Studies of Insulin and Growth Hormone Secretion in a Subject with Hepatoma and Hypoglycemia

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SUMMARY

Studies of immunoreactive insulin (IRI) and human growth hormone (HGH) secretion were undertaken in a subject with chronic hypoglycemia associated with hepatoma. Fasting IRI concentrations were below 3 μU./ml. at glucose concentrations of 30 mg./100 ml. IRI secretion was not stimulated by acutely administered glucose, tolbutamide, glucagon or arginine. IRI concentrations rose after tolbutamide when plasma glucose was raised to 100 mg./100 ml. by sustained glucose infusion. Fasting ILA (muscle, adipose) levels were not elevated.

Fasting HGH concentrations were below 5 mμg./ml.; secretion was stimulated by arginine despite hypoglycemia. During glucose administration, HGH concentrations rose to 45 mμg./ml. as FFA levels fell from 2,250 to 233 μEq./L. Estimated total glucose utilization rate was high (7.5 mg./kg./hr. at a plasma glucose concentration of 100 mg./100 ml.).

Conclusions: (1) Glucose, or some metabolic product is required for human insulin secretion; (2) HGH secretion after arginine is not inhibited at low glucose concentrations; (3) FFA may exert an inhibitory role in HGH regulation; (4) Hypoglycemia in our subject with hepatoma was associated with increased glucose utilization, not with hyperinsulinism. DIABETES *20*:607-14, September, 1971.

The importance of glucose metabolism for insulin secretion has been documented abundantly under experimental conditions.[1-4] The occurrence of chronic hypoglycemia in a patient with hepatoma provided an opportunity to acquire evidence in vivo regarding the role of glucose in human insulin release. It is generally accepted that acute hypoglycemia stimulates growth hormone (HGH) release.[5] Recent studies suggest, however, an inhibitory effect of free fatty acids (FFA) on HGH release.[6] Studies were devised to examine in the present patient the influence of FFA in HGH regulation, since chronic insulinopenia resulted in an unusual dissociation between glucose and FFA effects upon plasma HGH. Finally, despite previous studies concerning the riddle of hepatoma hypoglycemia, mechanisms have not been evaluated entirely. Therefore, studies were performed in our patient to evaluate rates of glucose utilization, integrity of glycogenolysis, gluconeogenesis, counterregulation, and plasma insulin concentrations. The results presented herein lend additional support to the concept that hypoglycemia in hepatoma is primarily due to increased utilization of glucose.

CASE REPORT

A thirty-eight-year-old Negro male was admitted to the hospital in coma. For one month before admission, he had recurrent upper abdominal pain, a twenty-pound weight loss, and several episodes of irrational behavior. For two days before admission, he had become more confused and drowsy; he gradually lapsed into coma several hours before admission.

On physical examination there were findings of pleural fluid at the right lung base and an enlarged, nontender liver, measuring 24 cm. in overall length. The spleen tip was palpable.

The hematocrit was 35 per cent. The blood glucose was 21 and the total bilirubin, 2 mg./100 ml. The glutamic oxalacetic transaminase (SGOT) was 100 U. and bromsulfalein retention was 30 per cent at forty-five minutes. Brain scan, carotid, and hepatic arteriographic studies and electroencephalograms were normal. X-ray films of the chest showed a pleural effusion at the right lung base and an elevated right diaphragm. A liver scan showed decreased uptake over the right lobe. Thoracentesis recovered a clear fluid with total protein of 4.6 gm./100 ml. There were no abnormal cells.

For ten days, early in his hospital course, the patient received a course of 16 mg. of dexamethasone daily in divided doses. Blood glucose rose to above 100 mg./100 ml. The hospital course of three months was marked by frequent episodes of hypoglycemia with response to oral or intravenous glucose. Weight fell from 155 to 118 pounds. Liver function deterio-

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rated, appetite fell, several generalized convulsions occurred, then he lapsed into a coma and died. Permission for autopsy was denied.

An open liver biopsy was performed on the seventieth hospital day. The left and right lobes of the liver were diffusely infiltrated by tumor. Microscopic findings were typical of hepatoma.

METHODS

Except where noted, all tests were performed after an overnight fast. The patient was maintained on a daily intake of at least 150 gm. carbohydrate.[7] Doses used were as follows: (1) oral glucose tolerance, 100 gm.; (2) intravenous glucose tolerance, 0.5 gm./kg. body weight over a two-min. period; (3) intravenous tolbutamide, 1.0 gm. over a two-min. period; (4) intravenous glucagon, 1 mg. over a 30-sec. period; (5) intravenous arginine, 20 gm. of arginine hydrochloride in 500 ml. of 0.9 per cent saline over a thirty-min. period.

All biochemical determinations were done on heparinized plasma samples drawn from an indwelling needle in the antecubital vein. Plasma was separated immediately and kept at —10° C. to —20° C. before it was analyzed in duplicate for glucose,[8] FFA,[9] lactate,[10] phosphate,[11] IRI[12,13] and/or HGH.[14]

Plasma insulin-like activity was estimated by a modification of the method described by Metz.[15] Hemidiaphragms weighing 20-35 mg. wet weight were carefully removed from male Sprague-Dawley rats who were fasted overnight. After a short preliminary period in chilled Gey and Gey buffer,[16] hemidiaphragms were incubated in 2 ml. of heparinized undiluted plasma or an equal volume of buffer with added insulin and 0.2 per cent gelatin. Medium glucose concentration was 300 mg./100 ml. in all cases. Incubation time was ninety minutes under 95 per cent O_2 and 5 per cent CO_2 at pH 7.4 and temperature of 37° C. A Dubnoff metabolic shaking incubator was used. In each assay, insulin in buffer and gelatin was incubated in separate flasks without tissue to estimate loss of insulin on glass. The immunoassayable insulin concentration determined at the end of incubation was accepted as equal to that to which control diaphragms were exposed. The medium glucose concentration was measured at the end of incubation and glucose uptake expressed as milligrams of glucose per gram wet weight of diaphragm per ninety minutes. In each run, the uptake of the hemidiaphragm incubated in the buffer with glucose was subtracted from the uptake of the paired hemidiaphragm incubated in plasma or standard insulin solution to give the increment in glucose uptake produced by insulin. Results from a minimum of three separate incubations were pooled.

Plasma insulin-like activity on adipose tissue was also determined, using the method of Beigelman.[17] Epididymal adipose tissue from Sprague-Dawley rats weighing from 123-174 gm. was employed. Rats were allowed free access to food until the time of decapitation. Weight of individual segments was from 60-286 mg. wet weight. Incubation conditions were as above except that a two-hour period of incubation was employed. A time course experiment established this as the time of maximal insulin action on adipose tissue under the conditions chosen. In each run, the glucose uptake of the adipose tissue segments incubated in buffer with glucose was subtracted from that of the segments incubated in plasma or standard insulin solution to give the increment in glucose uptake produced by insulin. This was expressed as excess glucose uptake, mg./gm./120 min. Results from a minimum of four separate incubations were pooled.

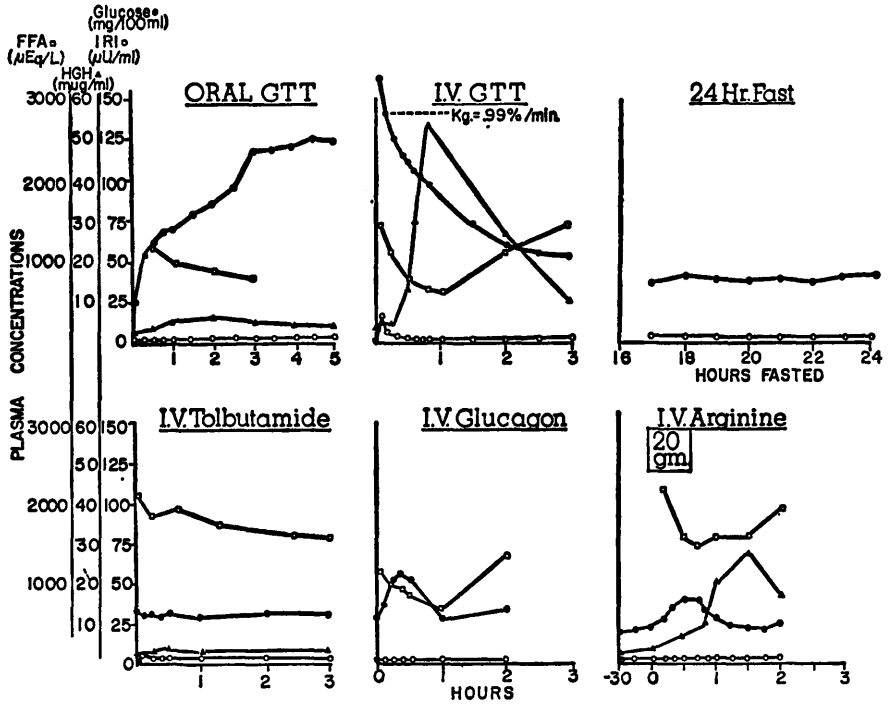

FIG. 1.

Plasma glucose, FFA, IRI, and HGH concentrations following various stimuli of insulin secretion and a twenty-four-hour fast in the presence of hypoglycemia.

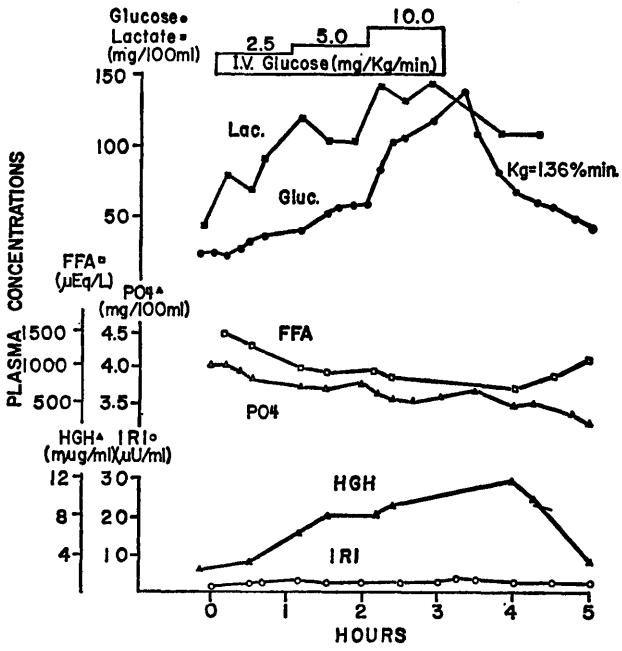

FIG. 2. Effect of intravenous glucose infusion on plasma concentrations of glucose, FFA, IRI, HGH, phosphate, and lactate.

RESULTS

In vivo

Studies were undertaken to explore IRI and HGH secretion after glucose, glucagon, arginine, and tolbutamide. Indices of glucose utilization, gluconeogenesis, glycogenolysis, and counter-regulation to hypoglycemia were also obtained.

1. *Insulin secretion.* Secretion of insulin was inhibited in the presence of hypoglycemia. This phenomenon was apparent with glucose given orally, intravenously as a single injection, or as a continuous infusion. Insulin release was not stimulated by intravenous tolbutamide, glucagon or arginine when blood glucose was low (figures 1 and 2). The decreased insulin release was not altered materially by the administration of phentolamine during intravenous glucose administration (figure 3).

In contrast, when a constant infusion of 20 per cent glucose was administered at a rate of 8-10 mg./kg./min. for twenty-four hours before testing, plasma glucose concentrations of 100 mg./100 ml. or above occurred and insulin release was seen with intravenous tolbutamide (figure 4).

2. *Growth hormone secretion.* In the presence of hypoglycemia, plasma FFA concentrations were high and HGH levels were not elevated (figures 1-3). When normoglycemia was achieved with constant glucose infusion, HGH levels rose as FFA concentrations decreased (figure 3). When plasma FFA levels were lowered to 233 μEq./L. with a two day infusion of 700 gm. of glucose daily, plasma glucose was 167 mg./100 ml. and HGH rose to 28 mμg./ml. The next day, high plasma HGH and low FFA levels were also present during a glucose infusion before the second tolbutamide test (figure 4). When glucose was injected rapidly into a vein, HGH levels rose in association with the rapidly falling plasma FFA concentrations (figure 1). Arginine infusion was followed by a significant rise in HGH, in spite of a minimal fall in plasma FFA levels. No measurable change in HGH levels occurred at high plasma FFA concentrations with oral glucose or after tolbutamide (figure 1).

Whereas plasma HGH levels bore no relationship to plasma glucose or insulin concentrations during insulinopenia, there was a negative curvilinear relationship between plasma FFA

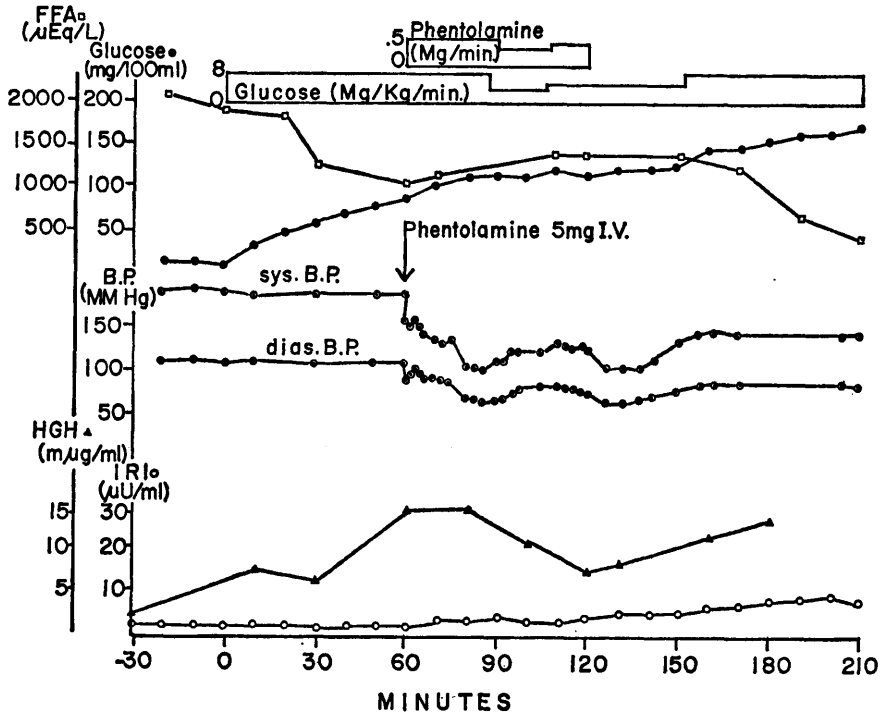

FIG. 3.

Effect of glucose infusion and alpha adrenergic receptor blockade on plasma glucose, FFA, IRI, and HGH concentrations.

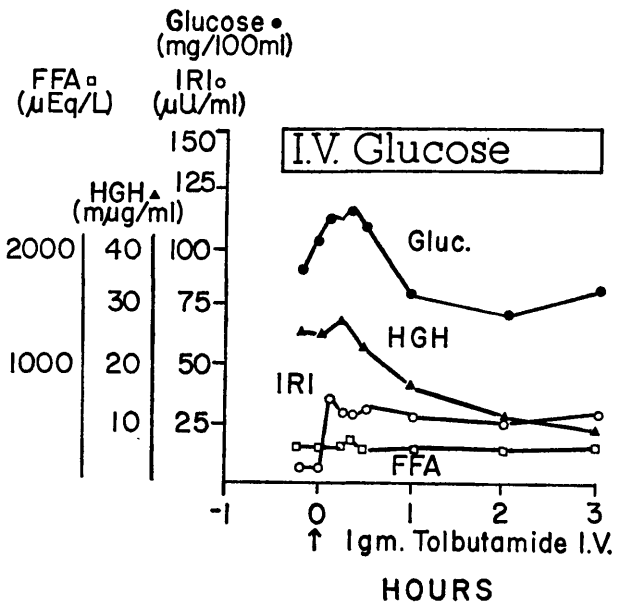

FIG. 4. Effects of tolbutamide on plasma glucose, FFA, and IRI levels during intravenous glucose infusion (8 mg./kg./min.).

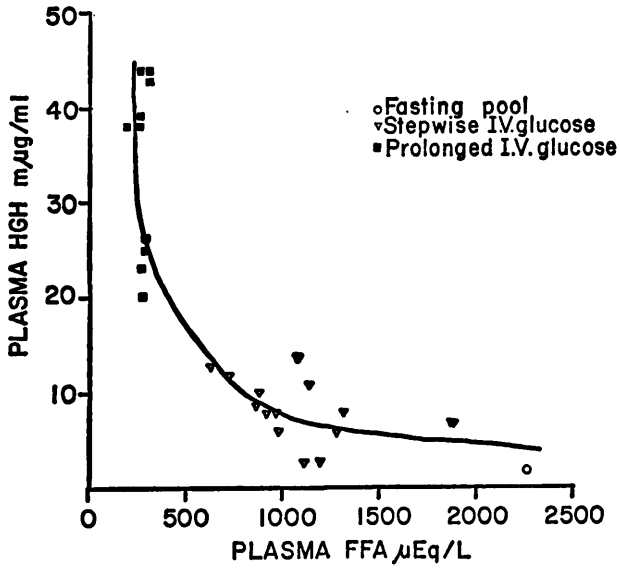

FIG. 5. Relationship between plasma FFA and HGH concentrations obtained under conditions noted in key.

and HGH levels obtained during fasting or steady state glucose infusion (figure 5). In contrast, after chronic glucose infusion, the rise in IRI after tolbutamide was attended by an HGH fall in the presence of hyperglycemia (figure 4).

3. *Glucose utilizaton.* Glucose disappearance rate (Kg calculated between 15 and 45 min.[18]), figure 1, was almost normal (normal range > 1.05 per cent/min.[18]) and was within normal limits following discontinuation of a glucose infusion (figure 2). Overall glucose assimilation was estimated by plotting steady state plasma glucose concentrations (figures 1-4) against glucose infusion rates (figure 6). The results suggest a glucose utilization rate of about 7.5 mg./kg./min. at plasma glucose levels of 100 mg./100 ml. This was achieved without measurable increase in immunoreactive insulin secretion (figure 2).

4. *Gluconeogenesis.* Plasma glucose levels at almost 40 mg./100 ml. were maintained in the presence of a twenty-four-hour fast (figure 1).

5. *Glycogenolysis.* Plasma glucose levels rose 30 mg./100 ml. after intravenous glucagon (figure 1). Upon infusion of arginine, plasma glucose levels rose from 20 to 40 mg./100 ml. and returned to the pre-infusion level after the infusion was stopped (figure 1).

6. *Counterregulation.* Twenty-four-hour urine determinations for total catecholamines were 142-148 μg. Intravenous phentolamine produced a fall in blood pressure (figure 2). Morning plasma cortisol levels were normal. Fasting plasma growth hormone concentrations were slightly elevated in the presence of hypoglycemia. Plasma free fatty acid concentrations were markedly elevated and fell moderately after small amounts of intravenous or oral glucose, tolbutamide, glucagon, and arginine (figure 1). After prolonged infusion of 20 per cent glucose, plasma FFA concentration was below 300 μEq./L.

In vitro

1. *Insulin recovery from plasma.* Extracted human insulin was added to heparinized fasting plasma from the patient. Recovery for insulin ranged from 100-106 per cent over insulin concentrations of 6-60 μU./ml. Plasma containing human insulin secreted endogenously after oral glucose was added to the patient's plasma. Recovery was 86-100 per cent over insulin concentration of 47-96 μU./ml.

2. *Glucose uptake by muscle.* Pooled plasma specimens obtained after arginine, overnight fast, oral glucose, tolbutamide, or intravenous glucose were incubated with rat hemidiaphragms (table 1). It is apparent that under no conditions did the plasma from the subject studied exert an action on glucose uptake by muscle like that observed with crystalline insulin.

3. *Glucose uptake by adipose tissue.* Pooled fasting plasma

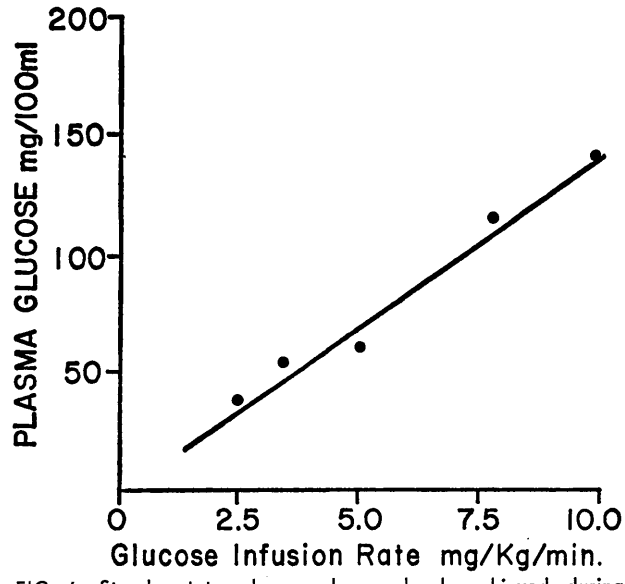

FIG. 6. Steady state plasma glucose levels achieved during variable glucose infusion rates.

TABLE 1

Insulin-like activity on rat hemidiaphragm

	N*	Excess Glucose Uptake mg./gm./90 min.
Insulin (40 μU./ml.)	11	4.05 ± 0.7†
Insulin (400 μU./ml.)	11	9.3 ± 2.9
Arginine	3	1.92
Fasting	4	1.83
Oral glucose	5	0.33
Tolbutamide	3	−1.34
IV glucose	5	−2.32

* Number of hemidiaphragms incubated
† S.D.

TABLE 2

Insulin-like activity on rat epididymal fat

	N*	Excess Glucose Uptake mg./gm./120 min.
Insulin (70 μU./ml.)	9	0.7 ± 0.4†
Insulin (350 μU./ml.)	14	2.24 ± 0.4†
Tolbutamide	4	2.29
IV glucose	4	1.65
Fasting	4	1.69

* Number of hemidiaphragms incubated
† S.D.

and plasma obtained after intravenous glucose exerted some activity on rat adipose tissue (table 2). By extrapolation, it could be estimated that this activity was about 150 μU./ml. Plasma obtained after tolbutamide had a greater activity, approximately 300 μU./ml.

DISCUSSION

Insulin secretion

Secretion of IRI was consistently depressed in the presence of hypoglycemia in the subject studied. In vitro observations have shown that stimulation of insulin secretion by glucose requires a minimum concentration of glucose, usually above 50 mg./100 ml.[1-4,19] Matchinsky and Ellerman[4] have related this to the rapid penetration and phosphorylation of glucose by the pancreatic beta cell. Only after plasma glucose levels above 100 mg./100 ml. were achieved did insulin secretion occur in the present subject after tolbutamide injection. These observations afford strong evidence that in man glucose or some product of its metabolism is necessary for the response of the pancreatic beta cell to these secretagogues.

A second possible explanation for the inhibition of insulin secretion in this subject was secretion of catecholamines in response to hypoglycemia. An excessive output of catecholamines was suggested by moderately elevated levels of urinary total catecholamines and the high plasma free fatty acid and lactate concentrations. However, the infusion of phentolamine in amounts sufficient to lower blood pressure, and presumably, to produce alpha receptor blockade, did not materially affect the pancreatic insulin response to intravenously administered glucose. A rise in insulin secretion has been shown when alpha receptor blockade is produced with phentolamine during epinephrine infusion[20] or in subjects with pheochromocytoma.[21,22]

A third possibility for the low insulin concentrations would be a high extraction of insulin by liver or tumor tissue. Since the disappearance rate of endogenously secreted insulin after tolbutamide was delayed in the presence of glucose loading (figure 4), such a possibility seems unlikely. However, our studies do not exclude an inordinately high rate of insulin extraction by liver or tumor which is dampened only after prolonged normoglycemia.

Hypoglycemia

Among the mechanisms explored most frequently to explain the riddle of hypoglycemia in hepatoma has been an increased rate of glucose utilization. The evidence for this concept has been reviewed by McFadzean et al.[23] The calculated glucose disposal in our subject supports this view, since over 500 gm. of glucose was needed on a daily basis to maintain normoglycemia. Similar findings have been reported by others.[24-28]

As has been reported by others,[24,27,29] the present study showed little evidence of a biologically active insulin-like material in the patient's plasma in the fasting state. There was no increase in ILA after the administration of glucose, arginine, or tolbutamide. Although insulin-like activity in excess of 70 μU./ml. was found in fasting plasma incubated with adipose tissue, the physiological significance of this must be questioned for several reasons. First, in vivo observations of high plasma FFA levels suggested that lipolysis was proceeding at a rapid rate. Second, rat adipose tissue is sensitive to a variety of hormones which may have been present in elevated amounts in fasting plasma. Although fasting HGH and cortisol levels were normal, other counterregulatory hormones such as epinephrine and glucagon may have been present in excess; both of these have been shown to produce an excess glucose uptake by adipose tissue in vitro.[30,31] The slight stimulatory effect of the plasma after tolbutamide on adipose tissue (table 2) had been seen with the drug alone in studies by Candela et al. on adipose tissue.[32]

A decreased rate of degradation of insulin has been postulated as a cause for hypoglycemia,[27] but is unlikely in view of the persistently low immunoreactive insulin levels found in our subjects. In vitro studies

showed that either exogenous (pork) insulin or endogenous (human) insulin could be recovered quantitatively from the patients' plasma. Thus, the persistently low plasma insulin levels were not due to plasma factors which might interfere with the determination of insulin immunoassay or cause destruction of insulin in plasma.

The quantity of available glucose is determined by the balance between the glucose production rate and rate of glucose utilization. Although glucose utilization is apparently accelerated in subjects with hypoglycemia and hepatoma, one must ask why glucose production is not adequate to meet the increased demands for glucose. The recent studies by McFadzean et al.[23] have shown that residual liver and tumor tissue from patients with hepatoma and prolonged hypoglycemia have abundant stores of glycogen which are remarkably stable upon incubation or storage. In addition, these observers noted that many subjects had a progressive reduction in the hyperglycemic response to glucagon as the tumor grew in size. These findings of an "acquired glycogenosis" in these subjects are partially supported by the present studies, since glucagon injection produced only a small rise in blood glucose. A greater rise was anticipated, particularly in the absence of insulin secretion. Studies in our laboratories have shown a mean peak plasma glucose rise of 50 ± 10 (S.E.M.) mg./100 ml. thirty minutes after this dosage of glucagon, preceded by a mean peak rise in plasma IRI of 60 ± 15 (S.E.M.) μU./ml. at five minutes after injection.[33]

Our studies failed to give definitive data regarding gluconeogenesis in this subject. Impairment of endogenous glucose production from lactate has been reported in one patient with hepatoma and hypoglycemia by Kreisberg.[34] Isolation of an antigluconeogenic substance from a retroperitoneal hemangiopericytoma has been reported by Ensinck.[35] The elevation of plasma lactate levels in our patient may have reflected defective incorporation of lactate into glucose. However, lactate acid production by glycolysis via the hexose monophosphate shunt may be increased in hepatoma.[27] The fact that plasma glucose levels were maintained in the presence of twenty-four-hour fasts suggests that some gluconeogenetic ability was maintained. However, arginine infusion stimulates immediate glucagon release,[36] and the rise in plasma glucose in our subject may have been due to glycogenolysis. It should be noted, however, that glucagon also accelerates gluconeogenesis.[37] Further, the rise in plasma glucose levels after glucocorticoid administration could be interpreted as reflecting responsive gluconeogenic pathways.

Some consideration must be given to the possibility that these tumors are associated with hypoglycemia because of decreased counterregulation to hypoglycemia. This explanation is unlikely, since in the present study, there is adequate evidence of catecholamines and cortisol secretion. Probable causes for the growth hormone responses are discussed below. Autopsy studies in other cases have revealed no metastic involvement of the pituitary or adrenal glands.

Growth hormone secretion

The present subject afforded a unique opportunity to study the influences of glucose, FFA, and insulin on growth hormone secretion in man. In the presence of chronic fasting hypoglycemia, plasma HGH concentrations were not elevated. When plasma glucose levels were raised acutely by slow intravenous infusion or oral ingestion of glucose, HGH levels rose, rather than fell. When intravenous glucose was given over a prolonged period, HGH levels were high, in the presence of an elevated plasma glucose level. These observations were paradoxical in view of the usual glucose-induced suppression and hypoglycemia-induced elevations of plasma HGH concentrations.[5] Another influence upon HGH release was therefore considered.

As shown in figure 5, plasma HGH levels bore a curvilinear relationship to plasma FFA levels under the experimental conditions used. Only briefly after the acute intravenous administration of glucose or after arginine infusion did this relationship deviate. Suppression of insulin-induced HGH secretion by elevation of plasma FFA levels has been shown in rhesus monkeys.[6] Further, nicotinic acid-induced suppression of plasma FFA in the absence of changes in blood glucose is followed by an increase in plasma HGH levels in man.[33] Blackard et al. have suggested an inhibitory effect of octanoate on growth hormone synthesis by the anterior pituitary.[34] The findings in our patients strongly support the concept of an FFA-induced suppression of HGH synthesis or release. Our studies do not differentiate between such variables as plasma vs intracellular FFA levels or rate of changes of FFA concentrations as primary control mechanisms. They provide no information on the site(s) of action of FFA in this regard.

In the subject studied, alpha receptor blockade with phentolamine was followed by a fall in HGH levels, suggesting that alpha receptor stimulation was at least partially responsible for the measurable plasma HGH level[35] in the face of the inhibitory action of FFA. However, the fall in HGH levels also paralleled a rise in plasma FFA induced by alpha receptor blockade; thus, the drop in plasma HGH levels may have been mediated

through beta adrenergic stimulation of lipolysis.

In view of the fact that glucose administration is usually followed by a fall in plasma HGH level, we must consider why the reverse response was noted under our experimental conditions. Removal of the inhibitory effects of elevated FFA levels cannot be the only reason, since when glucose and insulin are given together, or when insulin secretion occurs appropriately following a glucose stimulus, plasma FFA and HGH levels usually fall in normals.[5] A paradoxical rise in HGH following glucose may also occur in diabetics,[36] acromegaly[37] and acute intermittent porphyria.[38] It is of interest that each of these may be accompanied by either a decreased rate of insulin secretion or peripheral resistance to the action of insulin. In view of these findings and those in our subject, it may be postulated that glucose-induced suppression of HGH secretion may be mediated through hypothalamic-pituitary centers which are insulin-sensitive.

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