MEDICAL GRAND ROUNDS

PARKLAND MEMORIAL HOSPITAL

February 17, 1966

"GLUCAGONOMA"

I. HISTORY OF GLUCAGON

1922 - Crude pancreatic extracts of insulin prepared by Banting and Best are observed to cause an initial rise in blood sugar preceding the hypoglycemia. Neither Collip, MacLeod, Banting, or Best correctly interpreted this hyperglycemic effect.

1923 - Murlin and associates demonstrate that the hyperglycemic and hypoglycemic activities of the pancreatic extracts are separate by destroying the latter with alkali. They suggest the hyperglycemic factor may be a hormone of glucoregulation which opposes insulin action, and they name it "glucagon".

1929 - Collens and Murlin suggest that hyperglycemic effect of glucagon is due to increased hepatic glycogenolysis.

1948 - Foa finds hyperglycemic activity in pancreatic venous blood of hypoglycemic dogs.

1950-62 - Numerous studies of the site of origin of glucagon point to the alpha cells as its principal source.

1953 - Staub, et al, at Eli Lilly prepare highly purified crystalline glucagon.

1957 - Bromer elucidates amino acid sequence of glucagon.

1957 - Gluconeogenic effect of glucagon established.

1958 - Sutherland establishes the biochemical site of glycogenolytic action of glucagon.

1959-65 - Radioimmunoassay for glucagon permits first measurements of plasma glucagon and makes possible physiologic and clinical studies of its role in health and disease (Unger 1959-1964).

1966 - First proven case of a glucagon disease, a "glucagonoma" (Patient [redacted]. Case #1 in this protocol).

II. THE ALPHA CELL

Alpha cell is a morphologic designation which refers to certain histochemical characteristics of its granules which differentiate it from other islet cells, beta and delta cells.

TABLE I - HISTOCHEMICAL CHARACTERISTICS OF ISLET CELLS

	ALPHA	BETA	DELTA
GRANULES			
a. Chromhematoxylin phloxin stain	Red	Blue	Negative
b. Silver stain	Positive	Negative	Negative
c. Tryptophane stain	Positive	Negative	Negative
d. Aldehyde fuchsin stain	Negative	Positive (purple)	Negative
DARKFIELD	Luminescence	No luminescence	No luminescence
ULTRASTRUCTURE			
a. Mitachondria	Not numerous	Numerous	-
b. Golgi	Not prominent	Prominent	-
c. Granules	Amorphous, dense round with tightly applied limiting membrane	Vary in shape, from species to species; electron-lucid halo between granule and the limiting membrane	-
LOCATION IN ISLET	Peripheral	Central	Scattered sparsely

COMPARATIVE ENDOCRINOLOGY OF ALPHA CELLS (Miller)

1. The earliest known equivalent of islet tissue is the "follicles of Langerhans" in the hagfish and lamprey embedded in intestinal wall but not connected to the gut lumen. Below this phylogenetic level (amphioxus) there is only exocrine tissue.

2. In cyclostomes, islet tissue is widely distributed throughout gut and in parts of the liver.

3. All vertebrates have beta cells but alpha cell population varies widely: Birds and reptiles have mostly alpha cells; they have infinite insulin tolerance (canaries can tolerate up to 4000 U/kg (LD-10,000 U/kg) and in lizards there is no LD for insulin). Pancreatectomy causes hypoglycemic death. Amphibians by contrast have only beta cells and are sensitive to insulin.

4. Immunoassayable insulin and glucagon found in Cottus and hagfish.

5. In man, as in most mammals, beta cells predominate, with alpha cell at the periphery of islets.

DEVELOPMENT OF ALPHA CELLS

a. Embryology: All islet cells develop from 1 dorsal and 2 ventral rudiments in gut wall which bud into pancreatic ducts; ducts give rise to cells, some of which retain duct connection and become exocrine cells, while others lose the connection and become islets.

b. Neogenesis: In the adult, new islet cells are probably derived from fully differentiated cells in the intralobular ductules which are multipotential. Thus mixed cell types may be seen in tumors (e.g., a beta cell tumor may contain 5% alpha cells).

EVIDENCE FOR ALPHA CELL ORIGIN OF GLUCAGON

a. Glucagon-like activity can be extracted from the duct-ligated, alloxan-treated pancreas devoid of both acinar and beta cells (Peden 1955).

b. Glucagon-like activity can be extracted from portions of dog pancreas which contain alpha cells but not from the uncinate process which is devoid of alpha cells (Bencosme 1955).

c. Alpha cells take a tryptophane stain; glucagon contains tryptophane.

d. Glucagon administration causes atrophy of alpha cells (see "Effects of Chronic Glucagon Administration").

e. Specific immunofluorescent studies show that antibodies to beef glucagon bind to alpha cells of the beef pancreas (Baum 1962).

EVIDENCE FOR EXTRA-INSULAR SITES OF GLUCAGON SYNTHESIS

a. Insulare gangorgan: These are cells in the pancreatic ducts which share some but not all of the staining characteristic of alpha cells (Feyrter 1943 Goede 1950). No other evidence that they secrete glucagon.

b. Gastrointestinal sites:

1) Upper G-I tract contains many chromaffin cells which stain with silver (Van Campenhout 1933). They are "argentaffine" (they release diamine salts of silver directly), whereas alpha cells are "argyrophyllic" (they become impregnated with silver only in the presence of reducing agents (Fodden 1953, Volk 1954).

2) The upper G-I tract contains a hyperglycemic, glycogenolytic factor similar to glucagon (Sutherland and de Duve 1948), which appears to be indistinguishable from glucagon in its mechanism of action in liver slices. By an adenyl cyclase assay Makman (1964) finds 5.5 ug/g of glucagon-like activity in both mucosa and muscle of stomach, 1-2 ug/g in gut, and 7.3 ug/g in pancreas.

However, other agents (epinephrine, serotonin) may duplicate the effects of glucagon on liver slice; specific studies of the G-I tract by glucagon radioimmunoassay have not been completed, and the issue remains in doubt.

III. GLUCAGON

STRUCTURE OF GLUCAGON

A straight-chained polypeptide composed of 29 amino acids with a M. W. of 3485.

METABOLISM OF GLUCAGON

Exogenous glucagon circulates in plasma in free form with a 5-10 minute half-time; it is rapidly taken up (bound) by liver and kidney and degraded.

A. EFFECTS OF GLUCAGON ON GLUCOSE METABOLISM*

a. Glycogenolysis: Hyperglycemic effect of glucagon can be accounted for by an increase in hepatic glucose production (Collens and Murlin 1929, Shoemaker et al 1959, 1960); this is accompanied by a reduction of liver glycogen in vitro (Sutherland and Cori 1948) and in vivo (Cahill 1957), indicating that glucagon hyperglycemia is at least in part due to augmentation of hepatic glycogenolysis.

Subsequent demonstration that glucagon causes activation of liver phosphorylase (Sutherland and Cori 1951), the rate-limiting step in glycogenolysis, led to the elucidation of the following mechanism by Sutherland and his group (1956-1960).

* The term "physiologic effects" is deliberately avoided, since in most studies pharmacologic doses of glucagon in the milligram range have been employed, producing glucagon levels far above the upper physiologic range. These effects may or may not provide insight into the physiology of endogenous glucagon.

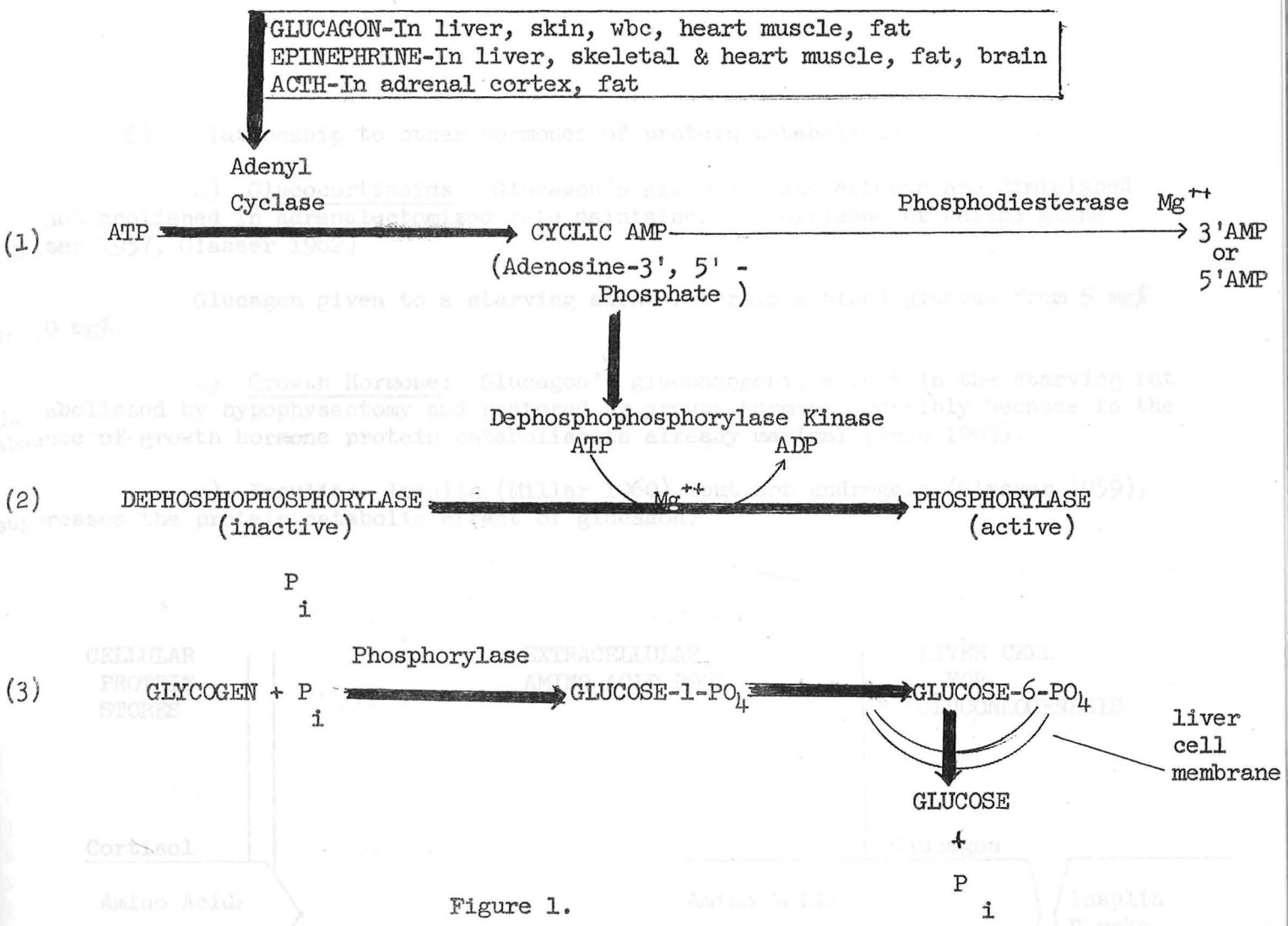

Figure 1.

b. Gluconeogenesis:

1) Glucagon causes increased nitrogen excretion in rabbits (Tyberghein 1952) in rats (Izzo 1957), in humans (Salter 1957).

2) Glucagon causes increased incorporation of C^{14} - labelled glycine into the glycogen of fasted rats after initially depleting liver glycogen. Subsequently, it appears as $C^{14}O_2$ (Kalant 1956).

3) Glucagon decreases blood amino acid concentration (Helmer 1957, Curry 1958, Izzo 1959, Weinges 1959, Bocek 1960) and causes a 4-fold rise in hepatic uptake of alpha amino nitrogen within 45 minutes of a single glucagon injection in dogs; although this effect begins promptly, it reaches a peak only when glycogenolytic effect is terminated, i.e., 45 minutes (Shoemaker 1960). In man within one hour of a 3-7 mg glucagon injection a doubling of splanchnic amino acid uptake was noted (Kibler 1964).

4) Perfusion of 1 ug of glucagon/hour increases urea production by the isolated perfused rat liver (Nutter 1960).

5) Although the precise biochemical site and mechanisms of the gluconeogenic action of glucagon is unknown, glucagon, epinephrine and cyclic AMP all increase pyruvate carboxylase activity.

6) Relationship to other hormones of protein metabolism:

a) Glucocorticoids: Glucagon's gluconeogenic effects are diminished but not abolished in adrenalectomized rats maintained on cortisone or saline alone (Salter 1957, Glasser 1962)

Glucagon given to a starving adrnx rat raises blood glucose from 5 mg% to 30 mg%.

b) Growth Hormone: Glucagon's gluconeogenic effect in the starving rat is abolished by hypophysectomy and restored by growth hormone, possibly because in the absence of growth hormone protein catabolism is already maximal (Izzo 1963).

c) Insulin: Insulin (Miller 1960), but not androgens (Glasser 1959), suppresses the protein catabolic effect of glucagon.

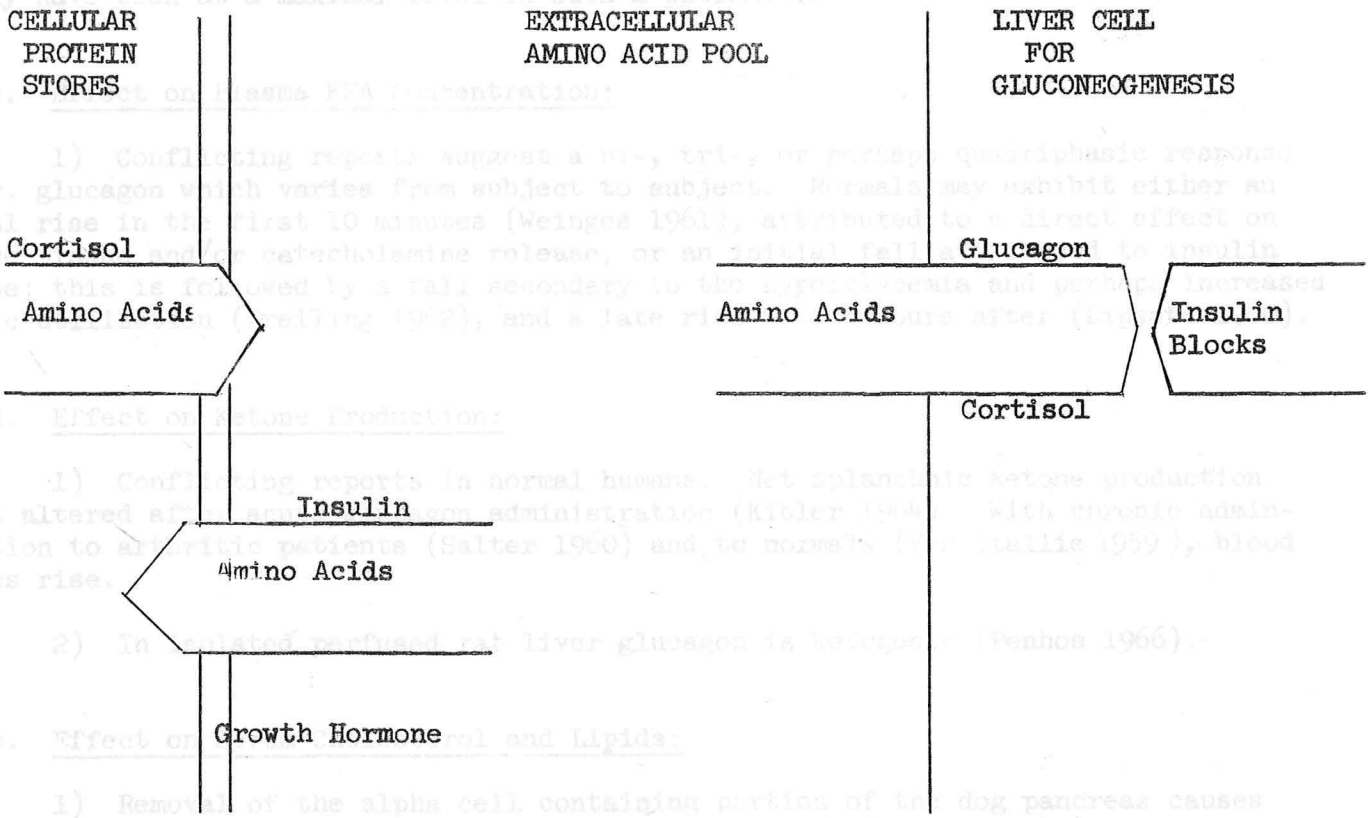

Figure 2. Postulated hormone interrelationships in gluconeogenesis.

B. EFFECTS OF GLUCAGON ON LIPID METABOLISM

a. Effect on Fat Tissues:

1) Glucagon (0.01 ug/ml) causes release of FFA and glycerol in vitro (Steinberg, Hagen 1961, Mahler 1964, Weinges 1961). This lipolytic effect is restrained by insulin at physiologic (100 uU/ml) concentrations independently of glucose uptake.

2) Glucagon also inhibits fatty acid synthesis (Orth 1960).

b. Effect on Hepatic Metabolism of FFA:

1) Its effect upon FFA utilization is uncertain.

2) Increases FFA utilization by isolated perfused rat liver (Penhos 1966).

3) Dreiling et al (1962) found a fall in FFA in a totally depancreatized subject who had received no insulin for 24 hours. This would have to be attributed to increased FFA utilization, probably by liver, exceeding any increase in FFA release (which might already have been at a maximal level in such a patient).

c. Effect on Plasma FFA Concentration:

1) Conflicting reports suggest a bi-, tri-, or perhaps quadriphasic response to i.v. glucagon which varies from subject to subject. Normals may exhibit either an initial rise in the first 10 minutes (Weinges 1961), attributed to a direct effect on adipose tissue and/or catecholamine release, or an initial fall attributed to insulin release; this is followed by a fall secondary to the hyperglycemia and perhaps increased hepatic utilization (Dreiling 1962), and a late rise 4 to 6 hours after (Lipsett 1960).

d. Effect on Ketone Production:

1) Conflicting reports in normal humans. Net splanchnic ketone production is not altered after acute glucagon administration (Kibler 1964). With chronic administration to arthritic patients (Salter 1960) and to normals (Van Itallie 1959), blood ketones rise.

2) In isolated perfused rat liver glucagon is ketogenic (Penhos 1966).

e. Effect on Serum Cholesterol and Lipids:

1) Removal of the alpha cell containing portion of the dog pancreas causes hyperlipemia which can be abolished by 10 mg of glucagon/d (Paloyan 1961).

2) Glucagon (1-5 mg/d) lowers cholesterol, triglycerides and phospholipids in essential hyperlipemia, and lowers cholesterol in female, but not male, hyperlipemics without change in diet (Amatuzio 1962).

3) Cobaltous chloride, a transient alphacytotoxin, raises cholesterol and causes transient lipemia which can be corrected by glucagon (Caren 1960).

C. EFFECTS OF GLUCAGON ON SECRETION OF OTHER HORMONES

a. Effect on Catecholamine Secretion:

1) Infusion of glucagon (0.1 mg/min x 10 min) in dogs causes a rise in epinephrine and norepinephrine in adrenal vein plasma; a single injection of 50 ug/kg. in dogs causes a rise in plasma epinephrine (Sarcione 1963). Glucagon (0.5-1 mg) caused a striking pressor response to two proven pheochromocytoma patients but not in control subjects (Lawrence 1964). It has been suggested by Lawrence that the catecholamine response to insulin-induced hypoglycemia is glucagon-mediated (doubtful).

b. Effect on Insulin Secretion:

1) Foa (1952) noted the hyperglycemic effect of glucagon was greater in the absence of the pancreas and that glucagon injection in a dog causes a blood-sugar lowering substance to appear in the pancreatic effluent. Weisenfeld (1959) suspected a direct glucagon effect on insulin release effect on the basis of enhanced glucose utilization following glucagon administration. Coore (1961) observed greater insulin concentration in media when pancreatic slices were incubated in the presence of glucagon; however, they ascribed this to diminished insulin degradation by the tissue, rather than to increased release from the beta cells.

2) Samols (1965) and Crockford et al (1966) demonstrate an insulin releasing effect of large milligram doses of glucagon.

3) Smaller doses (as little as 4 mug) also cause insulin release, provided BS concentration is not low (Ketterer 1966).

D. MISCELLANEOUS EFFECTS

a. Electrolytes:

1) Potassium: Increases serum potassium (Wolfsen 1956) probably via release from the liver during glycogenolysis, but unlike glycogenolysis it can be blocked by dibenamine or dihydroergotamine (Ellis 1957), and may therefore be an epinephrine mediated effect. Potassium excretion is increased by glucagon (Elrick 1958).

2) Phosphate: Glucagon decreases serum inorganic phosphate (Bondy 1956), probably because of phosphorylation of the glucose released from glycogen. However, this fall may occur without hyperglycemia, so increased urinary excretion may play a role.

3) Sodium: Increased excretion of sodium, chloride, uric acid, bicarbonate and water have been attributed to increased glomerular filtration (Elrick 1958).

b. Gastrointestinal Tract:

1) Glucagon decreases G-I tract contractility, causing anorexia and nausea (Stunkard 1955, Sporn 1956, Ezrin 1957, Morrison 1958, Robinson 1957). It decreases hunger (Stunkard 1955, Sudsanch 1959) and hunger contractions, an action which is eliminated by destruction of ventromedial area of hypothalamus. Glucagon might be a physiologic factor in hunger regulation (Penick 1961).

2) Volume and HCL content of gastric secretion (Robinson 1957) is decreased by glucagon, even when it is administered intraportally (von Heimberg).

c. On Tumor Growth:

1) Glucagon inhibits growth of Walker 256 adenocarcinoma in vivo (Salter 1959), but has no effect in vitro. Effect could be secondary to an increased protein catabolism in the host secondary to increased gluconeogenesis.

EXPERIMENTAL GLUCAGON DEFICIENCY

A. Effects on Lipids:

1. Cobaltous chloride, a transient alpha cell toxin, causes transient hypercholesterolemia and hyperlipemia (Caren).

2. Removal of body and tail of dog pancreas, the portions which contain most of the alpha cells, causes hyperlipemia (Paloyan 1961).

B. Effects on Blood Sugar:

1. Removal of predominantly alpha cell islets of the fowl causes hypoglycemia, convulsions, and death in 12-36 hours. Glucagon prevents this (Mialhe, Mikani 1958).

2. In lizards total pancreatectomy causes severe hypoglycemia for 12 hours to 2 weeks, followed by permanent diabetes. Same is true in snakes. In tortoise, blood sugar goes up immediately (Penhos).

3. Although apparently an essential hormone in certain reptiles and birds, no such vital function has been established in mammals. However, the removal of the remnant of the pancreas in duct-ligated, alloxan-diabetic animal causes amelioration of the diabetes.

EXPERIMENTAL HYPERGLUCAGONEMIA PRODUCED BY CHRONIC GLUCAGON ADMINISTRATION

a. Effects on Blood Sugar:

1) Very difficult to produce diabetes in intact rats even with large doses of glucagon.

2) In partially depancreatized rats diabetes is aggravated by glucagon (Ingle), and it will cause glycosuria in force fed rats (Cavellero, Salter, Lacy). In guinea pigs initially severe glycosuria is not maintained by continued glucagon therapy (Lazarus).

3) In rabbits 2 mg/kg of glucagon daily, started on second post-natal day, will cause intermittent hyperglycemia and mild glycosuria. While 60% return to normal, a few metaglucagon diabetes remains 18-63 days after injections stop (Logothetopoulos). Adult rabbits given glucagon plus cortisone show adaptation with waning of hyperglycemia and histologic evidence of neogenesis of beta cells (Lazarus 1959).

4) In man Salter (1960) reports that glucagon given in doses of 30 mg/d to six patients with rheumatoid arthritis was associated with FBS of 75-106 mg%, post-prandial hyperglycemia to 340 mg%, and glycosuria. Van Itallie (1959) gave glucagon, 1.3-4.0 mg IM q 6 hr, and a high CHO diet to young normals for 2-5 days and found not fasting hyperglycemia but fasting hypoglycemia and impaired glucose tolerance. Glycosuria ranged from 1-28 g/d. According to van Itallie, glucagon diabetes in man is associated with a normal or low FBS like "hunger diabetes".

b. Effects on Ketones:

1) Blood ketones increase (Van Itallie 1959).

c. Effects on Islet Cells:

1) Alpha cells show marked atrophy or degranulation in rats and guinea pigs (Lazarus, Petersen 1963) and rabbits (Logothetopolous) after 30 days of glucagon. According to the Uppsala group this atrophy is limited to the silver-negative A_2 cells while the silver-stained A_1 cells are unaltered.

2) Beta cells show degranulation after chronic glucagon administration with glycogenation, and hypertrophy (Logothetopolous, Lacy, and Lazarus) and increased ergostoplasm and cytoplasmic processes extending into intercapillary spaces, all suggesting increased-secretory activity (Lacy 1959).

EVIDENCE OF THE PHYSIOLOGIC ROLE OF ENDOGENOUS GLUCAGON

A ROLE IN GLUCOPENIA:

1. Foa demonstrated by cross circulation experiments that the pancreatic venous effluent from dogs made hypoglycemic with insulin causes hyperglycemia in a recipient dog, while blood of the mesenteric vein does not.

2. Development of a radioimmunoassay for glucagon made possible specific measurement of glucagon in plasma and suggested that glucagon is a hormone of glucopenia (Unger 1959-64).

a. Glucagon secretion rose during insulin hypoglycemia.

b. Rose during phloridzin hypoglycemia.

c. Rose during 3 days of complete starvation.

d. Fell after rapid intravenous glucose loading during both insulin and phloridzin-induced hypoglycemia.

It was, therefore, concluded that glucagon is one of several hormones of glucose need, its role being to maximize hepatic glucose production, thus serving the needs of the glucose-dependent central nervous system.

A ROLE IN GLUCOSE ABUNDANCE (?):

1. Burger in 1947 described the appearance of a hyperglycemic material in human plasma during the first phase of alimentary hyperglycemia, and concluded that glucagon is released after a glucose load.

2. Samols (1965) confirmed this by radioimmunoassay, showing that glucagon is released after an oral, but not after an intravenous load, and proposed that, by stimulating insulin release, it may account for the much greater insulin release after oral as opposed to intravenous administration of glucose.

NOTE: Studies now in progress in several laboratories should resolve the apparent conflict as to whether glucagon is a glucopenic hormone, a hormone of glucose abundance, or both.

V. ISLET CELL NEOPLASMS

ORIGIN

Probably originate either from differentiated islet cells or from an undifferentiated islet cell precursor in the ductular epithelium. The multipotentiality of the latter cell may account for the fact that mixed islet cell tumors are not uncommon.

HISTOLOGY

a. Grading: Islet cell tumors are considered malignant 1) if carcinoma extends beyond the capsule, or 2) if metastases are present. As in other endocrine neoplasms, morphologic criteria are deceptive; benign lesions may appear quite anaplastic while malignant lesions may resemble normal islets.

b. Cellular patterns: Islet tumors may assume 1) cord or trabecular or ribbon pattern - 2 cells in width and are separated from each other by thin septae containing sinusoids (Frantz 2) medullary pattern with occasional tubule or rosette formation.

TABLE II CORRELATION OF ISLET CELL TUMOR MORPHOLOGY WITH SECRETORY PRODUCT

	SECRETORY PRODUCT				
Principal Cell Type	Insulin	Gastrin or Gastrinoid	Small Bowel stimulant	Glucagon (?)	Alphacytotropin (?)
Alpha	-	-	-	+	-
Beta	+	-	-	-	-
Non-specific	-	+	+	-	-
Unclassifiable	-	-	-	-	+

TABLE III

PREVIOUS CASES WITH POSSIBLE ALPHA CELL TUMOR

	Histologic Evidence	Clinical Manifestations	Glucagon Assay	Age	Sex
Hess (1946)	Silver stain positive.	Symptoms of gastritis. Widespread metastases except in liver. Blood sugar normal.	Patient's plasma caused glucose release from liver slice (4 x normal).	24	F
Hamperel (1952)	Bodian silver stain positive.	Ulcers and abdominal meso-hepatic metastases. Blood sugar elevated.	Not done.	63	M
Malandra (1957)	Some cells had argyrophilic granules.	Heavy sensation in LUQ. No carbohydrate disturbance.	Not done.	54	F
Gossner (1960)	Argyrophilia. Gomori positive. Tryptophane positive.	Eczematoid pemphigus foliaceous--no response to steroids. Abnormal GTT and glycosuria (35g) when on steroids. FBS <50 mg%. Abdominal pressure due to hepatic metastases.	Acid alcohol extract of the tumor → ↑BS in rabbit (110 mg% rise)	51	M
Keen (1962)	No granules.	Wildly fluctuating blood sugar--60 to 40 mg% without explanation. Liver metastases. Post-traumatic erysipelas of face.	Negative liver slice assay. Extracts caused hyperglycemia.	59	M
Behrendt (1962)	Argyrophilia. Gomori positive.	Pain from hepatic metastases--died with jaundice, ascites and hepatic coma.	Not done.	60	M
Yoshinaga (1966)	Argyrophilia. Gomori positive. Tryptophane positive.	Upper abdominal pain and mass and sudden onset of severe diabetes; complete replacement of pancreas by tumor.	Makman assay shows glucagon-like activity	26	F

CASE #I:

[redacted] is a 42 year old [redacted] female first seen in the [redacted], St. Louis, Missouri in [redacted] 1963, complaining of a skin lesion involving her extremities, perineum, trunk, and face. Her recent and past history was unremarkable save for anorexia, rapid satiety while eating, and a probable, though unverified, weight loss for more than 6 months. She denied knowledge of familial diabetes, but her mother died at age 36 and she has had no contact with her siblings for many years.

Physical examination revealed only a "bullous eczematoid" lesion involving the previously mentioned areas.

LABORATORY FINDINGS: Hemoglobin 11 g., hematocrit 33%, WBC and differential normal; urinalysis normal except for glycosuria 2+. The 100 gram glucose tolerance was: FBS 107, 1/2 hour - 220, 1 hour - 272, 2 hour - 193, 3 hour - 165. Cholesterol 140, BUN 10, Na K CO_2, Cl were all normal. Chest x-ray normal. Special fundus examination with photography revealed no microaneurysms or other abnormalities.

HOSPITAL COURSE: The diabetes was controlled readily with a diet of 1500 calories; protein 72 grams, carbohydrate 171 and fat 60. The skin lesions were resistant to conservative therapy and constituted the patient's major complaint. The persistance of the dermatitis necessitated admission to the hospital in [redacted] of 1963, at which time repeated cultures and scrappings of the skin revealed no fungal organisms; no specific dermatologic diagnosis was made.

Eighteen months ([redacted] 1964) later, because of the development of right pleuritic pain, the patient was admitted to Barnes for study. There had been no known weight loss or significant complaint other than the dermatitis prior to this symptom. Physical examination revealed the persistance of the dermatitis, an elevated right diaphragm, and a markedly enlarged stoney hard liver 7 cm. below the costal margin. Laboratory work-up revealed a hemoglobin 10.2 grams, normal WBC, differential, platelets, and a reticulocyte count of 3-5.3%. Liver function tests: BSP 5% retention, alkaline phosphatase 4.5, ceph. floc. negative, pro-time 100%, serum albumin 3.2, globulin 2.4. The serum iron was 61, the total iron-binding capacity 304. The bone marrow examination was interpreted as non-diagnostic marrow with slightly decreased cellularity of all elements and small amounts of extracellular iron.

The chest x-ray disclosed a markedly elevated right hemidiaphragm with evidence of hepatomegaly. A liver scan showed the presence of at least two large masses in the liver and celiac axis arteriogram suggested the possibility of a tumor in the tail of the pancreas. As a result of these findings, a laparotomy was performed. Needle biopsy on the pancreatic mass and the right lobe of the liver were obtained. The histologic diagnosis on H and E stain was undifferentiated carcinoma of the pancreas. The patient was discharged with a grave prognosis. During the succeeding eight months the patient did surprisingly well, manifesting no significant complaints other than the dermatitis. The remarkedly benign course, without signs of progressive deterioration, prompted a review of the microsections of the tumor. This time the tumor was thought to be of islet origin. Since the prognosis for islet tumors is known to be better than that of acinar tumors of the pancreas, an attempt to resect the primary tumor and, if possible, the hepatic metastases was made in [redacted], 1965. A large firm tumor was removed in toto from the tail of the pancreas,

but it was not possible to remove the hepatic metastases. Because of blood loss during the surgery, the patient required fifteen transfusions. After recovery from the acute postoperative complications the patient was followed in the clinic and was seen again in [redacted] of 1965. At that time the significant findings included the development of edema of the legs, marked intensification of the dermatitis and progressive anemia. She was re-admitted for special studies. The hemoglobin was 9.9 grams, WBC and differential normal. Serum electrolytes and BUN were normal, alkaline phosphatase normal. Serum albumin was now 3.0 and the globulin 2.0. Overnight gastric secretion study revealed normal volume, pH and acid content. The 24 hour excretion of 5 hydroxyindole acetic acid was normal. A repeat liver scan was unchanged. Studies of her normocytic, normochronic anemia revealed completely normal absorption of iron with an increased iron turn over but a normal red cell utilization of the iron. A bone marrow now showed marked increase in iron and many siderocytes were observed. It was not felt that the fifteen blood transfusions per se could have produced this degree of iron deposition in the marrow.

The nature of her dermatitis remains uncertain. It responded dramatically, with almost total clearing, following an oral administration of 40 mgms. of Prednisone a day only to recrudesce as the dose was diminished. Control of the glycosuria, required 20 or more units of insulin per day while the patient was on steroids. Presently she is receiving Prednisone, insulin, B complex vitamins, diuretics, and is on a diabetic diet.

Additional significant findings were: normal volume pH and acid content of overnight gastric secretions; normal serum potassium; normal radiographic appearance of the stomach, duodenum, and small bowel; normal 24 hour urinary excretion of 5 hydroxyindole acetic acid, 3 methoxy, 5 hydroxy-vanyl mandelic acid, epinephrine, and nor-epinephrine. These provide <u>in vivo</u> evidence that excludes the Zollinger-Ellison syndrome, carcinoid tumor and pheochromocytoma. Special studies are recorded below.

TABLE IV

HISTOCHEMICAL REACTIVITY OF GLUCAGON-CONTAINING ISLET CELL TUMOR AND NORMAL ISLET CELLS

	Alpha Cells	Tumor of A.H.	Beta Cells
Argyrophilia	+	+	-
Tryptophane (pDMAB Reaction)	+	+	-
Chromhematoxylin phloxin	red	red	blue
Aldehyde fuschin	-	-	+

TABLE V

EXTRACTABLE GLUCAGON AND INSULIN CONTENT OF TUMOR OF [redacted]

PANCREAS TISSUES AND OTHER ISLET CELL TUMORS

SPECIMEN	GLUCAGON (μg/g)	INSULIN (U/g)
TUMOR OF [redacted] (SURGICAL SPECIMEN)	14.0	0.029
TAIL OF HUMAN PANCREAS (SURGICAL SPECIMEN)	9.2	0.5
OTHER NON-BETA ISLET CELL TUMORS (4)*	0.002-0.064	0.001-0.016
INSULINOMA (SURGICAL SPECIMEN)	0.3	0.3

* 2 of the 4 were obtained post-mortem.

TABLE VI

FASTING PLASMA GLUCAGON OF PATIENT [redacted] (mμg/ml)

[redacted]	55.0
	46.0
NORMAL SUBJECTS	<2.0
A NORMAL SUBJECT 2.5 MINUTES AFTER 3 MG. OF CRYSTALLINE GLUCAGON I.V.	38.5

HYPERGLYCEMIC EFFECTS OF TUMOR EXTRACTS AND CRYSTALLINE GLUCAGON IN A DOG

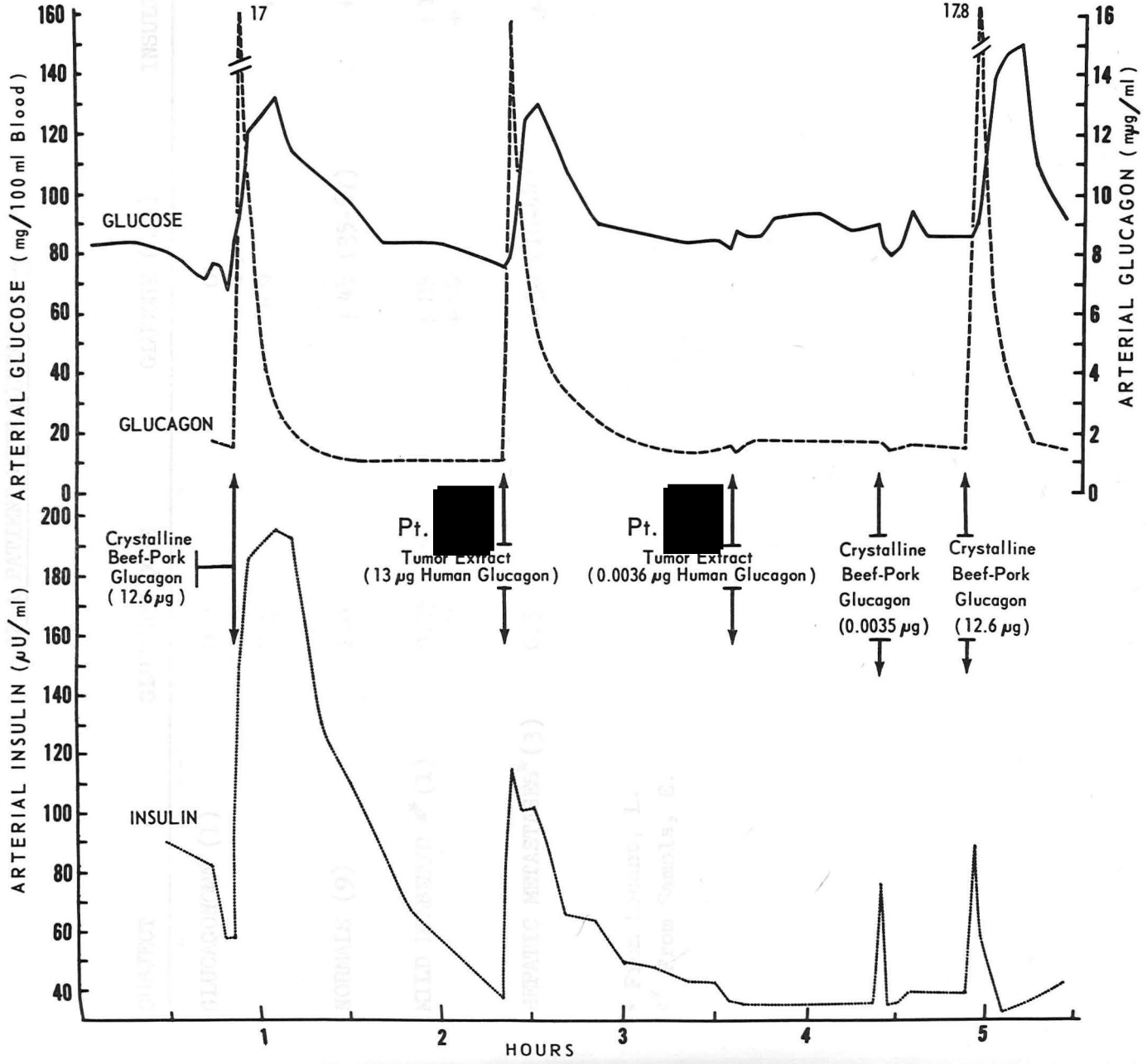

TABLE VII

RELATIVE RESPONSIVENESS TO INTRAVENOUS GLUCAGON

OF A PATIENT WITH GLUCAGONOMA

SUBJECT	GLUCAGON DOSE	GLUCOSE (mg%)	INSULIN (μU/ml)
GLUCAGONOMA* (1)	0.25	0	0
	0.50	+ 4	0
	2.5	+ 9	+10
NORMALS (9)	1.0	+ 45 (35-67)	+63 (41-84)
MILD DIABETIC ** (1)	0.25	+ 25	+104
	1.0	+ 50	+ 82
HEPATIC METASTASES* (3)	0.5	+ 30 (16-44)	+ 55 (34-80)

* From Recant, L.

** From Samols, E.

TABLE VIII

URINARY CATECHOLAMINE EXCRETION* IN A PATIENT ([redacted] WITH A GLUCAGONOMA

	[redacted]	NORMAL
VMA (mg/24 hr.)	3.3	2.0-6.0
Nor-metanephrine (mg/24 hr.)	0.35	0.2-1.3
Epinephrine (ug/24 hr.)	6.0	0-15
Nor-epinephrine	99.0	10-70

Kindly performed by Dr. Richard Crout.

CRITERIA OF PROOF OF ENDOCRINE HYPERACTIVITY IN A TUMOR

1. Appropriate anatomic location of the primary lesion.
2. Morphologic similarity of the tumor cells and the cells of the parent endocrine tissue.
3. Presence of specifically identifiable, biologically active hormone in tumor.
4. Presence of increased plasma concentration of the hormone.
5. Presence of a clinical abnormality in the patient attributable to hormonal excess.

HOW PATIENT [redacted]. MEETS CRITERIA FOR A GLUCAGONOMA

1. Location	Tail of Pancreas
2. Morphology	Identical to Alpha Cells (See Table IV).
3. Identification of Glucagon in tumor.	
a. Specific assay for glucagon.	Positive - (See Table V).
b. Biological Glucagon-like activity.	Positive - (See Figure 3).
4. Identification of Hormone in Plasma.	Positive - 55 mug/ml (See Table VI).
5. Abnormalities Anticipated in Chronic Hyperglucagonemia	
a. Hyporesponsiveness to test doses of exogenous glucagon.	
1) Hyperglycemic response.	Markedly diminished, even with 2.5 mg of glucagon. (See Table VII).
2) Hyperinsulinemic response.	Markedly diminished, even with 2.5 mg of glucagon. (See Table VII).
b. Abnormal Glucose Tolerance	Present but this is extremely non-specific: 1) may be seen in any pt with ca of pancreas. 2) this pt may be a genetic diabetic since her MCBM >1500Å.
c. Increased Insulin Secretion	Fairly high fasting insulin levels-about 40uU/ml.
d. Negative nitrogen balance	Not studied.
e. Increased catecholamine secretion (?)	Not present. (See Table VIII)
f. G-I symptoms	Non-specific symptoms present.
g. Degranulation of alpha cells	Present.

CONCLUSIONS CONCERNING THE CLINICAL SYNDROME OF GLUCAGONOMA

It is not possible to delineate a syndrome on the basis of a single case. However,

1. Suspicion of some type of islet cell tumor should always be aroused by the presence of a slowly growing pancreatic tumor with or without hepatic metastases.

2. Suspicion that an islet cell tumor may be a glucagonoma should be aroused if both the Zollinger-Ellison syndrome and the diarrheal syndrome are absent.

3. Skin lesions may possibly be a mysteriously related part of the syndrome since 2 of the 4 probable glucagonoma cases, had skin lesions. A third case of islet cell tumor (Case #2 in this protocol) had a generalized skin disease, although she probably did not have a glucagonoma. This then may be a feature of islet cell tumors, although a coincidental association of unrelated diseases, or a dermatitis 2^{o} to malignancy, is possible. "Pellagra", secondary to diversion tryptophane to glucagon, also deserves mention, although this is quantitatively improbable.

4. Presence or absence of abnormal glucose tolerance test does not affect the diagnosis, because:

   a) Any malignancy, chronic illness, or extensive liver involvement may be accompanied by an abnormal iv and oral GTT.

   b) Carcinoma of pancreas of acinar origin is commonly associated with abnormal GTT.

   c) It is not certain that prolonged glucagon administration in man causes abnormal GTT; compensation may occur in the non-diabetic subject as it probably does in acromegaly.

5. IV glucagon response test is probably the best screening test.

6. Glucagon radioimmunoassay of plasma or tumor is the only real proof of diagnosis.

CASE #2:

The patient, [redacted] female is now 42 years old. At age 27 she had a 9 pound baby. Her other previous children were smaller. There was no known family history of diabetes mellitus. At age 33 ([redacted] 1956) an itching purulent rash appeared on the ankles, lower legs, and finally on the body, particularly around the groins. This resisted all locally applied medicines, and she was finally hospitalized at the Mayo Clinic in [redacted], 1957. An admission urine specimen showed no sugar. The fasting blood sugar on [redacted] was 74 mg.%. On [redacted] treatment was begun with prednisone (delta 1 cortisone), 10 mg. per day by mouth. On [redacted] glycosuria was noted for the first time. On [redacted] the fasting blood sugar was found to be 220 mg%. NPH insulin was begun and continued in a daily dosage of 30-36 units a day. Between [redacted] and [redacted] her fasting blood sugar on this program varied between 104 and 144 mg.%.

On [redacted] she was referred to [redacted]. By this time the dermatitis had improved considerably, though it was not completely gone. A skin biopsy at this time showed "non-specific inflammation with sub-corneal pustule formation; not characteristic of psoriasis or pemphigus". Hydrocortisone in a dosage of 50 mg. had been substituted for prednisone. As the skin improved, hydrocortisone and insulin were both slowly decreased in dosage, and on December 17 they were omitted altogether. Her skin was much improved, although still not completely healed.

In [redacted], 1958 attention was first turned to complaints of indigestion and intermittent diarrhea which actually had been present for nearly a year. X-ray examination at this time showed marked narrowing of the second portion of the duodenum and various laboratory studies of intestinal fat absorption showed a marked deficiency in this regard, restored completely to normal by pancreatic extract by mouth. Her dermatitis now flared up again.

An oral glucose tolerance test on [redacted] gave the following results: fasting blood sugar, 100 mg%; 1/2 hour, 158; 1 hour, 188; 2 hours, 134; 3 hours, 85 mg%. The normal fasting values for our laboratory are 65-90 mg%. Therefore, this is a very slightly diabetic curve.

Because of the x-ray findings and the steatorrhea, abdominal exploration was performed by Dr. Alan Thal. At operation he found a 5 centimeter rounded indurated mass in the head of the pancreas. The remainder of the pancreas was extremely attenuated and rather leaflike in character, measuring only 2 or 3 mm. in thickness, and being nearly translucent, and not indurated. The pancreatic duct was dilated.

A pancreatoduodenectomy was performed. The tail of the pancreas remained and the duct was anastomosed to the remaining bowel. Some amylase was detectable in the pancreatic duct fluid.

Since the operation the patient has felt very well. She has gained about 25 pounds in weight, and her skin, which cleared completely shortly after the operation, has remained entirely clear.

The glucose tolerance curve on March 11 was as follows: fasting, 85; 1/2 hour, 187; 1 hour, 175; 2 hours, 107; 3 hours, 58. This curve still shows a slightly high peak value and a slightly high 2 hour value, though it is nearly normal.

Histologic examination of the tumor with nematoxylin and eosin stain showed carcinoma consistent in appearance with islet cells. With Gomori's aldehyde fuchsin stain there was no staining, suggesting the cells either were not beta cells or else were degranulated beta cells. The indole stain, also performed in Dr. Arnold Lazarow's department, was interpreted as positive. This is consistent with the behavior of alpha cells or of any cells containing tryptophane-bearing protein.

An acid alcohol extract of the tumor was prepared according to the method used for extraction of insulin from the pancreas suggested by Wrenshall. When injected into fasting mice, this extract showed slight blood sugar lowering activity. However, when 2 cc. of the extract were injected intravenously into a fed rabbit, the rabbit's blood sugar rose from 112 to 182 mg% in 18 minutes. On a subsequent day this same amount of extract was given intravenously to the same rabbit 10 minutes after a subcutaneous injection of 1 mg. of dihydroergotamine tartrate. Moderate hyperglycemic activity was still seen, the blood sugar rising from 140 to 172 mg% in 20 minutes.

On prednisone, previous dose, for 48 hours, October, 1958: Glucose tolerance frankly diabetic the following day.

Bioassay of the tumor extract revealed 10-15 ug. of glucagon-like activity per g. of wet tumor weight, but no radioimmunoassay studies were performed.

Currently there is no evidence of recurrence of the tumor, and her problems include the skin disease, pancreatic insufficiency, and diabetes requiring 55 U/d.

TEXT BOOKS

1. Brolin, S. E., Hellman, B., Knutson, H.: The Structure and Metabolism of the Pancreatic Islets. Wenner-Gren Center International Symposium Series, Volume 3, 1964.

NOTE: Clearly the most complete review and bibliography on alpha cell morphology and function.

2. Miller, M. R., and Wursten, D. H.: Comparative Endocrinology, Wiley, N. Y. 1959.

3. Frantz, V. K.: Tumors of the Pancreas. Atlas of Tumor Pathology, Section VII, Fascicles 27 and 28, pp. 79-134, Subcommittee on Oncology of the Committee on Pathology of the National Research Council, A.F.I.P., Washington, D. C., 1959.

4. Foa, P., and Galansino, G.: Glucagon: Chemistry and Function in Health and Disease. Charles C. Thomas, Springfield, Ill., 1962.

5. Foa, P., and Galansino, G.: Ciba Colloquia on Endocrinology, Vol. IX.

I. POSSIBLE ALPHA CELL TUMORS

Doubtful (Probably Delta Cell or Non-Specific)

1. Espiner, E. A., Beaven, D. W.: Non-Specific Islet-Cell Tumor of the Pancreas with Diarrhoea. Quarterly Journal of Medicine, New Series XXXI, #124, October 1962.

2. Bloodworth, J.M.B., and Elliott, D. W.: The Histochemistry of Pancreatic Islet Cell Lesions. Journal of the American Medical Assn, March 1963, 183:1011-1015.

3. Singleton, John W., Kern, Fred, and Waddell, Wm.R.: Diarrhea and Pancreatic Islet Cell Tumor. Gastroenterology 49:#2 1965.

4. Veer, J., Choufoer, J.C., Querido, A., van der Heul, R. O., Hollander, C. F. and van Rijssel, T. G.: Metastasising Islet-Cell Tumor of the Pancreas Associated with Hypoglycemia and Carcinoid Syndrome. The Lancet, June 27, 1964, pp. 1416-1419.

5. Shafer, Wm. H.: Nonbeta Islet-Cell Carcinoma of the Pancreas Presenting as Diarrhea. Annals 61:#3, Sept 1964.

6. Chears, W. C., Thompson, J.E., Hutcheson, James B., and Patterson, C.O.: Pancreatic Islet Tumor with Severe Diarrhea. Amer. Jour. Med. XXIX:#3, pp. 529-533, 1960.

7. Morrison, A. B., Rawson, A. J., and Fitts, Wm. T. Jr.: The Syndrome of Refractory Watery Diarrhea and Hypokalemia in Patients with a Non-Insulin - Secreting Islet Cell Tumor. Amer. Jour. Med. 32: January 1964.

8. Kernen, J. A., et al: Metastastic Islet Cell Carcinoma of the Pancreas with Long Survival. Amer. J. Gastroenterology 44:54-56, July 1965.

9. Singleton, J. W., et al: Diarrhea and Pancreatic Islet Cell Tumor; Report of a Case with a Severe Jejunal Muscosal Lesion: Gastroenterology 49:197-208, August 1965.

10. Hindle, W. L., et al: Watery Diarrhea and an Islet Cell Tumor. J. Amer. Med. Assn. 145:1198-1203, Dec 15, 1964.

11. Sendrail, M. et al: Non-Functional Nesidioblastoma in an Aberrant Pancreas. Ann. Endocrinology 25:657-660, Nov-Dec.1964.

12. Vague, J. et al: Two Cases of Pancreatic Nesidioblastomas, Clinical Biological and Atomic Study. Marseille Med. 102:45-50, 1965.

II. PROBABLE ALPHA CELL TUMORS

1. Hess, W.: Ueber ein endokrin inaktives Carcinom der Langerhansschen Inselin. Schweiz Med. Wschr 76:802-804, 1946.

2. Hamperl, H.: Uber Argyrophile Zellen. Virchows Archiv, Bd. 321, S.482-507, 1952.

3. Malandra, B.: Sui Tumori Delle Isole Pancreatiche. Rivista Anatomia Pathologica e dt Oncologia 12:1956-67, TXU-M.

4. Grossner, W., and Korting, W. W.: Metastasierendes Inselzell Karzinom von A-Zelltyp bei einem Fall von Pemphigus Foliaceus mit Diabetes Renalis. Deutsch. Med. Wschr. 85: 434-437, 1960.

5. Keen, H.: Fluctuating Hyper- and Hypoglycemia Associated with Multiple Hepatic Tumors. Guy's Hosp. Reports 111: 1962.

6. Behrendt, W.: Ein Beitraz zur Pathologie des A Zellkarzenouis., Zbl. Allg. Path. 104: 199-204, 1963.

7. Yoshinaga. In press. Diabetes, 1966.

III. POSSIBLE ALPHACYTOTROPIC TUMOR

1. Verne, J., Gueniot, R.: Tumeur Diabetogene Du Pancreas. Second International Congress of the International Diabetes Federation, Cambridge, England, July 1955.

IV. GLUCAGON DEFICIENCY SYNDROMES

1. McQuarrie, I., Bell, E. T., Zimmerman, B., and Wright, W. S.: Deficiency of Alpha Cells of Pancreas as possible etiological factor in familial hypoglycemosis. Fed. Proc. 9:337, 1950.

2. Grollman, A., McCaleb, W. W., and White, F. N.: Glucagon Deficiency as a Cause of Hypoglycemia. Metabolism 13:6 86, 1964.

V. GLUCAGON EXCESS SYNDROMES
(See also "Probable Alpha Cell Tumors)

1. Ferner, H.: The A and B-cells of the pancreatic islets as sources of the antagonistic hormones, glucagon and insulin. The shift of the AB-Relation in diabetes mellitus. Amer. J. Digest. Dis. 20:301, 1953.

2. Ferner, H.: Studien uber die Histiophysiologic des Inselsytems de Bauchspeicheldruse und den Diabetes mellitus. Virchow's Arch. 319:390, 1951.

VI. EMBRYOLOGY AND COMPARATIVE ENDOCRINOLOGY

1. Miller, M. R., and Wursten, D. H.: Comparative Endocrinology, Wiley, N. Y. 1959.

VII. EXTRA-INSULAR SITES OF GLUCAGON SYNTHESIS

1. Feyrter, F.: Uber das Inelorgan des Menschen. Erg. Path. 36:3, 1943.

2. Gaede, K., Ferner, H., Insulares Gangorganes, Klin. Wschr. 28:631, 1950.

3. Sutherland, E. W., de Duve, C.: Origin of HGF., J. Biol. Chem. 175:663, 1948.

4. Makman, M. H., Sutherland, E. W.: Liver Adenyl cyclase assay for glucagon in G-I tract and pancrease. Endocrinology 75:127, 1964.

5. Fodden, J.H.: Enterochromaffin and Pancreatic Alpha Cells: Comparison. Amer. J. Clin. Path. 66:10, 1958.

6. Volk, B. W., Lazarus, S. S., Goldner, M. G.: Alpha Cells of the Pancreas. Morphologic and Physiologic Considerations. Arch. Int. Med. 93:87, 1954.

VIII. GLUCAGON AND GLUCOSE METABOLISM

Glycogenolysis

1. Burger, M.: Das Glukagon. Fortschr. Diag. u. Ther., 1:1, 1950.

2. Cornblath, M., Levin, E. Y., and Marquetti, E.: Studies of carbohydrate metabolism in the newborn. II. The effect of glucagon on the concentration of sugar in capillary blood of the newborn infant. Pediatrics, 12:885, 1958.

3. Loube, S. D., Campbell, E. D., and Mirsky, I. A.: Administration of the hyperglycemic-glycogenolytic factor of the pancreas to non-anesthetized and anesthetized subjects. Proc. Soc. Exp. Biol. Med., 75:161, 1950.

4. Bondy, P. K., and Cardillo, L. R.: The effect of glucagon on carbohydrate metabolism in normal human beings. J. Clin. Invest., 35:494, 1956.

5. Butterfield, W. J. H., Kelsey Fry, I., and Whichelow, M. J.: The Effect of small doses of glucagon in normal and diabetic subjects. Guy's Hosp. Reports., 109:95, 1960.

6. Vuylsteke, C. A., and De Duve, C.: Influence du glucagon sur le dosage de l'insuline. Arch. Internat. Physiol., 61:275, 1953.

7. Kibler, R. G., Taylor, W. J., and Myers, J. D.: Effects in man of the hypoglycemic glycogenolytic factor of the pancreas. Amer. J. Med. 13:647, 1952.

8. Shoemaker, W. C., Van Itallie, T. B., and Walker, W. F., Measurement of hepatic glucose output and hepatic blood flow in response to glucagon. Amer. J. Physiol., 196:315, 1959.

9. Landau, B. R., Leonards, J. R., and Barry, F. M.: A quantitative study of glucagon-induced hepatic glycogenolysis. Amer. J. Physiol., 199:231, 1960.

10. Shoemaker, W. C., and Van Itallie, T. B., The hepatic response to glucagon in the unanesthetized dog. Endocrinology 66:260, 1960.

11. Shoemaker, W. C., Van Itallie, T.B., and Walker, W. F., Measurement of hepatic glucose output and hepatic blood flow in response to glucagon. Amer. J. Physiol., 196:315, 1959.

12. Burger, M., and Kramer, H.: Primare Hyperglykamie und Glykogenverarmung der Leber als Folge Intraportaler Insulininjekton nach Untersuchungen am Hund. Zeit. ges. exp. Med. 67:441, 1929.

13. Costa, E., Galansino, G., and Foa, P. P.: Glycogen stores in glucagon-treated rats. I. Time Factors. Proc. Soc. Exp. Biol. Med. 91:308, 1956.

14. Foa, P. P., Galansino, G., and Pozza, G.: Glucagon, a second pancreatic hormone. Recent Progress Hormone Res., 13:473, 1957.

15. Longley, R. W.: The in vivo effect of purity and mode of administration of glucagon on glycogenesis. Endocrinology 63: 383, 1958.

16. Okuno, G.: Studies on the delayed increase of liver glycogen by glucagon. Med. J. Osaka Univ., 10:483, 1960.

17. Sutherland, E. W., and Cori, C. F.: Influence of insulin preparation on glycogenolysis in liver slices. J. Biol. Chem. 172:737, 1948.

18. Vuylsteke, C. A., and De Duve, C.: The assay of glucagon on isolated liver slices. Arch. Internat. Pharmacodyn., 111:437, 1957.

19. Miller, L. L., Sokal, J. E., and Sarcione, E. J.: Effects of glucagon and tolbutamide on glycogen in isolated perfused rat liver. Amer. J. Physiol., 199:286, 1959.

20. Cameron, J. S. et al: The effect of small doses of insulin and glucagon on the human liver. Clin. Sci. 27:67-76, August 1964.

21. Sokal, J. E. et al: Relative potency of glucagon and epinephrine as hepatic glycogenolytic agents: Studies with the isolated perfused rat liver. Endocr. 74:930-938, June 1964.

22. Miller, W. M. et al. Comparative expiratory and oxidative effects of glucagon and epinephrine in mice. Proc. Soc. Exp. Biol. Med. 113:784-788, July 1963.

Gluconeogenesis

1. Bocek, R. M., Peterson, R. D., and Beatty, C. H.: Effect of glucagon on amino-acid metabolism. Feder. Proc., 19:149, 1960.

2. Curry, D. M., and Beaton, G. H.: Glucagon administration in pregnant rats. Endocr. 63:252, 1958.

3. Glasser, S. R., and Izzo, J. L.: Interrelationships of growth hormone and glucagon on protein metabolism in fasting hypophysectomized rats. 41st Meet. Endocrine Soc., 1959, p. 76.

4. Helmer, O. M., Kirtley, W. R., and Ridolfo, A. S.: Clinical and Metabolic changes induced by glucagon in patients with rheumatoid arthritis. J. Lab. Clin. Med. 50:824, 1957.

5. Weinges, K. F.: Die Wirkung des Glucagons auf die Gesamtaminosauren im Serum. Arch. exp. Path. u. Pharmakol., 237:17, 1959.

6. Weinges, K. F.: Der Einfluss eines protrahiert wirkenden Glucagons auf den Blutzucker das anorganische Serumphosphat und die Gesamtaminosauren im Serum. Arch. exp. Path. u. Pharmakol., 237:22, 1959.

7. Shoemaker, W. C., and Van Itallie, T. B., The hepatic response to glucagon in the unanesthetized dog. Endocrinology, 66:260, 1960.

8. Kalant, N.: The effect of glucagon on metabolism of glycine-1-C^{14}. Arch. Biochem. Biophys., 65:469, 1956.

9. Lipsett, M. B., Engel, H. R., and Bergenstal, D. M.: Effects of glucagon on plasma unesterified fatty acids and in nitrogen metabolism. J. Lab. Clin. Med., 56:342, 1960.

10. Okuno, G.: Studies on the delayed increase of liver glycogen by glucagon. Med. J. Osaka Univ., 10:483, 1960.

11. Miller, L. L.: Glucagon: a protein catabolic hormone in the isolated perfused rat liver. Nature 185:248, 1960.

12. Miller, L. L.: Direct actions of insulin and glucagon on the isolated perfused rat liver. Feder. Proc., 20:191, 1961.

13. Cahill, G. F., Jr., Zottu, S., and Earle, A. S.: In vivo effects of glucagon on hepatic glycogen, phosphorylase and glucose-6-phosphatase. Endocrinology, 60:265, 1957.

14. Exton, J. H. and Park, C. R.: Gluconeogenesis in Liver; effect of adrenolectomy cortisol and glucagon. Fed. Proc. 24:537, 1965.

15. Mahler, R., et al: The Effects of Insulin on Lipolysis. Diabetes 13:297-302, May-June 1964.

16. Shrago, E. et al: Metabolic and Hormonal control of phosphoenolpyruvate carboxykinase and malic enzyme in rat liver. J. Biol. Chem. 238:3188-92, October 1963.

17. Izzo, J. L. et al: Interrelationship of actions of glucagon and somatotropin on protein metabolism in the fasted hypophysectomized rat. Endocr. 72:701-708, May 1963.

IX. GLUCAGON AND LIPID METABOLISM

1. De Duve, C., and Berthet, J.: Le glucagon. 4e Reun. Endocrinol., Ann. Endocrinol. 18:333, 1957.

2. Berthet, J.: Action du glucagon et de l'adrenaline sur le metabolisme des lipides dans le tissu hepatique. 4th Internat. Congr. Biochem., Vienna, 1958, p. 107.

3. Orth, R. D., Odell, W. D., Williams, R. H.: Some hormonal effects on the metabolism of acetate-1-C^{14} by rat adipose tissue. Amer. J. Physiol., 198:640, 1960.

4. Davoren, P. R., and Bornstein, J.: Effect of glucagon on metabolism of glucose and acetate by isolated rat liver. Amer. J. Physiol., 197:887, 1959.

5. Steinberg, D. M., Shafrir, E., and Vaughan, M., Direct effect of glucagon on release of unesterified fatty acids (UFA) from adipose tissue. Clin. Res., 7:250, 1959.

6. Weinges, K. F.: Der Einfluss von Glukagon und Insulin auf den Stoffwechsel der nicht veresterten Fettsauren am isolierten Fettgewebe der Ratte in vitro. Klin. Wschr., 39: 293, 1961.

7. Hagen, J. H.: Effect of glucagon on the metabolism of adipose tissue. J. Biol. Chem., 236:1023, 1961.

8. Vaughan, M.: Effect of hormones on glucose metabolism in adipose tissue. J. Biol. Chem., 236:2196, 1961.

9. Lipsett, M. B., Engel, H. R., and Bergenstal, D. M.: Effects of glucagon on plasma unesterified fatty acids and in nitrogen metabolism. J. Lab. Clin. Med., 56:342, 1960.

10. Giacovazzo, M., and Bianchi, P.: Comportamento del colesterolo totale ed esterificato del siero durante e dopo trattamento glucagonico. Contributo sperimentale. Boll. Soc. It. Biol. Sper., 34:597, 1958.

11. Goodman, D. S., and Gordon, R. S., Jr., The metabolism of plasma unesterified fatty acid. Amer. J. Clin. Nutr., 6:669, 1958.

12. Campanacci, D., and Butturini, U.: Il Glucagone in Biologia ed in Clinica. Pacini Mariotti, Pisa, 1957.

13. Caren, R., and Corbo, L.: Glucagon and cholesterol metabolism. Metabolism 9:938, 1960.

14. Bondy, P. K., and Cardillo, L. R.: The effect of glucagon on carbohydrate metabolism in normal human beings. J. Clin. Invest., 35:494, 1956.

15. Rutman, J. Z., Pincus, I. J., Brown, L., and Scott, M.: Effect of HGF on acidosis and lipids in intact and depancreatized dogs. Feder. Proc., 12:121, 1953.

16. Ezrin, C., Salter, J. M., Ogryzlo, M. A., and Best, C. H.: The clinical and metabolic effects of glucagon. Canad. M. A. J., 78:96, 1958.

17. Salter, J. M., Ezrin, C., Laidlaw, J. C., and Gornall, A. G.: Metabolic effects of glucagon in human subjects. Metabolism, 9:753, 1960.

18. Haugaard, E. S., and Haugaard, N.: The effect of hyperglycemic-glycogenolytic factor on fat metabolism of liver. J. Biol. Chem., 206:641, 1954.

19. Dole, V. P.: The significance of non-esterified fatty acids in plasma. A. M. A. Arch. Int. Med. 101:1005, 1958.

20. Aydin, A. et al: Uptake of plasma free fatty acids by the isolated rat liver; Effect of glucagon. Amer. J. Physiol. 205:667-670, Oct 1963.

21. Amatuzio, D. S. et al: Essential Hypolipemia: The effect of glucagon. Minnesota Med. 46:1088-91, November 1963.

22. Koyama, S. : Effect of glucagon on blood glucose and plasma nonestrified fatty acids concentrations. J. Jap. Soc. Intern. Med. 53:9-20, April 1964.

23. Amatuzio, D. S., Grande, F., Wada, S.: Effect of glucagon on the Serum Lipids in essential hyperlypemia and hypercholesterolemia. Metabolism 11:1240-9, December 1962.

24. Bierman, E. L., Hamlin, J. T.: The effect of insulin and glucagon on the removal of C-14-labeled particulate triglyceride from plasma in man. Metabolism 12:666-72, July 1963.

25. Rudman, D. et al: Adipokinetic action of adrenocorticotropin, thyroid-stimulating hormone, vasopressin, Alpha- and Beta-melanocyte-stimulating hormones fraction H, epinephrine and norepinephrine in the rabbit, guinea pig, hamster, rat, pig and dog. Endocr. 72:527-43, April 1963.

26. Persson, B. et al: Effect of glucagon on the plasma level of free fatty acids. Acta Paediat. (upps) 51 (Suppl) 135:165-70, June 1962.

27. Penhos, L. R.: In press. Diabetes, 1966.

28. Caren, R. et al: Glucagon and Plasma arachidonic Acid. Metabolism 14:684-92, June 1965.

29. Steinberg, D. et al: Effects of the prostaglandins on hormone-induced mobilization of free fatty acids. J. Clin. Invest. 43:1533-1540, Aug. 1964.

30. Vaughn, M. et al: Hormone-sensitive lipase and monoglyceride lipase activities in adipose tissue. J. Biol. Chem. 239:401-9, Feb. 1964.

31. Kibler, R. F. et al: The effect of glucagon on net spanchnic balances of glucose, amino acid, nitrogen, urea, ketones and oxygen in man. J. Clin. Invest. 43:904-15, May 1964.

32. Rudman, D.: The adipokinetic action of polypeptide and amine hormones upon the adipose tissue of various animal species. J. Lipid. Res. 4:119-29, April 1963.

33. Vaughan, M. et al: Effect of hormones on lipolysis and estrification of free fatty acids during incubation of adipose in vitro. J. Lipid Res. 4:193-9, April 1963.

X. GLUCAGON AND CATECHOLAMINE SECRETION

1. Sarcione, E. J. et al: Elevation of plasma epinephrine levels produced by glucagon in vivo. Endocr. 72:523-6, April 1963.

2. Lawrence, A. M., and Forland, M.: Glucagon Provacative test for pheochromocytoma. J. Clin. and Lab. Med. P. 878, 1964.

3. Sarcione, E. J., Sokal, J. E., and Gerszi, K. E.: Relationship of the adrenal medulla to the hyperglycemic effect of glucagon. Endocrinology, 67:337, 1960.

4. Scian, L. F., Westermann, C. D., Verdesca, A. S., and Hilton, J. G.: Adrenocortical and medullary effects of glucagon. Amer. J. Physiol. 199:867, 1960.

XI. GLUCAGON AND INSULIN SECTION

1. Crockford, P. M., Porte, D., Wood, F. C., and Williams, R. H.: Effect of Glucagon on serum insulin, plasma glucose, FFA in man. Metabolism January 1966.

2. Samols, E., Marri, G., and Marks, V.: Promotion of Insulin Secretion by Glucagon. The Lancet, August 28, 1965, pp. 415-416.

XII. GLUCAGON AND ELECTROLYTES

1. Wolfson, S. K., Jr., and Ellis, S.: Effects of glucagon on plasma potassium. Proc. Soc. Exp. Biol. Med., 91:226, 1956.

2. Ellis, S., Beckett, S. B., and Boutwell, J. H.: Dibenamine blockage of epinephrine and glucagon hyperkalemias. Proc. Soc. Exp. Biol. Med., 94:343, 1957.

3. Elrick, H., Huffman, E. R., Hlad, C. J., Jr., Whipple, N., and Staub, A.: Effects of glucagon on renal function in man. J. Clin. Endocrinol. Metab. 18:813, 1958.

4. Bondy, P. K., and Cardillo, L. R.: The effect of glucagon on carbohydrate metabolism in normal human beings. Yale J. Biol. Med., 26:263, 1954.

5. Finder, A. G. et al: Relationship of hepatic potassium efflux to phosphorylase activation induced by glucagon. Amer. J. Physiol. 206:738-42, April 1964.

6. Ellis, S. et al: Mechanism of the potassium mobilizing action of epinephrine and glucagon. J. Pharmacol. Exp. Ther. 142:318-326, December 1963.

XIII. GLUCAGON AND THE G-I TRACT

1. Ezrin, C., Salter, J. M., Ogryzlo, M. A., and Best, C. H.: The clinical and metabolic effects of glucagon. Canad. M. A. J., 78:96, 1958.

2. Morrison, S. D., Lin, H. J., Eckel, H. E., Van Itallie, T. B., and Mayer, J.: Gastric contractions in the rat. Amer. J. Physiol. 193:4, 1958.

3. Penick, S. B., and Hinkle, L. E., Jr.: Depression of food intake induced in healthy subjects by glucagon. New Eng. J. Med. 264 893, 1961.

4. Robinson, R. M., Harris, K., Hlad, C. J., and Eiseman, B.: Effect of glucagon on gastric secretion. Proc. Soc. Exp. Biol. Med., 96: 518, 1957.

5. Stunkard, A. J., Van Itallie, T. B., and Reis, B. B.: The mechanism of satiety: effect of glucagon on gastric hunger contractions in man. Proc. Soc. Exp. Biol. Med. 89:258, 1955.

6. Sporn, J., and Necheles, H., Effect of glucagon on gastrointestinal motility. Amer. J. Physiol., 187:634, 1956.

7. Sudsaneh, S., and Mayer, J.: Relation of metabolic events to gastric contractions in the rat. Amer. J. Physiol. 197:269, 1959.

8. Von Heimburg, R. L. et al: Inhibition of gastric secretion in dogs by glucagon given intraportally. Gastroenterology 47:531-535, Nov 1964.

9. Penick, S., et al: The effect of glucagon phenmetrazine and epinephrine on hunger, food intake and plasma nonestrified fatty acid. Amer. J. Clin. Metr. 13:110-4, August 1963.

10. Dotevall, G. et al: The effect of glucagon on intestinal motility in man. Gastroenter. 45:364-7, Sept. 1963.

11. Aylett, P.: Gastric emptying and change of blood glucose level as affected by glucagon and insulin. Clin. Sci. 22:171-8, April 1962.

12. Dreiling, D. A. et al: Effect of ACTH hydrocortisin and glucagon on plasma nonestrified fatty acid concentration (NEFA) in normal subjects and in patients with liver disease. Metabolism 11:572-8, June 1962.

XIV. GLUCAGON AND TUMOR GROWTH

1. Salter, J. M., De Meyer, R., and Best, C. H.: Effect of insulin and glucagon on tumor growth. Brit. Med. J., 2:5, 1958.

XV. GLUCAGON DEFICIENCY (Experimental)

1. Caren, R., and Corbo, L.: Glucagon and cholesterol metabolism. Metabolism, 9:938, 1960.

2. Paloyan, E., and Harper, P. V., Jr.: Glucagon as a regulating factor of plasma lipids. Metabolism 10:315, 1961.

3. Mialhe, P.: Mise en evidence d'une hormone hyperglycemiante dans le sang veineux pancreatique du canard normal a jeun. C. Rend. Soc. Biol., 154:1867, 1960.

4. Mialhe, P., and Agid, R.: Diminution de la tolerance a l'insuline chez la ratte pancreatectomisee totalement maintenue a un niveau glycemique normal. C. Rend. Acad. Sci., 249: 2622, 1959.

5. Mialhe, P.: Evolution de la glycemie chez le canard pancreatectomize totalement: influence de la nourriture. J. Physiol. (Paris) 48:647, 1956.

6. Mialhe, P.: Glucagon insuline et regulation endocrine de la glycemie chez le canard. Acta Endocrinol., 28, Suppl, 36, 1958.

7. Penhos, J. C. et al: Total pancreaticectomy in toads; effect of hypophysiosectomy an d glucagon. Gen. Comp. Endocr. 4:264-270, June 1964.

8. Mikami, S. I. et al: Glucagon deficiency induced by extirpation of alpha islets of the fowl pancreas. Endocr. 71:464-73, September 1962.

XX. GLUCAGON EXCESS (Experimental)

1. Ingle, D. J., Beary, D. G., and Purmalis, A.: Effect of continuous injection of glucagon upon glycosuria of partially depancreatized rats. Proc. Soc. Exp. Biol. Med. 85:432, 1954.

2. Ingle, D. J., Nezamis, J. E., and Humphrey, L. M.: Absence of hyperglycemic effect of glucagon in the eviscerate rat. Proc. Soc. Exp. Biol. Med. 84:232, 1953.

3. Salter, J. M.: Metabolic effects of glucagon in the Wistar rat. Amer. J. Clin. Nutr. 8:535, 1960.

4. Salter, J. M., Ezrin, C., Laidlaw, J. C., and Gornall, A. G.: Metabolic effects of glucagon in human subjects. Metabolism 9:753, 1960.

5. Lacy, P. E., Cardeza, A. F., and Wilson, W. D.: Electron microscopy of the rat pancreas. Effects of glucagon administration. Diabetes 8:36, 1959.

6. Lazarus, S. S., and Volk, B. W.: The effect of protracted glucagon administration on blood glucose and on pancreatic morphology. Endocrinology 63:359, 1958.

7. Lazarus, S. S., and Volk, B. W.: Effect of prolonged administration of glucagon in guinea pigs. Diabetes 8:294, 1959.

8. Cavallero, C., and Malandra, B.: Hyperglycemic and glycosuric effect of glucagon in the intact force-fed rat. Acta Endocrinol., 13:79, 1953.

9. Logothetopoulos, J., Sharma, B. B., Salter, J. M., and Best, C. H.: Glucagon and metaglucagon diabetes in rabbits. Diabetes 9:278, 1960.

10. Peterson, B. et al: Effects of long-term administration of glucagon on the pancreatic islet tissue of rats and guinea pigs. Acta Endocr. (kobenhavn) 44:139-49, Sept. 1963.

11. Van Itallie, T. B., Felber, J.P., Hoet, J., and Renold, A. E.: Impaired glucose tolerance and glucosuria induced in man by repeated injections of glucagon. Diabetes 8:94, 1959.

12. Van Itallie, T. B., Morgan, M. C., and Dotti, L. B.: Effect of glucagon on peripheral utilization of glucose in man. J. Clin. Endocrinol. Metab. 15:28, 1955.

13. Verne, J. et al: Histological picture of the endocrine pancreas under the action of glucagon. Ann. Endocr. (Paris) 22:965, 1961.

XXI. PHYSIOLOGIC ROLE OF ENDOGENOUS GLUCAGON

1. Unger, R. H., Eisentraut, A., McCall, M., and Madison, L.: Demonstration of the Hormonal Status of Endogenous Glucagon. Ciba Colloquia on Endocrinology, 14:212-221 1962.

2. Unger, R. H., Eisentraut, A., and Madison, L.: The effects of total starvation upon the levels of Circulating glucagon and insulin in man. J. Clin. Invest. 42:1031, 1963.

3. Unger, R. H., Eisentraut, A., McCall, M. S., Keller, S., Lanz, H. and Madison, L.: Glucagon Antibodies and Their Use for Immunoassay for Glucagon. Proc. Soc. Exp. Biol. Med. 102:621-623, 1959.

4. Unger, R. H., Eisentraut, A., McCall, M. S. and Madison, L.: Glucagon Antibodies and an Immunoassay for Glucagon. J. Clin. Invest. 40:1280-1289, 1961.

5. Unger, R. H., Eisentraut, A., Lochner, J. de v., Baum, B. E., Simons, Jr., and Madison, L.: Immunologic Studies of A-cell Function. Proc. 3rd International Symposium, Stockholm August 1963.

6. Baum, J., Simons, B. E., Unger, R. H., and Madison, L.: Localization of Glucagon in the Alpha Cells in the Pancreatic Islet by Immunofluorescent Technics. Diabetes 11:371-374 Sept-Oct 1962.

7. Unger, R. H., Eisentraut, A., McCall, M. S. and Madison, L.: Measurements of Endogenous Glucagon in Plasma and the Influence of Blood Glucose Concentration upon its Secretion. J. Clin. Invest. 41:682-689, April 1962.

8. Unger, R. H., Eisentraut, A.: Studies of the Physiologic Role of Glucagon. Diabetes 13:563-568, Nov-Dec, 1964.

9. Samols, E., Tyler, J., Marri, G. and Marks, V.: Stimulation of glucagon secretion by oral glucose. The Lancet, Dec 18, 1965 p. 1257-1259.

10. Samols, E., Marri, G. Marks, V.: Promotion of Insulin Secretion by Glucagon. The Lancet, August 28, 1965. p. 415-416.

11. Burger, M., and Brandt, W.: Uber das Glukagon (die hyperglykamisierende Substanz des Pankreas). Zeit. ges. exp. Med., 96:375, 1935.

12. Burger, M., and Klotzbucher, E.: Der Nachweis zuckerregelnder Hormone (Insulin, Glukagon) im menschlichen Blute durch Transfusion. Zeit. ges. inn. Med. 2:43, 1947.

13. Burger, M., and Kramer, H.: Uber den hepatischen Angriffspunkt des Insulins. Die primare paradoxe Insulinhyperglykamie. Zeit. ges. exp. Med., 65:487, 1928.

14. Burger, M., and Kramer, H.: Primare Hyperglykamie und Glykogenverarmung der Leber als Folge Intraportaler Insulininjektion nach Untersuchungen am Hund. Zeit. ges. exp. Med., 67:441, 1929.

15. Lawrence, A. M.: Reduction in radioimmunoassayable glucagon following glucose loading in man. (Abstract) Jour. Clin. Lab. Med. p.46-47, 1965.

XXII. GLUCAGON METABOLISM

1. Merimee, T. J. et al: Studies on glucagon I-131 in normal and human serum. J. Lab. Clin. Med. 64:412-417 Sept. 1964.

XXIII. DERMATITIS AND MALIGNANCY

1. Arnould, G.: On a Case of Dermatomyositis revealing bronchial cancer. Ann. Med.3:668, 1964.

2. Kilby, P. E.: Carcinoma of Rectum presenting with exfoliative erythrodermia. Brit. J. Cancer 19:317, 1965.