MEDICAL GRAND ROUNDS

PARKLAND MEMORIAL HOSPITAL

February 17, 1966

"GLUCAGONOMA"

GLUCAGONOMA -- TWO DECADES LATER

Roger H. Unger, M.D.

Medical Grand Rounds
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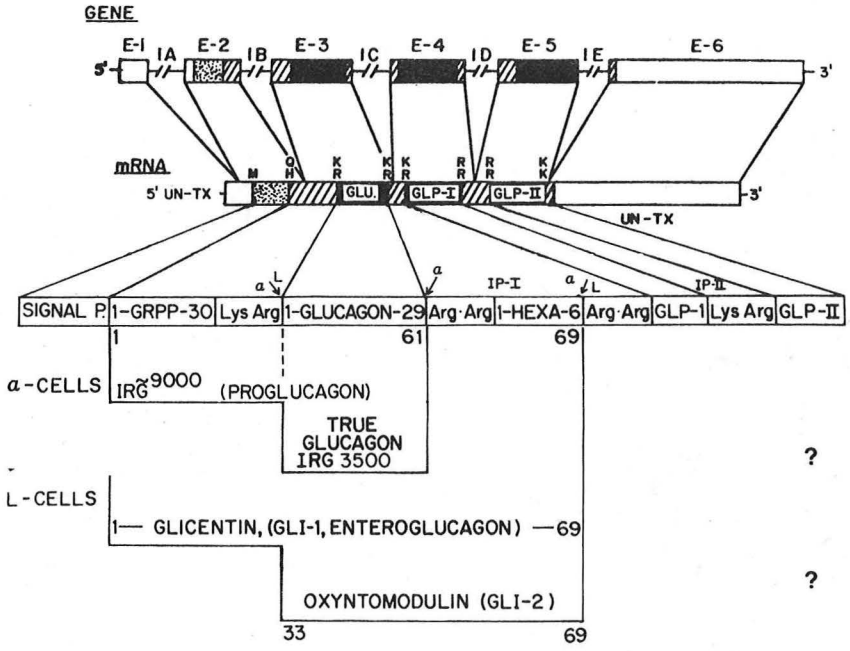

THE FIRST PROVEN CASE OF GLUCAGONOMA SYNDROME

Twenty years ago this month the first proven case of glucagonoma syndrome was in the process of being worked up at this center and at Barnes Hospital. The patient, A.H., was a 42-year-old white female first seen in the Hospital Out Patient Clinic in St. Louis, Missouri, in January, 1963, complaining of skin lesions involving her extremities, perineum, trunk and face. Her recent and past history was unremarkable save for anorexia, rapid satiety and weight loss for the past six months. She denied a family history of diabetes but her knowledge of her family was meager.

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 2+ glycosuria. 100 gm OGTT: FBS 107; 1/2 hour 220; 1 hour 272; 2 hours 193; 3 hours 165. Cholesterol 140; BUN 10; electrolytes normal. Chest xray was normal. Her diabetes was controlled with a diet of 1500 calories. The skin lesions were resistant to conservative therapy and constituted the patient's major complaint. Repeated cultures and scraping of skin revealed no fungal organisms and no specific dermatologic diagnosis was made. Eighteen months later in December, 1964, she developed right pleuritic pain and was admitted to Barnes Hospital for study. Physical examination revealed dermatitis, an elevated right diaphragm and markedly enlarged stoney-hard liver 7 cm below the costal margin. Hemoglobin 10.2 gm; WBC diff, platelets normal; retic count 3 to 5.3%. Liver function tests, including alkaline phosphatase, were normal. Chest x-ray showed a markedly elevated right hemidiaphragm and hepatomegaly. Liver scan revealed at least two large metastases. Celiac arteriogram disclosed a tumor blush in the tail of the pancreas. Needle biopsies of the pancreatic mass and right lobe of liver obtained at laparotomy were read as showing the histologic diagnosis undifferentiated carcinoma of the pancreas on H and E stain. The patient was discharged with a grave prognosis.

During the ensuing eight months the patient did surprisingly well and was without complaints other than dermatitis. The lack of expected progressive deterioration prompted a review of the microsections of the tumor by Dr. Malcolm McGavran. He now thought the tumor was of islet origin. Plasma sent to Dallas for glucagon assay was markedly hyperglucagonemic (Table 1).

Table 1
Fasting plasma glucagon of patient A.H. (ng/ml)

A.H.	55.0
	46.0
Normal subjects	<0.25
A normal subject 2.5 min after 3 mg crystalline glucagon i.v.	38.5

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 the hepatic metastases was made in May, 1965. A large firm tumor was removed in toto from the tail of the pancreas and sent to Dallas but the hepatic metastases could not be removed. The tumor contained large amounts of immunoreactive glucagon (Table 2; Figure 1) which was biologically similar to purified glucagon (Figure 1).

Table 2
Extractable glucagon and insulin content of tumor of A.H., pancreas tissues and other islet cell tumors

Specimen	Glucagon ug/g	Insulin U/g
Tumor of A.H. (surgical specimen)	14.0	0.029
Tail of human pancreas (surgical specimen)	9.2	0.5
Other non-beta islet cell tumors*	0.002-0.064	0.001-0.016
Insulinoma (surgical specimen)	0.3	0.3

*Two of the four were obtained post-mortem

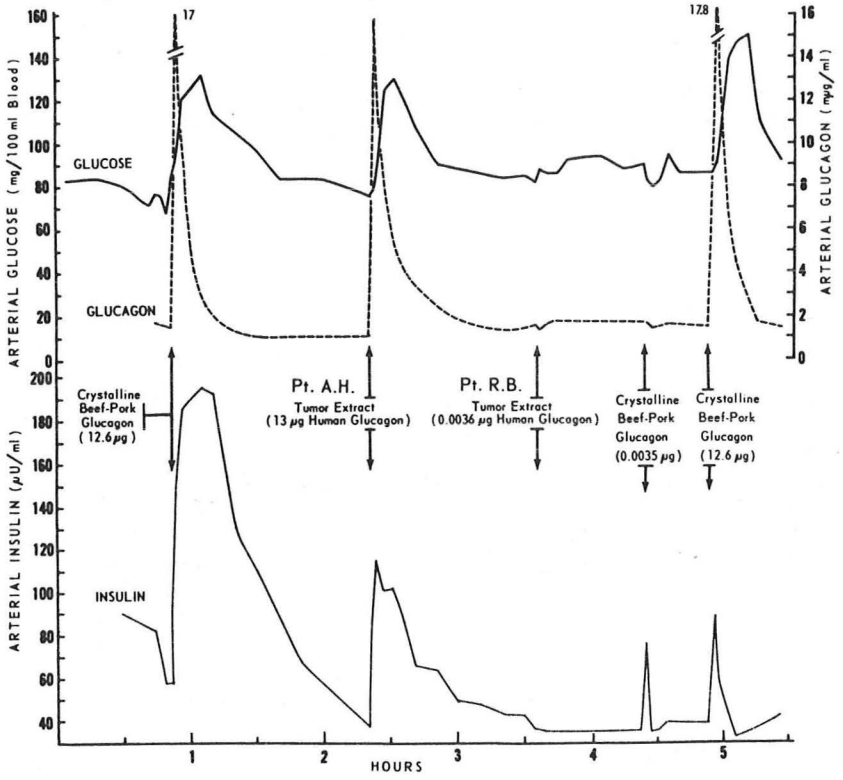

Fig. 1: The effect of i.v. glucagon, glucagonoma extract from Patient A.H., a non-glucagonoma pancreatic tumor extract (Patient R.B.) and repeat injections of crystalline glucagon on the arterial concentrations of glucose, glucagon and insulin in a normal dog.

The patient was followed in the clinic and in October, 1965, exhibited pedal edema, marked worsening of the dermatitis and progressive anemia. Alkaline phosphatase was still normal, but the hemoglobin had fallen to 9.9 gm. The nature of her dermatitis was uncertain. It responded dramatically with almost total clearing to oral administration of 40 mg of prednisone per day but recrudesced as the dose was diminished. On steroids she required 20 units of NPH insulin to control the glycosuria.

She left St. Louis and was lost to follow-up.

On February 17, 1966, when she was presented here at Medical Grand Rounds, the literature contained only four probable and 12 possible cases of glucagonoma (Becker et al., 1942; Hess, 1946; Gossner and Korting, 1960; Behrendt, 1963) --all unproven since they antedated the development of glucagon radioimmunoassay (Unger et al., 1959) and glucagon immunocytochemistry technics (Baum et al., 1962). Because two of the four probable cases had skin lesions, the possibility that skin involvement was a part of a glucagonoma syndrome was raised at the conference (Figure 2), but the editors of the NEJM discouraged emphasis of this possibility in the report of the case that followed (McGavran et al., 1966).

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^{0} 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.

Fig. 2

Since then the availability of radioimmunoassay (Unger et al., 1959; 1961) and immunocytochemical technics for glucagon (Baum et al., 1962) have permitted a specific definition of the glucagonoma syndrome by Mallinson (1974b) and others (Bhathena et al., 1981; Wood et al., 1983). It is caused by a pancreatic alpha cell tumor, almost always a malignant one, that secretes large quantities of glucagon and its biosynthetic precursors, the GLIs. It is often associated with a painful pruritic rash called "necrolytic migrating erythema", glossitis, hypoaminoacidemia, weight loss, depression, anemia and the consequences of tumor metastases. Over 100 cases have been reported. The latest case, J.H., is the first such patient to be observed at this institution.

PATIENT J.H.

This 40-year-old white woman was electively admitted to Parkland Hospital in June, 1985, for evaluation of a generalized dermatitis, abdominal mass and weight loss.

She was in apparent good health until August, 1984, when she was admitted to Jones Hospital, Sherman, Texas, for evaluation of watery, nonbloody diarrhea, dehydration and facial dermatitis of two weeks duration. UGI, barium enema, stool guaiac, examination for ova and parasites, upper endoscopy with duodenal biopsies and aspirates for Giardia were negative. The diarrhea was attributed to an occult Giardia infection and the patient responded to high dose Flagyl. The rash was characterized as seborrheic dermatitis and was treated with topical steroids.

The patient did well for the next two months, but was readmitted January, 1985, with a two-month history of an eruption of her lower extremities with a gradual progression to her trunks, arms and genitalia. Topical steroids were ineffective. Physical examination revealed a beefy red tongue, a 2-3 cm mass in the right upper quadrant just below the costophrenic angle and a generalized eruption of the legs, hips, arms, lower back and abdomen with scattered crusted bullous erosions on the nasal area, around the mouth and genital regions. Skin biopsy revealed a superficial perivascular lymphocytic infiltrate. Direct immunofluorescence was negative for immunoglobulin. The patient was given high dose prednisone with improvement but subsequent tapering of steroids resulting in recurrence of her lesions.

The patient was readmitted March, 1985, with excoriated, erythematous patches covered with scaling crusts of the distal portions of her hands and feet, hips and thighs. Erythematous, edematous erosions of the labia majora and inner thighs were noted. Serum zinc level was 88 (normal 76-138). Upon admission the patient was treated with prednisone 40 mg/day, erythromycin and Lotrimin Cream. Nearly all lesions resolved.

They recurred in June, 1985, and she was referred to Parkland Hospital. In addition to the painful dermatitis, she had lost 40 pounds over the preceeding six months. She noted no further diarrhea, fevers, chills or abdominal pain. Physical examination was significant for a slightly reddened tongue with angular cheilitis, a liver span of 10 cm with an edge palpable 1 cm below the right costal margin and widespread dermatitic papules, patches and plaques over the posterior scalp, painful erythroderma of both antecubital areas, trunk, lower extremities, forearms and hands that resembled necrotizing migratory erythema.

Admission CXR was within normal limits.

There was proteinuria of 30 mg%. NA = 139, K = 3.6, CL = 107, CO_2 = 25, Ca^{++} = 9.1, P = 3.5, **GLU = 156**, CREAT = 0.6, T.B. = 0.6, **ALK PHOS = 127**, OT = 9, Mg^{++} = 1.2, T.P. = 4.3, Alb = 2.1, WBC + 7.7, HGB = 12.5, HCT = 37.4, PLAT = 317, CK = 27, LDH = 114, PT = 11.3, PTT = 24.2. SPEP = mild increased in alpha-2-globulin. OGTT was not performed. Serum **IRG** (immunoreactive glucagon) was **1500 pg/ml** (normal 50-250 pg/ml) with about 55% in the 3500 M.W. range and 45% in 9000 M.W. range (Figure 3); **GLI was 2.1 ng/ml** (normal 0.6-1.2 ng/ml). Insulin and C-peptide levels were normal to high. The levels of 14 **amino acids** were diminished or absent.

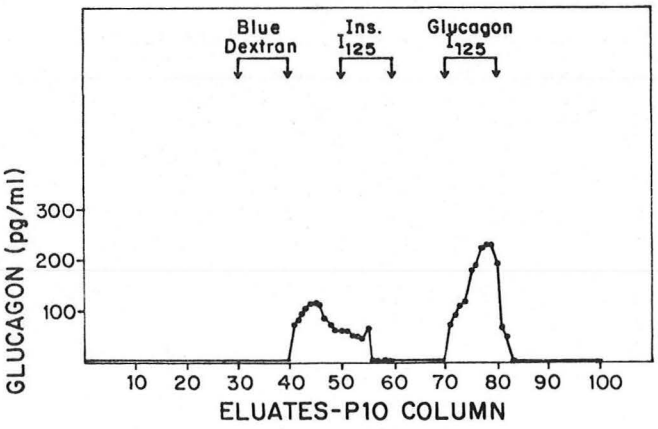

Fig. 3: Bio-Gel P10 chromatogram of plasma from J.H. containing 1500 pg/ml of glucagon.

Abdominal CT scan demonstrated a vascular mass in the right lower lobe of the liver. No pancreatic lesions were seen. Celiac angiography confirmed a mass in the right lobe of the liver. The pancreatic lesion was not noticed until reexamination of the films post-operatively.

She was treated with 200 mg/day of the somatostatin analog SMS 201-995. There was prompt relief of pain and pruritis and the rash disappeared within 72 hours. There was little effect on the hyperglucagonemia (Figure 4).

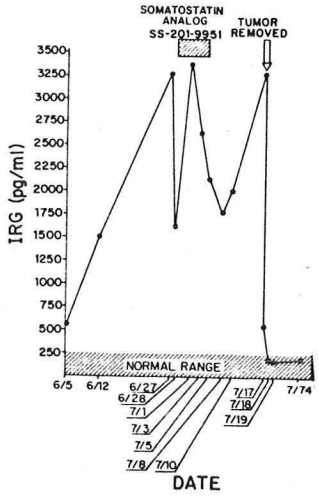

Fig. 4: IRG profile before, during and after somatostatin analog treatment and resection of the tumors.

On July 24, 1985, a 5 cm tumor of the tail of the pancreas regional lymph nodes were removed and a portion of the right lobe of the liver containing a single 6 cm metastasis and a hepatic metastasis were resected. Her IRG levels fell promptly to normal (Figure 5) and her skin disease did not recur.

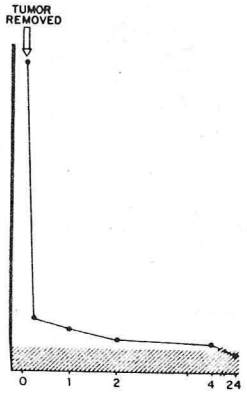

Fig. 5: IRG levels during and after resection of the tumor.

As shown in Table 3, the tumor was positive by immunofluorescent staining technics for glucagon, glicentin, pancreatic polypeptide and prealbumin. There were a few insulin and somatostatin cells that may have been islets "trapped" by the tumor. It was negative for all other peptide hormones tested.

Table 3 *
Immunofluorescence reactions on Bouin's fixed, paraffin-embedded tissues of J.H.

Antiserum	Dilution	Result
Primary Pancreatic Tumor		
Anti-insulin (Lot 573 from Dr. Wright)	1:400	Few positive cells
Anti-somatostatin (Lot 19.578 from Dr. Dubois)	1:200	Few positive cells
Anti-N-terminal glucagon (Lot K6248 from Dr. Heding)	1:50	Positive cells, single or in small groups
	1:50 + glucagon	Inhibited
Anti-C-terminal glucagon (Lot K6251 from Dr. Heding)	1:100	Brightly positive cells, single or in small groups
	1:100 + glucagon	Inhibited
Anti-glicentin (Lot R64 from Dr. Moody)	1:100	Brightly positive cells, same distribution as glucagon cells
	1:100 + glicentin	Inhibited
Anti-GLP1 (from Dr. Habener)	1:100	Positive cells, less numerous than glucagon cells
Anti-BPP (Lot 615-R110-146-14 from Dr. Chance)	1:500	Positive cells, much less numerous than glucagon cells
	1:500 + BPP	Inhibited
Anti-human prealbumin (from DAKO)	1:10	Virtually all the tumoral cells appeared positive
	1:10 + prealbumin	Inhibited
Other Tumors		
The tumor in the liver and in lymph node did not react with anti-insulin and anti-somatostatin antisera. The immunoreactions with the other antisera were similar to those obtained on the pancreatic tumor. - Glicentin cells appeared more numerous than glucagon cells. The same antisera were tested on the skin lesion with negative results.		

The diagnosis of glucagonoma was proven beyond question by the demonstration of 1) hyperglucagonemia; 2) an elevated GLI; 3) the return of IRG to normal following removal of the tumor; and 4) the demonstration of glucagon and glicentin immunoreactivity in the tumor cells.

*Rawnsley and Orci

THE PATHOPHYSIOLOGY OF THE GLUCAGONOMA SYNDROME (Table 4)

Table 4
Clinical Configuration of the Glucagonoma Syndrome

Demography	
Age:	20-73 years
Sex:	62% female
Cardinal clinical features	
Necrolytic migratory erythema	100% (by definition) (69% of all glucagonomas)
Hypoaminoacidemia	100% (of patients in which it is measured)
NIDDM or impaired OGTT (IDDM occurs if tumor destroys pancreas)	100%
Weight loss	<100%
Anemia	100%
Glossitis	80%
Less common clinical features	
Venous thrombosis	30%
Depression	?
Diarrhea	20%
Hypertrophy of duodenum and/or jejunal mucosa	?
Polyglandular syndromes	?
Multihormonal syndromes	?

I. **THE SKIN DISEASE - NECROLYTIC MIGRATORY ERYTHEMA (Wilkinson, 1973):** The most dramatic and specific feature of the glucagonoma syndrome is skin disease, described as a figurate erythema which migrates rapidly and leaves behind necrolytic areas which histologically resemble toxic epidermal necrolysis (Sweet, 1974). The painful involvement of the tongue and the angles of the mouth interfere with food intake. Skin lesions may be agonizingly pruritic and painful, particularly in the perineum and genital areas. (Patient JH could barely move in bed when her rash was at its worst.)

A. Gross Appearance: A very recognizable characteristic lesion easily diagnosed by dermatologists (Tharp, personal communication). The rash normally involves buttocks, groin, perineum, thighs and distal extremities but may occur anywhere (Freedberg and Galdabini, 1976; Binnick et al., 1977; Gatrelli-Beltzer, 1980). Skin contact and trauma may play an important part in this distribution. Pale brown macules and papules with superficial scaling give it an eczematous psoriasiform appearance. Erythematous areas appear and enlarge, forming central blisters that break down and encrust. They heal over a 14-day period leaving a trail of pigmentation but no scarring. New lesions appear in crops and tend to coalesce into extensive circinate or gyrate patterns. There is a characteristic waxing and waning of the rash. Complications include bacterial and fungal superinfection, atrophic glossitis, cheilitis, stomatitis, ungular dystrophy and thinning of hair (Binnick et al., 1977). The rash may be present for >12 years prior to diagnosis (Stevens et al., 1984).

B. Histologic Appearance: Less helpful than clinical appearance (Tharp, personal communication). Lesions are distinctive only at the edge of a new lesion (Wilkinson, 1973): 1) Focal parakeratosis with superficial epidermal vesicles leading to subcorneal separation; 2) Swollen pale vacuolated cells with pyknotic nuclei; 3) Absence of acantholysis (Wilkinson, 1973; Binnick et al., 1977), marked perivascular infiltration and immunofluorescent staining for immunoglobulin or complement (Wilkinson, 1973; Kahan et al.,

1977). The lack of acantholysis is particularly helpful (Kahan et al., 1977; Swenson et al., 1978).

C. Possible etiologies: The cause is a mystery.

1. Glucagon: Glucagon has never been identified in the skin. The high glucagon levels that are present in mixed hormone-secreting tumors are generally unassociated with the rash. Also, the rash has been reported in a nonglucagonoma malignant islet cell tumor (Verbov, 1981).

2. Glicentin or PP effects: Although glicentin and PP are almost always increased in glucagonoma syndrome, it is difficult to imagine how they could produce a skin lesion.

3. Hypoaminoacidemia: Hypoaminoacidemia is invariably associated with glucagonoma syndrome. The hypoaminoacidemia of protein malnutrition (Kwashiorkor) is associated with a rash similar to necrolytic migratory erythema (Hennington et al., 1958). Histidine and tryptophane deficiency each cause scaling rashes (Leicher, 1980; Scully and McNeely, 1975). Moreover, dramatic improvement of necrolytic migratory erythema by intravenous amino acids has been reported by Norton et al. (1979) and Mallinson et al. (1977). However, glucagonomas without skin disease are also associated with hypoaminoacidemia.

4. Zinc deficiency: Amon et al. (1976), Mallinson et al. (1977) and Horrobin and Cunnane (1980) report low plasma zinc levels in glucagonoma syndrome and response in some cases to intravenous and topically administered zinc (Mallinson et al., 1977). However, normal zinc levels are common in glucagonoma syndrome.

5. Tryptophan deficiency: The pellagra-like appearance of the skin rash has suggested (Goldstein and Brown, personal communication) that glucagon might cause a Hartnup-like syndrome by decreasing tryptophan absorption and/or increasing tryptophan excretion and thus cause a niacin deficiency (Figure 6). A therapeutic trial of 200 mg/day of niacin, the dose recommended for Hartnup's disease, was inconclusive in patient JH, largely because she proved intolerant to the substance. No other support for this hypothesis is available. Normal tryptophane levels have been reported in the syndrome (Peterson et al., 1984).

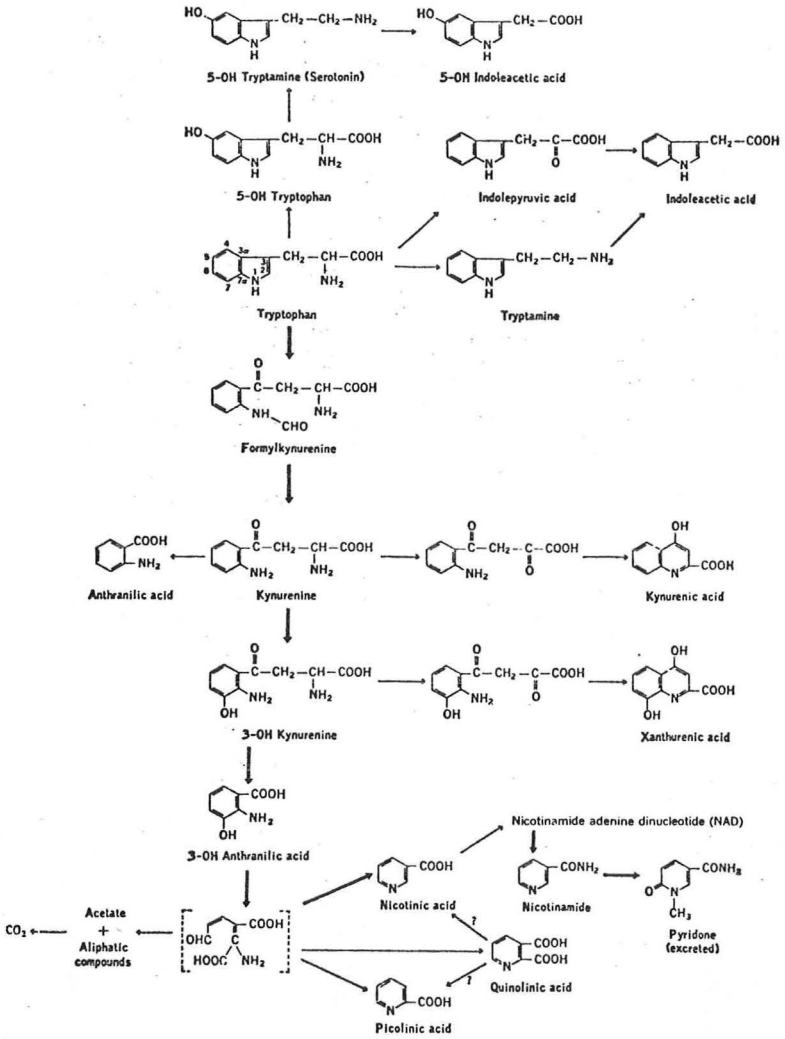

Fig. 6: Metabolic pathways which are open to tryptophan. Main pathways are shown in bold arrows. [From Jepson JB: Hartnup disease. In: Stanbury, Wyngaarden, Fredrickson (eds) Metabolic Basis of Inherited Disease, McGraw Hill, New York, p 1567, 1978.]

6. Essential fatty acid deficiency: This can cause an erythematous rash (Reilla et al., 1975). However, normal levels of essential fatty acids have been reported in the glucagonoma syndrome (Peterson et al., 1984).

7. Increased epidermal arachidonic acid and its metabolites: Peterson et al. (1984) report a significant increase in arachadonic acid in cultured keratinocytes (CK) exposed to glucagonoma plasma or normal plasma with added purified glucagon.

Table 5
Arachidonic acid (AA) levels

Test Specimen	AA (% of total fatty acids)
Normal pool plasma	5.2
Glucagonoma plasma	4.3
CK fed with normal pooled plasma	3.1
CK fed with normal pooled plasma with Sigma glucagon added	6.0
CK fed with glucagonoma patient's plasma	9.6

CK = cultured keratinocytes

They propose (despite a lack of compelling evidence) that glucagon increases arachadonic acid in the skin and thus causes the lesions (Peterson et al., 1984). Prostaglandin E_2 causes erythema, increased vascular permeability and potentiation of carrageenan-induced edema (Kingston and Greaves, 1985).

II. THE DIABETES MELLITUS: Although the prevalence of diabetes in the glucagonoma syndrome has been placed at 100% (Bhathena et al., 1981), this probably reflects generous diagnostic criteria for an abnormal glucose tolerance test. It should be kept in mind that in any illness as stressful as the glucagonoma syndrome glucose tolerance may be abnormal and stress hyperglycemia may be present.

A. Stress hyperglycemia vs. diabetes: Stress hyperglycemia, a compensatory response that maintains cerebral glucose delivery during life-threatening disorders that reduce cerebral blood flow (Lindsey et al., 1973; 1974; 1975; Willerson et al., 1974; Unger, 1981), is the consequence of hyperglucagonemia stimulated by increased levels of the hormones of stress [catecholamines (Marliss et al., 1970), cortisol (Marco et al., 1973), β-endorphin (Ipp et al., 1978), vasopressin (Dunning et al., 1982), growth hormone (Sirek et al., 1979), and quite possibly stress-induced release of neurotransmitters]. Morphine-like agents administered in such circumstances also stimulate glucagon and may exaggerate the hyperglycemia (Ipp et al., 1980). At the same time the normal compensatory response of insulin to hyperglycemia is reduced or inhibited by catecholamine release and correction of hyperglycemia is thus prevented (Porte et al., 1973). While such abnormalities are generally considered to be an acute feature of stressful events, in severely burned patients the hyperglucagonemia persists until healing (Figure 7) (Wilmore et al., 1974). Thus, serious involvement of a substantial portion of the skin may cause sustained hyperglucagonemia. This has import in interpreting the specificity of an elevated glucagon level in a dermatologic disease.

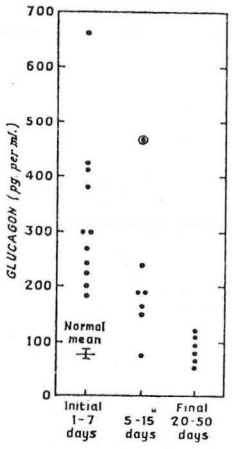

Fig. 7: Glucagon levels at various times after thermal injury. Circled dot indicates patient who had persistent infection.

B. Why overt diabetes is rare in hyperglucagonemic glucagonoma patients: The specific actions of glucagon are discussed below. Glucagon is necessary, but not sufficient for the production of diabetes; the insulin levels must be too low to overcome glucagon-induced overproduction of glucose and ketones by the liver. Most glucagonoma patients have enough normal islets to mount an adequate compensatory response of insulin and thus to prevent overt diabetes. However, the patient of Yoshinaga et al. (1966) had a massive alpha cell tumor that destroyed most of the pancreas, thereby preventing adequate compensatory insulin secretion; this patient had florid diabetic ketoacidosis.

There may be other reasons why the hyperglucagonemia of a glucagon-producing tumor does not often cause a florid diabetic state: 1) Much of the immunoassayable secretory product has reduced or absent biologic activity (Figures 8 and 9; see below); 2) Glucagon resistance: there is down-regulation of glucagon receptors (Srikant et al., 1977b) (Figure 10) that is compensated by enhanced cAMP response per occupied glucagon receptor (Figure 11), placing any glucagon resistance distal to adenylate cyclase; 3) The glycogenolytic effects of sustained hyperglucagonemia tend to be short-lived, although the gluconeogenic and ketogenic effects of sustained hyperglucagonemia do not wane (Cherrington and Liljenquist, 1981). When glucagon fluctuates rapidly, as in uncontrolled diabetes, its glycogenolytic activity persists (Fradkin et al., 1979). Thus, the fact that biologically active glucagon secreted by glucagonoma is always at very high levels would minimize its glycogenolytic activity, even if glycogen stores were adequate.

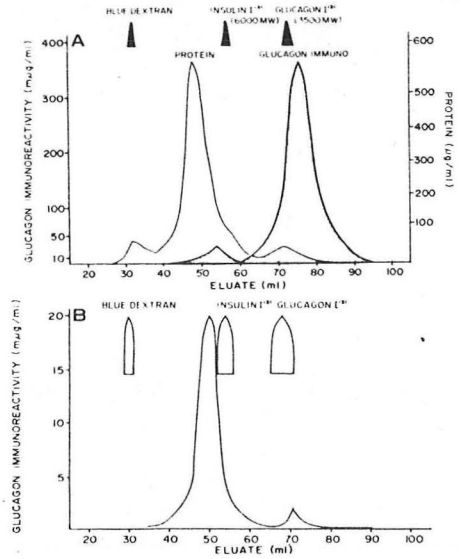

FIG. 8 *A (upper)*, elution pattern of glucagon immunoreactivity in extracts of canine pancreas. A typical pattern showing a small early appearing peak of immunoreactivity, LGI. *B (lower)*, elution pattern of immunoreactivity after the rechromatography of the LGI containing eluates. No shift in elution volume is noted.

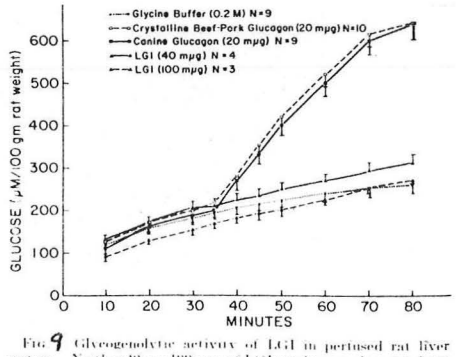

FIG. 9 Glycogenolytic activity of LGI in perfused rat liver system. Neither 10 nor 100 mμg of LGI produces a glycogenolytic response significantly different from the buffer control, in contrast to the dramatic effect of 20 mμg of both crystalline beef pork glucagon and canine glucagon. N, number of experiments.

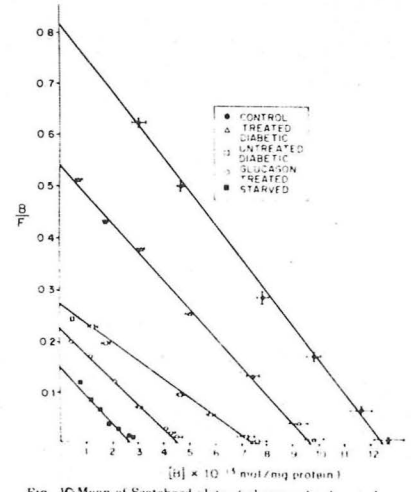

FIG. 10 Mean of Scatchard plots of glucagon binding to liver cell membranes of the normal and chronically hyperglucagonemic groups of rats. The ratio of the concentrations of bound and free ^{125}I-glucagon (*B/F*) is plotted on the *ordinate* against the concentration of membrane bound unlabeled glucagon (*B*) on the *abscissa*. The receptor concentration R_0 was calculated for each membrane preparation individually, but only the mean values of (*B/F*) and (*B*) are shown here.

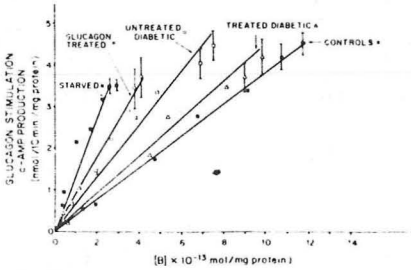

FIG. 11 Relationship between the concentration of membrane-bound glucagon and glucagon-stimulated cAMP production in normal and hyperglucagonemic rats. [*B*] is the concentration of membrane-bound glucagon. At higher glucagon concentrations, the cAMP levels obtained are shown as mean ± S.E.

cyclase through a pair of guanine nucleotide binding regulatory proteins (G proteins), one of which stimulates the cyclase (G_s), and one which inhibits its activity (G_i). In the absence of G_s, adenylate cyclase cannot be activated. (For reviews of these interactions see Gilman, 1984 and Rodbell, 1983). Within seconds after the administration of glucagon, the cyclic-AMP level rises in hepatocytes. (The level of cAMP is controlled by the relative activities of adenylate cyclase, which catalyzes the production of cyclic-AMP from ATP, and of phosphodiesterase, which degrades cAMP to AMP.) cAMP in the cytosol binds to and activates cAMP-dependent protein kinase. By phosphorylating certain key proteins in the hepatocyte and thus altering their functional activity, this enzyme initiates almost all of the known actions of glucagon (Figure 12a).

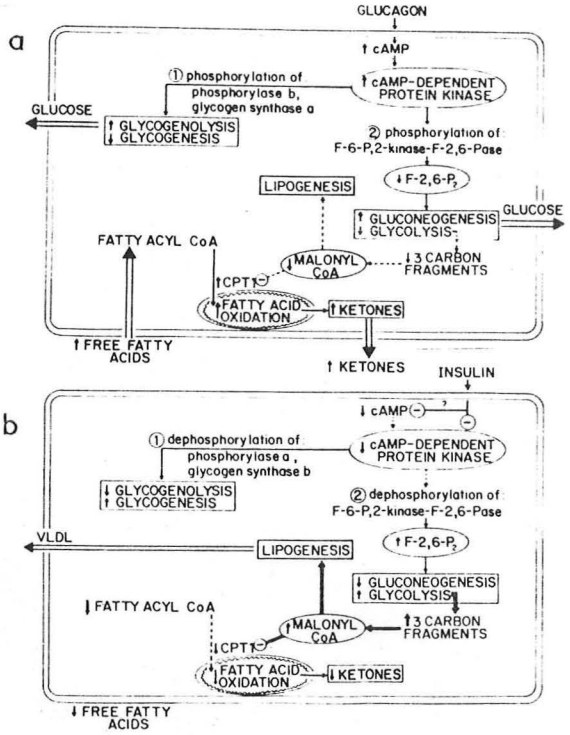

Fig. 12: The biochemical basis for insulin-glucagon interactions on fuel metabolism in the liver. See text. (From Unger and Foster, 1985)

There may also be a cAMP-independent mechanism for glucagon action comparable to that of alpha adrenergic agonists, vasopressin and angiotensin which activate liver phosphorylase by increasing cytosolic Ca^{2+} concentration (De Wulf et al., 1980).

2. Effects on hepatic glycogenolysis and glycogenesis (Figure 12a): Phosphorylation of phosphorylase-b kinase enables it to phosphorylate phosphorylase-b to the active form, phosphorylase-a, the rate-limiting enzyme for glycogenolysis in the liver. This accounts for the glycogenolytic action of glucagon and its short-term enhancement of glucose production. At the same time phosphorylation of active glycogen synthase-a inactivates it to the b form and thereby reduces glycogen formation (see Stalmans, 1983 for review).

3. Effects on hepatic gluconeogenesis and glycolysis (Figure 12a): Activated cyclic-AMP-dependent protein kinase also increases hepatic

gluconeogenesis and ketogenesis via phosphorylation mechanisms, and is thereby responsible for its long-term enhancement of hepatic fuel production. This is in large part mediated by changes in the level of fructose-2,6-bisphosphate (F-2,6-P_2) (Furuya and Uyeda, 1981; Van Schaftingen et al., 1981; El-Maghrabi et al., 1982a), a key regulator of glycolysis and gluconeogenesis (Figure 12a). F-2,6-P_2 allosterically stimulates the rate-limiting enzyme for glycolysis, 6-phosphofructo-1-kinase (PFK-1) and inhibits the rate-limiting enzyme of gluconeogenesis, fructose-1,6-bisphosphatase (FBPase-1). When fructose-2,6-bisphosphate levels in the hepatocytes are low, glycolysis is inhibited and gluconeogenesis is stimulated (see El-Maghrabi et al., 1982b, and Hers and Van Schaftingen, 1982, for reviews). The glucagon-mediated increase in cAMP-dependent protein kinase activity lowers F-2,6-P_2 levels by phosphorylating a bifunctional enzyme (Furuya and Uyeda, 1981; El-Maghrabi et al., 1982b), which, depending on its phosphorylation state, acts either as a kinase 6-phosphofructo-2-kinase (PFK-2) to form F-2,6-P_2 from F-6-P, or as a fructose-2,6-bisphosphatase (FBP2) to degrade F-2,6-P_2 to F-6-P. When in its phosphorylated state, the bifunctional enzyme assumes the role of degrader of F-2,6-P_2, thus blocking glycolysis and promoting gluconeogenesis. The sequence is as follows:

$$\uparrow \text{cAMP} \rightarrow \uparrow \text{cAMP-dependent protein kinase} \rightarrow$$
$$\uparrow \text{phosphorylation of the bifunctional enzyme} \rightarrow$$
$$\downarrow \text{F-2,6-}P_2 \rightarrow \downarrow \text{glycolysis and} \uparrow \text{gluconeogenesis}$$

This extremely important reaction is exquisitely sensitive to glucagon which reportedly can lower fructose-2,6-bisphosphate in concentrations as low as 10^{-13} (Richards et al., 1981). Phosphorylation of pyruvate kinase may also participate in the glycolytic block (see Claus et al., 1983, for review).

Insulin (Figure 12b) opposes the foregoing sequence of glucagon-mediated events largely by deactivating the cAMP-dependent protein kinase, thereby reducing the phosphorylation state of the above enzymes and reversing the glucagon-mediated reduction of gluconeogenesis and enhancement of glycolysis (Gabbay and Lardy, 1984) (Figure 12b). To a lesser degree it reduces cyclic-AMP levels via increased phosphodiesterase activity.

4. Effects on hepatic ketogenesis and lipogenesis (Figure 12a) (see Foster and McGarry, 1983, for review): The above schemes also help to explain the powerful ketogenic and anti-lipogenic actions of glucagon. By blocking the flow of carbon from glucose to acetyl-CoA, the substrate for lipogenesis, the fall in F-2,6-P_2 reduces glucose-derived fatty acid synthesis. Malonyl-CoA, the first product in the lipogenic pathway, inhibits carnitine palmitoyl transferase-1 (CPT-1). This is the enzyme that transesterifies fatty acyl CoA to fatty acyl carnitine, thus enabling it to enter the mitochondria, the site of fatty acid oxidation to ketones (McGarry et al., 1978). Glucagon reduces malonyl-CoA levels both by blocking glycolysis, as discussed above, and by inhibiting acetyl-CoA carboxylase (Cook et al., 1977), also through a phosphorylation mechanism (Lent et al., 1978). The liver is thereby converted into a potentially ketogenic organ.

However, the actual rate of hepatic ketone production will also depend on the availability to the liver of free fatty acids, which are the substrates for ketogenesis, and of carnitine. Low levels of the anti-lipolytic hormone insulin enhance the release of free fatty acids from adipocytes, providing an abundance for the liver (McGarry and Foster, 1980). Concomitantly, glucagon increases hepatic carnitine levels via a mechanism that has yet to be elucidated. To summarize, when glucagon levels are increased and insulin levels reduced, as in starvation or in insulin deficiency, three factors assure a high rate of ketogenesis: 1) high intrahepatic levels of fatty acyl CoA; 2) high intrahepatic levels of carnitine; and 3) activation of CPT-1. It is emphasized that in this circumstance de novo hepatic lipogenesis from products of glycolysis has been blocked and that the increased intrahepatic fatty acid CoA is derived from lipolysis of extrahepatic fat stores.

III. THE HYPOAMINOACIDEMIA

A. The mechanism; is it all an hepatic effect? It has been assumed that the mechanism of hyperaminoacidemia, perhaps the most common and specific metabolic lesion in glucagonoma, is at the level of the liver. Glucagon increases inward transport of certain amino acids into the hepatocyte (Freychet and LeCam, 1977; Fehlmann et al., 1979a and 1979b; Kelley et al., 1980a and 1980b). and plays an important role in hepatic retention of ingested amino acids via cAMP (LeCam and Freychet, 1976; Freychet and LeCam 1977; Fehlmann et al., 1979a and 1979b; Kelley et al., 1980a and 1980b). Hyperaminoacidemia occurs in glucagon deficient duodenopancreatectomized patients (Boden et al., 1980). Glucagon inhibits net protein synthesis (Pryor and Berthet, 1960) and increases protein catabolism (Mallette et al. 1969b), ureogenesis (Snodgrass et al., 1978) and gluconeogenesis (Fehlmann et al., 1979b). There is a 3-fold increase in alanine to glucose conversion by glucagon and by cAMP (Mallette et al., 1969a), again mediated by cAMP. Mallette et al. (1969b) found that glucagon increased intracellular utilization of glycine, alanine, glutamate, phenylalanine, but increased intracellular production of leucine, isoleucine, valine. Snodgrass et al. (1978) showed glucagon effects on amino acid catabolism with significant increases in serine dehydratase, homoserine dehydratase, tyrosine transaminase and ornithine aspartate and alanine transaminases. All of these effects are opposed by insulin.

However, glucagon, a stimulator of hepatic lysosomal activity (Ashford and Porter, 1962; Schworer and Mortimore, 1979; Amherdt et al., 1974) (Figure 13), and gluconeogenesis from hepatic protein, increases hepatic output of branched chain amino acids, an index of increased hepatic proteolysis. (NOTE: The lysosome level in a liver biopsy might have corroborative diagnostic value but has never been checked.)

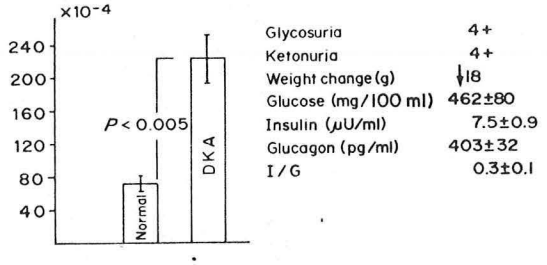

Fig. 13: A comparison of volume density of hepatic lysosomes of six rats with severe diabetic ketoacidosis with that of four normal rats, together with the laboratory data of the former.

B. The case for extrahepatic sites of glucagon-induced hypoaminoacidemia: Since glucagonoma patients, including patient J.H., have low, not high, levels of branched chain amino acids, one is virtually compelled to search for an extrahepatic explanation for the panhypoaminoacidemia. There are no studies of glucagon effects on either amino acid absorption or excretion. However, glucagon in pharmacologic quantities and glucagon precursors and derivatives are known to have profound effects upon both gastrointestinal and renal function.

1. Gastrointestinal actions: Glucagon inhibits gastrointestinal motility (Stunkard et al., 1955) independently of its effect on carbohydrate metabolism. It retards gastric emptying, inhibits gastric hydrogen ion secretion, but the only known effect upon absorption are to increase fluid and sugar transport. Its major actions are enterotrophic (cf below) and spasmolytic. The 1-21 derivative of glucagon, which is devoid of adenylate cyclase activating activity in adipocytes, is equipotent with glucagon in its spasmolytic action on the electrically stimulated guinea pig ileum (Diamant and Picazo, 1983). To summarize, while none of the documented actions of glucagon or its precursors or products are known to alter amino acid absorption, they do have biological effects on gastrointestinal tissues that may warrant a direct examination of this issue.

2. Renal actions: The kidney, the major physiologic site of glucagon degradation, is also a probable target of glucagon action. Pharmacologic levels of glucagon cause a strong diuretic effect in dogs with enhanced renal excretion of sodium, chloride, phosphate and potassium (Staub et al., 1956; 1957) via a stimulatory effect on prostaglandin synthesis by the kidney (Kirschenbaum and Zawada, 1980). Glucagon increases glomerular filtration. Thus, while there is no present experimental evidence to support an aminoaciduric effect of glucagon, the possibility warrants direct study.

IV. THE WEIGHT LOSS: Weight loss ranges from the trivial to the extreme (27 kg), but averages about 12.6 kg. Its reported occurence with a relatively localized glucagon-producing tumor suggests that the anorexic and catabolic effects of glucagon contribute to weight loss above and beyond the effect of the malignant disorder (Bhathena et al., 1981).

V. THE ANEMIA: Normocytic, normochromic anemia is present in most cases and varies from mild to severe. No abnormalities have been found in the marrow with respect to cellular turnover and iron stores (Mallinson et al., 1974b; Valverde et al., 1976).

VI. THE VENOUS THROMBOSES: Venous thrombosis is observed in about 30% of patients. The mechanism is unknown (Mallinson et al., 1974b; Pedersen et al., 1976).

VII. THE DEPRESSION: Psychiatric disturbances, in particular depression, are frequent. The etiology is unknown.

VIII THE DIARRHEA: Nonhypokalemic intermittent diarrhea occurs in about 20% of patients. Steatorrhea is not part of the syndrome. Coarse folds of the duodenum are common and in one case large jejunal villae were found (Stevens et

al., 1984). This has also been reported in a GLI-secreting enteroglucagonoma (Gleeson et al., 1971; Mallinson et al., 1974b).

It may well be that glucagon and/or its GLI forms, which are reasonably well established as co-trophic factors for liver regeneration (Leffert et al., 1975; 1983) and may well have enterotrophic activity as well. Mucosal thickening in the small bowel can be observed on CT and seem most associated with enteroglucagon (Jones et al., 1983).

IX. THE ASSOCIATED CLINICAL SYNDROMES

A. Polyglandular syndromes: Familial multiple endocrine neoplasia Type I (Stackpoole et al., 1981): In a MEA-I family three members with alpha cell tumors are described. Two of these had classic glucagonoma syndrome.

B. Insulinoma only: While increased levels of one islet cell secretory product, pancreatic polypeptide, produced by F-cells which replace alpha cells in the peripheral rim of islets located in the posterior portion of the head of the pancreas, are very common in glucagonoma, hypersecretion of other islet cell hormones is relatively rare. Somatostatin hypersecretion by a glucagonoma has yet to be reported, but several authors have noticed association of glucagonoma and insulinoma syndromes. Ohneda et al. (1979) reported conversion of a malignant insulinoma into a glucagonoma syndrome. Another patient, who had been diagnosised as having a malignant insulinoma with metastases to the liver, developed a glucagonoma syndrome 17 years after discovery of the original metastatic islet cell tumor (D'Arcangues et al., 1984). A third patient reportedly had three pancreatic tumors resected over an 8-year period of which two were immunocytochemically diagnosed as insulinoma and one as a glucagonoma. Glucagon cells were found in lymph nodes and multiple glucagon-containing nodules were found throughout the pancreas. It appears that the development of neoplasia in a remarkably tissue-specific manner can be induced by introducing foreign DNA into the genome with certain virus genes such as the simian virus 40 T antigen genes (Messing et al., 1985; Hanahan, 1985).

Among nonislet cell hormones, hypersecretion of parathormone, gastrin and calcitonin, cortisol and ACTH has been reported with glucagonomas (Bhathena et al., 1981).

ALPHA CELL TUMORS

I. BENIGN ALPHA CELL TUMORS

A. Subclassification: Benign alpha cell tumors fall into two groups: 1) predominantly alpha cell adenoma originating from a single islet or from more than one islet. These invariably are clinically asymptomatic (or at least clinically undiagnosable) and are discovered only at autopsy or in the course of abdominal surgery for some other reason. These tumors may well be the precursors of malignant glucagonomas. 2) benign alpha cell tumors in association with other endocrine adenomas that produce symptoms of excess of other hormones (gastrin, insulin, parathormone, etc.). These alpha cell tumors are usually unassociated with the devastating components of the glucagonoma syndrome, i.e., the necrolytic erythema migrans, perhaps because symptoms caused by the other hormones bring them to medical attention before malignant transformation has occurred.

B. Immunocytochemistry: In contrast to the more malignant tumors that are associated with the glucagonoma syndrome, the majority of cells in benign adenomata stain strongly positive with C-terminally directed anti-glucagon antiserum. These cells also react positively with antiglicentin antiserum. However, the number of cells that are glicentin-positive and glucagon-negative cells are very low if not absent - in marked contrast to the malignant tumors of the glucagonoma syndrome.

C. Electronmicroscopy: At the electron microscopic level the secretory granules are typical alpha granules with an electron-dense central core surrounded by a less dense halo -- again in contrast to the atypical granules observed in malignant alpha cell tumors associated with the glucagonoma syndrome.

D. Functional behavior: The cells of a benign adenoma may well be under normal functional control inasmuch as hyperglucagonemia has not been present in the few instances in which this has been measured. Normally, alpha cells are suppressed by an increase in glucose (Unger et al., 1962), insulin (Samols et al., 1972), somatostatin (Koerker et al., 1974) and secretin (Santeusanio et al., 1972) and are stimulated by amino acids (Unger et al., 1969) by most of the gastrointestinal hormones [CCK, GIP (Unger et al., 1967), gastrin], and by virtually all of the hormones of stress (growth hormone, β-endorphin, vasopressin, catecholamines, cortisol). It would appear that a benign adenoma obeys commands appropriately and fails to produce a hormonal disequilibrium or metabolic manifestations thereof.

NOTE: The best guess is that the benign adenoma is an undiagnosable lesion unless it is associated with other endocrine adenomas that produce clinical manifestations and bring the patient to medical attention. The reason that when associated with multiple endocrine adenomata benign alpha cell tumors do not exhibit the full glucagonoma syndrome may well be because the excess of other hormones brings them to medical attention before malignant transformation of the benign alpha cell tumor has occurred. This is an unfortunate probability, inasmuch as the syndrome would at this stage be easily curable; by the time the skin manifestations associated with hyperglucagonemia and hyperglicentinemia appear the chance for curability hs declined precipitously.

II. MALIGNANT ALPHA CELL TUMORS

A. Gross characteristics: Usually single and large (78% are greater than 5 cm in their major axis).

B. Histology: The variable cellular arrangements may take the form of trabecular and solid or diffuse patterns of growth, and occasionally anastamosing thin ribbons of cells or glandular formations. There is little or no histologic evidence of malignancy.

C. Evidence of malignancy: Local infiltration of blood vessels, adjacent tissues and/or metastases to liver, lymph nodes and spine, the former being the most common site of metastases.

D. Immunofluorescent staining: With specific (C-terminally directed) antiglucagon serum the distribution of glucagon-containing cells is uneven,

with unreactive cells predominating. Some of the glucagon-containing cells also react with antiglicentin antiserum, but the number of glicentin-positive cells usually exceeds cells that react with antisera specific for the C-terminal region of glucagon. This indicates that the cleavage of the C-terminal extension of the glucagon molecule did not take place in those cells (see below) and/or that the post-cleavage secretory product was emptied from the cell. It is quite likely that many of these malignant cells have a decreased capacity to convert glicentin to glucagon.

E. Electron microscopy: The morphology of the secretory granules in the tumor cell is very heterogeneous with only a minority of granules resembling the typical alpha granules of adult human islets (granules with a round dense core surrounded by an eccentric lighter halo). The majority of granules resemble neither alpha, beta, delta or PP granules. These atypical features of the granules may reflect the presence of glicentin without true glucagon. One would like to be able to claim that the so-called atypical granules are actually glicentin-containing L-cell granules (see below), but the evidence for this is not now available.

HOW NORMAL ALPHA CELLS AND L-CELLS FUNCTION: GLUCAGON, GLI-1 (GLICENTIN) AND GLI-2 (OXYNTOMODULIN)

I. **ALPHA CELLS; NORMAL SECRETION OF GLUCAGON:** Normally the 29 amino acid polypeptide glucagon (M.W. 3485) is secreted by alpha cells located in specific regions of the islets of Langerhans. In the rat alpha cells are arrayed in the periphery of the islet (Figure 14). In man the peripherally arrayed cellular elements form septum-like perivascular extensions towards its center (Figure 14). In the dog alpha cells are also present in the fundus of the stomach; they have typical alpha secretory granules containing glucagon (Baetens et al., 1976) that is immunologically, physicochemically and biologically indistinguishable from pancreatic glucagon (Sasaki et al., 1975; Srikant et al., 1977a). In normal human adults extrapancreatic glucagon-producing cells are sparsely distributed in the gastrointestinal tract (Munoz-Barragan et al., 1977).

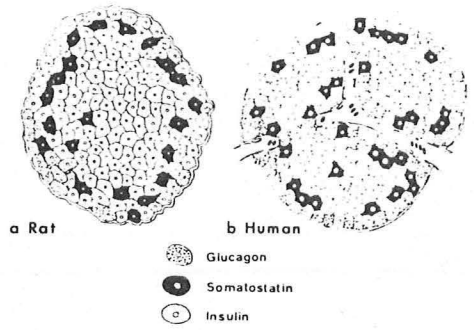

Fig. 14

II. **L-CELLS; NORMAL SECRETION OF GLI:** L-cells (A-like cells) were first discovered in 1968 (Orci et al., 1968) at the time of the discovery of GLI (Unger et al., 1968) (Figure 15). A relationship between alpha and L-cells was first suspected on morphologic grounds and more compellingly established by immunocytochemical identification (Ravazzola and Orci, 1980). Early in the course of the development of the radioimmunoassay for glucagon it was found that while most glucagon antisera reacted with both pancreatic and jejunal

extracts, a rare antisera would react only with pancreatic extracts (Unger et al., 1968; Valverde et al., 1970a). Antiserum 30K is the most famous example of the latter. It was unreactive with des-26-29-glucagon, whereas the antisera that reacted with jejunal extracts were equally reactive with 1-29 and 1-26 and 1-21 glucagon. Consequently, the terms "C-terminally reactive" or "pancreas-specific" antiglucagon serum were applied to the 30K type of antibody and "N-terminal" or "centrally directed" or "nonspecific" antiserum were applied to the other antibody. Such information also led to the conclusion that the jejunum contains a molecule which crossreacts with N-terminally directed antiserum for glucagon. At first we named this molecule "enteroglucagon", but because its structure at least at its C terminus and its secretory responses were so different from pancreatic glucagon (Figure 16), we renamed it "glucagon-like immunoreactivity" or "GLI". However, the name enteroglucagon proved more popular and a confusing dual nomenclature resulted. During the late 1960s and early 1970s we learned that GLI consisted of a large and small fraction, GLI-1 and GLI-2 (Valverde et al., 1968; 1970a; 1970b). Workers in the Novo Institute in Copenhagen initially believed that GLI-1 consisted of 100 amino acids and they therefore gave it the name "glicentin" (Jacobsen et al., 1977). Although they subsequently found that it contains only 69 amino acids (Thim and Moody, 1981), the name "glicentin" has survived. Glicentin includes the entire 29 amino acid sequence of glucagon extended at its C terminus by a hexapeptide linked to glucagon by a dibasic amino acid pair and at its N terminus by a similarly attached peptide called glicentin-related peptide or GRP (cover figure). In alpha cells, but not in L-cells, the C-terminal extension is cleaved while the N-terminal extension is cleaved both in L cells and in alpha cells (cover figures). Thus, L cells, it is now believed, release both intact glicentin (GLI-1) and glicentin minus the N-terminal GRP segment but with the C-terminal extension; we used to call this GLI-2, but it is now oten referred to as "oxyntomodulin" because it increases cAMP levels in parietal cells (Bataille et al., 1981; 1982). Both glicentin and oxyntomodulin are secreted by L-cells during any absorptive event (Valverde et al., 1970a). The intraduodenal instillation of monosaccharides (Unger et al., 1968), amino acids (Unger et al., 1969), fat (Bottger et al., 1973) and salts (Bottger et al., 1972) was observed many years ago to produce an outpouring of GLI measurable with glucagon antisera directed against the N-terminal portion of the glucagon molecule but not with antisera directed against the C terminus (Figure 16). Thus a carbohydrate meal will suppress true glucagon (Muller et al., 1970) as measured with C-terminally directed antiglucagon antibody but stimulate GLI measured with N-terminally direct antiglucagon (Bottger et al., 1972). C-terminally directed antiglucagon serum measures in addition to true pancreatic glucagon, a larger biosynthetic intermediate (Rigopoulou et al., 1970) with a fully exposed C terminus of glucagon but with an apparent molecular weight of 9000, which suggests that the N-terminal extension is uncleaved, i.e., its GRP is still attached (Figure 8 and cover figure). It was originally named "proglucagon" but is now called IRG^{9000}. IRG^{9000} does not bind to glucagon receptors of purified rat hepatocyte membranes (Figure 17a), increase cAMP therein (Figure 17b) or increase glucose production by the isolated perfused rat liver (Rigopoulou et al., 1970) (Figure 9) and is presumably inactive (Srikant et al., 1977a). This would support the view of Rodbell (1983) that the N terminus of glucagon binds to the glucagon receptor, although integrity of the C-terminal region appears to determine the level of biologic activity of the molecule at least in hepatocytes.

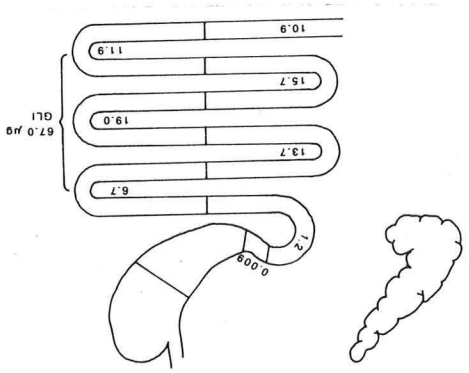

Fig. 15: The distribution of glucagon-like immunoreactivity acid-alcohol extracts of the tissues of the upper gastrointestinal tract of a dog.

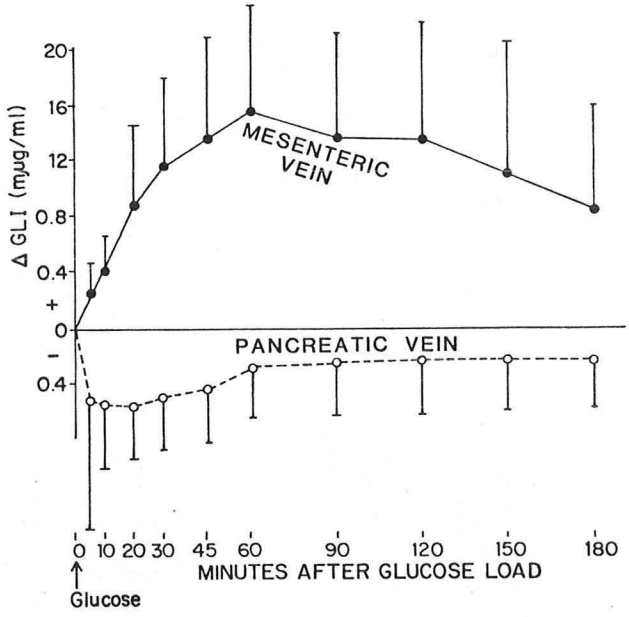

Fig. 16: The change in total glucagon-like immunoreactivity in the mesenteric vein and pancreatic vein of normal dogs following an intraduodenal glucose load. Measurements were made with a "nonspecific" or N-terminally directed glucagon antiserum which could not discriminate between true pancreatic glucagon and glucagon-like immunoreactivity from the intestine. The fact that pancreatic vein immunoreactivity was suppressed while mesenteric vein activity rose markedly provided the first evidence that one was dealing with two physiologically different sources of glucagon-like immunoreactivity (Unger et al., 1968 adaptation). The subsequent development of a C-terminally directed antibody against glucagon (antibody 30K) made it possible to assay true pancreatic glucagon without simultaneously measuring intestinal GLI.

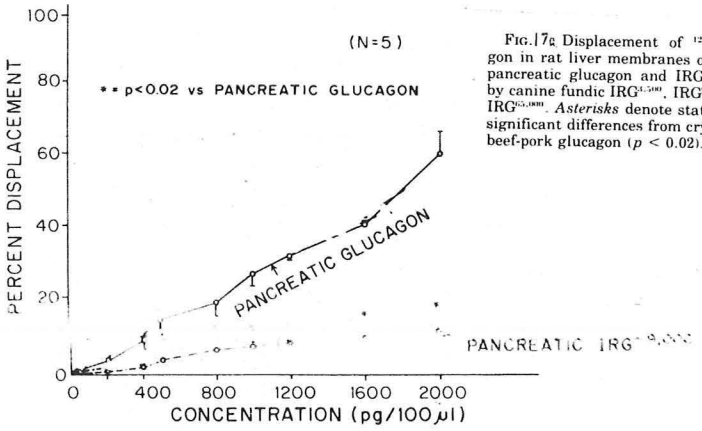

FIG. 17a Displacement of ^{125}I-glucagon in rat liver membranes of canine pancreatic glucagon and IRG^{9,000} and by canine fundic IRG^{4,500}, IRG^{9,000}, and IRG^{65,000}. *Asterisks* denote statistically significant differences from crystalline beef-pork glucagon ($p < 0.02$).

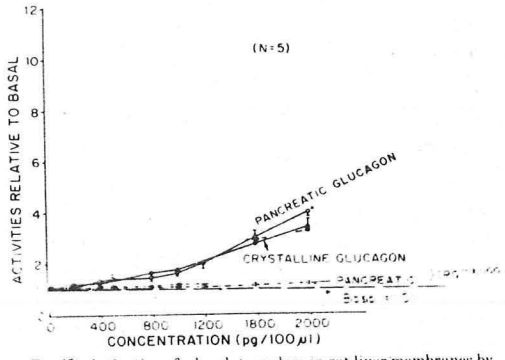

FIG. 17b Activation of adenylate cyclase in rat liver membranes by crystalline beef-pork glucagon, canine pancreatic glucagon, and IRG^{9,000}, and canine fundic IRG^{4,500}, IRG^{9,000}, and IRG^{65,000}. Activities are expressed relative to basal level taken as 1.0. *Asterisks* signify statistically significant differences from crystalline beef-pork glucagon ($p < 0.01$). Both fundic IRG^{9,000} and pancreatic IRG^{9,000} differ significantly from the crystalline glucagon at the three highest concentration points ($p < 0.02$). Immunometrically equivalent concentrations of the IRGs are expressed in picograms/100 µl.

III. ALPHA CELL AND L-CELL SECRETORY GRANULES (Figure 18): The typical alpha granules in human alpha cells have a dense glucagon-containing core and a less dense glicentin-containing halo. The granules of L-cells contain no true glucagon and consist of homogeneous glicentin-positive material (Figure 18).

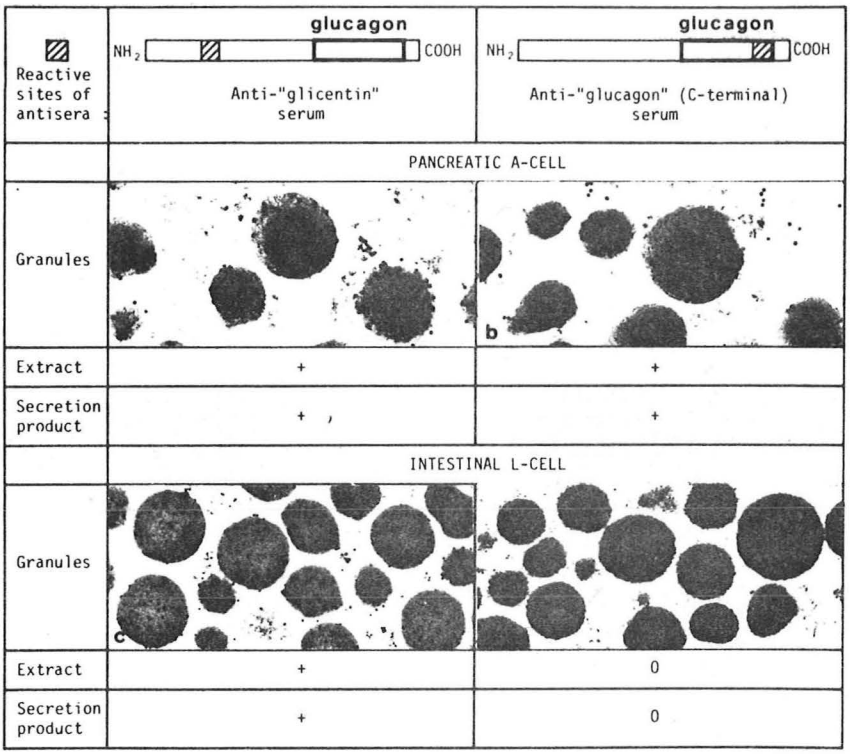

Fig. 18: Reactivity of an anti-"glicentin" serum and an anti-"glucagon" (C-terminal) serum. Both sera are shown in reaction with the precursor molecule of glucagon biosynthesis (a), with secretory granules of pancreatic A cells (b), and with secretory granules of small intestine L cells (c). The black particles in b and c represent antigenic sites revealed by the protein A-gold method.

PHYSIOLOGIC ROLE OF GLUCAGON AND THE FUNCTION OF PANCREATIC ALPHA CELLS
(For a complete review see Unger and Orci, 1981)

The function of glucagon is to prevent hypoglycemia between meals, during carbohydrate deprivation and during exercise, thereby maintaining adequate source of fuel for the glucose-dependent central nervous system. Consequently, normal alpha cells respond to fasting, carbohydrate-free meals and exercise with an increase in glucagon secretion. The increase in glucagon is mediated both by changes in circulating levels of influential nutrients such as glucose and amino acids, but also by humoral and neurotransmitted signals.

During carbohydrate abundance glucagon's action is unnecessary and would tend to interfere with normal storage of ingested carbohydrate. Consequently, during carbohydrate meals glucagon levels are normally suppressed.

ALPHA CELL FUNCTION IN DISEASE

Diabetes: Alpha cell function is abnormal in all forms of diabetes (Unger et al., 1970; Unger and Orci, 1981), by far the most common disorder of alpha cell function. The following abnormalities are observed: 1) relative or absolute hyperglucagonemia; 2) exaggerated response to all stimuli (amino acids, exercise, humoral secretagogues); 3) loss of alpha cell suppression during a rise in glucose concentration. The etiology of these abnormalities are multiple and extremely complex and the details are not relevant to this discussion (see Unger and Foster, 1985, for review). Suffice it to say that the islets of Type I diabetic subjects resemble alpha cell microadenomata with more than 75% of the islet composed of glucagon-containing alpha cells. Hypersecretion of glucagon by these cells is primarily a consequence of insulin lack (Figure 19) (Maruyama et al., 1984) and can be completely suppressed by insulin repletion (Braaten et al., 1974).

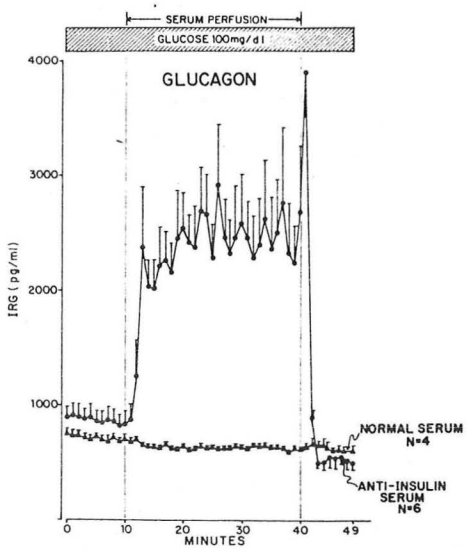

Fig. 19: The effect of anti-insulin serum (closed circles) on glucagon secretion (mean ± SEM) in the isolated perfused rat pancreas. Normal guinea pig serum (closed triangles) was used as a control. (From Maruyama et al., 1984).

THE LABORATORY DIAGNOSIS OF GLUCAGONOMA

The diagnosis requires: 1) Fasting or non-fasting hyperglucagonemia with total IRG (with C-terminally directed antiglucagon antibody) greater than 800 pg/ml in the absence of diabetes, cirrhosis, renal insufficiency or acute stressful disease. (As mentioned, any extensive skin disease may cause nonspecific hyperglucagonemia, but unless glucagonoma is present, a pharmacologically induced remission of the skin disease will be associated with a decline in secondary hyperglucagonemia.) 2) A relatively high percentage of total IRG may be due to IRG^{9000}. While IRG^{9000} is present normally and may be high in the rare patient with familial hyperproglucagonemia and in renal failure, an IRG^{9000} fraction of more than 50 percent of the total IRG favors a diagnosis of glucagonoma. 3) Functional tests are not very helpful. In most cases glucagon levels are suppressed by a rise in glucose [our first patient (A.H.) exhibited a decline from 50,000 pg/ml to 25,000 pg/ml during i.v. GTT]. Insulin suppresses glucagon secreted from tumors, which may explain the variable response to glucose. Secretin, which normally suppresses glucagon, is said to stimulate glucagon from glucagonomas. Other functional tests have been proposed but none of them seem of value. 4) Hyperglicentinemia - High levels of glicentin as measured with an antiglicentin serum (not available outside of Denmark) or with an N-terminally directed antiglucagon serum are extremely important markers of the syndrome. Unfortunately, not enough data is available to evaluate the diagnostic

importance of high levels of GLI. On the basis of what evidence is available, a high GLI level is virtually diagnostic of glucagonoma syndrome. If glucagon is normal in the presence of a high GLI level, one would suspect a glipoma. Only one glipoma patient has been described with the primary tumor in the kidney. There was severe intestinal stasis and hypertrophy of the small intestinal mucosa, all of which disappeared when the tumor was removed (Gleeson et al., 1971; Bloom, 1972).

Identification of the tumor: Since glucagonomas have thus far arisen only in the pancreas, demonstration of primary pancreatic tumor is essential for the diagnosis and for appropriate treatment. Celiac angiography has been the most commonly used diagnostic procedure for this purpose but Breatnach et al. (1985) maintain that CT is the mainstay in the identification, localization and staging of tumors. They have detected tumors ranging from 2.5 to 6 cm in maximal diameter. The primary tumors are solid and all are hypervascular. Calcification has been observed in the primary tumor. Metastases should be expected if the patient has a glucagonoma syndrome. The liver is the most common site of metastases. Large cystic masses have been observed, including one tumor measuring 14 x 15 cm.

TREATMENT

Treatment of the tumor:

Surgical: Surgery is at present the only means for a complete cure. Because the tumor will almost always have metastasized at the time of diagnosis one must resect both the primary lesion and the metastases to effect a cure. Only rarely will this be possible. Nevertheless the debulking may cause a gratifying remission in the disease which may be long-lasting because of the usually slow rate of tumor growth.

Chemotherapy: Although streptozotocin has been employed, the results have not been encouraging. The most useful chemotherapeutic agent thus far studied has been dimethyltriazenoimidazole carboxamide (Marynick et al., 1980). Streptozotocin in combination with 5-fluoracil (Khandekar, 1979; Moertel et al., 1980) is under investigation in the treatment of all islet cell tumors. Chlorozotocin, which is related to streptozotocin and streptozotocin plus doxyrubicin are similarly under investigation.

Hepatic artery embolization: This has been used in patients with hepatic metastases when surgery is impossible and chemotherapy ineffective. The purpose is to obliterate the arterial blood supply to the metastases.

Treatment of the necrotizing migratory erythema:

Treatment of the skin disease may become an urgent necessity because of the chronic agony, the catabolic effects of extensive skin disease and the reluctance of surgeons to operate through severely diseased skin. Although steroids have produced temporary remissions, this is not a recommended therapeutic option. While gratifying responses have been reported with both oral and topical zinc preparations and with intravenous amino acid infusion, the somatostatin analogs, such as SMS 995-201, have been dramatically effective in clearing the skin disorder. Although the rationale for their administration was suppression of glucagon and/or GLI secretion, in fact they appear to be ineffective in this respect (Figure 4).

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