

The Effect of Administration of Human Growth Hormone on the Plasma Growth Hormone, Cortisol, Glucose, and Free Fatty Acid Response to Insulin: Evidence for Growth Hormone Autoregulation in Man

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ABSTRACT The effect of administration of human growth hormone (HGH) (3 mg every 6 hr for 6 days) on the endogenous GH response to insulin-induced hypoglycemia at 8, 12, 24, and 48 hr posttreatment was studied in 11 healthy male adults. Free fatty acid, cortisol, and glucose responses pre- and posttreatment with HGH were evaluated concurrently. Control subjects received saline injections to evaluate relationship of GH responses to the periodicity of insulin tolerance tests. The data were compared for each subject pre- and posttreatment with HGH as well as by comparison of the results of the saline-treated group with those of the HGH-treated group.

The mean maximal GH concentration in response to insulin-induced hypoglycemia for all the subjects ($n = 16$) was 31.1 ± 3.6 ng/ml (\pm SEM) on day 1 of the control period and 23.4 ± 3.1 (SEM) on day 2, not statistically significant.

A significant decrease in the maximal peak GH response ($n = 8$) after insulin-induced hypoglycemia was observed at 8 and 12 hr after HGH administration was terminated with mean peak values for GH of 4.6 ± 1.3 ng/ml and 10.4 ± 1.9 ng/ml, respectively ($P < 0.01$). A progressive return to control values was noted between 12 and 24 hr. The GH responsiveness of the saline-treated group ($n = 5$) was unchanged from that observed during the control period.

The fasting glucose values were unchanged in the GH-treated group from those of the control period or of the saline-treated controls. Insulin resistance was ap-

parent at 8 hr posttreatment with HGH. No differences in FFA response after insulin-induced hypoglycemia were observed in GH-treated or saline-treated subjects. The rise in plasma cortisol after insulin-induced hypoglycemia was comparable in the GH-treated and saline-treated group. Diurnal variation in plasma cortisol was maintained during the period of GH suppression.

These observations support the concept that GH can modulate its secretion by means of an auto-feedback mechanism.

INTRODUCTION

Experimental data in rats suggest that growth hormone (GH) may participate in the modulation of its secretion by means of an auto-feedback mechanism operative at the level of the hypothalamus or pituitary gland (2-10); however, evidence for a similar mechanism in man is limited. Women in the third trimester of pregnancy and during the first few weeks postpartum have a reduced GH response to insulin-induced hypoglycemia and to arginine (11-14). This behavior is coincident with high circulating levels of human chorionic somatomammotropin (HCS) (15), a placental hormone which is immunologically and physicochemically similar to GH and exhibits many of its metabolic effects, but at a considerably lower order of potency (16). The decreased GH response to insulin and arginine provocation observed in late pregnancy and the immediate postpartum period may be a consequence, at least in part, of the high concentration of plasma HCS. Further, we have observed in children that growth hormone release after provocative stimuli may be diminished or absent when the pretest concentration of plasma GH is elevated (17).

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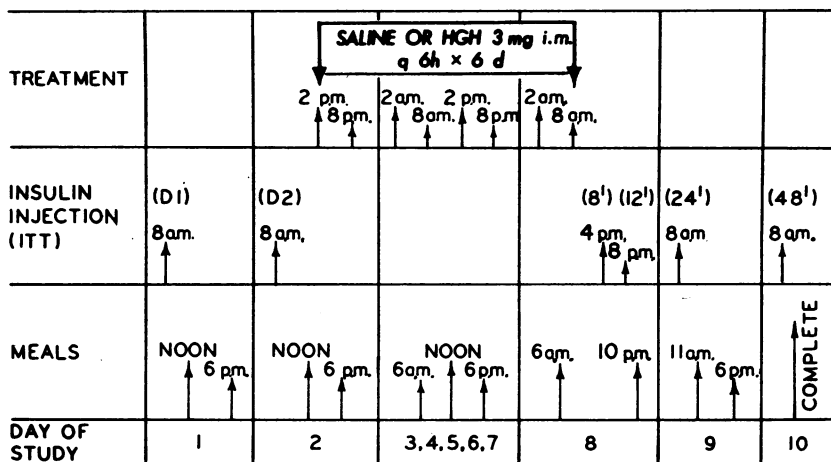


FIGURE 1 Diagram of the protocol utilized in the study showing the relationships between meals, treatment, and testing procedures.

The present study is an attempt to (a) define GH autoregulation in man by utilizing the GH response to insulin-induced hypoglycemia as a method of assessing this phenomenon, (b) clarify the individual variability in GH response to a hypoglycemic stimulus in a homogeneous population under stringently controlled experimental conditions, and (c) elucidate concurrent changes in the concentration of plasma cortisol and free fatty acid (FFA), substances which have been implicated in the control of GH secretion. The results indicate the presence of GH-induced inhibition of the GH response to insulin-induced hypoglycemia in man.

METHODS

Subjects for this study were 16 male volunteers, aged 24–30 yr, who were hospitalized for treatment of traumatic orthopedic injury (Table I), but at the time had convalesced fully. All were ambulatory but restricted to the ward during the study. None had a personal or family history of endocrine or metabolic disease or were obese. None had taken any medication for at least 1 wk before admission to the study.

All studies were carried out on a metabolic ward at a constant temperature (72°–74°F) in a comfortable, yet stringently controlled, setting in which environmental conditions and subject routine varied little from day to day. The patients were permitted no strenuous exercise and slept only during the night (10 p.m. to 6 a.m.) during which time lights were turned off. Meals were served at standard hours (Fig. 1); the diets were isocaloric and contained at least 250 g carbohydrate and approximately 27% fat. All subjects were weighed daily and showed no appreciable weight variation during the course of the study.

Hypoglycemia was induced by intravenous injection of crystalline insulin (ITT) 0.1 U/kg body weight at 8 a.m., 24 hr after admission to the ward. Blood samples were collected from an indwelling venous catheter which was inserted at least 4 hr before ITT (patency of the catheters was maintained by a very slow infusion of normal saline). Samples

were collected immediately before injection and 15, 30, 45, 60, 90, and 120 min after insulin administration. A second ITT was done on day 2 to establish base line responses and assess day-to-day variation.

Control period. The subjects were separated into two groups (Table I) on the basis of alternate admission to the study. Group I (n=5) received normal saline (0.6 cc) intramuscularly every 6 hr for 6 days (days 2–8) and group II (n=11) received human GH (National Pituitary Agency No. 3-C) dissolved in pyrogen-free water (0.6 cc = 3 mg GH) intramuscularly every 6 hr for 6 days (Fig. 1). Injection sites were rotated in both groups to avoid excessive local irritation and discomfort. Vials of GH and

TABLE I
Clinical Data

	Subjects	Age	Ht.	Wt.
		yr	cm	kg
Saline treated	L. E. A.	26	177.8	73.8
	B. O. T.	24	174.0	78.7
	B. O. Y.	28	195.6	68.2
	V. W. I.	30	176.5	64.6
	B. R. O.	29	171.5	66.3
GH treated	B. O. D.	25	170.2	64.7
	Y. E. L.	25	171.5	71.9
	S. U. T.	27	185.4	83.8
	R. Y. A.	24	172.7	73.3
	H. O. O.	28	182.2	76.0
	M. A. C.	30	175.9	81.7
	V. A. F.	29	184.2	88.6
	R. I. C.	26	182.9	72.9
	W. E. G.	24	175.3	76.4
	W. I. L.	24	175.3	62.7
	P. E. N.	25	180.3	83.0

TABLE II
Summary of Data for All Subjects ($n = 16$) during Control Period, Mean \pm SEM

	Day 1	Day 2	Averaged (D1 + D2)
Fasting plasma glucose, mg/100 ml	95.4 \pm 2.3	94.1 \pm 0.8	93.8 \pm 1.2
Maximal per cent fall of plasma glucose, %	62.1 \pm 2.2	65.8 \pm 1.5	63.9 \pm 1.7
Fasting plasma GH, ng/ml	1.2 \pm 0.2	1.7 \pm 0.7	1.4 \pm 0.4
Peak Plasma GH, ng/ml			
All subjects	31.1 \pm 3.6	23.4 \pm 3.1	26.7 \pm 3.3
Saline treated			24.3 \pm 4.2
GH treated			28.1 \pm 4.9
Fasting plasma FFA, μ Eq/liter	1193.9 \pm 111.1	1033.8 \pm 71.2	1113.8 \pm 83
Maximal per cent fall of plasma FFA, %	46.1 \pm 4.3	50.7 \pm 4.2	48.4 \pm 2.9
Per cent recovery of plasma FFA at 120 min, %	96.5 \pm 11.5	79.4 \pm 7.9	88.0 \pm 8.8

saline were identified only by code; contents were unknown to the subject or the person who administered the injections.

Posttreatment period. Posttreatment ITT's were performed 8, 12, 24, and 48 hr after the last injection of either saline or GH (Fig. 1). Three group II subjects (W.E.G., W.I.L., and P.E.N.) received a higher dose of regular insulin (0.15 U/kg body weight) for the first post-GH treatment ITT (8 hr after the previous injection). All patients were fasted for 8 hr before an ITT, but on day 8 they fasted until the completion of both the 8 and 12 hr post-treatment ITT.

The concentration of plasma cortisol was measured in four saline-treated and eight GH-treated subjects at 0 and 60 min after insulin injection on the 2nd control day, and at 8 and 12 hr after completion of treatment.

Plasma glucose in all patients was measured by a glucose oxidase method (18), plasma GH by a modification of the double antibody method (19), plasma FFA by a modification of the method of Dole (20), and plasma corticoids by the competitive protein-binding method (21). Antibody formation to GH was tested by the method of Berson, Yalow, Bauman, Rothschild, and Newerly (22). All samples from any individual patient were analyzed during the same assay.

Results were analyzed by nonparametric criteria, which have advantages of (a) independence of the distribution of values from the test population and (b) comparison of data derived from different or independent experimental populations without distortion. The Kruskal-Wallis one-way analysis of variance (23) was used to convert individual scores into ranks, thereby providing high sensitivity as to the direction of the data without introduction of distortion caused by numerical differences (cf. sample calculation, Appendix).

RESULTS

Control period (days 1 and 2)

Glucose response. Fasting plasma glucose concentrations on days 1 and 2 were normal in all patients except W.E.G., in whom a fasting value of 120 mg/100 ml was obtained on day 1, but was 97 mg/100 ml on day 2; he demonstrated a normal hypoglycemic response

to insulin on both days. Plasma glucose concentration was higher in 8 of the 16 patients on day 1 and in seven different subjects on day 2; 1 patient had the same fasting value on both days. The mean difference between the day 1 and day 2 fasting values was 4.3 ± 0.8 mg/100 ml ($\bar{x} \pm$ SEM, $n = 15$), exclusive of W.E.G. Comparison of the mean plasma glucose for the entire group on day 1 and day 2 further dampened this small individual subject variation, 95.4 ± 2.3 ($\bar{x} \pm$ SEM, $n = 16$) on day 1 and 94.1 ± 0.8 , $n = 16$, on day 2. No significant individual variation was observed between the 2 control days.

Maximal fall in plasma glucose concentration occurred within 30 min of insulin administration in all subjects, on both control days. The variation in maximal hypoglycemic response of individual subjects was minimal (mean difference in lowest plasma glucose concentration between day 1 and 2 was 5.3 ± 1.4 mg/100 ml ($\bar{x} \pm$ SEM, $n = 16$)). The group mean maximal per cent fall in plasma glucose was $62.1 \pm 2.2\%$ on day 1 and $65.8 \pm 1.5\%$ on day 2 ($\bar{x} \pm$ SEM, $n = 16$), a negligible variation (Table II).

GH response. Fasting GH values of 1.0 ng/ml or less were observed on both control days in 13 subjects. 2 subjects (R.Y.A. and Y.E.L.) had a fasting level of 1.2 and 3.2 ng/ml, respectively, on day 1, and a fasting level of 1.0 ng/ml on day 2. Subject R.I.C., whose fasting concentration was 1.0 ng/ml on day 1, had a fasting value of 21.0 ng/ml (on repeated assays) and a 15 min (postinsulin) level of 1.0 ng/ml on day 2. The peak concentration of plasma GH after insulin injection was noted at 60 min in 8 of 16 subjects on day 1 and 9 of 16 subjects on day 2. In P.E.N., peak value was at 120 min on both control tests. In the remaining patients, maximal values occurred at 45 or 90 min. The group

TABLE III
Plasma Concentrations of GH during Saline or GH Treatment

	Time after Injection		
	3 hr	5 hr	6 hr
Saline treated			
I. E. A.	1.0 (2)	1.0 (2)	1.0 (2,6)
B. O. Y.	1.0 (2)	1.0 (2)	1.0 (2,6)
B. O. T.	1.0 (2)	18.0 (2)	1.0 (2,6)
V. W. I.	1.0 (2)	1.0 (2)	1.0 (6)
B. R. O.	1.0 (2)	1.0 (2)	1.0 (2,6)
GH treated			
B. O. D.	7.5 (2)	5.0 (2)	4.5 (2)
S. U. T.	8.0 (2)	5.0 (2)	3.0, 1.0 (2,6)
R. Y. A.	4.0 (2)	7.5 (2)	1.0 (6)
H. O. O.	10.5 (2)	—	8.0, 7.0 (2,6)
M. A. C.	6.8 (2)	—	4.0, 5.2 (2,6)
V. A. F.	6.5 (2)	6.5 (2)	5.5, 7.5 (2,6)
R. I. C.	3.0 (2)	3.0 (2)	4.5 (2)

Parentheses indicate day of treatment; plasma concentrations given in nanograms per milliliter.

mean maximal plasma GH concentration was 31.1 ± 3.6 ng/ml ($\bar{x} \pm \text{SEM}$, $n = 16$) on day 1 and 23.4 ± 3.1 ($n = 16$) on day 2, not statistically significant (Table II). The mean difference in peak plasma GH concentration for an individual subject between day 1 and 2 was 12.1 ± 2.3 ($n = 16$). This variation is greater than the $\pm 10\%$ intra-assay and between-assay variation for the GH radioimmunoassay method utilized in this study. In 4 subjects, the peak values on the two tests fell within 10% of the higher value; 8 of the remaining 12 subjects showed higher peak GH concentrations on day 1. Mild symptoms of hypoglycemia were noted in less than two-thirds of the subjects; when they occurred they conformed temporally to the lowest concentration of plasma glucose.

FFA response. 6 subjects had fasting plasma FFA levels $> 1000 \mu\text{Eq/liter}$ on both control days; 2 others had high levels only on day 1, and 2 others only on day 2. The high values were limited to the subjects who were cigarette smokers, as noted by others.¹ Prohibition of cigarettes was successful only for the 1 hr preceding each ITT.

The mean difference in concentration between the 2 control days for an individual subject was $313.9 \pm 48.0 \mu\text{Eq/liter}$, $n = 16$; 75% had lower fasting values on day 2. The mean fasting value for the group was 1193.9 ± 111.1 , $n = 16$, on day 1 and 1033.8 ± 71.2 , $n = 16$, on day 2 (Table II). These values were not significantly different. Changes in plasma FFA levels after insulin administration were calculated as per cent changes from

the fasting value. The maximal per cent fall occurred on day 1 by 30 min in 14 of 16 subjects and by 45 min in the remaining 2 subjects. A similar pattern of response was observed on day 2. The mean maximal per cent decrease for the group was $46.1 \pm 4.3\%$, $n = 16$, and $50.7 \pm 4.2\%$, $n = 16$, on days 1 and 2, respectively. Restoration of plasma FFA values from insulin-induced depression was calculated as the per cent of fasting value achieved at 120 min. The mean group rise was $96.5 \pm 11.5\%$, $n = 16$, on day 1 and $79.4 \pm 7.9\%$, $n = 16$, on day 2; these values were not significantly different.

The pattern of plasma FFA response to insulin ad-

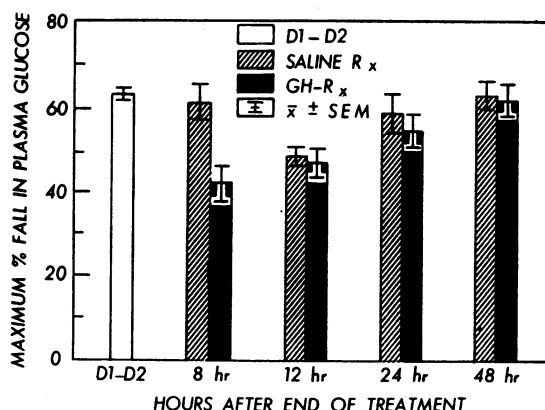


FIGURE 2 The height of the bar represents the maximal per cent depression of plasma glucose before and after treatment with saline or GH. Moderate insulin resistance was noted in the GH-treated group at 8 hr as compared with saline-treated group and the control period data.

¹Feldman, E. B. Personal communication.

TABLE IV
Effect of 6 days of GH Treatment on Fasting Plasma Glucose, GH, and FFA

	Fasting values, control D1 - D2	After 6 days treatment			
		8 hr	12 hr	24 hr	48 hr
Plasma glucose (mg/100 ml)	93.8 ± 1.2				
Saline treated		89.8 ± 0.7	96.4 ± 2.3	108.0 ± 11.6	94.8 ± 2.4
GH treated		100.1 ± 4.9	96.9 ± 2.4	87.5 ± 2.4	87.9 ± 0.8
Plasma GH (ng/ml)	1.4 ± 0.4				
Saline treated		3.0 ± 1.1	1.1 ± 0.1	1.0 ± 0.0	1.0 ± 0.0
GH treated		3.5 ± 0.5	1.7 ± 0.4	4.0 ± 2.6	2.9 ± 1.3
Plasma FFA (μEq/liter)	1113.8 ± 83.1				
Saline treated		1466.8 ± 350.9	2238.2 ± 249.0	1171.6 ± 203.6	1054.8 ± 103.5
GH treated		1348.0 ± 215.2	1161.8 ± 159.3	914.6 ± 128.9	790.8 ± 173.6

ministration was characterized by a fall in FFA level to approximately 50% of the fasting value, usually maximal by 30 min, followed by minor oscillations which terminated in a rise to approximately fasting value by 120 min.

Day-to-day variation was observed for most of the variables measured, although the differences were not statistically significant. Hence, the data obtained for each subject on days 1 and 2 were averaged (Table II). The results of posttreatment ITT's were compared to the averaged control data for statistical analysis.

When the concentration of plasma GH was measured 3, 5, and 6 hr after an injection of saline, $n = 5$, or of GH, $n = 7$, on different days during the post-control period, the slope of decrease in the concentration of plasma GH during this slow phase was duplicated closely on different treatment days (Table III).

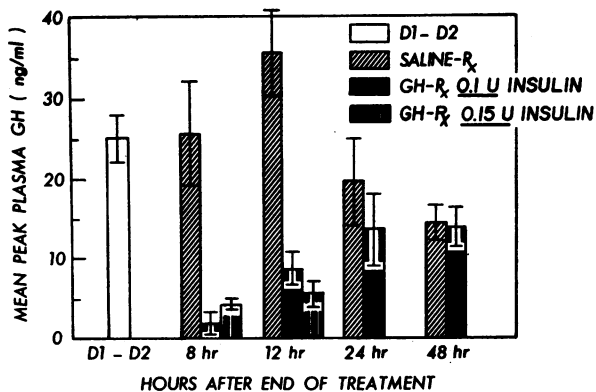


FIGURE 3 The mean maximal plasma GH concentration (ng/ml) after insulin-induced hypoglycemia at 8, 12, 24, and 48 hr after 6 days of saline or GH administration. The data for the control period (D1-D2) have been averaged. Note the marked inhibition of GH response at 8 and 12 hr post-GH administration.

Posttreatment period (days 8-10)

Glucose response. Fasting plasma glucose levels were comparable in both groups throughout the post-treatment period and were not significantly different from the mean control value. Insulin resistance after GH treatment was noted during the first postinjection ITT (8 hr) (Fig. 2). The mean maximal per cent fall in the GH-treated group was 34% less than the comparable control value ($P < 0.001$) and 30.5% less than the mean maximal per cent fall in the saline-treated group ($P < 0.02$) at 8 hr. The mean maximal per cent fall for the saline-treated group at 12 hr was 23% less than the control value ($P < 0.01$). Moderate insulin resistance at that time of day (8 p.m.) was anticipated on the basis of a previously described circadian variation in glucose-insulin interrelationships (24). The mean decrease in plasma glucose at 12 hr was 49.2% in the saline-treated and 47.6% in the GH-treated subjects, suggesting that there was no superimposed GH-induced insulin resistance at that time. The mean maximal per cent decrease in plasma glucose was comparable in both treatment groups at 24 hr (saline group 59.6%, GH group 55.6%) and 48 hr (saline group 63.4%, GH group 62.5%), and was comparable to the control value (Fig. 2).

In the three GH-treated subjects (W.E.G., W.I.L., and P.E.N.) who were given regular insulin 0.15 U/kg intravenously at 8 hr (a 50% increase in the insulin dose) the mean maximal per cent fall in plasma glucose was $34.7 \pm 5.8\%$, $n = 3$, not significantly different from the per cent fall noted in those GH-treated subjects given only 0.10 U/kg ($42.0 \pm 4.2\%$). The results (glucose, GH, FFA, and cortisol) obtained in these 3 subjects in all posttreatment ITT's were not included in the statistical data for the GH-treated group, inasmuch as they were given a larger dose of insulin at 8 hr.

GH response. Mean fasting plasma concentrations of

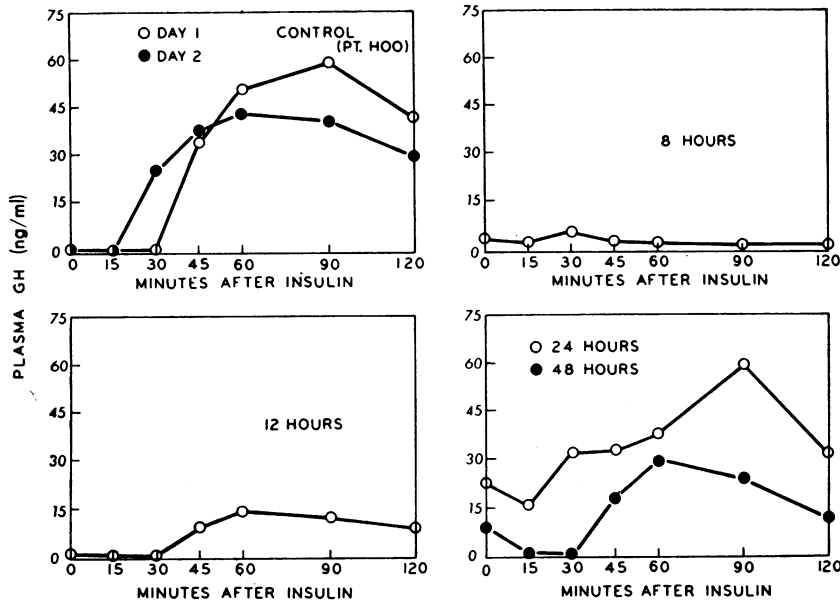


FIGURE 4 The GH response to insulin-induced hypoglycemia during the control period and at timed intervals after 6 days of GH administration is indicated for subject H.O.O. GH responsiveness comparable to the control period was not observed until 24 hr after discontinuation of GH treatment.

GH were similar (3.0 ± 1.1 , 3.5 ± 0.5 ng/ml) in both treatment groups at 8 hr, and in all succeeding ITT's (Table IV). Posttreatment results were analyzed in two different ways to minimize artifacts introduced by design of study resulting from biologic variation: data were compared to control data for the same subjects and, alternately, to data for the saline-treated subjects obtained at each test period.

When tested at 8 hr the maximal concentration of plasma GH after insulin-induced hypoglycemia (0.1 U/kg) in subjects given GH (4.6 ± 1.3 ng/ml) was significantly less than the control value (28.1 ± 4.9 , $P < 0.01$) (Fig. 3). A similar suppression in concentration of GH was noted at 12 hr (10.4 ± 1.9 ng/ml versus control of 28.1 ± 4.9 , $P < 0.01$). The mean maximal concentration at 24 and 48 hr, while 11.1 and 12.0 less than the control value, was not significantly less. Mean maximal concentration was 84% less than that of the saline-treated subjects at 8 hr ($P < 0.01$) (Fig. 3). The difference between the mean concentration attained by each group at 12 hr (10.4 versus 36.5 ± 5.6 ng/ml) was equally significant ($P < 0.01$). The mean maximal GH level of the saline-treated subjects at 24 and 48 hr was 3.5 and 9.3 ng/ml less, but not statistically significantly less, than the saline group control value (24.3 ± 4.2 ng/ml). The mean peak concentration of the GH-treated subjects at 24 hr (17.0 ± 6.5 ng/ml) and 48 hr (16.1 ± 2.9 ng/ml) was not significantly different from the value obtained for the saline-treated subjects (20.8 ± 5.2

and 15.0 ± 2.1 ng/ml, respectively). In the 3 patients who received the higher dose of insulin (0.15 U/kg) at 8 hr, the mean GH values were 4.3 ± 0.75 ng/ml (8 hr), 5.6 ± 1.6 ng/ml (12 hr), 12.0 ± 5.4 ng/ml (24 hr), and 12.5 ± 4.7 ng/ml (48 hr). Progressive "recovery" of the GH response to insulin-induced hypoglycemia was observed in many of the GH-treated subjects. The usual course of "recovery" of the GH response is shown for patient H.O.O. (Fig. 4). 7 of the 11 GH-treated men attained a maximal GH response greater than 8.0 ng/ml

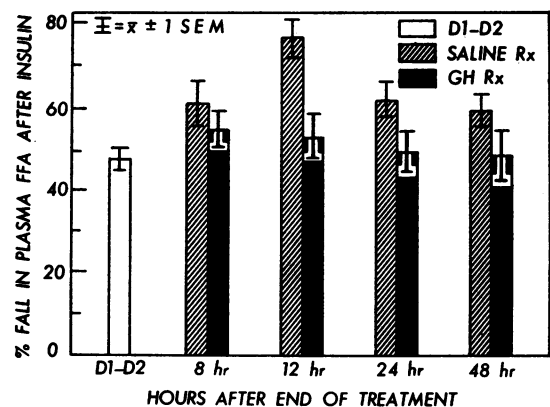


FIGURE 5 The maximal per cent depression in plasma FFA after insulin-induced hypoglycemia is compared during control period and at 6, 12, 24, and 48 hr after saline or GH administration. No significant differences were observed.

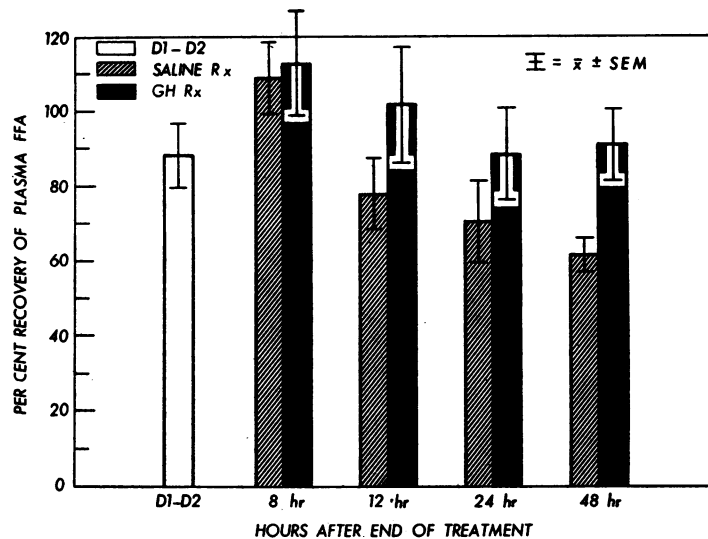


FIGURE 6 The terminal recovery of concentration of plasma FFA after insulin-induced hypoglycemia is represented as a percentage of the fasting concentration of FFA. A slight rebound phenomenon was noted only at 8 hr postsaline or GH treatment.

at 24 hr; 3 had lower maximum values when tested at 48 hr.

Antibodies to GH were not detectable in the GH-treated subjects after the 6 day treatment.

FFA response. Mean fasting concentrations of plasma FFA were similar in both treatment groups in all posttreatment ITT's (Table IV). The decrease after insulin administration was similar in the GH-treated subjects ($55.3 \pm 4.2\%$) and the saline-treated subjects ($62.0 \pm 5.1\%$) at 8 hr (Fig. 5). The decrease in the GH-treated subjects at 12 hr was similar to that noted for the GH- and saline-treated subjects at 8, 24, and 48 hr. The maximal per cent fall for the saline-treated subjects at 12 hr was 29.1% greater than the control value ($P < 0.01$), the reason for which is obscure. The fall in the GH-treated subjects at 12 hr, while not different from the control value, was significantly less than the unaccountably high value ($77.5 \pm 4.9\%$) obtained in the saline-treated subject at 12 hr ($P < 0.02$). The degree of terminal recovery was not different from the control value in any posttreatment period in either treatment group (Fig. 6).

Cortisol response. Fasting cortisol values were normal in both treatment groups before and after treatment (Fig. 7); diurnal variation was preserved during the period of GH suppression. The rise in plasma cortisol in response to insulin-induced hypoglycemia for the GH-treated subjects was not statistically significantly different from that observed for the saline-treated subjects, before and after the treatment period.

DISCUSSION

This study demonstrates that insulin administration repeated two to three times within 24 hr exerts no significant distortion on the fasting concentration or on the response of plasma glucose to insulin. The magnitude of the GH response to insulin-induced hypoglycemia varied as widely as 30 ng/ml in healthy adult males from day to day despite unchanged study conditions. Comparison of group mean values obtained on different days did not reveal the same variability as the observation of mean individual-subject differences on these days inasmuch as the individual-subject value may change in either a positive or negative direction. The absence of a definite directional trend of this variable GH response with short-interval repetitive insulin administration, suggests that the disparity in daily GH response is a manifestation of intrinsic "biologic variation" and not a consequence of experimental design. The time-course pattern of the GH response was uniformly consistent throughout the series of insulin tolerance tests. A similar individual variation in the FFA response to insulin was encountered. The randomness of the variability makes doubtful a prime distorting influence of repetitive insulin injection.

The maximum GH response of the saline-treated group tended to diminish during the late posttreatment period. The GH response of these subjects at 24 and 48 hr was less than the control or the initial posttreatment responses. A correlation between symptoms of hypoglycemic stress and the magnitude of the rise in plasma

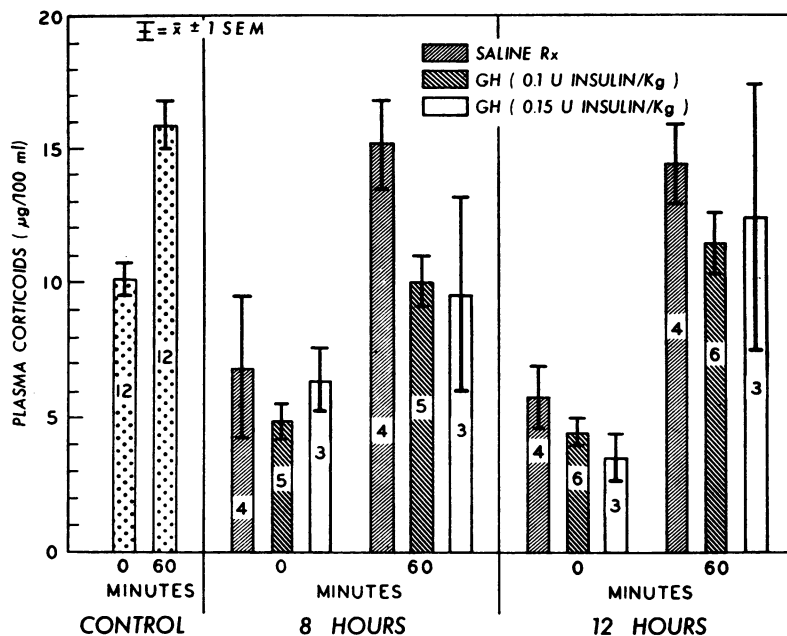


FIGURE 7 The concentration of plasma cortisol ($\mu\text{g}/100\text{ ml}$) before and at 60 min after insulin-induced hypoglycemia, during the control period, and 8 hr and 12 hr after saline or GH administration. The numbers enclosed in the bars refer to the number of subjects in whom cortisol levels were determined. Diurnal variation is maintained in the GH-treated group. The cortisol responsiveness to hypoglycemia is unchanged from control data after treatment with saline or GH.

GH was not apparent in this study, nor did the concentrations of plasma cortisol suggest a greater stress effect in the early insulin tolerance tests. Further, the mean GH response of these control subjects at 12 hr was somewhat greater than during the earlier ITT's. For these reasons, it is difficult to ascribe the diminished GH release late in the posttreatment period to increased test sophistication. Equally unlikely is the possibility that the test intervals impinged on the time required for replenishment of pituitary GH. A diminished GH response after repeated insulin-induced hypoglycemia at the intervals utilized in this study would be in accord with a decreasing sensitivity and resetting of a variable set point receptor which responds to the substrate deprivation caused by insulin. The theory of hormone control, based on such hypothalamic variable set point receptors, has been discussed by Yates and Urquhart (25).

The usual GH response to insulin-induced hypoglycemia is absent in subjects who have received 6 days of exogenous GH. This suppression persists many half-lives beyond the time at which exogenous GH is contributing significantly to the concentration of plasma GH. The diabetogenic effect of administered GH is apparent 8 hr after the last GH injection. A close correlation of the magnitude of the hypoglycemic stimulus and the rise in plasma GH was suggested by the studies

of Frantz and Rabkin (26), but has not been substantiated by subsequent reports (27-29). Glick (27) demonstrated that a decrease of 26-39 mg/100 ml in blood glucose after insulin administration leads to a normal GH response. At the 8 hr insulin tolerance test a decrease in blood glucose of 35-40 mg/100 ml was achieved in all the GH-treated subjects. It is doubtful that the diminished release of GH in response to hypoglycemia at the time was due exclusively to a smaller decrease in plasma glucose, since a similar magnitude of glucose depression in saline-treated subjects at 12 hr evoked a substantial GH response. The GH response is significantly inhibited for 12 hr after the completion of GH treatment. Comparison of the GH-treated subjects to themselves as controls indicates that the rise in plasma GH after insulin-induced hypoglycemia is restored to normal in most subjects by 24 hr.

Inhibition of the GH response to a stimulus solely by administration of GH is consistent with the action of a negative "short-loop" feedback mechanism. Autoregulation of adrenocorticotrophic hormone (ACTH) was first suggested by the observations of Gemzell and Heijkenskjold (30), Kitay, Holub, and Jailer (31), and Hodges and Vernikos (32). Characterization of this type of control mechanism for ACTH was described by Motta, Mangili, and Martini in 1965 (33). Similar

mechanisms have been proposed for luteinizing hormone (LH) (34, 35) and follicle-stimulating hormone (FSH) (36, 37).

Supporting precedent for GH autoregulation has been obtained in animal experiments. Implants of bovine GH into the rat hypothalamus reduce pituitary weight and decrease pituitary GH concentration (5, 7, 8). Decreases in pituitary GH content are noted after transplantation of GH-secreting tumors into rats (2-4, 9, 10). Sawano, Arimura, Bowers, and Schally (6) have shown that pretreatment of rats with exogenous GH blocks the depletion of pituitary GH content which ordinarily is induced by administration of purified GH-releasing factor (GHRF) or by insulin-induced hypoglycemia (3). Parenteral administration of GH inhibits the release of pituitary GH in rats, as measured by tibial epiphyseal plate responses (4). McCann and Porter (38) and Muller, Sawano, Arimura, and Schally (7) suggest that GH auto-feedback is directed at the level of the pituitary, based on their observation of decreased GH secretion in the face of undiminished GHRF activity in rats given large doses of GH. More recently, inhibition of GH responsiveness to insulin-induced hypoglycemia after a 2 hr infusion of GH has been reported in the monkey by Sakuma and Knobil (39).

The present study, demonstrating auto-regulation of growth hormone secretion in man (1) does not provide information on the precise functional anatomical locus for this phenomenon, although the results of previous studies point toward involvement of the hypothalamus. GH response to insulin-induced hypoglycemia is blocked in monkeys with ablative lesions of the hypothalamic median eminence (40) and, in man, after pituitary stalk section (41, 42) or hypothalamic disease (43, 44). Also, microinjection of glucose into the hypothalamus, coincident with systemic hypoglycemia, prevents the normal GH response to hypoglycemia (45). The administration of insulin, therefore, apparently provokes GH secretion by way of a neural arc involving hypothalamic glucoreceptors, and the median eminence as a final common pathway. Further, monkeys with median eminence lesions have normal, detectable fasting levels of plasma GH during inhibition of the GH response to hypoglycemia, just as the human subjects of the present study; this similarity suggests that the pituitary continues tonic function during suppression of a hypothalamic mechanism.

The basic nature of the GH aut feedback mechanism remains obscure. Irie, Sakuma, Tsushima, Shizume, and Nakao (46) and Muller and Pecile (3) have suggested that modulation of GH secretion is not a function of the polypeptide acting directly on the pituitary gland or hypothalamus, but rather is a result of variation in the concentration of plasma FFA. These findings were not confirmed by Blackard and Heidingsfelder, who could

not demonstrate a correlation between changes in plasma FFA and GH concentrations in the monkey (47). The present study indicates that if an intermediate substance is responsible for GH auto-regulation, the effective intermediate is probably not plasma FFA. The fasting concentration of plasma FFA and the pattern of FFA decrease and recovery after insulin administration were not different in the GH-treated subjects during inhibition of the GH response from that observed in saline-treated subjects.

The similar basal plasma cortisol values obtained before and after treatment with saline or GH are inconsistent with a significant effect of acute "stress" in auto-regulation of GH secretion in man. Circadian patterns of plasma cortisol were unaffected by GH treatment. The increase in plasma cortisol after insulin-induced hypoglycemia is similar to the results described by Landon, Wynn, and James (48). The suppression of GH hypersecretion by parenteral administration of GH was achieved without interference with, or unusual stimulation of, the hypothalamic-pituitary-adrenal axis.

APPENDIX A

Kruskal-Wallis one-way analysis of variance: an example.

$$\text{Formula: } H = \frac{12}{N(N+1)} \sum_{j=1}^k \frac{R_j^2}{n_j} - 3(N+1) \left[1 - \frac{\sum T}{N^2 - N} \right]$$

where; H = sampling distribution, the probability of which is determined from a table of critical values of chi square; k = number of samples; n_j = number of cases in jth sample; N = ∑n_j, the number of cases in all samples combined;

R_j = sum of ranks in jth sample (column); ∑ directs one to sum over the k samples (columns); j = 1; T = t³ - t (when t is the number of tied observations in a tied group of scores); and degrees of freedom = k - 1.

Data. Maximal per cent depression of plasma glucose at 12 hr:

	Saline treated	GH treated
	51	63
	57	39
	47	43
	47	52
	44	43
		39
		40
		62
Ranks.		
	k_1	k_2
	Saline treated	GH treated
	9.0	13.0
	11.0	1.5
	7.5	4.5
	7.5	10.0
	6.0	4.5
	—	1.5
	41.0	3.0
		12.0
		50.0

$$k = 2$$

$$N = 13(8 + 5)$$

$$R_1 = 41, R_2 = 50 \quad H = \frac{12}{13(14)} \left[\frac{(40^2)}{(5)} + \frac{(50^2)}{(8)} \right] - 3(14)$$

$$T = 210$$

$$d_f = 1$$

$H = 0.77567, P > 0.5$, not significant.

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REFERENCES

- Abrams, R. L., S. L. Kaplan, and M. M. Grumbach. 1969. Growth hormone autoregulation in man. Abstracts of the 51st Meeting of the Endocrine Society, New York. 106.
- Bates, R. W., S. Milkovic, and M. M. Garrison. 1962. Concentration of prolactin, growth hormone and ACTH in blood and tumor of rats with transplantable mammatropic pituitary tumors. *Endocrinology*. 71: 943.
- Müller, E., and A. Pecile. 1966. Influence of exogenous growth hormone on endogenous growth hormone release. *Proc. Soc. Exp. Biol. Med.* 122: 1289.
- MacLeod, R. M., M. C. Smith, and G. W. DeWitt. 1966. Hormonal properties of transplanted pituitary tumors and their relation to the pituitary gland. *Endocrinology*. 79: 1149.
- Krulich, L., and S. M. McCann. 1966. Influence of growth hormone (GH) on content of GH in the pituitaries of normal rats. *Proc. Soc. Exp. Biol. Med.* 121: 1114.
- Sawano, S., A. Arimura, C. Y. Bowers, and A. V. Schally. 1967. Effect of CNS-depressants, dexamethasone and growth hormone on the response to growth hormone-releasing factor. *Endocrinology*. 81: 1410.
- Muller, E. E., S. Sawano, A. Arimura, and A. V. Schally. 1967. Mechanism of action of growth hormone in altering its own secretion rate: comparison with the action of dexamethasone. *Acta Endocrinol.* 56: 499.
- Katz, S., M. Molitch, and S. M. McCann. 1967. Feedback of hypothalamic growth hormone (GH) implants upon anterior pituitary (AP). Abstracts of the 49th Meeting of the Endocrine Society. 86.
- Peake, G. T., I. K. Mariz, and W. H. Daughaday. 1968. Radioimmunoassay of growth hormone in rats bearing somatotropin producing tumors. *Endocrinology*. 83: 714.
- MacLeod, R. M., G. W. DeWitt, and M. C. Smith. 1968. Suppression of pituitary gland hormone content by pituitary tumor hormones. *Endocrinology*. 82: 889.
- Katz, H. P., M. M. Grumbach, and S. L. Kaplan. 1969. Diminished growth hormone response to arginine in the puerperium. *J. Clin. Endocrinol.* 29: 1414.
- Yen, S. S. C., N. Samaan, and O. H. Pearson. 1967. Growth hormone levels in pregnancy. *J. Clin. Endocrinol.* 27: 1341.
- Mintz, D. H., R. Stock, J. L. Finster, and A. L. Taylor. 1968. The effect of normal and diabetic pregnancies on growth hormone responses to hypoglycemia. *Metabolism*. 17: 54.
- Tyson, J. E., D. Rabinowitz, T. J. Merimee, and H. Friesen. 1969. Response of plasma insulin and human growth hormone to arginine in pregnant and postpartum females. *Amer. J. Obstet. Gynecol.* 103: 313.
- Li, C. H., M. M. Grumbach, S. L. Kaplan, J. B. Josimovich, H. Friesen, and K. J. Catt. 1968. Human chorionic somato-mammotropin (HCS), proposed terminology for designation of a placental hormone. *Experientia*. 24: 1288.
- Grumbach, M. M., S. L. Kaplan, J. J. Sciarra, and I. M. Burr. 1968. Chorionic growth hormone-prolactin (CGP): secretion, disposition, biologic activity in man, and postulated function as the "growth hormone" of the second half of pregnancy. *Ann. N. Y. Acad. Sci.* 148: 501.
- Kaplan, S. L., C. A. L. Abrams, J. J. Bell, F. A. Conte, and M. M. Grumbach. 1968. Growth and growth hormone. I. Changes in serum level of growth hormone following hypoglycemia in 134 children with growth retardation. *Pediat. Res.* 2: 43.
- Saifer, A., and S. Gerstenfeld. 1958. The photometric microdetermination of blood glucose with glucose oxidase. *J. Lab. Clin. Med.* 51: 448.
- Youlton, R., S. L. Kaplan, and M. M. Grumbach. 1969. Growth and growth hormone. IV. Limitations of the growth hormone response to insulin and arginine and of the immunoreactive insulin response to arginine in the assessment of growth hormone deficiency in children. *Pediatrics*. 43: 989.
- Dole, V. P. 1956. A relation between non-esterified fatty acids in plasma and the metabolism of glucose. *J. Clin. Invest.* 35: 150.
- Murphy, B. E. P. 1967. Some studies of the protein-binding of steroids and their application to routine micro and ultramicro measurement of various steroids in body fluids by competitive protein-binding radioassay. *J. Clin. Endocrinol.* 27: 973.
- Berson, S. A., R. S. Yalow, A. Bauman, M. A. Rothschild, and K. Newerly. 1956. Insulin-¹²⁵I metabolism in human subjects: demonstration of insulin binding globulin in the circulation of insulin treated subjects. *J. Clin. Invest.* 35: 170.
- Siegel, S. 1956. Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill Book Company, New York. 184.
- Abrams, R. L., G. M. Cerchio, and A. L. Graber. 1968. Circadian variation of intravenous glucose tolerance in man. *Diabetes*. 17(Suppl. 1): 314. (Abstr.)
- Yates, F. E., and J. Urquhart. 1962. Control of plasma concentrations of adrenocortical hormones. *Physiol. Rev.* 42: 359.
- Frantz, A. G., and M. T. Rabkin. 1964. Human growth hormone: clinical measurement, response to hypoglycemia and suppression by corticosteroids. *N. Engl. J. Med.* 271: 1375.
- Glick, S. M. 1970. Hypoglycemic threshold for human growth hormone release. *J. Clin. Endocrinol.* 30: 619.
- Koh, C. S., J. Kohn, K. J. Catt, and H. G. Burger. 1968. Lack of relation between plasma growth-hormone levels and small decrements in blood-sugar. *Lancet*. 1: 13.
- Kaplan, S. L., C. A. L. Abrams, J. J. Bell, F. A. Conte, and M. M. Grumbach. 1968. Growth and growth hormone. I. Changes in serum level of growth hormone following hypoglycemia in 134 children with growth retardation. *Pediat. Res.* 2: 43.

30. Gemzell, C. A., and F. Heijkenskjöld. 1957. Effect of corticotrophin on the content of corticotrophin in the pituitary glands of adrenalectomized rats. *Acta Endocrinol.* **24**: 249.
31. Kitay, J. I., D. A. Holub, and J. W. Jailer. 1959. Inhibition of pituitary ACTH release: an extra-adrenal action of exogenous ACTH. *Endocrinology.* **64**: 475.
32. Hodges, J. R., and J. Vernikos. 1958. Influence of circulating adrenocorticotrophin on the pituitary adrenocorticotrophic response to stress in the adrenalectomized rat. *Nature (London).* **182**: 725.
33. Motta, M., G. Mangili, and L. Martini. 1965. A "short" feedback loop in the control of ACTH secretion. *Endocrinology.* **77**: 392.
34. Corbin, A. 1966. Pituitary and plasma LH of ovariectomized rats with median eminence implants of LH. *Endocrinology.* **78**: 893.
35. David, M. A., F. Fraschini, and L. Martini. 1966. Control of LH secretion: role of a "short" feedback mechanism. *Endocrinology.* **78**: 55.
36. Corbin, A., and J. C. Story. 1967. "Internal" feedback mechanism: response of pituitary FSH and of stalk-median eminence follicle stimulating hormone-releasing factor to median eminence implants of FSH. *Endocrinology.* **80**: 1006.
37. Fraschini, F., M. Motta, and L. Martini. 1968. A "short" feedback mechanism controlling FSH secretion. *Experientia.* **24**: 270.
38. McCann, S. M., and J. C. Porter. 1969. Hypothalamic pituitary stimulating and inhibitory hormones. *Physiol. Rev.* **49**: 240.
39. Sakuma, M., and E. Knobil. 1970. Inhibition of endogenous growth hormone secretion by exogenous growth hormone infusion in the Rhesus monkey. *Endocrinology.* **86**: 890.
40. Abrams, R. L., M. L. Parker, S. Blanco, S. Reichlin, and W. H. Daughaday. 1966. Hypothalamic regulation of growth hormone secretion. *Endocrinology.* **78**: 605.
41. Roth, J., S. M. Glick, R. S. Yalow, and S. A. Berson. 1963. Secretion of human growth hormone: physiologic and experimental modification. *Metabolism.* **12**: 577.
42. Antony, G. J., J. J. Van Wyk, F. S. French, R. P. Weaver, G. S. Dugger, R. L. Timmons, and J. F. Newson. 1969. Influence of pituitary stalk section on growth hormone, insulin and TSH secretion in women with metastatic breast cancer. *J. Clin. Endocrinol.* **29**: 1238.
43. Landon, J., F. C. Greenwood, T. C. B. Stamp, and V. Wynn. 1966. The plasma sugar, free fatty acid, cortisol, and growth hormone response to insulin and the comparison of this procedure with other tests of pituitary and adrenal function. II. In patients with hypothalamic or pituitary dysfunction or anorexia nervosa. *J. Clin. Invest.* **45**: 437.
44. Krieger, D. T., S. Glick, A. Silverberg, and H. Krieger. 1968. A comparative study of endocrine tests in hypothalamic disease. Circadian periodicity of plasma 11-OHCS levels, plasma 11-OHCS and growth hormone response to insulin hypoglycemia and metyrapone responsiveness. *J. Clin. Endocrinol.* **28**: 1589.
45. Blanco, S., D. S. Schalch, and S. Reichlin. 1966. Control of GH secretion by glucoreceptors in the hypothalamic-pituitary unit. *Fed. Proc.* **25**: 191.
46. Irie, M., M. Sakuma, T. Tsushima, K. Shizume, and K. Nakao. 1967. Effect of nicotinic acid administration on plasma growth hormone concentrations. *Proc. Soc. Exp. Biol. Med.* **126**: 708.
47. Blackard, W. G., and S. A. Heidingsfelder. 1969. Effect of adrenergic receptor blockade on nicotinic acid-induced plasma FFA rebound. *Metabolism.* **18**: 226.
48. Landon, J., V. Wynn, and V. H. T. James. 1963. The adrenocortical response to insulin-induced hypoglycemia. *J. Endocrinol.* **27**: 183.