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MEDICAL GRAND ROUNDS

PARKLAND MEMORIAL HOSPITAL

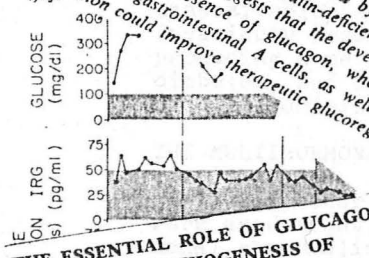
October 20, 1977

STATUS REPORT: THE GLUCAGON CONTROVERSY

[Roger H. Unger]

Glucagon: Role in the Hyperglycemia of Diabetes Mellitus

Abstract. Glucagon suppression by somatostatin reduces or abolishes ... glycemia in dogs made insulin-deficient by somatostatin, alloxan, or ... createctomy. This suggests that the development of severe diabetic hyp... requires the presence of glucagon, whether secreted by pancreatic ... identified gastrointestinal A cells, as well as a lack of insulin. Glucagon ... pression could improve therapeutic glucoregulation in diabetes.



THE ESSENTIAL ROLE OF GLUCAGON IN THE PATHOGENESIS OF DIABETES MELLITUS

Summary The following evidence suggests that diabetes mellitus may not be the simple consequence of relative or absolute insulin deficiency by itself, but may require the presence of glucagon: (1) relative or absolute hyperglucagonæmia has been identified in every form of endogenous hyperglycæmia, including total pancreatectomy in dogs; (2) insulin lack in the absence of glucagon does not cause endogenous hyperglycæmia, but when endogenous or ...ogenous glucagon is present, it quickly appears, ...ective of insulin levels at the time. These facts ...tible with a bihormonal-abnormality hypothesis holds that the major consequence of ...ve insulin lack is glucose under-...olute or relative glucagon excess ...e over-production of glucose

PREVENTION OF HUMAN DIABETIC KETOACIDOSIS BY SOMATOSTATIN

Evidence for an Essential Role of Glucagon

Abstract To evaluate the role of glucagon in the pathogenesis of diabetic ketoacidosis in man, we studied the effect of suppression of glucagon secretion by somatostatin on changes in plasma β-hydroxybutyrate and glucose concentrations (as well as changes in their precursors) after acute withdrawal of insulin from seven patients with juvenile-type diabetes.

...pression of glucagon secretion prevented the de... ...ent of ketoacidosis for 18 hours after ac... ...rawal, whereas in control stu...

dosis occurred 10 hours after insulin was sto... Plasma β-hydroxybutyrate, glucose, free fatty acid, glycerol levels were all markedly lower during suppression of glucagon secretion ($p < 0.001$), whereas plasma alanine levels were higher ($p < 0.001$). These studies indicate that ... not lead to t... that ...

...sulin lack per se does ...'oacidosis in man and ...gluconeogenic, keto... ...erequisite to the d...

PRIMACY OF INSULIN DEFICIENCY RATHER THAN GLUCAGON EXCESS IN THE PATHOGENESIS OF DIABETES. Robert Sherwin, M.D., William Tamborlane, M.D., Rosa Hendler, M.D., and Philip Felig, M.D.

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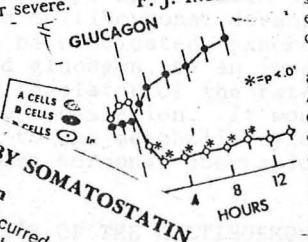
This attractive hypothesis of the bi-hormonal gover... ...nance and of the role of the glucagon:insulin ratio in the maintenance of normal glucose tolerance is still difficult to accept wholly because of a number of contradictory facts. Since it is highly unlikely that insulin and glucagon exert their seemingly opposing effects by acting in a plus-minus fashion on the same biochemical mechanism, it might clear the air if one did not expect metabolic effects from a ratio of ... two peptides ... ...ured in serum.

...ACHMIEL LEVINE, M.D.

DEBATES ON DIABETES

Finally, there is the argument abou... Does this agent, by inhibiting the rele... help therapy of diabetic ketoacidosis? ...statin is not generally available, this p... at present more academic than prac... less, as the recent editorial by Roger ... glucagon as a contributory factor in c... ticularly ketoacidosis cannot be entir... the basis of present evidence, althou... ologists appear to believe that the i... agent is relatively minor, whether th... or severe.

F. J. INGELFINGER,



Glucagon Role in Diabetes Mellitus Described as Minor

WASHINGTON, D.C.—The bihormonal theory of diabetes accords star roles to both insulin and glucagon. But Yale researchers now challenge that billing, claiming that insulin plays the lead in the disease process while glucagon has little more than a supporting role. Somatostatin, meanwhile, has been relegated to the wings by Dr. Robert Sherwin and colleagues, who assert that this agent, which suppresses glucagon and insulin, has no place in treating diabetics—especially those with maturity-onset conditions—and may even be deleterious.

Dr. Sherwin, reporting on a series of trials at the annual meeting of the American Association of Physicians here, clearly challenged the hypothesis that diabetes depends equally on a glucagon excess and an insulin deficit. Proponents of this theory hold that an excess of glucagon is a critical component of hyperglycemia, and cite the fact that blood glucose falls when glucagon is suppressed by somatostatin, despite an

Glucagon's Effect 'Evanescent'

In fact, Dr. Sherwin told DIABETES OUTLOOK, "the elevating effect of glucagon excess on blood glucose is an evanescent one." The New Haven team found that raising glucagon levels in normal humans induced only a mild and transient increase in plasma glucose. Furthermore, "somatostatin produces only transient hypoglycemia in normal subjects and maturity-onset diabetics," according to Dr. Sherwin, an assistant professor of medicine. Indeed, after an initial dip, plasma glucose starts to rise, reaching hyperglycemic levels despite

THE UNIHORMONAL ABNORMALITY CONCEPT OF THE PATHOGENESIS OF DIABETES

The traditional unihormonal concept of the pathogenesis of diabetes holds that all of the metabolic aberrations of severe diabetes are the direct consequence of insulin deficiency. This view is derived from the experiments of von Mering and Minkowski (59), demonstrating that pancreatectomy produces severe diabetes, and those of Banting and Best (4) showing that the severe diabetes responds to injections of insulin-containing pancreatic extracts. This concept would be unassailable -- unless 1) the insulin deficient state were invariably associated with a second abnormality capable of producing some or all of the metabolic derangements of severe diabetes, and 2) this second abnormality were reversible with insulin administration.

THE MULTIHORMONAL ABNORMALITY CONCEPT

It is now clear that insulin deficiency is, with extremely rare exceptions, 1) invariably associated with a second hormonal abnormality, relative* or absolute hyperglucagonemia (54), and 2) that the postpancreatectomy hyperglucagonemia is readily correctable with insulin treatment. Since glucagon is a powerful glycogenolytic, gluconeogenic, lipolytic and ketogenic hormone, and its actions are opposed by insulin and intensified by insulin deficiency, a deleterious contribution by the hormone to the metabolic syndrome of diabetes would, on *a priori* grounds, be expected. The potential therapeutic benefit of reducing diabetic hyperglucagonemia makes this issue one of practical as well as of pathophysiologic importance.

It is also clear that the functions of other islet cells, the somatostatin-secreting D-cells and the pancreatic polypeptide (PP) secreting F- or D_1-cells, are abnormal in diabetes. Thus, diabetes must be viewed as a multihormonal derangement. While the actions of PP remain to be elucidated, pancreatic somatostatin may, like insulin and glucagon, be an important regulator of nutrient flux -- a regulator of the rate at which ingested nutrients enter the circulation. It would not be surprising if contributions to the metabolic syndrome of diabetes attributable to these hormonal aberrations were one day to be recognized.

THE CHARACTER AND PATHOGENESIS OF THE MULTIHORMONAL ABNORMALITIES OF DIABETES MELLITUS

A. Abnormal A-cell Function

1. Loss of glucose sensing function: In all forms of diabetes thus far studied, with the possible exception of the somatostatinoma syndrome (15, 23), measurable levels of immunoreactive glucagon (IRG)

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* relative to the ambient plasma glucose concentration

have been high relative both to glucose and insulin levels. In functional terms in diabetics neither steady-state hyperglycemia nor sudden increases in hyperglycemia elicit the normal reduction in glucagon secretion that characterizes the nondiabetic state -- in fact, hyperglycemia may cause a paradoxical rise in plasma IRG concentrations in diabetic subjects. [Yet a rise in free fatty acids will reduce plasma IRG levels in diabetics much as in nondiabetics, suggesting selective impairment of glucose sensing by the A-cells of diabetes (18). Also in diabetics, hypoglycemia appears incapable of eliciting a rise in IRG levels as it does in nondiabetics (17).

2. Hyperresponsiveness to stimulation: In addition to the loss of glucose sensing by the diabetic A-cell, there appears to be a hyperresponsiveness to stimulation by intravenous amino acids and by protein meals. Whereas in nondiabetics the protein-induced rise in glucagon is completely abolished by hyperglycemia, in diabetics hyperglycemia fails to modify the protein-induced rise in glucagon (34). The ingestion of protein by diabetics could, therefore, cause a glucagon-mediated rise in glucose levels.

3. Response of abnormalities to insulin: In the juvenile-type diabetic glucose plus insulin can abolish protein-stimulated increases in glucagon secretion, but it fails to restore the normal relationship between changes in glucose concentration and changes in glucagon secretion -- i.e. in such patients one must vary the insulin concentration so as to simulate normal patterns of insulin secretion in relation to glycemic change in order to produce normal patterns of glucagon secretion.

   In contrast to the effectiveness of insulin in lowering glucagon levels in juvenile-type diabetics, in maturity onset type diabetics insulin fails to reduce glucagon secretion -- even when administered in supraphysiologic doses. This clear difference in the ability of insulin to influence A-cell function in the two forms of diabetes suggests a difference in their pathogenesis; this is further supported by the marked difference in the pathology of the islets (vide infra) in these two forms of the disease.

B. Abnormal D-cell Secretion

Extractable somatostatin-like immunoreactivity (SLI) and somatostatin-containing D-cells are increased in the islets of rodents (38) with experimentally induced destruction of B-cells and in juvenile-type diabetics there is a 2.5 fold increase in the D-cell population per islet (36). More

recently, fasting plasma somatostatin-like immunoreactivity has been reported to be increased in alloxan diabetic dogs (Figure 1) and in juvenile-type diabetics (45).

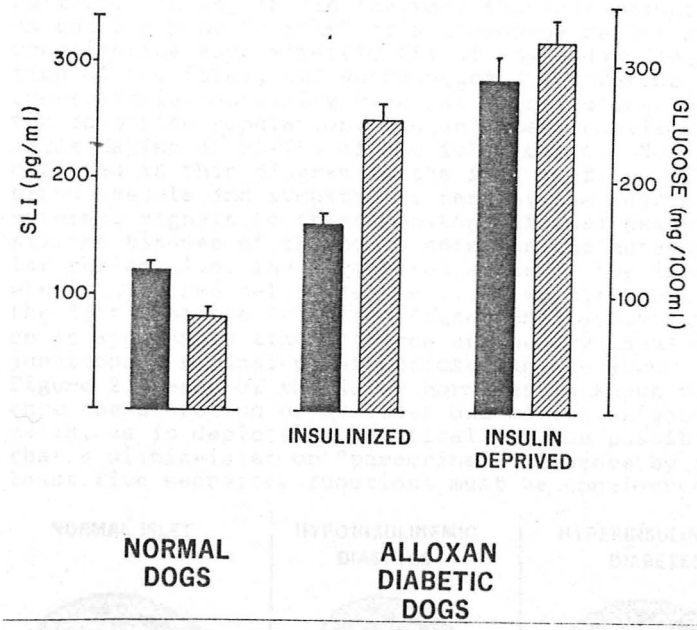


FIGURE 1: Fasting levels of somatostatin-like immunoreactivity (SLI) (closed bars) and glucose (hatched bars) in normal dogs and alloxan diabetic dogs receiving their usual maintenance dose of insulin and dogs deprived of insulin for 48 hours. Statistically significant differences in SLI were observed between all groups. These differences are qualitatively similar to differences in fasting glucagon in the groups.

In the dogs the meal-induced rise in plasma SLI was markedly delayed and the intravenous infusion of glucose, which in nondiabetic dogs reduces the meal-induced rise in somatostatin, fails to do so in diabetics. The metabolic implications of these abnormalities remain to be established, but they could signify a breakdown in the normal coordination between D-cell and B-cell function which may be necessary for coordination between the entry rate of ingested nutrients into the circulation and their efflux from the extracellular space into tissues.

C. Pathology of the Islets in Diabetes

1. Morphology of the normal islets: As depicted schematically in Figure 2, the cells of the normal islets of Langerhans are arranged in a specific topographical pattern. In man and in the rat, the islet consists of an outer rim or "cortex" of glucagon-secreting A-cells, constituting approximately 25% of the endocrine population of the islet, and surrounding a scattering of somatostatin-containing D-cells, constituting 10% of the endocrine population, and an inner medulla of B-cells making up 60-70% of the islet cells. Not depicted in this diagram is the fact that the afferent blood vessels and sympathetic nerves, the sources of external signals to these monitors of fuel needs for all the tissues of the body, enter in the heterocellular region, i.e. the peripheral areas of the islet where the three cell types meet. Also not shown is the fact that the islet is a functional syncytium, all cells apparently linked to one another by means of gap junctions. A final point depicted in the lower area of Figure 2: each of the islet hormones is known to influence the secretion of at least one of its neighboring cells, as is depicted schematically. The possibility that a within-islet or "paracrine" influence by at least five secretory functions must be considered.

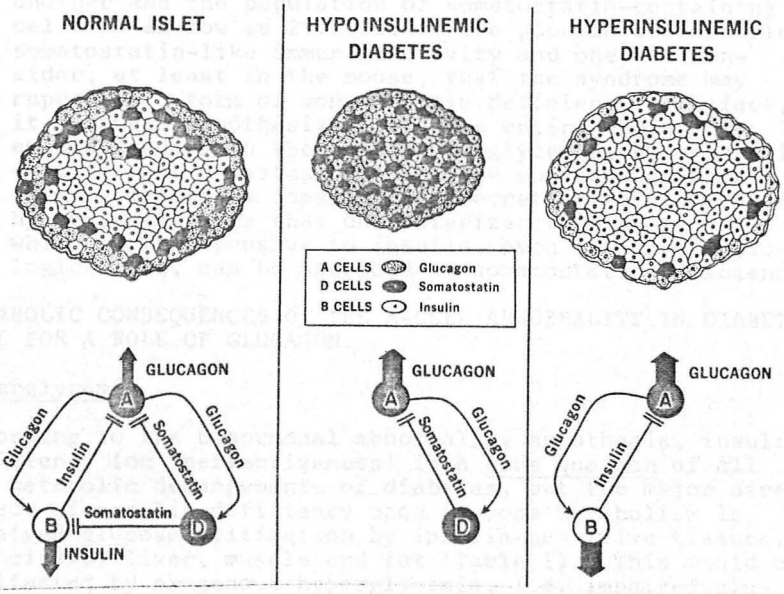


FIGURE 2: Schematization of the anatomy of normal, insulin-deficient diabetic, and insulin-abundant diabetic islets of Langerhans and of the hypothetical "within islet" interactions of individual secretory products upon secretion by neighboring cells.

2. Diabetes with insulin deficiency: The islet in diabetes characterized by paucity or absence of B-cells is depicted in the center panel of Figure 2. The islet is shrunken by the lack of B-cells and its topographical relationships are disrupted. A-cells now make up 75% and D-cells 25% of the islet cell population. The effect of this disruption upon the vasculature and neural interrelationship is not known, but may be profound, nor is the effect of this disruption on the intercellular syncytial system known. If a paracrine interrelationship between the hormones does normally exist, it is clear that the effects of insulin are missing; if they are important in down-regulating locally the function of A- and/or D-cells, the absence of insulin in the islet could explain the hyperglucagonemia and hypersomatostatinemia of this form of diabetes.

3. Diabetes without insulin deficiency: In the right panel of Figure 1 is depicted the islet of the ob/ob mouse in which obesity, hyperglycemia and hyperinsulinemia suggest a resemblance to many human patients with maturity onset diabetes. In the ob/ob mouse, the beta cell mass is increased while a relative diminution in the within-islet A-cell and D-cell population is observed. A- and D-cells are separated from one another and the population of somatostatin-containing cells is as low as 2%. Islets are poor in extractable somatostatin-like immunoreactivity and one can consider, at least in the mouse, that the syndrome may represent a form of somatostatin deficiency. In fact, it has been hypothesized that the entire chain of events leading to the obese hyperglycemic, hyperinsulinemic form of diabetes could be the consequence of a primary defect in somatostatin secretion (57). Perhaps the hyperglucagonemia that characterizes this state and which is unresponsive to insulin, even in supraphysiologic doses, can be ascribed to somatostatin deficiency.

THE METABOLIC CONSEQUENCES OF THE A-CELL ABNORMALITY IN DIABETES: THE CASE FOR A ROLE OF GLUCAGON

A. Hyperglycemia

According to the bihormonal abnormality hypothesis, insulin deficiency (or ineffectiveness) is a sine qua non of all the metabolic derangements of diabetes, but the major direct effect of insulin deficiency upon glucose metabolism is impaired glucose utilization by insulin-sensitive tissues, principally liver, muscle and fat (Table I). This would be manifested by exogenous hyperglycemia, i.e. impaired glucose tolerance or postprandial hyperglycemia, but by only very slight endogenous hyperglycemia (<170 mg%). While insulin lack also directly increases hepatic glucose production (25), this increase is modest and is probably insuffi-

TABLE I: Contribution of hormonal abnormalities to the metabolic derangements of severe diabetes.

Derangement	Insulin Deficiency	Glucagon Excess
1) Underutilization of glucose	++++	0
2) Overproduction of glucose	+	++++
3) Increased release of amino acids	++++	0
4) Increased gluconeogenesis	++++	++++
5) Increased lipolysis	++++	++(?)
6) Increased ketogenesis	+(?)	++++

cient to cause the rapid severe endogenous hyperglycemia (>200 mg/100 ml) that characterizes acute insulin deprivation (7, 11, 43).

B. Hyperketonemia

With respect to ketogenesis, McGarry and Foster and their coworkers (29-31) have shown that the major direct consequence of insulin deficiency is increased lipolysis with elevation of free fatty acids, the substrate for ketogenesis; however, in the absence of glucagon the ketogenic capacity of the liver is not sufficient to generate the massive ketone production required to produce ketoacidosis (19, 29-31). They have characterized glucagon's role in the excessive glucose and ketone production of severe diabetes (29) and their concepts have been substantiated by the in vivo studies of Gerich et al. (19, 20) (Figure 3), Liljenquist et al. (24), and Cherrington and his colleagues (9). Insulin deficiency without hyperglucagonemia has been induced in nondiabetics by means of somatostatin infusion and is unassociated with either severe hyperglycemia (the glucose levels rise only to between 135 and 170 mg/100 ml) or severe hyperketonemia (48). Similar studies conducted in maturity onset diabetics reveal that during suppression of both insulin and glucagon with somatostatin, plasma glucose rises by only 10 mg/100 ml and β-hydroxybutyrate by only 0.2 mM (52). By contrast, insulin deficiency with hyperglucagonemia is characterized by severe endogenous hyperglycemia that may exceed 300 mg/100 ml within a few hours and by a more rapidly rising plasma level of ketones (Figure 2) (19, 20).

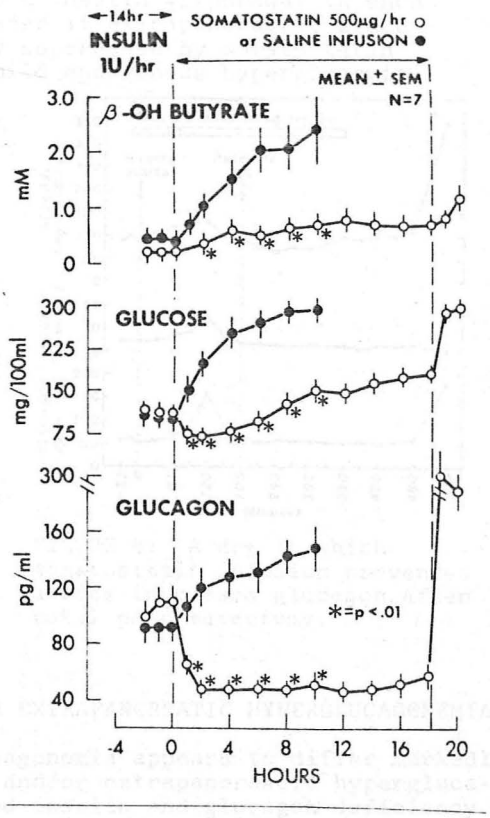


FIGURE 3: The effect of acute insulin deprivation in juvenile diabetic patients during either infusion of a saline control or infusion of somatostatin. Somatostatin-mediated suppression of glucagon following insulin withdrawal greatly obtunds the endogenous hyperglycemia and the hyperketonemia, and both increase when the somatostatin is continued and hyperglucagonemia reappears.

Courtesy of J.E. Gerich, M.D.

DIABETES WITHOUT PANCREATIC GLUCAGON

The diabetes produced by total pancreatectomy is characterized by severe endogenous hyperglycemia and ketoacidosis in the total absence of pancreatic glucagon, but circulating nonpancreatic glucagon is present both in totally depancreatized dogs (27, 28, 60) and in humans (32, 33, 37) and the glucagon levels rise during insulin deprivation and decline when insulin is provided (11). In the dog the circulating nonpancreatic glucagon originates in the fundus of the stomach. A-cells indistinguishable by electron microscopy from pancreatic A-cells have been identified in the gastric fundus of dogs (3) and a polypeptide has been extracted which is physicochemically, immunochemically and biologically indistinguishable from pancreatic glucagon (44, 50). Direct measurements of glucagon in the venous effluent of the gastric fundus indicate that it is the principal source of extrapancreatic glucagon secretion in depancreatized dogs (6). The severe endogenous

hyperglycemia that follows acute insulin withdrawal in such dogs can be dramatically obtunded if extrapancreatic glucagon secretion is concomitantly suppressed by somatostatin infusion, in which case only mild endogenous hyperglycemia (<170 mg/100 ml) occurs (43) (Figure 4). Replacement infusions of glucagon during somatostatin administration result in severe endogenous hyperglycemia (43).

Qualitatively similar results have been observed in depancreatized humans. Barnes and coworkers reported that in totally depancreatized patients without hyperglucagonemia acute insulin withdrawal induces less severe endogenous hyperglycemia and hyperketonemia than in juvenile-type diabetics with marked hyperglucagonemia (5).
(see page 11)

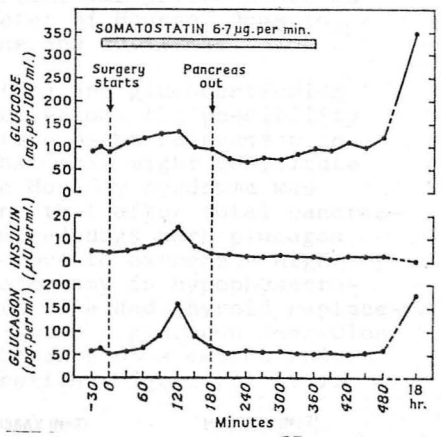


FIGURE 4: A dog in which somatostatin infusion prevented a rise in plasma glucagon after total pancreatectomy.

DIABETES WITHOUT PANCREATIC OR EXTRAPANCREATIC HYPERGLUCAGONEMIA

Diabetes without any hyperglucagonemia appears to differ markedly from diabetes with pancreatic and/or extrapancreatic hyperglucagonemia. Two forms of combined insulin and glucagon deficiency have now been reported: the somatostatinoma syndrome and the Houssay syndrome.

A. Somatostatinoma

Two patients with a functioning somatostatinoma have been reported (23, 15). In both patients plasma insulin and glucagon levels were low and in both, although glucose tolerance was impaired (exogenous hyperglucagonemia), severe endogenous hyperglycemia in excess of 170 mg% and hyperketonemia were both absent.

B. Houssay Syndrome

In 1930 Houssay and Biassotti (21) reported that resection of the pancreas in previously hypophysectomized dogs resulted in a mild form of diabetes that contrasted sharply with the virulent diabetes that followed total pancreatectomy in dogs with an intact hypophysis. The Houssay dogs required little

or no insulin and yet their blood glucose levels were frequently normal or low. Houssay concluded that the anterior hypophysis was essential for severe diabetes and ascribed the milder diabetes of Houssay dogs to deficiencies of growth hormone and cortisol.

Because both growth hormone (51) and glucocorticoids (26, 62) increase glucagon secretion, the possibility that a glucagon deficiency state might be present in hypophysectomized dogs and that this might constitute an ameliorating factor in the Houssay syndrome was recently tested. It was found that after total pancreatectomy in sham hypophysectomized dogs both glucagon secretion and glucose levels rose to extremely high values, whereas after pancreatectomy in hypophysectomized dogs receiving hydrocortisone and thyroid replacement both glucose levels and gastric glucagon secretion were far lower (Figure 5A), because of a marked reduction in gastric glucagon secretion (Figure 5B) (35).

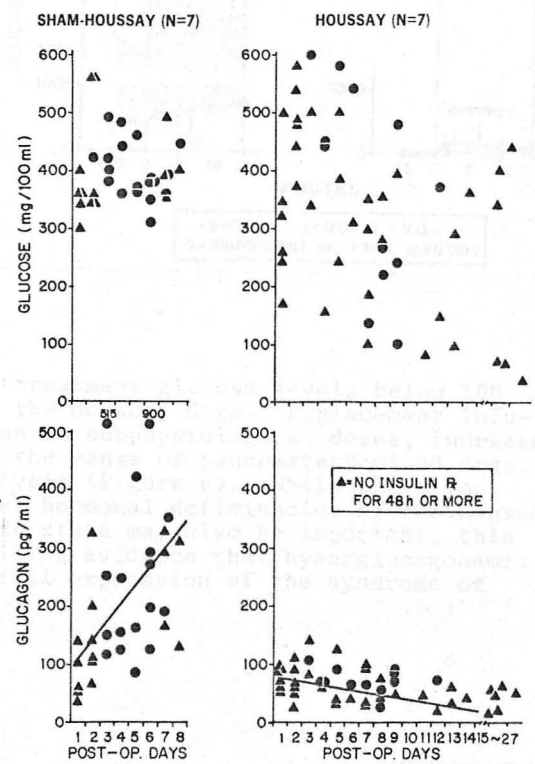
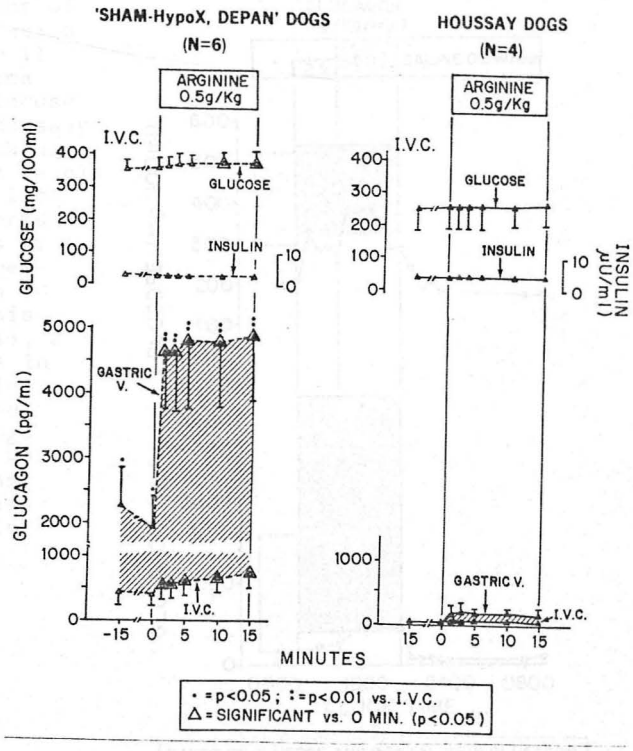


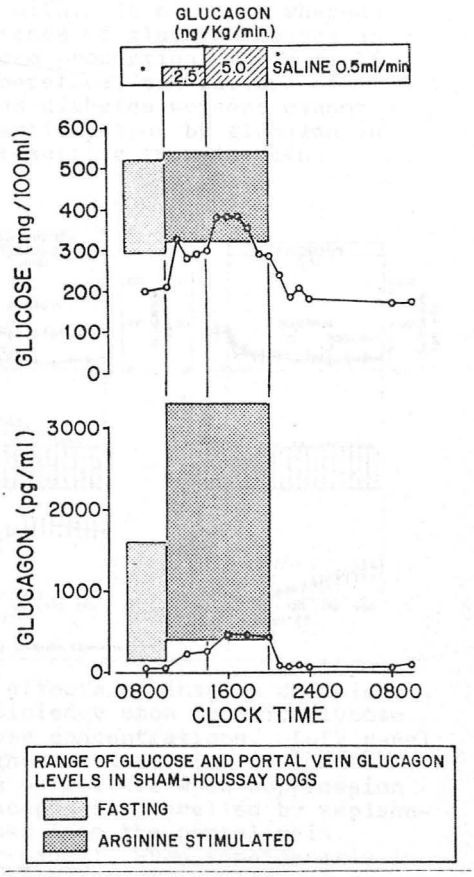
FIGURE 5A: Fasting glucose and glucagon levels in sham hypophysectomized, depancreatized dogs and in Houssay dogs. Note the frequency of fasting glucose levels below 300 in the latter group, including some hypoglycemic values in Houssay animals receiving no insulin. Note too the remarkedly low levels of glucagon two weeks or more after pancreatectomy, which, when the hypophysis is intact, results in increasing levels of extrapancreatic hyperglucagonemia.

FIGURE 5B: Gastric vein and inferior vena caval glucagon levels before and during arginine infusion in sham hypophysectomized, depancreatized dogs and in Houssay animals. The marked hypoglucagonemia is clearly attributable in large measure to the remarkable reduction in gastric glucagon secretion.



Even without insulin treatment glucose levels below 100 mg% were observed in the Houssay dogs. Replacement infusion of glucagon, even in subphysiological doses, increased the hyperglycemia to the range of pancreatectomized dogs with an intact hypophysis (Figure 6). While the direct influence of the other hormonal deficiencies of the Houssay dog upon the metabolic state may also be important, this study provides compelling evidence that hyperglucagonemia is required for the full expression of the syndrome of severe diabetes.

FIGURE 6: Effect of replacement infusion of glucagon for 11 hours upon plasma glucagon and glucose levels in the Houssay animal. Even though plasma glucagon levels were not quite into the range of portal glucagon levels of totally depancreatized dogs with intact hypophysis (stippled areas), a prompt increase in hyperglycemia into the range of the sham Houssay dogs was observed. Not shown here is an accompanying massive increase in glycosuria.



ARGUMENTS AGAINST GLUCAGON'S ROLE IN DIABETES

A. Does glucagon mediate certain of the metabolic consequences of insulin deficiency? (a pathophysiologic issue)

Argument #1: Discontinuation of insulin therapy in juvenile-type diabetics may give rise to endogenous hyperglycemia and an increase in plasma ketones without a concomitant rise in glucagon levels (1).

NOTE: These workers failed to consider that a reduction of insulin levels greatly enhances the biologic activity of a fixed level of glucagon, a fact which forms the basis for the use of the insulin:glucagon ratio (53) as an index of net bihormonal metabolic effects. Indeed, Cherrington and coworkers (9) have found that insulin deficiency induced

in the presence of constant basal glucagon levels causes glucose production to double within 30 minutes, whereas insulin deficiency in the absence of glucagon causes an initial drop in hepatic glucose production, and a small late increase (Figure 7). Therefore, the failure of glucagon levels to increase as diabetes worsens cannot be taken as evidence of nonparticipation by glucagon in the metabolic deterioration resulting from insulin deprivation.

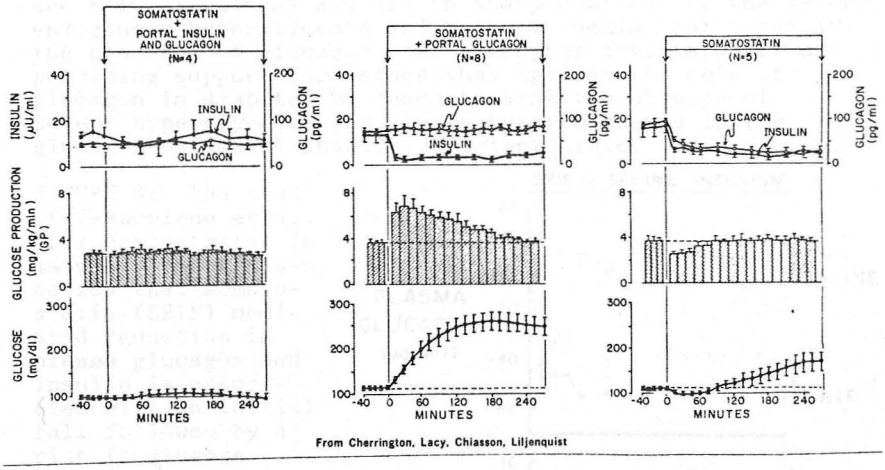


FIGURE 7: Comparison of the effects of insulin deficiency with and without glucagon deficiency upon hepatic glucose production and arterial glucose concentrations. Left panel: This is a control study designed to determine if somatostatin has effects upon these parameters when suppression of endogenous insulin and glucagon is cancelled by replacement infusions of both hormones into the portal vein. Clearly, it does not. Center panel: When somatostatin is infused with intraportal glucagon but no insulin, producing insulin deficiency without modification of glucagon levels, there appears a substantial and prompt increase in hepatic glucose production which begins to wane within one hour and returns to basal levels within three hours. However, the arterial hyperglycemia is not transient because clearance of the endogenous glucose from the extracellular space is delayed in the hypoinsulinemic animal. This experiment emphasizes two important points: 1) Although the glucagon-mediated increase in hepatic glucose production is transient, its hyperglycemic consequences persist well beyond the period of glucose overproduction. 2) The effects of glucagon may be enhanced without the rise in glucagon concentration simply by lowering the insulin concentration. Right panel: This demonstrates the effect of combined glucagon and insulin deficiency. When glucagon is absent there is no increase in hepatic glucose production nor does marked endogenous hyperglycemia appear. The rise in glucose concentration rarely exceeds 170 mg% and is at least in part the consequence of a late fall in peripheral glucose utilization (not shown here).

Argument #2: When one gives somatostatin to nondiabetics or maturity-onset diabetics for several hours, hyperglycemia and hyperketonemia occur despite continued suppression of glucagon (48, 52).

NOTE: These authors use the term "hyperglycemia" for a rise in glucose of 10 mg% (Figure 8) to a level of 120 mg% and "hyperketonemia" for a 0.2 mM rise in ketones. These are trivial changes and are in sharp contrast to the severe endogenous hyperglycemia and hyperketonemia that occur in the presence of glucagon. We interpret their studies as providing support for rather than against the role of glucagon in diabetes by demonstrating the absence of severe hyperglycemia and severe hyperketonemia in the glucagon-deficient insulin-deficient state.

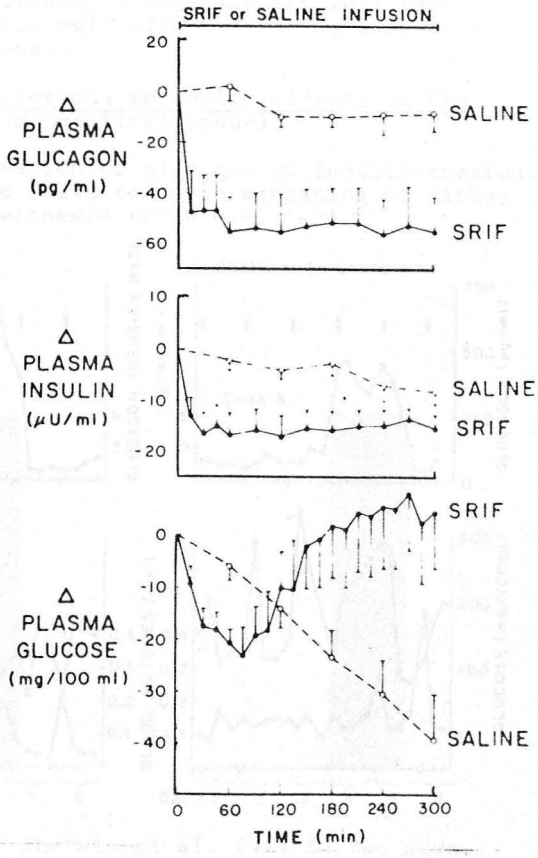


FIGURE 8: The study of Tamborlane et al. (52) demonstrates in maturity onset diabetics that somatostatin (SRIF) mediated reduction in plasma glucagon and insulin is associated with an initial fall followed by a rise in glucose levels to approximately 10 mg% above the basal value (not significant). They conclude from this that "hyperglycemia" may occur despite glucagon suppression.

Argument #3: Endogenous hyperglycemia and hyperketonemia develop following insulin withdrawal in totally depancreatized patients with unmeasurable plasma glucagon levels (5).

NOTE: It is doubtful that patients with total pancreatectomy really have zero levels of circulating immunoreactive glucagon (IRG) and related polypeptides. All four other groups (8, 32, 33, 37) that have studied such patients have detected circulating IRG in totally depancreatized diabetics using conventional radioimmunoassays for glucagon. Barnes' assay procedure differs uniquely from all other groups in the field in that he adds the "glucagon stripped" plasma of each patient to his standard tubes. Failure to demonstrate immunoreactive glucagon in the plasma of depancreatized patients may reflect the presence in the standard tubes of circulating glucagon-like polypeptides not removed by the "stripping" procedure, many of which have biologic activity. Although glucagon-like biologic activity is probably reduced in depancreatized patients, the residual unmeasured amounts of glucagon-like immunoreactive moieties may well have exerted glucagon-like biologic effects that were exaggerated by insulin withdrawal.

B. Does glucagon have deleterious metabolic effects in the presence of insulin? (a practical issue)

Argument #1: Administration of glucagon to insulin-treated juvenile-type diabetics fails to cause worsening of either the hyperglycemia or ketonemia (Figure 9) (47).

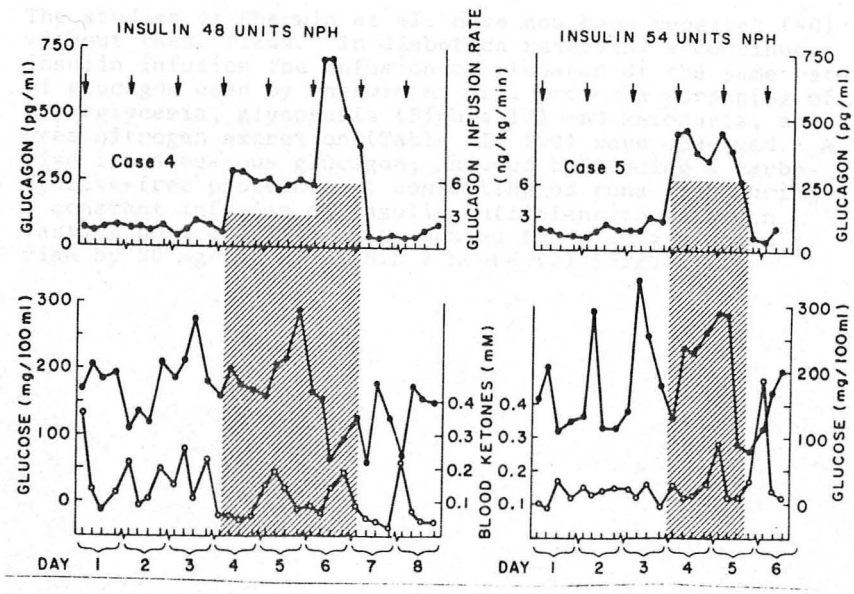


FIGURE 9: The study of Sherwin et al. (47) in two juvenile-type diabetics said to demonstrate that constant infusion of glucagon is without effect on either hyperglycemia or ketonemia.

This means that in diabetics glucagon is biologically impotent in the presence of insulin, that abnormal A-cell function in diabetic patients is not deleterious, and that efforts to find a pharmacologic glucagon-suppressing drug as a means of improving control of hyperglycemia are pointless.

NOTE: This study of Sherwin et al. (47) has itself been challenged on several grounds: 1) In selecting an infusion rate of glucagon that would simulate the portal vein glucagon levels presumed to be present in poorly controlled diabetics, they reckoned that hepatic extraction of glucagon was low (13); recent studies by Jaspan et al. (22) reveal the hepatic extraction of glucagon to be much higher than had been estimated by Felig et al. (13) so that the dose of glucagon they employed was probably low. 2) They premixed the infused glucagon with each patient's own blood, a maneuver which has been shown to cause a 60% loss of biologic activity in four hours and 100% loss in eight hours (55). 3) The absorption of depot insulin was clearly erratic since hypoglycemia and wide swings in glycemia were observed. 4) Glycosuria, ketonuria and other sensitive indices of glucagon action were not measured.

The studies of Sherwin et al. have now been repeated (40) without these flaws. In diabetics receiving a continuous insulin infusion the infusion of glucagon at the same rate of glucagon used by Sherwin et al., profound worsening of hyperglycemia, glycosuria (Figure 10) and ketonuria, and urea nitrogen excretion (Table II) (40) were observed. A rise in endogenous glucagon, induced by feeding a carbohydrate-free protein meal consisting of tuna fish during a constant infusion of insulin sufficient to maintain fasting normoglycemia, also causes the plasma glucose to rise by 50 mg/100 ml within 2 hours (2) (Figure 11).

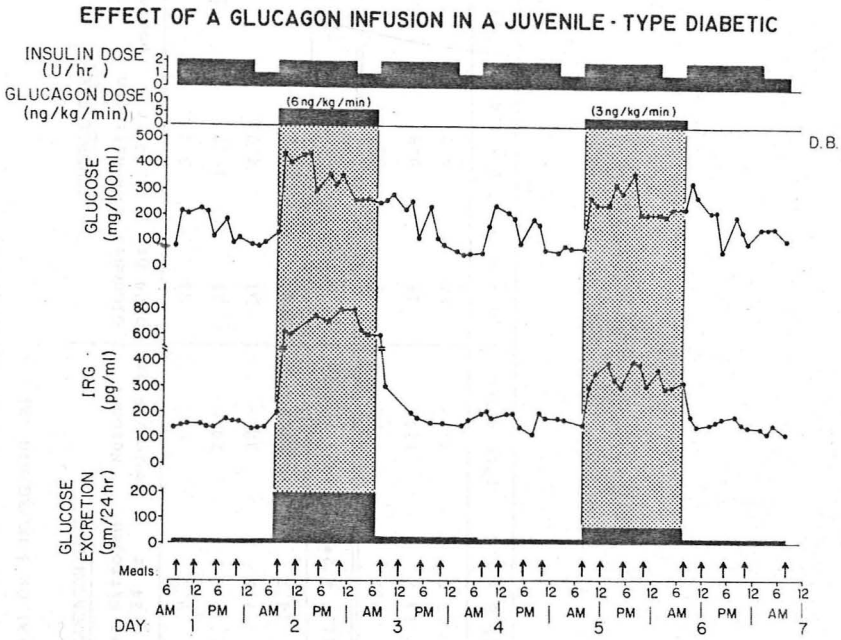


FIGURE 10: The effect of the intravenous administration of glucagon in doses of 3 ng/kg/min and 6 ng/kg/min on plasma glucose and IRG concentration and 24-hour glucose excretion in a juvenile diabetic patient during intravenous insulin infusion. The insulin infusion rate was decreased from 1 a.m. to 7 a.m. each morning to avoid hypoglycemia.

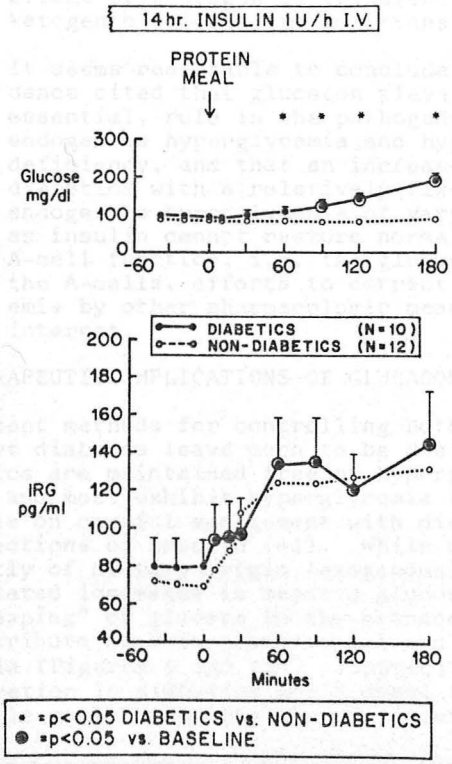
TABLE II

24-HOUR URINARY GLUCOSE, UREA NITROGEN, AND KETONE EXCRETION BEFORE, DURING, AND AFTER INTRAVENOUS ADMINISTRATION OF GLUCAGON TO DIABETICS AT A RATE OF 6 NG/KG/MIN (A) OR 3 NG/KG/MIN (B)

PATIENT	CONTROL DAY			GLUCAGON DAY			CONTROL DAY		
	Glucose g/24 hr	Urea Nitrogen g/24 hr	Ketones μmoles/24 hr	Glucose g/24 hr	Urea Nitrogen g/24 hr	Ketones μmoles/24 hr	Glucose g/24 hr	Urea Nitrogen g/24 hr	Ketones μmoles/24 hr
C.P.	44	5.4	340	158	9.2	690	33	5.7	320
D.B.	5	9.6	553	188	15.4	2445	21	10.7	1286
P.T.	69	4.3	296	163	7.6	3265	51	4.7	753
V.E.	26	8.9	738	99	14.7	1331	19	12.2	609
Mean	36 ± 14	7.0 ± 1	482 ± 102	152 ± 19*	11.7 ± 2*	1933 ± 573*	31 ± 7*	8.3 ± 2*	742 ± 202*
C.P.	5	5.6	307	18	9.1	708	6	5.5	346
D.B.	3	10.2	936	59	13.7	1308	16	9.8	867
P.T.	44	10.2	506	168	12.6	3248	36	5.7	616
Mean	17 ± 13	8.7 ± 1.5	583 ± 186	82 ± 45	11.8 ± 1.4	1755 ± 766	19 ± 9	7.0 ± 1.4	550 ± 104

* $p<0.05$ vs. preceding day

FIGURE 11A: The effect of a rise in glucagon induced by the ingestion of a tuna fish meal in adult-type diabetics during the constant overnight infusion of insulin. Note that despite the administration of insulin in quantities sufficient to normalize the fasting glucose level, the rise in endogenous glucagon stimulated by the protein meal is accompanied by a 50 mg% increase in glucose concentration. This is believed to be the consequence of an increase in glucagon at a time when insulin levels do not rise. Note too the statistically significant differences between the diabetic and the nondiabetic glucagon (IRG) levels.



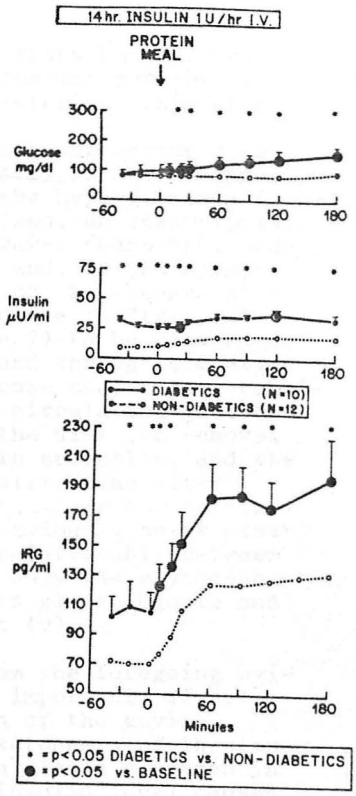


FIGURE 11B: The identical study carried out in juvenile-type diabetics. Although the rise in glucagon is no different from nondiabetics when these patients are maintained with an overnight insulin infusion, glucose levels rise approximately 50 mg% during the 2 hours after the ingestion of the tuna fish, attributed to a rise in glucagon when the insulin level is fixed.

Argument #2: Glucagon's action is transient (7, 10, 14, 49) so the hyperglucagonemia of diabetics must be devoid of any persistent metabolically deleterious influence.

NOTE: The waning of glucagon's acute augmenting effect on glucose production (glycogenolysis) is not necessarily reflected by a parallel waning of the hyperglycemia it has caused. When insulin levels are fixed, an inappropriate increase in glucose production, however transient, adds glucose to the extracellular space and, in the absence of a rise in insulin, this glucose cannot be cleared at a normally rapid rate. Thus, the glucose is "trapped" in the extracellular space (9) (Figure 7) to be cleared at a fixed subnormal rate by tissues and through excretion -- just as is the case after a glucose meal (also transient). In other words, whether the circulating glucose is derived from the liver or from the diet its removal is retarded by the defect in insulin secretion, and the worsening of the hyperglycemia persists even after increase in glucose influx (from the liver or the diet) has ceased. Increased glycosuria obviously helps clear the glucose and a certain inverse relationship between the two may be expected. However, only the glycogenolytic effect of glucagon is transient; its gluconeogenic and ketogenic effects are not transient (9).

It seems reasonable to conclude from the foregoing evidence cited that glucagon plays an important, if not essential, role in the pathogenesis of the severe endogenous hyperglycemia and hyperketonemia of insulin deficiency, and that an increase in plasma glucagon in diabetics with a relatively fixed insulin level causes endogenous hyperglycemia of varying severity. Inasmuch as insulin cannot restore normal glycemic control of A-cell function, i.e. the glucose-sensing function of the A-cells, efforts to correct diabetic hyperglucagonemia by other pharmacologic means become of therapeutic interest.

THERAPEUTIC IMPLICATIONS OF GLUCAGON SUPPRESSION IN DIABETES

Present methods for controlling both juvenile-type and adult-onset diabetes leave much to be desired. Few, if any, diabetics are maintained free of hyperglycemia throughout the day and most exhibit hyperglycemia for 8-16 hours per day while on careful management with diet and multiple daily injections of insulin (41). While daytime hyperglycemia is mostly of dietary origin (exogenous), inappropriate glucagon-mediated increases in hepatic glucose production, with "trapping" of glucose in the extracellular space, may well contribute to both postprandial and postabsorptive hyperglycemia (Figures 6 and 10). Suppression of excess glucagon secretion in diabetics would seem, therefore, to be a justifiable goal in the therapy of diabetic hyperglycemia.

In juvenile-type diabetics insulin reduces the hyperglucagonemia, but the quantities of insulin required to do so are high and increase the risk of hypoglycemia (2). In adult-type diabetics, by contrast, even supraphysiological doses of insulin fail to reduce elevated levels of glucagon (2). Therefore, insulin therapy alone is generally ineffective in or unsuitable for suppressing glucagon secretion in human diabetes. The ideal glucagon-suppressing agent would be one without glucoregulatory influences of its own and without insulin-suppressing activity or influence upon other organ systems.

Although somatostatin has widespread gastrointestinal actions and also suppresses insulin and growth hormone release, it may be the prototype of more suitable glucagon-suppressing agents. Gerich and coworkers (16) and Raskin (39) (Figure 12) have observed remarkable improvement in the control of hyperglycemia in some juvenile diabetics when somatostatin was administered as adjunct to insulin therapy. In severe alloxan diabetic dogs Schusdziarra et al. (46) have demonstrated improved control of hyperglycemia and triglyceridemia (Figure 13) when somatostatin analogs were administered subcutaneously as a supplement to suboptimal insulin therapy (12).

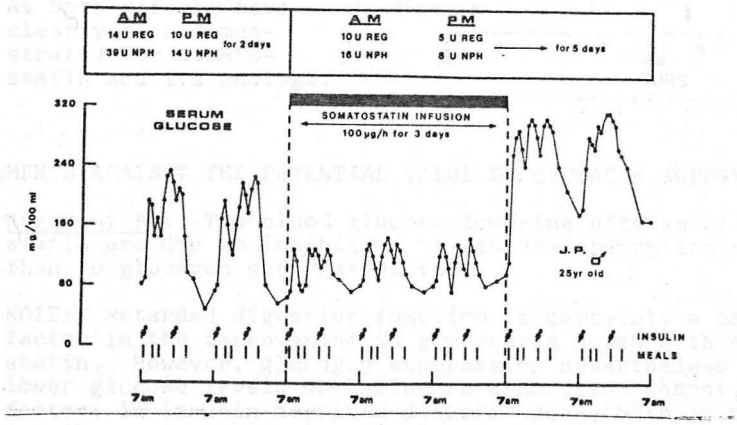


FIGURE 12: Effects of 3-day somatostatin infusion on diurnal serum glucose levels in an insulin-dependent diabetic subject. Courtesy of J.E. Gerich, M.D.

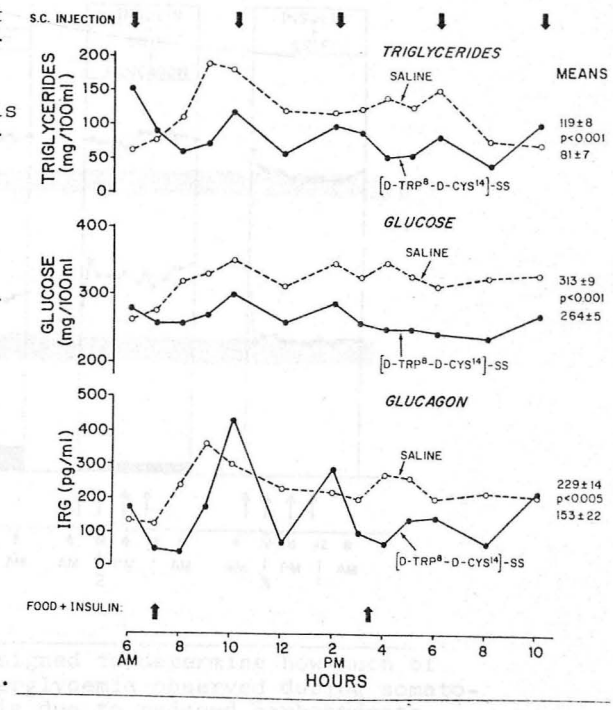


FIGURE 13: The effect of a D-amino acid substituted analog of somatostatin on around the clock levels of triglycerides, glucose and glucagon in chronic alloxan diabetic dogs receiving a suboptimal dose of insulin. Despite the inadequacy of the insulin dose, a significant lowering of plasma glucose and triglyceride levels is observed. (It is not clear how much of this is attributable to reduced rate of triglyceride absorption and how much is due to a lowering of endogenous triglyceride levels, inasmuch as both effects have clearly been demonstrated for somatostatin and its analogs.

ARGUMENTS AGAINST THE POTENTIAL VALUE OF GLUCAGON SUPPRESSION

Argument #1: The blood glucose lowering effects of somatostatin are due to inhibition of glucose absorption rather than to glucagon suppression (61).

NOTE: Retarded digestive function is certainly a major factor in the improvement in glycosuria noted with somatostatin. However, glucagon suppression nevertheless can lower glucose levels when glucose absorption cannot be a factor; in insulin-deprived diabetic dogs, both in the fasting state and during IV alanine infusion, glucose declines at a rate of about 1 mg%/minute during somatostatin-induced glucagon suppression and rises when glucagon is replaced by infusion (42). And human diabetics placed on a virtually carbohydrate-free diet remain hyperglycemic despite constant IV insulin, but become normoglycemic when glucagon is suppressed by somatostatin; when glucagon infusion is added to the insulin and somatostatin infusions they again become hyperglycemic (41) (Figure 14).

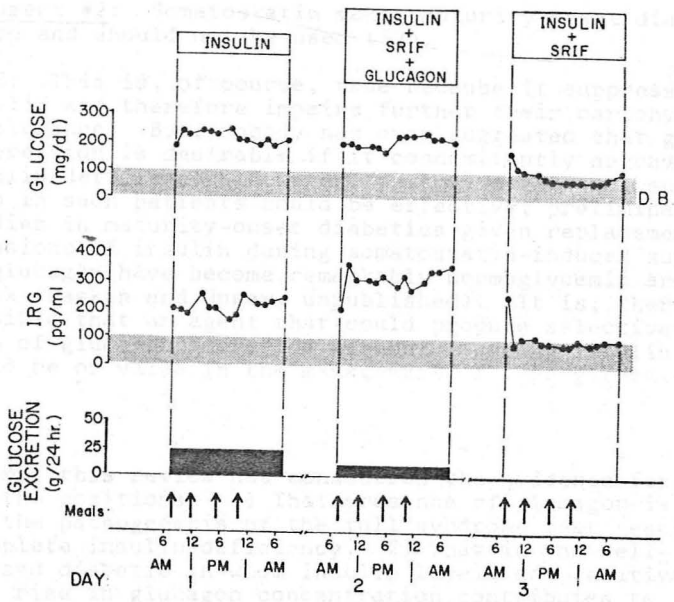


FIGURE 14: A study designed to determine how much of the improvement in hyperglycemia observed during somatostatin administration is due to reduced carbohydrate absorption as opposed to reduced levels of glucagon. The patients are placed on a virtually carbohydrate-free diet (less than 30 g/day) so that dietary contribution to hyperglycemia may be regarded as trivial. Despite this diet, constant infusion of insulin fails to normalize hyperglycemia and abnormally high glucagon levels are present (left panel). The addition of somatostatin (SRIF) to the insulin infusion lowers plasma glucagon (IRG) and brings glucose levels into the normal range (right panel). If glucagon is infused with the SRIF both hyperglucagonemia and hyperglycemia are present (center panel).

Argument #2: Somatostatin makes maturity-onset diabetics worse and should not be used (52).

NOTE: This is, of course, true because it suppresses their insulin and therefore impairs further their carbohydrate intolerance. But, nobody has ever suggested that glucagon suppression is desirable if it concomitantly aggravates insulin deficiency. However, selective glucagon suppression in such patients could be effective; preliminary studies in maturity-onset diabetics given replacement infusions of insulin during somatostatin-induced suppression of glucagon have become remarkably normoglycemic around the clock (Raskin and Unger, unpublished). It is, therefore, possible that an agent that could produce selective reduction of glucagon secretion without lowering insulin levels could be of value in the management of hyperglycemia.

SUMMARY

In summary, this review has considered the evidence for and against the positions: 1) That presence of glucagon is essential in the pathogenesis of the full syndrome that results from complete insulin deficiency. 2) That in the well-insulinized diabetic in whom insulin levels are relatively fixed, a rise in glucagon concentration contributes to endogenous hyperglycemia. 3) That conventional methods of treatment of diabetes do not fully correct either the abnormal glucagon levels or the hyperglycemia, but when insulin therapy is supplemented with somatostatin, both are corrected, facts providing a rationale for efforts to find a means of suppressing glucagon secretion in the management of human diabetes. As of now, there seems to be basis for modification of any of the foregoing positions.

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