, Rabinowitz, Cavalli-Sporza, and McKusick (4) reported that the effects of exogenous HGH (human growth hormone), 4–5 mg per

[1] *Abbreviations used in this paper:* BGH, bovine growth hormone; BSA, body surface area; BW, body weight; GH, growth hormone; HGH, human growth hormone; RBC, red blood cell count; T_4-I, serum thyroxine-iodine concentrations.

day for 1–5 days, on plasma concentrations of free fatty acids, blood urea nitrogen, and endogenous insulin, regularly produced in normal or hypopituitary human subjects, did not occur in African pygmies. Subresponsiveness of the pygmies' tissues to HGH was postulated. Another example of peripheral unresponsiveness to HGH is a sexually mature dwarf (5), who possessed normal-plasma levels of (endogenous) HGH, and exhibited little or no change in N balance during treatment with 5 mg exogenous HGH daily. (*c*) Prader, Illig, Szeky, and Wagner (6) and Brown, Stimmler, and Lines (7) reported that the magnitude of N retention produced by 10 mg HGH per day was two to six times greater in HGH-deficient children than in children with normal endogenous HGH. The widening effect of BGH (0.8–8 mg daily for 10 days) on the rat's tibial epiphysis is 3 to 7 times greater in the hypophysectomized than in the normal animal (3). (*d*) Morris, Jorgensen, Elvick, and Goldsmith (8) found that children treated with glucocorticoids showed little or no positive balance of N, K, or P in response to 10–30 mg HGH/day. Children not treated with glucocorticoids regularly exhibited positive balances in response to the same doses of hormone.

These observations suggest that age, deficiency of endogenous HGH, hyperadrenalism, and unidentified genetic factors may influence a subject's responsiveness to HGH. But the differing species, doses of HGH, and indices of response employed in these studies make it difficult to draw quantitative conclusions applicable to man.

Therefore, we have undertaken quantitative studies on the response of a series of subjects to HGH. Three doses of the hormone were used and the effect of each dose on N, P, Na, and K balance was measured. We initially visualized two groups of subjects: group I with GH deficiency, varying in age from the 1st to the 8th decade; group II with normal endogenous GH function, covering a similar span of age. In the early phase of the work, a patient with myotonic dystrophy was studied and the responsiveness of this patient to the hormone differed from that of members of either group I or II. Therefore, four cases of myotonic dystrophy were investigated under group III.

METHODS

1. *Subjects.* Subjects were referred to the Clinical Research Facility by physicians of Egleston, Grady Memorial, and Atlanta Veterans Administration Hospitals. The clinical data are summarized in Table I, which arranges the subjects into: group I, nine cases with GH deficiency; group II, eight cases with normal pituitary function; group III, four patients with myotonic dystrophy.

2. *Endocrine evaluation.* (*a*) Plasma HGH was determined by radioimmunoassay during insulin-induced hypoglycemia (0.1 U per kg intravenously) and during infusion of arginine (9, 10). The latter test in male subjects was preceded by 2-days' treatment with stilbestrol (2.5 mg twice a day). Two such provocative tests were done with insulin. Blood glucose concentration declined below 40% of the zero hour value in each such procedure. In group I subjects, two provocative tests with arginine were also done. Subjects in group I had less than 2.0 mμg HGH/ml plasma in all four-sample tests. Subjects in group II and III showed a peak HGH value of > 10 mμg/ml in at least one insulin test. Diagnosis of HGH deficiency in subjects 17 yr or younger was supported in every instance by retardation of growth (height more than three standard deviations below the mean of the population of their age; growth rate less than 2 cm per yr; bone age less than 75% of chronological age by the criteria of Greulich and Pyle [11]).

(*b*) TSH (thyroid-stimulating hormone) function was evaluated in all subjects by serum thyroxine-iodine concentration (T_4-I) and by thyroidal uptake of ^{131}I at 24 hr. Subnormal T_4-I and ^{131}I uptake occurred in five members of group I, leading to diagnosis of TSH deficiency and replacement therapy with desiccated thyroid (60–180 mg daily). T_4-I in all subjects was within the normal range (2.9–6.4 μg per 100 ml) during the period of this study.

(*c*) ACTH function was evaluated in all subjects by fasting plasma-cortisol concentration and 24 hr urinary excretion of 17-hydroxycorticosteroids; in group I subjects, in addition, we measured the urinary 17-hydroxycorticosteroid response to metyrapone, utilizing the technique and criteria of Goodman, Grumbach, and Kaplan (12). Subnormal response to metyrapone by the criteria cited and in some cases subnormal plasma-cortisol concentration and subnormal 24 hr urinary 17-hydroxycorticosteroid content, occurred in five members of group I and led to replacement treatment with hydrocortisone, 10–20 mg/day. In all cases, plasma-cortisol concentration and urinary 17-hydroxycorticosteroid excretion were in the normal range during the period of this study.

(*d*) Gonadotropin deficiency was considered to be present in subjects over 16 yr old when secondary sexual characteristics had failed to develop and less than 6-mouse uterine U of gonadotropin activity were present in a 24-hr urine sample.

(*e*) Diabetes insipidus was diagnosed in three cases of group I after excision of craniopharyngioma on the basis of polydypsia, excretion of over 5 liter urine a day with specific gravity less than 1.010, and correction of polyuria and polydypsia by intramuscular injection of 2.5–5.0 U pitressin tannate in oil. Such injections every 48–72 hr were used to treat the condition.

3. *Evaluation of dystrophy patients.* Diagnosis of myotonic dystrophy in the four (unrelated) patients of group III was based upon the presence of clinical myotonia, a distribution of muscular weakness involving neck flexors, distal upper extremity muscles, and anterior tibial muscles, and a positive family history of the disease. Electromyographic evidence of myotonia and myopathy was present in all four cases; EKG (electrocardiogram) abnormalities consistent with myotonic dystrophy were present in three. All four patients exhibited punctate posterior cortical cataracts in both eyes. One patient (No. 21) had a diabetic glucose tolerance test. No clinical evidence of testicular atrophy or central nervous-system degenerative changes were observed in this group.

4. *Experimental protocol.* Three doses of HGH were employed: 0.0168, 0.0532, and 0.168 U HGH/kg $BW^{3/4}$ per day. For a 50 kg subject, these doses represent 0.3162, 1.0, and 3.162 U/day; the log doses are -0.5, 0, and $+0.5$ re-

TABLE I

Clinical Data on the Subjects Studied

Group	Case	Age	Sex	Height	Weight	Bone age	Diagnosis	Duration since diagnosis	Pituitary hormone deficiencies	Hormone replacement treatment
		yrs		*cm*	*kg*			*yrs*		
I	1	6	M	89	12	2	Idiopathic GH deficiency	3	GH	None
	2	6	M	108	18	2	Idiopathic GH deficiency	3	GH	None
	3	15	F	129	28	11	Idiopathic GH deficiency	7	GH	None
	4	16	M	145	33	11	Idiopathic hypopituitarism	9	GH, FSH, LH, GH, ACTH	Cortisol, thyroid
	5	16	M	152	61	12	Hypopituitarism secondary to craniopharyngioma, excised	7	GH, ACTH, TSH, FSH, LH, ADH	Cortisol, thyroid, ADH
	6	17	F	136	37	12	Hypopituitarism secondary to craniopharyngioma, excised	8	GH, ACTH, TSH, FSH, LH, ADH	Cortisol, thyroid, ADH
	7	17	F	148	36	14	Hypopituitarism secondary to craniopharyngioma, excised	3	GH, ACTH, TSH, FSH, LH, ADH	Cortisol, thyroid, ADH
	8	42	F	136	36	Adult	Idiopathic deficiency of HGH and gonadotropins	25	GH, FSH, LH	None
	9	69	F	158	57	Adult	Sheehan's syndrome	29	GH, ACTH, TSH, FSH, LH	Cortisol, thyroid
II										
	10	9	M	115	19	8	Normal	—	None	None
	11	11	M	126	22	11	Normal	—	None	None
	12	12	F	130	25	12	Normal	—	None	None
	13	13	M	142	33	12	Normal	—	None	None
	14	52	M	160	46	Adult	Multiple sclerosis	19	None	None
	15	62	M	171	58	Adult	Normal	—	None	None
	16	73	M	171	60	Adult	Normal	—	None	None
	17	79	M	173	69	Adult	Normal	—	None	None
III										
	18	45	M	186	80	Adult	Myotonic dystrophy	12	None	None
	19	48	M	168	83	Adult	Myotonic dystrophy	15	None	None
	20	51	M	170	73	Adult	Myotonic dystrophy	16	None	None
	21	51	M	172	89	Adult	Myotonic dystrophy	20	None	None

spectively. The dosages were adjusted to kilogram $BW^{3/4}$ because of the evidence (13) that this unit is more closely correlated to the subject's metabolic rate than is BW or body surface area (BSA) (which is proportional to $BW^{2/3}$). The three dosages specified were selected because (*a*) the response to GH in the two most widely used rat assays (14, 15) is linearly related to *log dose* rather than to *dose;* (*b*) preliminary experiments here showed that GH-deficient children exhibited a progressively increasing response to HGH within this range of hormone dosage. The hormone, lot 4 *C,* was generously donated by the National Pituitary Agency.

To measure the response to each dose, a subject was observed for 14 days under conditions of metabolic balance study (16). During this period the patient ate a constant diet with the following daily content: 32–35 calories/kg

TABLE II

Measurement of Response of Case No. 2 to Dose C

		Intake				Output				Balance				
	Dose of HGH	N	P	Na	K	N	P	Na	K	N	P	Na	K	Wt
		g	*g*	*mEq*	*mEq*	*g*	*g*	*mEq*	*mEq*	*g*	*g*	*mEq*	*mEq*	*kg*
Day 1	0	9.0	1.03	66	50	7.8	0.85	77	40	+1.2	+0.18	−11	+10	11.3
Day 2	0	9.0	1.03	66	50	7.7	0.83	70	46	+1.3	+0.22	−4	+4	11.2
Day 3	0	9.0	1.03	66	50	8.1	0.92	66	48	+0.9	+0.11	0	+2	11.1
Day 4	0	9.0	1.03	66	50	8.6	0.98	64	47	+0.4	+0.05	+2	+3	10.8
Day 5	0	9.0	1.03	66	50	7.7	0.93	56	41	+1.3	+0.10	+10	+9	11.1
Day 6	0	9.0	1.03	66	50	7.7	0.88	66	48	+1.3	+0.15	0	+2	11.0
Day 7	0	9.0	1.03	66	50	7.9	0.90	58	50	+1.1	+0.13	+8	0	11.0
Average I (days 3–7)						8.0 ±0.18	0.92 ±0.002	62 ±2.1	47 ±1.6	+1.0 ±0.13	+0.11 ±0.02	+4 ±3.0	+3 ±1.5	
Day 8	2.1 unit (dose C)	9.0	1.03	66	50	7.0	0.85	49	45	+2.0	+0.18	+17	+5	11.2
Day 9	2.1 unit (dose C)	9.0	1.03	66	50	6.4	0.81	45	45	+2.6	+0.22	+21	+5	11.3
Day 10	2.1 unit (dose C)	9.0	1.03	66	50	6.2	0.78	44	40	+2.8	+0.25	+22	+10	11.5
Day 11	2.1 unit (dose C)	9.0	1.03	66	50	5.9	0.73	40	36	+3.1	+0.30	+26	+14	11.5
Day 12	2.1 unit (dose C)	9.0	1.03	66	50	6.7	0.83	56	41	+2.3	+0.20	+10	+9	11.9
Day 13	2.1 unit (dose C)	9.0	1.03	66	50	6.0	0.78	46	42	+3.0	+0.25	+20	+8	12.0
Day 14	2.1 unit (dose C)	9.0	1.03	66	50	5.5	0.73	32	35	+3.5	+0.30	+34	+15	12.1
Average II (days 10–14)						6.1 ±0.2	0.77 ±0.19	44 ±4.0	49 ±5.4	+2.9 ±0.17	+0.26 ±0.10	+22 ±3.2	+11 ±1.7	
Absolute Response (mean ±SE)										+1.9 ±0.21	+0.15 ±0.10	+18 ±4.3	+8 ±2.2	0.16
Response per M^2BSA (mean ±SE)										+2.6 ±0.29	+0.2 ±0.13	+25 ±6.0	+11 ±3.0	0.22
Response per (kg $BW^{\frac{3}{4}} \times 10^{-1}$) (mean ±SE)										+2.2 ±0.24	+0.16 ±0.11	+20 ±6.6	+9 ±2.5	0.19

BW; 1.5–2.0 g protein/kg BW for patients < 12 yr, and 1.0–1.5 g protein/kg BW for those > 12 yr; 45–80 mg Na/kg, 40–90 mg K/kg, 0.020–0.035 g P/kg, and 0.020–0.040 g Ca/kg. Fat furnished 37–45% of the calories, and carbohydrate, 36–55%. To promote "equilibration" of the patient with this diet, the subject was generally hospitalized for 17 days. During the first 3 hospital days, he ate a diet closely similar to the "metabolic diet" in content of calories, protein, carbohydrate, fat, Na, K, Ca, and P. On the 4th hospital day (day 1 of the 14-day metabolic balance study), the metabolic diet was begun; this diet was prepared throughout the 14-study days from the same lots of food items. On 4 of the study days, the menus were prepared in duplicate, one set being consumed by the patient and the other analyzed for N, P, Na, and K content. All urine was collected in 24-hr pools, and all stools in 3–5 day pools. After 7 days without HGH treatment, the selected dose was administered for 7 days (the hormone, dissolved in 0.9% NaCl at a concentration of 1–2 U/ml, was injected intramuscularly at 8 a.m.). The data of the first 2 days of the control and experimental periods were considered to represent equilibration (see Table II); the effects of the specified dose of HGH upon the subject's N, P, Na, and K balances were therefore calculated from the data of the last 5 days of the control and experimental periods. Patients were weighed daily before breakfast in order to evaluate weight gain (the basis of a popular GH assay method in the rat [15]) as a possible index of response to HGH.

Thus the subject's response to one dose of HGH was measured on each hospitalization (involving 14 days of metabolic balance study); to determine the "full" dose response curve to all three doses, the patient was hospitalized three times at 2- to 3-month intervals. In 11 patients, after completion of three admissions, the response to two or three doses of HGH was determined again on subsequent admissions, in order to evaluate the reproducibility of the measurement of response. All replicate experiments in a given subject were done within a 14 month period.

N, P, Na, and K in diet, urine, and stool were measured according to References 17–19.

RESULTS

Balance data

A representative protocol is shown in Table II. The response of this 6 yr old boy with isolated GH deficiency to dose *C* was calculated as (average daily balance for N, P, Na, or K during days 10–14) minus (average daily balance during days 3–7). Average daily BW increase during 7 days of treatment (weight on day 14 minus

weight on day 7, divided by 7) was also calculated. These "absolute" responses were: + 1.9 g N, + 0.15 g P, + 18 mEq Na, + 8 mEq K, + 0.16 kg BW. To normalize the response to the size of the subject, the absolute responses were divided by ($BW^{3/4} \times 10^{-1}$), giving values of + 2.2 g, + 0.16 g, + 20 mEq, + 9 mEq, and + 0.19 kg, respectively. (Similar normalization results from dividing by m^2 of BSA [2] with values + 2.16 g, + 0.2 g, + 25 mEq, + 11 mEq, and + 0.19 kg). 8 months later the experiment shown in Table II was repeated. Response per (kg $BW^{3/4} \times 10^{-1}$) was: + 2.6 g N, + 0.20 g P, + 25 mEq Na, + 21 mEq K, and + 0.21 kg BW. 23 other repetitions of earlier experiments showed a similar degree of reproducibility.[3]

The responses of all nine subjects in group I are shown in Fig. 1. In general, the magnitude of the responses (normalized to $BW^{3/4} \times 10^{-1}$) to each dose declined with age. Only the two 6 yr old children responded significantly [4] to all three doses of HGH. Among the five subjects 15–17 yr old, three responded to doses *B* and *C*, and two to dose *C* only.

In group II, no definite relationship between age and responsiveness was apparent. All eight subjects, age 9–79, responded to dose *C* to a similar extent (N response, + 0.15 to + 0.8 g/day per kg/$BW^{3/4} \times 10^{-1}$); only one subject (case 12, age 12) responded to dose *B*, and none to dose *A*. The responses of group II at all ages to dose *C* were only 30–50% as great as those of group I at comparable ages.

In group III, all four responded to doses *B* and *C*, and all but one responded to dose *A*. The magnitude of these responses was similar to those of the 6 yr old children in group I, and was greater than those of all other subjects in the study.

The responses of the members of the three groups are compared in Fig. 1.[3]

Other observations

A. *Electroencephalograms.* All patients in group I had abnormal EEG's (electroencephalogram) before HGH treatment was begun. A variety of abnormalities were observed, usually including: bursts of 14 and 6 per second positive-spike activity in sleep; intermittent high amplitude slow activity of 4–5 or 1–3 per second; marked response to hyperventilation consisting of diffuse high amplitude slow activity of 1–3 per second. EEG's were repeated on the 5th to 7th day of each course of HGH and also 20–30 days later. No consistent effect of HGH was observed.

B. *Hematocrit, red blood cell count (RBC), and hemoglobin.* Hematocrit, RBC and hemoglobin were subnormal in all members of group I (range 31–38%, 3.0–4.1 million/mm^3, and 9.5–12.5 g per 100 ml respectively). At the completion of a 7 day course of HGH at dose *C*, these values were unchanged. But in all four cases reexamined in this respect 20–30 days later, hematocrit, RBC, and hemoglobin values had improved by an average of 15, 14, and 13% respectively.

C. *Electrocardiographic changes.* Three of the four cases in group III (cases 18, 19, and 21) had first degree heart block; during treatment with dose *C*, the PR interval shortened in all three of these subjects (average change − 17%), and the amplitude of the QRS complexes increased in most leads (average changes in leads I, II, and III, + 32, + 44, and + 89%, respectively). These alterations were apparent by the 3rd day of treatment with dose *C* and most marked at 7 days. The fourth case of dystrophy, and subjects in groups I and II, showed no change in EKG during treatment with dose *C*.

D. *Muscle strength.* In the four cases of myotonic dystrophy, the strength of 49 muscle groups was measured before, at the completion of, and 30 days after completion of treatment with dose *C* and with dose *B*. No consistent change in score, calculated according to Lilienfeld, Jacobs, and Willis (21), was found. The degree of myotonia of the hands also seemed unchanged. All four patients, nevertheless, reported that they fatigued less easily and felt "stronger" while being treated with dose *C* and occasionally with dose *B*.

Measurements of motor performances, such as ambulation and stair-climbing times, degrees of active range of motion of selected joints, and the timed capacity to rise from a chair and from the floor, were all inconclusive.

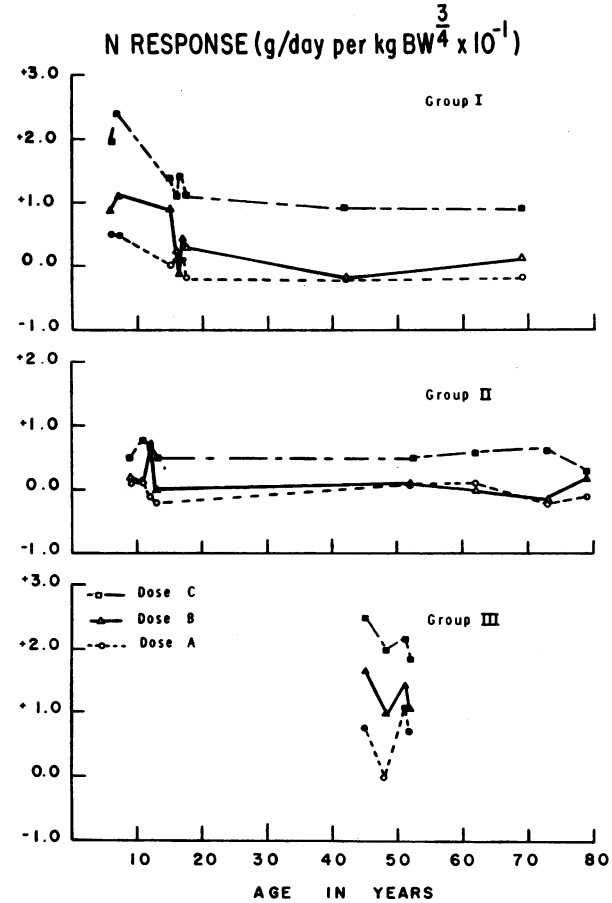
DISCUSSION

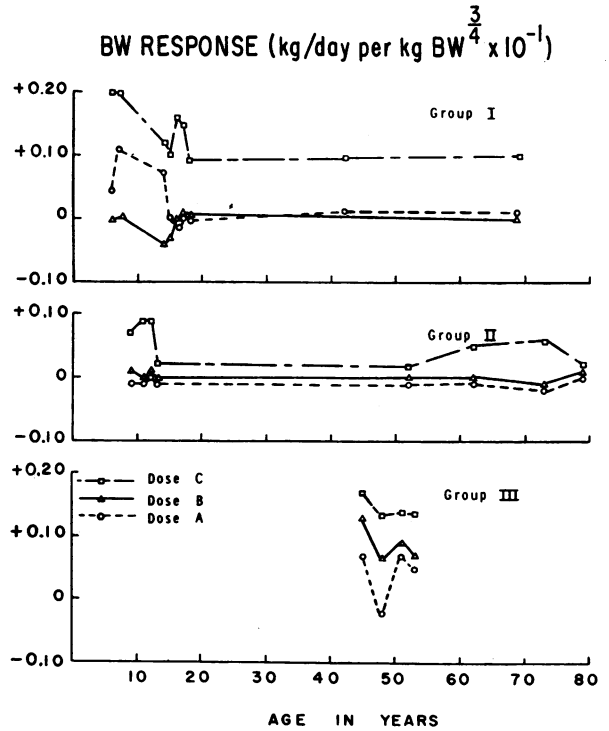
We have measured five indices of the anabolic effect of HGH: increments (Δ) in daily balance of N, P, Na, and K, and in BW. The quantitative relationships between the five indices are of interest. If the positive balances and weight gain reflect solely an HGH-stimulated formation of fat-free muscle protoplasm, the ratios ΔBW/ΔK/ΔNa/ΔP/ΔN would be 0.0291/2.7/0.77/0.067/1 (16). The averages which were actually found are given in

[2] BSA was calculated from the formula (20) $BSA = 71.84\ BW^{0.425} \times H^{0.725}$, where H is height in cm. Since $H = K_1 \times BW^{1/3}$, it can readily be calculated that $BSA = K_2\ BW^{2/3}$ (13).

[3] The numerical data showing N, P, Na, K, and BW responses to doses *A*, *B*, and *C* for all 21 subjects, including replicate experiments, have been deposited with the National Auxiliary Publication Service, No. 01406. This appendix expresses the response as average ±SE, and also gives *P* value for the significance of each response.

[4] $P < 0.05$ for the increase in N balance was used as a criterion for a significant response. In 84% of instances, such a N response was associated with a significant ($P < 0.05$) increase in phosphorus balance; in 86 and 88% of instances, with a significant ($P < 0.05$) response in balances of Na and K, respectively.





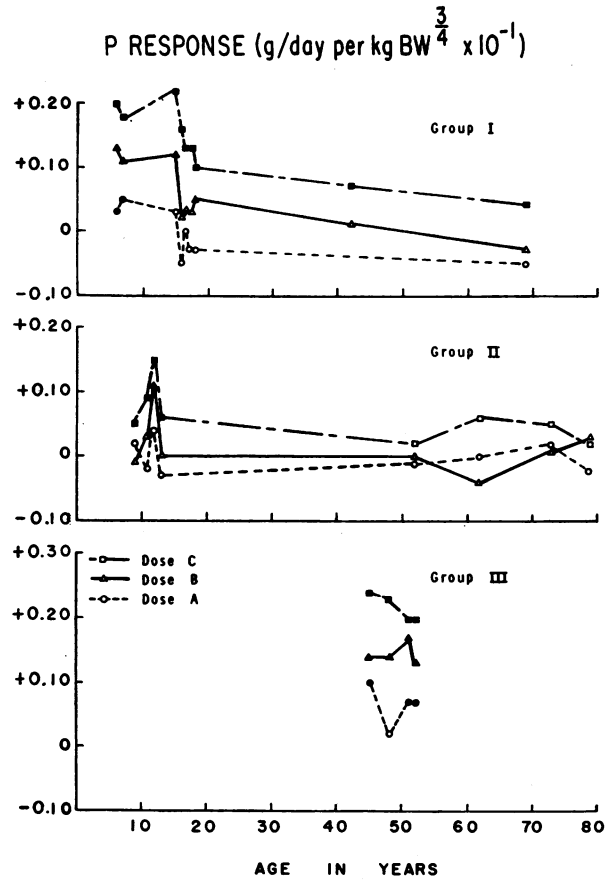
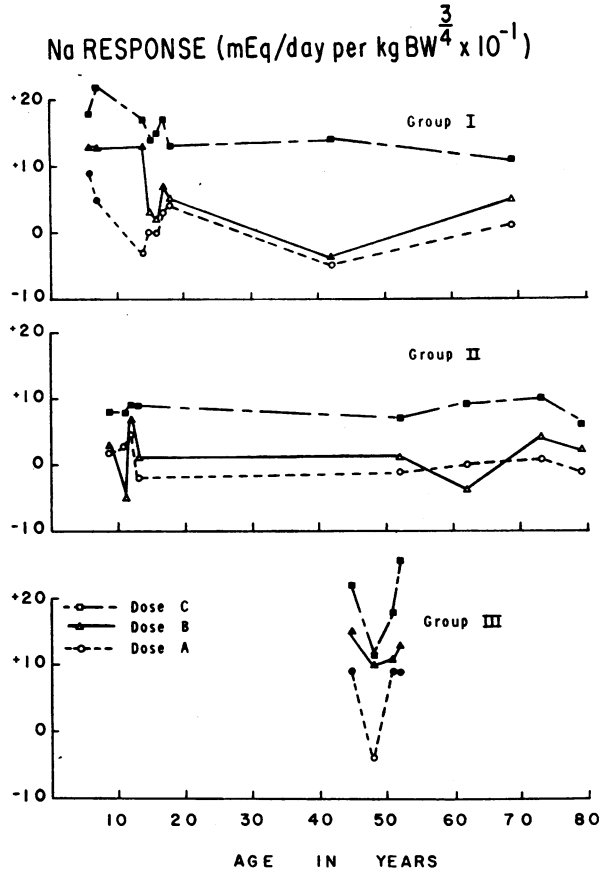
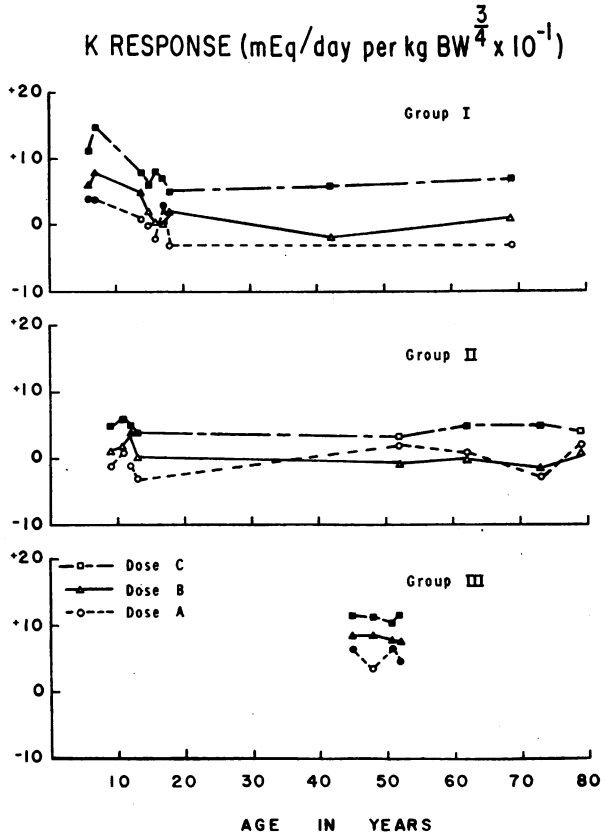


Table III. Inspection of this table shows that the theoretical ratios for ΔP/ΔN, and ΔK/ΔN, fall within the 95% confidence limits of the means of the observed ratios, while ΔNa/ΔN and ΔBW/ΔN are outside these limits. Na was retained, and BW increased, out of proportion to the quantities of N, P, and K retained. This disproportionate retention of Na probably reflects an increase in extracellular fluid volume, which has been observed in rats treated with BGH (23). The observed ratios of ΔBW/ΔN and ΔBW/ΔNa are consistent with this conclusion. The increase in extracellular volume explains the disproportion between ΔBW and ΔN. Only about 25% of the ΔBW can be accounted for by the deposition of protoplasm (16). The remaining 75% can largely be accounted for by the increase in extracellular fluid (compare theoretical ΔBW/ΔNa of 0.007 for *expansion of extracellular fluid* with the observed value

FIGURE 1 Responses of 21 subjects to doses A, B, and C of HGH. Where response to a given dose was measured twice, the average of the two measurements is shown. Closed symbols indicate $P < 0.05$ for significance of the response. P value could not be calculated for ΔBW, since this response was calculated as change in BW over 7 day period divided by 7, without estimate of variance.





of 0.004–0.007; the theoretical ΔBW/ΔNa for *deposition of protoplasm,* in contrast, is 0.38). Retention of more Na than K in human subjects during treatment with HGH has been observed previously (10, 24, 25).

The ratios among ΔBW, ΔNa, ΔK, ΔP, and ΔN do not differ significantly between groups I, II, and III, or between subjects < 17 yr old and > 17 yr old within group I or group II (Table III). This suggests that the

TABLE III

Comparison of Observed and Theoretical Ratios between ΔP(g), ΔN(g), ΔNa(mEq), ΔK(mEq), and ΔBW(kg)
Each Δ is expressed per day per ([kg BW$^{\frac{3}{4}}$] × 10^{-1})

	ΔP/ΔN	ΔNa/ΔN	ΔK/ΔN	ΔBW/ΔN	ΔBW/ΔNa
Observed for group I					
Mean	0.13	14.3	6.2	0.051	0.0045
95% confidence limits	0.05–0.21	8.5–20.1	1.2–11.2	0.030–0.072	0.0030–0.0060
Observed for group II					
Mean	0.18	15.4	7.8	0.093	0.0064
95% confidence limits	0.04–0.32	9.4–21.4	2.0–13.6	0.045–1.41	0.0019–0.0109
Observed for group III					
Mean	0.10	10.1	6.1	0.072	0.0070
95% confidence limits	0.02–0.18	8.0–12.2	2.0–10.2	0.040–1.04	0.0030–0.0100
Theoretical (on basis of deposition of muscle protoplasm)	0.067	0.77	2.7	0.029	0.38

composition of the protoplasm, and the ratio of extracellular fluid to protoplasm, accumulated in response to HGH, was generally similar in the various types of subject under study.

While the *nature* of the response thus appeared similar in all the subjects, the *magnitude* of the response varied with age, endocrine status, and presence of muscle disease (Fig. 1): (*a*) In group I, advancing age tended to be associated with diminishing responsiveness to all three dosages of HGH. (*b*) The members of group II exhibited a smaller response to dose *A, B,* or *C* than members of group I of comparable age. (*c*) The responses of the subjects of Group III (aged 45–51) were comparable to those of the 6-yr old GH–deficient subjects of group I and greater than those of all other persons tested in group I or group II.

1. In the GH-deficient subjects (group I), the correlation coefficients for the relationship between age and ΔN, ΔP, ΔNa, or ΔK at each dose of HGH were uniformly negative, ranging between -0.358 and -0.761. In 8 of 12 instances (4 indicators of response at 3 dosages), the P value for significance of this negative correlation was < 0.05. In groups II and III, no statistically significant correlation between age and response was evident. While these findings raise the *possibility* that in GH-deficient subjects, the responsiveness to exogenous HGH may diminish with age, there are several reasons why a firm conclusion on this matter is not warranted at present. The statistical significance of the inverse correlation was not convincing, generally not reaching the 1% level. Furthermore, the nine subjects of group I did not represent a balanced sampling of the age span under consideration, since five patients were clustered at age 15–17, and two patients at age 6. Finally, the apparent decline in responsiveness might not be an effect of age per se, but could instead be related to the duration of exogenous cortisol therapy or to the length of time during which endogenous GH had been secreted at a normal rate. Accordingly, a larger series will need to be studied before final conclusions can be made about the possible age-dependence of responsiveness to HGH in GH-deficient subjects.

2. A greater responsiveness to exogenous HGH of the individual lacking endogenous HGH was evident at all dosages of HGH up to the age of 17. Our subjects over 40 (2 in group I vs. 3 in group II) did not show this difference. Further cases will need to be tested before conclusions can be made about the relationship between endogenous HGH function and responsiveness to exogenous HGH during adulthood. The present evidence that GH deficiency enhances responsiveness of children to exogenous HGH confirms the conclusions of Prader et al. (6) and Brown and coworkers (7).

3. The hyperresponsiveness of adults with myotonic dystrophy was an unanticipated finding but was uniform in all four (unrelated) cases studied. The significance of this characteristic of these patients will not be known until further work is done. Muscle cells of the rat are highly sensitive to BGH, which stimulates amino acid uptake and synthesis of a variety of proteins by these cells (26). The major portion of the positive N, P, and K balance produced by BGH in the hypopituitary rat reflects the deposition of muscle protoplasm (27). The marked increments in these balances in the myotonic-dystrophy patients may therefore reflect a hyperresponsiveness of their muscle cells to HGH. The simultaneous retention of Na in a ΔNa/ΔN ratio of about 10, suggests a greater than normal responsiveness of these patients to the effects of HGH on extracellular volume as well as on formation of protoplasm. The insulin-provocative tests indicated normal endogenous GH function in these patients,[5] so that hyperresponsiveness cannot be ascribed to deficiency of endogenous GH; in any case, their responses far exceed those of even GH-deficient subjects > 20 yr in age. Perhaps the unknown biochemical lesion in the myotonic-dystrophy muscle cell is palliated or corrected by HGH with resulting rapid deposition of protein in these cells. If this interpretation should be correct, then long-term treatment of these patients with HGH might benefit the muscle disease. Improvement in the prolonged PR interval in the three myotonic dystrophy cases with first degree heart block, supports this conjecture. On the other hand, one could reason that an abnormal hyperresponsiveness of muscle cells to HGH may have predisposed to, or played a role in the development of, the degenerative disease of the muscle cells. Should this be the case, treatment with HGH could prove to have a detrimental rather than beneficial effect on myotonic dystrophy. Finally, the hyperresponsiveness to HGH may be a nonspecific consequence of atrophy or disuse of muscle, rather than a unique characteristic of the myotonic dystrophy patient. To examine this possibility, patients with other types of atrophic muscle disease are currently under study.

The presence of anemia in all GH deficient children studied, and improvement in the anemia in four cases reexamined 23–30 days after dose *C*, suggest that HGH may stimulate erythropoiesis in man. This possibility is supported by the observations of Meineke and Crafts (28) and of Fruhman, Gertsner, and Gordon (29) with BGH in the hypophysectomized rat.

[5] Peak serum HGH-levels during insulin-induced hypoglycemia were 12.7 ± 3.0 (average $\pm$SE, $n = 9$) for group III and 14.6 ± 3.4 (average $\pm$SE, $n = 18$) for group II.

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