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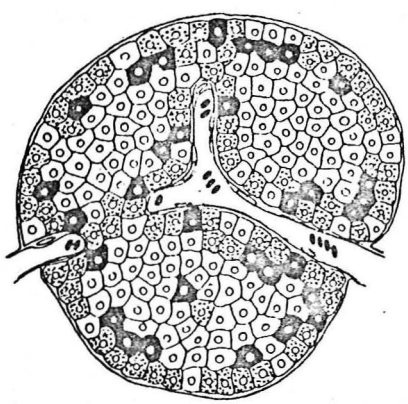
MEDICAL GRAND ROUNDS

PARKLAND MEMORIAL HOSPITAL

December 23, 1976

[Roger H. Unger]

DISEASES OF THE PANCREATIC ISLETS



THE ISLETS OF LANGERHANS IN 1907

". . . In the Islets of Langerhans in the guinea pig's pancreas, two types of cells, morphologically and physiologically distinct, are demonstrable. These cells show constant reactions to constant chemical tests. I have called these cells A and β-cells respectively . . ."

". . . In drawing conclusions from these facts, one is led to the conviction that the Islets of Langerhans are structures which, in all probability, have the function of producing a two-fold substance which, poured into the blood stream, has an important effect upon metabolism . . ."

Lane, M.A., Hull Laboratory of Anatomy, University of Chicago. From: The Cytological Characteristics of the Areas of Langerhans. Am. J. Anat., Volume 7, 1907.

THE ISLETS OF LANGERHANS IN 1976

TABLE I

CELL TYPE	PRODUCT	FUNCTION(S)		EXTRAINSULAR DISTRIBUTION
		Paracrine (?)	Endocrine	
B-, β-	Insulin	Restrains glucagon secretion (185)	Promotes nutrient ECF efflux and anabolism (Table II)	none
	C-peptide	? Inhibits insulin secretion*	?	?
A-, α_2-	Glucagon**	Enhances insulin (186) and somatostatin (159) secretion	Promotes production of endogenous fuels (Table II)	Oxyntic tissue of stomach (12)
	GLI***	?	?	Postduodenal gut (147)
D-, Δ, α_1-	Somatostatin (108, 44)	Restrains glucagon and insulin secretion (88)	Restrains nutrient absorption from gut (hypothetical)	CNS, gastric antrum, small bowel
F-cell ?	Pancreatic polypeptide (PP)	?	?	?
?	Gastrin ?	?	↑ HCl secretion	Gastric antrum, CNS

* Toyota, T., Abe, K., Kudo, M., Kimura, K., and Goto, Y.: Inhibitory effects of synthetic rat C-peptide 1 on insulin secretion in the isolated perfused rat pancreas. Tohoku J. exp. Med. 117:79-83, 1975.

** Baum, J., Simons, B.E., Unger, R.H., and Madison, L.L.: Localization of glucagon in the alpha cells in the pancreatic islet by immunofluorescent technics. Diabetes 11:371-374, 1962.

*** Srikant, C.B., and Unger, R.H.: Evidence for the presence of glucagon-like immunoreactivity (GLI) in the pancreas. Endocrinol. 99:1655-1658, 1976.
Moody, A., Frandsen, E.K., Jacobsen, H., Sundby, F., and Orci, L.: Glucagon Symposium. Metab. 25 (Suppl. 1):1336-1337, 1976.

TABLE II

METABOLIC ACTIONS OF INSULIN AND GLUCAGON (PHYSIOLOGIC)

INSULIN (Anabolic, anti-catabolic)		GLUCAGON (Catabolic, anti-anabolic)
Glycogenesis	↑	↓ Glycogenesis (hepatic)
Glycogenolysis	↓	↑ Glycogenolysis (hepatic) (109, 156)
Protein Synthesis	↑	↓* Protein Synthesis
Gluconeogenesis	↓	↑ Gluconeogenesis (156)
Lipogenesis	↑	↓ Lipogenesis
Lipolysis	↓	↑ Lipolysis (191)
Ketogenesis	↓	↑ Ketogenesis (122, 123)
Hepatic Lysosomes	↓	↑ Hepatic Lysosomes (4)

* Indirect by preempting potential substrate for gluconeogenesis

FIGURE 1

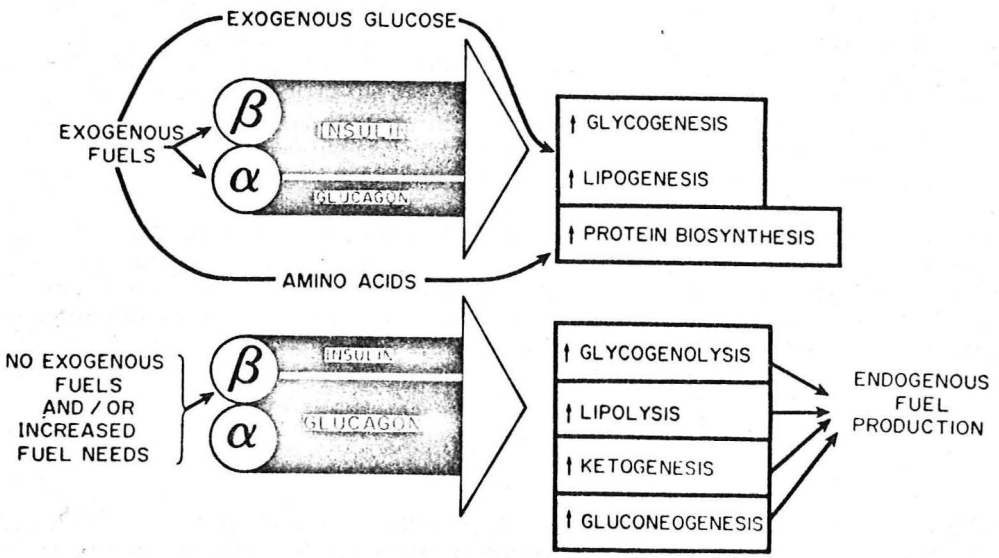


Figure I: Normally, as depicted in the upper panel, an influx of exogenous nutrients is greeted by an insulin-rich bihormonal mixture, thereby promoting the anabolic disposition of the ingested nutrients into macromolecules - the glucose into glycogen and fat, and the amino acids into protein. When nutrients are not available and/or when fuel needs increase, circumstances that often occur simultaneously, the normal islets of Langerhans respond by releasing a bihormonal mixture rich in glucagon relative to insulin. This promotes the retrieval of fuels from macromolecular storage forms: first, a breakdown of hepatic glycogen to maintain glucose production and, as glycogen stores are reduced, increasing glucose

production from non-glucose precursors including amino acids. To prevent excessive loss of amino acids via gluconeogenesis as fuel for the glucose-requiring brain, the low insulin: high glucagon mixture also promotes lipolysis, thereby providing increasing availability of free fatty acids and glycerol for general use, but, in addition, delivering to the liver the substrate for ketone formation. In the presence of glucagon, via a mechanism that appears to involve both glycogen depletion and increased presence of carnitine, the increased ketogenic capacity of the liver can furnish the brain with sufficient alternative fuel (122, 123) to reduce the need for gluconeogenesis and thereby spare protein (29).

THE INSULIN/GLUCAGON RATIO (I/G) (217)

If, as is now generally agreed, insulin and glucagon are biologic antagonists on common target cells, the direction and rate of metabolic processes which they both influence in such cells will be determined to a large degree by the relative concentrations of the two hormones that bind to such cells. The insulin: glucagon molar ratio (uU/ml of insulin/ pg/ml of glucagon x 23.3) in peripheral plasma probably reflects roughly in semiquantitative terms the islet hormone mixture perfusing the liver, the central organ of fuel disposal. The value of the I/G is limited by the facts, first that hormones in the peripheral plasma have escaped first circulation binding to the liver, their prime target organ, and second, that their actions on the liver are influenced by variables, such as other hormones which alter target organ sensitivity (cortisol) (156, 112, 240), changes in hormone receptor concentration and affinity, which alter relative biologic responses to the two hormones, and substrate availability. Nevertheless, the I/G provides a plasma index which correlates well with the metabolic behavior of the liver (157, 194) and with various anabolic or catabolic states of the organism. Whenever the molar concentration of glucagon is equal to or exceeds that of insulin, i.e. the I/G is 1.0 or less, a catabolic state is present with clinical manifestations thereof (217). Virtually every disorder characterized by negative nitrogen balance is associated with a low I/G (239, 106, 107, 177, 124); raising of the I/G by increasing the load of circulating insulin - either by stimulating with glucose* or by infusing insulin - will improve the nitrogen balance (46, 75) (hyperalimentation regimens) and constitute a means of augmenting the insulin: glucagon ratio so as to reduce the catabolic state and promote anabolism (46, 75, 111). It is of teleologic interest that in fetal life, the ultimate anabolic state, there is an abundance of both glucagon and insulin. But the developing liver lacks glucagon receptors, whereas it has receptors for insulin, the anabolic hormone**. The unlimited fuel supply from the mother makes glucagon responsiveness by the fetus both unnecessary and detrimental (anti-anabolic), while the need for growth makes responsiveness to its own insulin crucial.

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*Walker, C., Peterson, W., Jr., and Unger, R.H.: Blood ammonia levels in advanced cirrhosis during therapeutic elevation of the insulin: glucagon ratio. N. Engl. J. Med. 291: 168-171, 1974.

**Blazquez, E., Rubalcava, B., Montesano, R., Orci, L., and Unger, R.H.: Development of insulin and glucagon binding and the adenylate cyclase response in liver membranes of the prenatal, postnatal, and adult rat: Evidence of glucagon "resistance". Endocrinol. 98: 1014-1023, 1976.

In acute stress, the I/G falls almost instantly, thereby causing a rise in glycemia to above the normal range which neither B- or A-cells correct. This "ignoring" of hyperglycemia by the islets, aided by increased cortisol, is believed to be a compensatory response to maintain glucose delivery to the brain in the face of actual or potential reduction in cerebral blood flow (218). The elaborate relationships between the brain involve not only adrenergic control from the ventromedial nucleus of the hypothalamus* to the islets by mixed autonomic nerves (115) with adrenergic junctional relationships with A-cells and B-cells**. Adrenergic, cholinergic, dopaminergic, serotonergic, neuroendocrine (substance P, neurotensin) (153), and other hormones*** place the islets as a synaptic ganglion, and "appendage" of the central nervous system which they are designed to serve above all other organ tissues, and from which they are embryologically derived****.

THE GLUCOREGULATORY FUNCTION OF THE ISLETS

Whereas the extracellular fluid concentrations of fuels other than glucose range widely, glucose concentrations are kept within a very narrow range irrespective of glucose flux rates through the extracellular space. Normally the islets do not permit the ECF glucose concentration to fall below 60 mg%; indeed, the B-cell becomes refractory to other stimuli whenever glucose concentrations approach this level. Nor do the normal islets of Langerhans permit hyperglycemia in excess of 150 mg% in the unstressed state; indeed, the A-cell becomes refractory to stimulation whenever glucose concentrations reach this level (140, 132, 216).

The reasons why nature maintains glycemia within such narrow limits are not entirely clear. Obviously, rapid hypoglycemia during exercise or protein ingestion, the two common circumstances in which glucose efflux is augmented, would be incompatible with normal function of the glucose-requiring brain. But the reason for avoidance of hyperglycemia is not clear, unless as Cahill believes, repeated transient hyperglycemia through the years would favor glycosylation of certain proteins (e.g. hemoglobin $A1^C$) and raises the question as to whether thickened basement membrane of capillary may not be a manifestation of the same process.

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*Frohman, L.A., and Bernardes, L.L.: Effect of hypothalamic stimulation on plasma glucose, insulin, and glucagon levels. Am. J. Physiol. 221:1596-1603, 1971.

**Orci, L., Perrelet, A., Ravazzola, M., Malaisse-Lagae, F., and Renold, A.E.: A specialized membrane junction between nerve endings and B-cells in islets of Langerhans. Eur. J. Clin. Invest. 3:443-445, 1973.

***Moltz, J.H., Fawcett, C.P., McCann, S.M., Dobbs, R.E., and Unger, R.H.: The hypothalamo-pancreatic axis: Evidence for a neurohormonal pathway in the control of the release of insulin and glucagon. Endo. Res. Comm. 2 (8): 537-547, 1975.

****Pearse, A.G.E.: Peptides in brain and intestine. Nature 262:92-94, 1976.

FIGURE 2

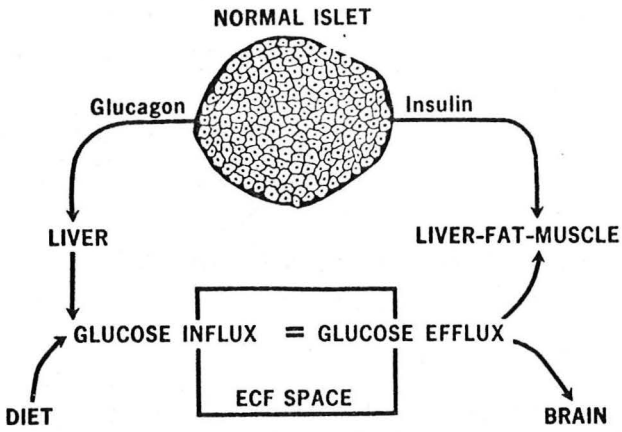

Figure 2: The simplest, if not only way, by which the concentration of a substance in a space can be maintained within narrow limits -- irrespective of changes in flux rates -- is by means of a push: pull system that permits, by means of a concentration sensor, the independent regulation of influx and efflux of that substance. The islets of Langerhans sense the glucose concentration and secrete the appropriate amount of its hormone of glucose influx (glucagon) and hormone of glucose efflux (insulin) to correct rapidly a change in glycemia. An increase in glucose efflux resulting either from protein ingestion (225), which, by causing the release of insulin required for incorporation of amino acids into protein, augments glucose uptake, or by exercise, which can increase muscle utilization of glucose ten-fold or more, an outpouring of glucagon (22) replaces precisely the outflowing glucose, thereby preventing hypoglycemia. Alternatively, an influx of dietary glucose elicits the secretion of just enough insulin to enhance glucose efflux into insulin sensitive tissues such as the liver (110)*, muscle, and fat, thus quickly bringing the glycemia back to basal levels. In other words, in a normal state, glucose influx and efflux are ultimately equal, any transient disequilibrium eliciting an appropriate corrective response by the B-cells and A-cells.

THE ENDOCRINE FUNCTIONS OF PANCREATIC SOMATOSTATIN (THIRD HORMONE HYPOTHESIS)

It is possible that somatostatin acts only locally within the islet and has no endocrine functions - i.e. it never enters the pancreatic venous effluent, or that, if it does, it is so rapidly destroyed that it never reaches any potential target cells (its biologic half-life is only three minutes). On the other hand, it could be a third islet hormone involved in the control of nutrient flux - influx from the gut.

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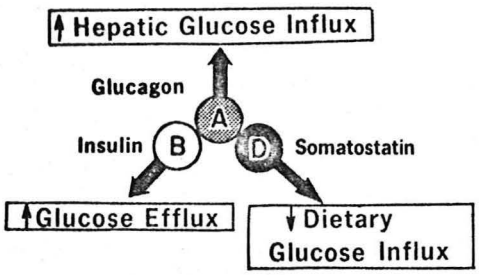
*Madison, L.L., Combes, B., Strickland, W., Unger, R.H., and Adams, R.: Evidence for a direct effect of insulin on hepatic glucose output. Metab. 8: 469-471, 1959.

FIGURE 3



The evidence: Patton et al. have demonstrated that somatostatin levels in venous effluent of the perfused canine pancreas increased several fold during the administration of arginine (160), alanine (Ipp et al., unpublished), and glucose (193; Weir et al., unpublished; Ipp et al., unpublished). While this could represent an unphysiologic "washout" of a locally active secretory product not normally exported from the pancreas, the likelihood is that this indicates that somatostatin is, indeed, a real hormone. Like insulin and glucagon, it could be involved in the control of nutrient flux; there is evidence that somatostatin has powerful actions on the gastrointestinal tract which would tend to reduce the influx of nutrients from the gut (233): 1) inhibition of gastrin secretion (Bloom); 2) inhibition of gastrin stimulated HCl secretion (Bloom); 3) inhibition of gastric emptying (Bloom); 4) inhibition of pancreozymin and secretin release (Creutzfeldt). While the doses of somatostatin used to demonstrate these actions were probably pharmacologic, they could, nevertheless, represent a physiologic effect of the peptide. The seemingly indiscriminate response to high perfusing levels of such diverse nutrients as glucose, arginine, and alanine would be compatible with such a hypothesis. Also IV glucose inhibits gastric emptying - perhaps by stimulating endogenous somatostatin. Perhaps it controls the rates at which nutrients enter the ECF from the gut so as to avoid entry rates that would outstrip disposal rates. The syncytial relationships of the D-cell to its insulin- and glucagon-secreting neighbors (Orci) would permit such coordinated action. Rapid entry would evoke a much higher I/G ratio response than a slow entry rate and could influence metabolic rates of nutrients. If so, the somatostatin-secreting D-cell may play an important role in a variety of common human disorders such as diabetes mellitus and obesity.

ISLET POLYPEPTIDES WITHOUT KNOWN FUNCTIONS

Pancreatic polypeptide: There is not even a good hypothesis for pancreatic polypeptide since its actions are unknown. It rises in response to the same circumstances that augment glucagon release: hypoglycemia, fasting, hyperaminoacidemia, meals, and exercise*. It is secreted by cells (probably F-cells) that substitute for the A-cell mantle of certain islets - generally those located near the duodenum**.

GLI: Unknown. Is it a precursor, derivative or product of a "bifurcation" in the biosynthetic pathway for glucagon? In the pancreas, it has been localized by immunofluorescense to the same cells that contain glucagon***. In the small bowel, it is present in A-like cells that contain no glucagon (147). It differs from glucagon biologically, immunologically, and physicochemically (190) (Table III).

TABLE III

COMPARISON OF PANCREATIC GLUCAGON, GASTROINTESTINAL (GI) GLUCAGON AND GLI

	PANCREATIC GLUCAGON	GI GLUCAGON	GLI
M.W.	3485	∿3500	∿2900
Isoelectric point	6.2	6.2	10
Immunoreactive ratio of 78J/30K*	1.0	0.9	61
Glycogenolytic Activity: % of 10 g of glucagon	100	100	50
70% of maximum adenylate cyclase stimulation	10^{-8} M	10^{-8} M	10^{-7} M
Affinity for rat liver membranes	4×10^{-9}	3×10^{-9}	5×10^{-8}

* Ratio of values obtained by measuring with antiserum 78J, which reacts with the 3-23 residues of glucagon thought to be contained in the GLI molecule and 30K, which reacts with 24-29 residues of glucagon, which are presumed to be absent or inaccessible in the GLI molecule.

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*Floyd et al.: Rec. Prog. Horm. Res., 1977 (In press).

**Orci, L.: Discussion of Floyd's paper. Rec. Prog. Horm. Res., 1977 (In press).

***Moody, A., Frandsen, E.K., Jacobsen, H., Sundby, F., and Orci, L.: Discussion. The structural and immunologic relationship between gut GLIs and glucagon. Metab. 25: 1336-1338 (Suppl. 1), 1976.

PARACRINE INTERACTIONS OF THE ISLETS

The possibility that the secreted polypeptides of islet cells may influence the secretory function of neighboring cells by direct peptide-cell contacts across the intercellular space has long been entertained. Such interactions via the intercellular spaces could be controlled and directed by the constantly changing network of tight junctions between islet cells described by Orci and coworkers (142, 146) and recently have been found to correlate with secretory activity (Orci et al., unpublished).

TABLE IV

EFFECTS OF ISLET SECRETORY PEPTIDES ON SECRETORY FUNCTION OF ISLET CELLS

PEPTIDE	B-CELL	A-CELL	D-CELL
Insulin	↓	↓	0(?)
Glucagon	↑	0	↑
Somatostatin	↓	↓	?
C-peptide	↓(?)	?	?
GLI	↑(?)	?	?
PP	0	0	?

FIGURE 4

NORMAL ISLET

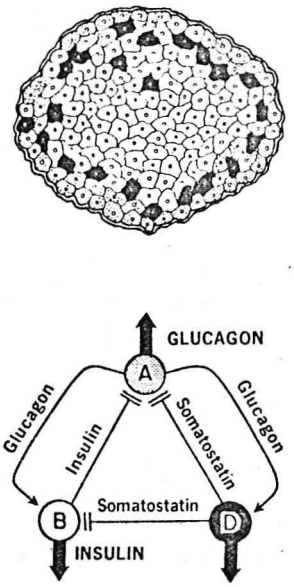

Figure 4: Based on Table IV, the paracrine system schematized to the left has been postulated: A-cells are restrained by insulin and somatostatin; glucagon stimulates both B- and D-cells and somatostatin restrains B-cells as well as A-cells.

Paracrine interactions between heterologous cells would probably be most active in the heterocellular region which, in the rat and human, forms the "cortex" of the islet. This consists of an outer mantle of A-cells ranging from one to three cells in thickness and constituting about 25% of the total endocrine population. Next are scattered somatostatin-secreting D-cells constituting approximately 10% of the normal endocrine population of the islets; they are arrayed in juxtaposition to the A-cells (or to F-cells in islets in which A-cells are replaced by PP-secreting F-cells). The insulin-secreting B-cells form the central mass of the islets at least 60% of the endocrine population.

It has now been established that all three major secretory products of the islets - insulin, glucagon, and somatostatin - can alter the secretory activity of at least one of the other islet cell types (Table IV). In 1965, Samols et al. (186) showed that glucagon could stimulate insulin secretion and, more recently, that insulin inhibits glucagon secretion (185), and he has long championed an insulin:glucagon feedback relationship (187). Koerker et al. (88) and other groups (129, 183) have demonstrated that somatostatin inhibits the release of both insulin and glucagon. Patton et al. (159) have reported that glucagon stimulates somatostatin secretion.

WITHIN-ISLET FUNCTIONS OF SECRETORY POLYPEPTIDES (HYPOTHETICAL)

Paracrine functions of somatostatin: Somatostatin release is markedly enhanced by every nutrient thus far tested - arginine (Figure 5), alanine, and glucose but not by insulin. Perhaps the sustained rise in somatostatin induced by arginine perfusion is intended to reduce the initial burst of insulin and glucagon secretion that greets abrupt increases in nutrient concentrations and shapes the contour of the so-called first phase of insulin and glucagon release. The reduced release of these hormones despite continuing high concentrations of nutrient could be mediated by the persistent release of somatostatin.

FIGURE 5

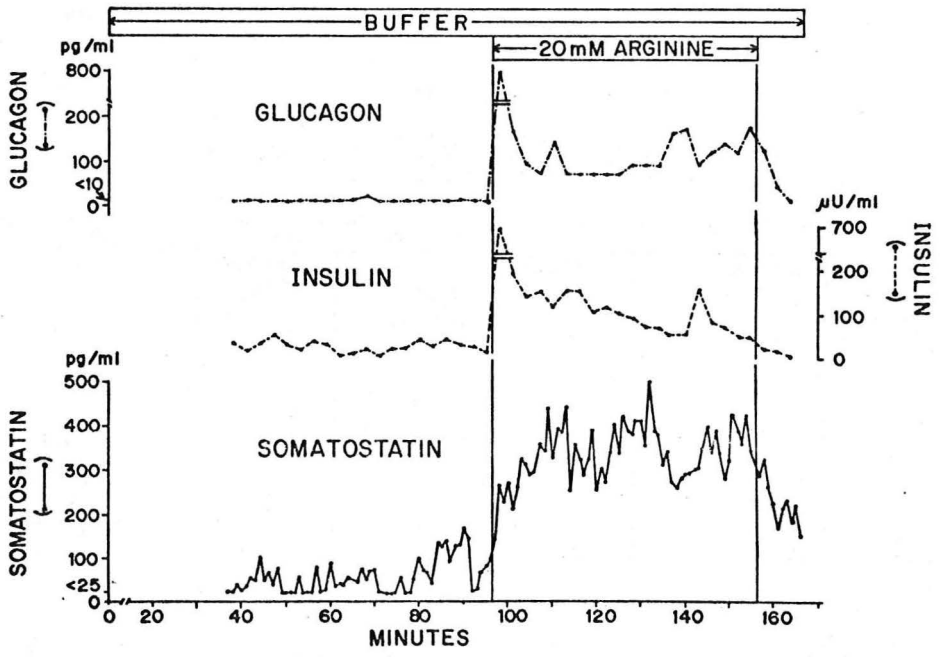

Figure 5: Immunoreactive somatostatin, insulin, and glucagon during perfusion of isolated dog pancreas with arginine.

Paracrine actions of glucagon: Glucagon may mediate somatostatin release in response to amino acids; high concentrations of glucagon, but not of insulin, have been shown to stimulate somatostatin secretion (159). It is not as yet known whether the D-cell responds directly to amino acids. The glucagon-mediated somatostatin release could result in down-modulation of both the glucagon and insulin responses to amino acids and minimize glucagon's direct stimulatory effect on insulin (eg. perhaps this is why alanine, a potent stimulator of glucagon release, elicits such secretion of insulin. If, under these circumstances, within islet glucagon were to elicit a strong release of insulin, the purpose of glucagon, to prevent hypoglycemia through glycogenolysis and gluconeogenesis, would be defeated by insulin).

Paracrine functions of insulin; "glucose sensing" by islet cells: The B-cell is believed to have glucoreceptors which enable insulin secretion to respond to changes in ambient glucose concentrations. However, the A-cell appears to lack its own glucose receptors; whenever A-cells lack contact with B-cells, as in the gastric fundus, glucagon-secreting tumors, and A-cells in the islets of juvenile diabetics, glucose is without effect on glucagon secretion unless insulin is concomitantly provided, but insulin and other responses are as in the normal islet (Table V).

TABLE V

RESPONSES OF GLUCAGON FROM A-CELLS
ADJACENT TO OR REMOTE FROM B-CELLS

CHALLENGE	ADJACENT		REMOTE		
	Normal A-cells	Adult Diabetic A-cells	Gastric A-cells	Glucagonoma A-cells	Juvenile Diabetic A-cells
Hyperglycemia	↓↓	0 or ↓	0	0 or ↑	↑
Arginine I.V.	↑	↑↑	↑	↑	↑↑
Insulin I.V. plus glucose (without hyperglycemia)	↓	↓	↓	↓	↓

Hyperglycemia cannot suppress glucagon secretion in the absence of insulin. In fact, it tends to cause a paradoxical rise in glucagon in insulin-deprived diabetics (28, 107, 8) and in glucagonomas (19, 212). When insulin rises together with glycemia, glucagon secretion by the gastric A-cells of insulin-deprived diabetic dogs (17, 18), glucagon secretion by the A-cells of insulin-deprived juvenile diabetics (170, 171, 174), and glucagon secretion by glucagon-producing tumors (107) is promptly suppressed. These facts raise the possibility that the "glucose sensor" for the normal A-cells of the islets of Langerhans is insulin released into the adjacent intercellular spaces. In the adult type diabetic in whom insulin is present in the islets of Langerhans, glucagon suppression by hyperglycemia, while reduced, is not abolished (171, 8).

MORPHOFUNCTIONAL RELATIONSHIPS OF NORMAL ISLETS

Morphologic Feature	Functional Implication
1) Deployment into separate micro-organs embedded into a firm retroperitoneal bed.	a) Reduced chance of total destruction. b) Possible unknown endocrine-exocrine interactions.
2) Heterocellular zones ("cortex").	Permits paracrine interactions (direct cell-to-cell interactions via secretory products) See Table IV and Figure 4 (149)
3) Entry of vessels and autonomic nerves in the heterocellular zones (56).	May be the control center for within islet signal transmission - the pacemaker.
4) Tight junctions (56, 150).	Create intercellular channels or compartments which may guide secretory products to appropriate destinations and prevent inappropriate interactions (such as glucagon-mediated stimulation of insulin secretion in time of glucose need).
5) Gap junctions (150, 145, 146).	Permit metabolic and electrotonic coupling between all islet cells, converting the islet into syncytium with coordination of all secretory products from asynchronously secreting cells.

TABLE VI

PRIMARY DISEASES OF THE ISLETS

Deficits of Islet Cells
Absence of islets
Glucagon deficiency (one case only)
Diabetes with absence of B-cells
Diabetes without absence of B-cells* (adult type)
Tumors and Hyperplasias of Islet Cells
Insulinoma (benign, malignant)
Glucagonoma GLI-oma
Nesidioblastosis

* Unknown pathology. Could be 1° or 2° D-cell deficiency if it resembles the pathology of the ob/ob mice as described by Baetens et al. (10).

CASE 1.

A.B. was a 49-year-old white female who was admitted with a 2-month history of exfoliative, migratory dermatitis of the extremities, buttocks, back and face, 25-pound weight loss, intermittent diarrhea and generalized weakness. A glucose tolerance test at the onset of this illness showed a fasting plasma glucose of 130 mg%, with a 2-hour value of 200 mg%. No family history of diabetes or endocrine abnormality. She had been in excellent health prior to this illness, and was taking no medication. On physical examination the most impressive finding was the rash, circumoral fissures and moderately severe glossitis. There were no palpable abnormalities in the abdomen. Fasting blood sugar on admission was 137 mg%; there was a moderate normochromic anemia; liver function was normal; routine chemistries, including thyroid and adrenal function tests were normal. Biopsy of a skin lesion showed a nonspecific perivascular, lymphocytic infiltrate. Fasting plasma glucagon (30K, Dr. Levin) = 3500 pg/ml.

Radiologic studies included an upper G.I. series, which showed swollen gastric and duodenal mucosal folds (apparently a common finding in patients with glucagonoma syndrome). Liver scan showed enlargement and a periportal filling effect. Celiac axis and superior mesenteric arteriography demonstrated abnormal vascularity in the head and tail of the pancreas and a single region of abnormal vessels in the right lobe of the liver.

At surgery a 3 x 3 cm spherical lesion was found in the head of the pancreas, with multiple lesions in an atrophic pancreatic tail. A single 7 cm spherical cystic liver metastasis was also found. Following a 95% pancreatectomy, an attempt was made to resect the isolated hepatic lesion. During this dissection the patient developed irreversiable cardiovascular collapse. She was maintained on automatic ventilation and circulatory support for the next 36 hours but expired.

Routine pathologic studies were said to show a tumor typical of islet cell carcinomas.

Special studies: There was no suppression of glucagon during a standard oral GTT or I.V. GTT. Arginine challenge (20 g over 20 min) elicited a doubling in plasma glucagon immunoreactivity. In addition, gastrin (Dr. M. Grossman's lab) was normal under all conditions. Gel filtration of the plasma (Dr. Unger's lab) showed the largest part of immunoreactivity in the true glucagon area, but a major peak in the 9000 M.W. zone.

Comment: In our lab plasma samples contained up to 8000 pg/ml of IRG and 9800 pg/ml of GLI. Dr. Orci's lab identified GLI and somatostatin but no glucagon in the tumor by immunofluorescent staining.

IMMUNOFLUORESCENT STAINING OF TUMOR OF PATIENT A.B.

anti-glucagon 15K (glucagon-specific)	--
anti-glucagon 30K (glucagon-specific)	--
anti-GLI (Moody)	+
anti-GLI + GLI	--
anti-GLI + somatostatin	+
anti-somatostatin (Patel)	++
anti-somatostatin + somatostatin	--
anti-insulin	--
anti-secretin	--
anti-gastrin	--

CASE 2.

A 42-year-old woman had a 7-year history of increasingly severe and recurrent duodenal and stomal ulcers for which she underwent a partial gastrectomy, gastrojejunal resection, vagotomy and finally total gastrectomy. Pancreatic exploration revealed two tumors (I and II), of 1.5 and 1.2 cm in diameter respectively. In addition, a diffuse spread of microadenomas was found in the resected body and tail of the pancreas, chiefly at the histological level. The patient is still well and symptomless 8 years after the last operation. An ultrastructural study on this case has previously been reported (Tardini, Anversa and Bordi, 1969).

IMMUNOFLUORESCENT STUDIES

	GASTRIN	GLUCAGON (15K)	GLI	SOMATOSTATIN	PP
Tumor I	--	+	+	+*	+
Tumor II	--	+	+	+**	+
Micro-adenoma	--	+	+	+*	+

* Positive cells are scarce and show only a weak fluorescence.
** Numerous positive cells.

CASE 3.

This female infant (A.M.) was born at full term. At 24 hours multiple generalized convulsions were noted, which were treated with diazepam (Valium). At 14 days of age, baby was referred because of continuing recurrent convulsions.

Upon admission the infant was pale, flaccid, showed no reactions and had a weak cry. The liver was palpable 2 cm below the costal margin. The plasma glucose concentration was 0 mg/100 ml, urinary actone was negative and the baby responded immediately to 4 ml of 40% glucose intravenously with a normal cry, normal movements and a rosy skin color. The hemoglobin was 19.1 g/100 ml, and bilirubin was 1 mg/100 ml.

Despite hourly administrations of 40% glucose followed by continuous 10 to 20% glucose infusion, the plasma glucose values fluctuated between 0 and 20 mg/100 ml. Diazoxide up to 16 mg/kg/day, prednisone up to 3 mg/kg/day, intramuscular human growth hormone (Raben) 1 mg/day, intramuscular chlorpromazine (Largactil) 1 mg/kg several times per day were without effect. Only continuous I.V. 30% glucose (300-400 ml/24 hours) in addition to oral feedings (300-450 ml Nan 14%/day) and repeated intravenous glucagon injections up to 2.5 mg every two hours prevented coma. Innumerable attempts to gradually diminish the glucose and glucagon dose were followed by a prompt drop of the glucose level and muscular hypotonia, pallor, sweating, tachycardia, and twitching of the eyes. 51 plasma glucose levels between 0 and 10 mg/100 ml were recorded during these attempts. Under the high glucose/glucagon regimen, the glucose level fluctuated between 30 and 150 mg/100 ml. The child received a mean of 610 calories per day, and the weight increased from 3800 to 4870 g in two months.

Of 10 insulin levels determined one-half to two hours after attempts to stop the glucose/glucagon therapy, six values were high with respect to the corresponding glucose levels. An I.V. glucagon test at three weeks of age showed a normal glucose increase, late hypoglycemia at 60 to 120 minutes and an only small insulin peak in the peripheral vein. An I.V. glucose test performed at three and one-half weeks of age, two hours after stopping the glucose/glucagon therapy, revealed an extremely rapid glucose disappearance rate ($K_{Glucose}$ = 9.9%/min) with severe hypoglycemia already after 30 minutes despite an only minimal insulin peak. A leucine test could not be performed because of the spontaneous drop of the glucose level whenever the infusion was stopped. Leucine sensitivity was unlikely since the glucose concentration rose from 38 to 71 mg/100 ml after oral administration of 100 ml of 14% Nan, which contains leucine. Intravenous fructose was well-tolerated, excluding hereditary fructose intolerance. At six weeks of age, stopping the glucose infusion induced a normal elevation in plasma growth hormone and cortisol (F), indicating that both hormones were secreted normally.

The relatively or absolutely high insulin concentrations and the results of the functional tests were compatible with an islet cell adenoma. At seven weeks, a laparotomy was performed with a continuous 30% glucose infusion. No tumor was found and a 2/3 pancreatectomy was done. During the operation serial bloods were taken for glucose and insulin determinations. A markedly higher insulin level was present in the portal vein (59 μU/ml) than in the caval circulation (30 μU/ml). Following the partial pancreatectomy this difference was still present, although at a lower level 39 vs. 8 μU/ml). Postoperatively, the plasma glucose concentration again dropped to 6 mg/100 ml one and one-half hours after replacing the glucose by a saline infusion.

Case 3. (cont'd)

Postoperatively, the baby continued with chronic intractable hypoglycemia and was treated with the same medical regimen. The "fasting" glucose and insulin levels improved slightly, but her general condition gradually deteriorated. Several EEGs were pathologic, showing dysrhythmic and paroxysmal 3-6 Hz waves bitemporally. The baby developed staphylococcal septicemia from a venous catheter site. Finally, a second laparotomy was performed at 11 weeks of age. Most of the remaining pancreatic tissue was removed, leaving some tissue at the duodenal border because of technical reasons. The child recovered well at first, and the glucose level remained normal with an infusion of only physiologic saline. One day after the second operation, her circulation deteriorated suddenly and the baby died with E. Coli septicemia 44 hours after the successful subtotal pancreatectomy.

The main findings at autopsy were E. Coli septicemia, endocrine cell hyperplasia, replacing most of exocrine tissue, and a markedly diminished weight (285 g, normally 411 g).

IMMUNOFLUORESCENT STAINING

INSULIN	GLUCAGON	GLI	SOMATOSTATIN	PP
++++	+	++++	++	+++

DIABETES 2° TO B-CELL DEFICIENCY

The Bihormonal Abnormality Hypothesis: The bihormonal abnormality hypothesis (226, 43) maintains that the full metabolic expression of diabetes mellitus is not solely the direct consequence of insulin lack by itself, but requires, in addition, an excess of glucagon relative to insulin. While insulin insufficiency is the sine qua non of diabetes mellitus, without which none of its metabolic abnormalities can occur, severe endogenous hypoglycemia and ketoacidosis require the presence of glucagon in addition to the insulin deficiency. The contribution of each hormonal abnormality is summarized in Table VII.

TABLE VII

CONTRIBUTION OF THE HORMONAL ABNORMALITIES
IN INSULIN DEFICIENCY DIABETES TO ITS METABOLIC ABNORMALITIES

	INSULIN LACK	GLUCAGON EXCESS
Glucose utilization	++++	0(?)
Glucose production	+	+++
Lipolysis	++++	0-+(?)
Ketogenesis	+(?)	++++

A. Metabolic consequences of the insulin abnormality:

On glucose metabolism:

1. The inability to change plasma insulin levels according to need is reflected by a rate of glucose disposal fixed by the level of exogenous insulin plus insulin-independent glucose removal such as uptake by the brain and excretion by the kidney.

2. In addition, as first shown by Madison et al.*, glucose production by the liver is increased by insulin lack, but in the absence of glucagon, this would cause only mild endogenous hyperglycemia (150-170 mg%).

On amino acid metabolism:

1. Insulin lack will favor increased mobilization of amino acids from body protein and present the liver increased quantities of gluconeogenic substrate.

2. Insulin lack removes a powerful brake upon gluconeogenesis.

On fat and ketones:

1. Insulin lack favors lipolysis, presenting the liver with increased substrate for ketogenesis.

2. Insulin lack probably favors hepatic ketogenesis, but only to a modest degree.

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* Madison, L.L., Combes, B., Strickland, W., Unger, R.H., and Adams, R.: Evidence for a direct effect of insulin on hepatic glucose output. Metab. 8:469-471, 1969.

B. Metabolic consequences of glucagon excess:

On glucose metabolism:

1. Probably without effect on exogenous glucose disposal although Madison and Lindsey reported some evidence to the contrary.

2. Powerful enhancer of hepatic glucose production - essential for severe endogenous hyperglycemia.

On amino acid metabolism:

1. Powerful gluconeogenic effect that increases with deficiency of insulin [insulin reduces substrate (amino acids) availability and opposes gluconeogenesis in the liver].

On fat and ketone metabolism:

1. Lipolytic effect of uncertain magnitude probably important only when insulin is very low.

2. Ketogenic action (121) essential for massive ketogenesis when FFA substrate is sufficient.

FIGURE 6

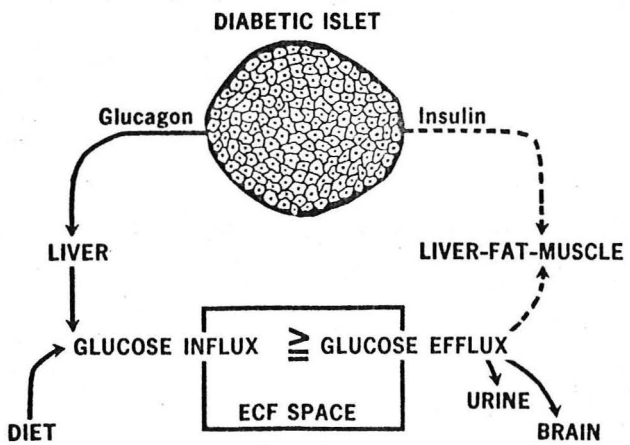

FIGURE 6: Glucoregulatory abnormality in diabetes: The islet, unable to sense or respond to change in glycemia by changing the insulin levels, can dispose of incoming glucose only at a rate pre-fixed by the amount of circulating exogenous insulin, and cerebral and renal clearance. This fixed reduced removal rate of glucose from the ECF - whether it is ingested glucose or glucose produced by the liver under the influence of inappropriate glucagon secretion - is autonomous of glycemic control.

Support for this hypothesis:

1. If one suppresses glucagon by infusing somatostatin in insulin deprived diabetic dogs (183, 184), hyperglycemia declines at a rate of ∿1 mg%/minute as the brain and the kidney continue to clear glucose in the absence of insulin at a rate which exceeds hepatic glucose production during hypoglucagonemia. Restoration of hyperglucagonemia causes prompt return of more severe hyperglycemia.

2. If one blocks glucagon secretion just before insulin deprivation - whether in insulin-requiring juvenile diabetics (62) or in totally depancreatized dogs (184, 43, 226) - the marked endogenous hyperglycemia that would otherwise occur is blocked; replacement infusion of glucagon restores hyperglycemia to the high control levels. This means that in the absence of hyperglucagonemia insulin deficiency fails to increase endogenous glucose production above the rate of glucose utilization by insulin-independent tissues.

3. If one blocks hyperglucagonemia following the discontinuation of insulin in juvenile-type diabetics by an infusion of somatostatin, one prevents the rise in β-hydroxybutyrate that would otherwise occur (62); this confirms at the clinical level the studies of McGarry and Foster (121, 122) ascribing to glucagon a key role in the augmentation of hepatic ketogenic capacity.

4. Glucagon is high in all forms of endogenous hyperglycemia - including total pancreatectomy in which condition glucagon is secreted by extrapancreatic A-cells, mainly in the stomach (11, 12, 18, 154, 155).

5. The islets of such diabetics contain hyperplastic glucagon-containing A-cells (144) (Table VIII).

TABLE VIII

Number of insulin-, glucagon-, and somatostatin-immunofluorescent cells in the islets of chronic juvenile-type diabetic subjects, and of non-diabetic subjects (expressed in % of the total number of immunofluorescent cells).

	Insulin	Glucagon	Somatostatin
Controls (N = 4)	61.2 ± 3.1	29.2 ± 3.0	9.7 ± 1.2
Diabetics (N = 2)	-	75.6 ± 2.7*	24.5 ± 2.7**

* $p<0.0005$ ** $p<0.0025$

Implications of the hypothesis: Its importance is predicated on the assumption that inappropriate hyperglucagonemia stimulated by food and/or hypoinsulinemia is causing the liver to "dump" glucose into the ECF space from which it cannot be promptly cleared because of the insulin defect. Although glucagon-mediated increases in hepatic glucose production are known to be transient (51, 195, 196), the hyperglycemic effect of the "glucose dump" is not so transient* because of the diabetic islets' inability to correct the hyperglycemia by an increase in insulin-mediated glucose uptake. Prevention of hyperglucagonemia could, therefore, theoretically reduce endogenous hyperglycemia (Table IX).

TABLE IX

RELATIONSHIP OF CHANGES IN GLUCAGON AND GLUCOSE IN INSULINIZED HUMAN DIABETICS

	Δ GLUCAGON pg/ml	Δ GLUCOSE mg%
Juvenile Diabetics		
Arginine infusion [Unger et al. (220)]	↑ 325	↑ 80
Arginine infusion [Barnes and Bloom (13)]	↑ 300	↑ 59
Mixed meal plus insulin [Gerich et al. (65)]	↑ 58	↑ 84
Mixed meal plus insulin plus somatostatin [Gerich et al. (65)]	↓ 55	↓ 50
Totally Depancreatized Patients		
Arginine infusion [Barnes and Bloom (13)]	0	0
Arginine infusion [Palmer et al. (155)]	↑ 61	↑ 48

Abnormalities in A-cell function:

1. Relative or absolute fasting hyperglucagonemia (220) despite fasting hyperglycemia.
2. Failure of postprandial hyperglycemia to suppress glucagon - rather a paradoxical rise occurs (130, 28, 174).
3. Exaggerated glucagon response to protein meal or I.V. amino acids (51, 130, 174).

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* Cherrington, A. et al. Personal communication.

Mechanisms: Probably secondary to within-islet insulin lack since correction of hypoinsulinemia does not restore normal glucagon-glucose relationships, but insulin in high doses (that raise plasma insulin to >300 μU/ml and thus may provide a physiologic "within-islet" insulin level while exposing non-islet tissues to supra-physiologic insulin concentrations) does normalize all abnormalities (although with daily doses subcutaneous insulin this is difficult to demonstrate in most patients with high doses - up to 200 U/d or more in some).

This means that if one tries to correct the A-cell abnormality with high doses of insulin, one is concomitantly augmenting glucose utilization to supraphysiologic levels while simultaneously reducing glucose production both via direct insulin action and low glucagon levels; hypoglycemia is inevitable, therefore, and, as in insulinoma patients, will not elicit endogenous glucagon secretion. Glucagon suppression by an agent that does not augment glucose utilization is theoretically more desirable. Somatostatin provides a prototype of such an agent (Figure

FIGURE 7

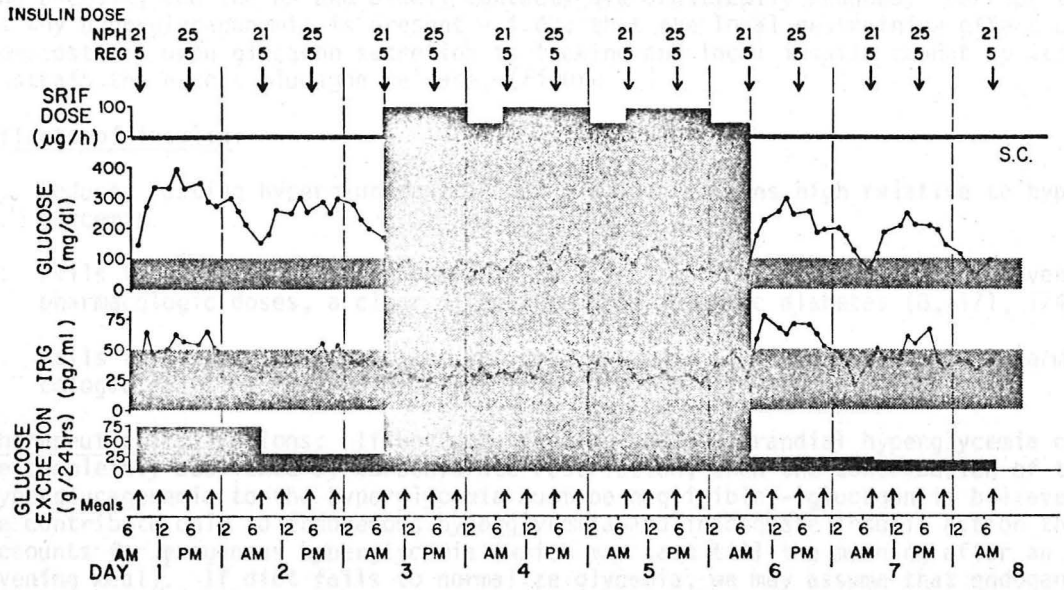

FIGURE 7: Therapeutic effects of somatostatin therapy in juvenile diabetes

Note: There is uncertainty as to whether the amelioration in hyperglycemia is secondary to glucagon suppression, reduced nutrient absorption, or both. We suspect both mechanisms and both may be desirable. Reduced rate of nutrient entry would fit well with the fixed therapeutic levels of exogenous insulin and reduce hypoglycemia (from Raskin et al., unpublished work).

DIABETES WITHOUT INSULIN DEFICIENCY

This is characterized in its most anabolic form by obesity and relative hyperinsulinemia and hyperglucagonemia, but the I/G is not low and the patient certainly not catabolic. Despite high plasma insulin levels the B-cell response is late and lacks the early first phase spike; however, target tissue insensitivity is generally considered to be the cause of the hyperglycemia, in addition to any B-cell dysfunction. Abnormalities in A-cell function are the same as in the other forms of diabetes:

1. Relative or absolute fasting hyperglucagonemia despite fasting hyperglycemia.

2. Only partial suppression by glucose.

3. Exaggerated glucagon response to protein meal or I.V. amino acid.

Mechanisms: No studies of human forms of hyperinsulinemic diabetes have yet been reported. In ob/ob mice, perhaps the murine counterpart of this human syndrome, the islets are characterized by an abundance of B-cells and reduced numbers of A- and D-cells, and the A- and D-cell contacts are drastically reduced. Perhaps this is why hyperglucagonemia is present - i.e., that the local restraining effect of somatostatin upon glucagon secretion is lacking and local insulin cannot by itself restrain the excess glucagon release. (Figure)

Effects of insulin:

1. Reduces fasting hyperglucagonemia but glucagon remains high relative to hyperglycemia.

2. Fails to alter exaggerated response to I.V. arginine or protein need - even in pharmacologic doses, a clear difference from juvenile diabetes (8, 171, 174).

3. Fails to restore to normal the abnormal response to glucose - even in pharmacologic doses, a clear difference from juvenile diabetes (8, 174).

Therapeutic implications: If both the fasting and postprandial hyperglycemia can be completely abolished by carbohydrate restriction, then the contribution of the hyperglucagonemia to the hyperglycemia must be negligible - glucagon is believed to contribute only to endogenous hyperglycemia and inadequate insulin action that accounts for exogenous hyperglycemia (which may last till the morning after an evening meal). If diet fails to normalize glycemia, we may assume that endogenous glucose production must be contributing to hyperglycemia and that glucagon suppression is a rational approach. (In practice stringent carbohydrate restriction is impractical and atherogenic, so glucagon suppression would warrant a trial in all cases that have hyperglycemia on an optimal diet.)

FIGURE 8

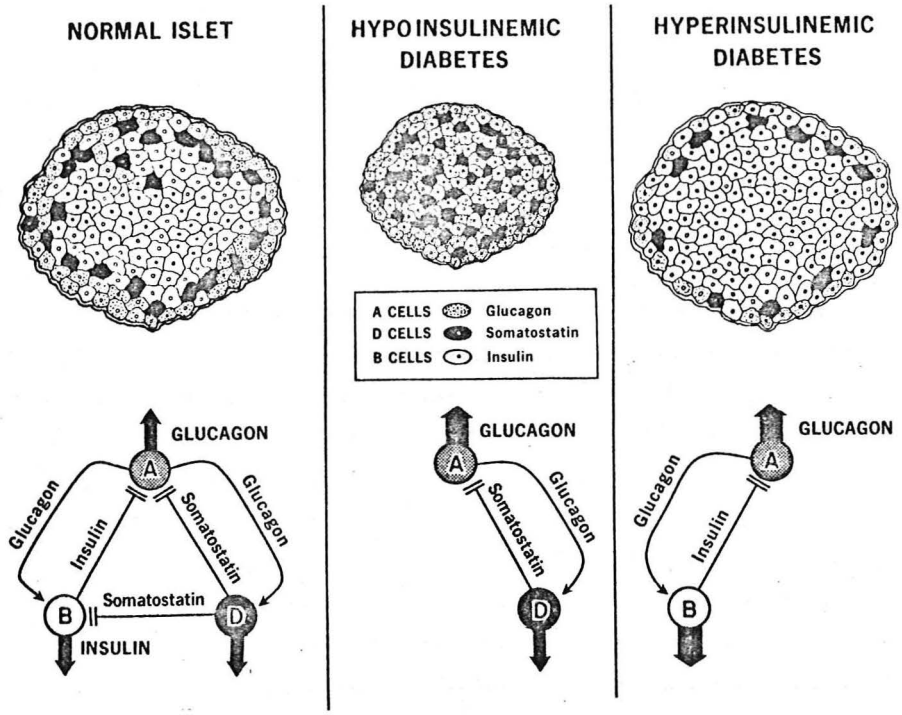

FIGURE 8: Hypothetical schema for the islets in diabetic disorders: The hyperglucagonemia of B-cell deficient diabetes (center panel) is secondary to within-islet insulin lack and correctable by large doses of insulin; the hyperglucagonemia of diabetes in which B-cells are abundant may be due to a paucity of somatostatin-containing cells and is not improved by insulin. Somatostatin deficiency might also contribute to a more rapid rate of gastric emptying and a greater food intake in the latter form of diabetes.

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