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G Boden, … , T E Lobe, O E Owen

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Research Article

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Glucagon Deficiency and Hyperaminoacidemia after Total Pancreatectomy

GUENTHER BODEN, RAVIDATT W. MASTER, IRAJ REZVANI, JERRY P. PALMER, THOM E. LOBE, and OLIVER E. OWEN, *Departments of Medicine and Pediatrics, and the General Clinical Research Center, Temple University Health Sciences Center, Philadelphia, Pennsylvania 19140; Department of Medicine and the Diabetes Research Center, University of Washington, Seattle, Washington 98101; Department of Surgery, Ohio State University Hospital, Columbus, Ohio 43201*

ABSTRACT The first goal of this study was to investigate whether totally pancreatectomized patients are glucagon deficient and if so, to what degree. Immunoreactive glucagon (IRG) concentrations in peripheral plasma of nine pancreatectomized patients were not significantly different from those of 10 normal controls as measured by two antisera (30-K and RCS-5) both detecting the COOH-terminal portion of the molecule and one (RCS-5) postulated to be specific for pancreatic glucagon. Plasma from six of nine pancreatectomized patients were fractionated over Sephadex G-50 and IRG was measured with both antisera in the column eluates. Using 30-K, 80.8±9% of the IRG eluted within the void volume. This material was rechromatographed on Sephadex G-200 and found to have an apparent mol wt of ~200,000. Only 18.3±9% eluted in the IRG^{3500} region. IRG^{3500} was significantly reduced in pancreatectomized patients as compared to normal controls (49±9 vs. 18±9 pg/ml, $P < 0.05$). Using RCS-5, all IRG (corresponding to 20±6 pg/ml of plasma) eluted in the IRG^{3500} region. The second goal of this study was to investigate the effects of chronic glucagon deficiency on plasma amino acids. In the nine pancreatectomized patients studied, postabsorptive plasma concentrations of serine, alanine, arginine, glycine, threonine, citrulline, α-aminobutyrate, and tryosine were significantly elevated compared to values obtained from 20 normal controls. Physiological glucagon increments produced in two pancreatectomized patients by infusion of glucagon (6.25 and 8.0 μg/h, respectively) resulted in normalization of the hyperaminoacidemia within 22 h. We conclude (*a*) that pancreatectomized patients are partially glucagon deficient because of diminished basal as well as diminished stimulated glucagon secretion; (*b*) that fasting concentrations of certain glucogenic amino acids are elevated in pancreatectomized patients probably as result of reduced hepatic gluconeogenesis; and (*c*) that the RCS-5 antiserum is not "pancreatic glucagon" specific.

INTRODUCTION

It has recently been shown that glucagon plays an important role in the control of glucose homeostasis (1) and ketone body production (2) in human subjects. Moreover, Unger and Orci (3) have described diabetes mellitus as a bihormonal disease characterized not only by insulin deficiency but also by glucagon excess. This view has been challenged by Barnes and Bloom (4) who found that totally pancreatectomized patients had no circulating plasma immunoreactive glucagon (IRG)[1] but readily developed hyperglycemia and ketoacidosis if deprived of exogenous insulin. Others, however, have detected varying amounts of glucagon in plasma of pancreatectomized patients (5–8). Thus, the solution to the question of whether or not glucagon remains in circulation of patients after total pancreatectomy has become important for the evaluation of the role of glucagon in the pathogenesis of diabetes mellitus.

There is also some evidence to suggest that glucagon, besides its effects on glucose and ketone metabolism, may play an important role in human amino acid metabolism. For instance, infusion of large glucagon doses lowers plasma amino acids (9); patients with glucagon-

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Please address requests for reprints to Dr. Boden.

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[1] *Abbreviations used in this paper*: IRG, immunoreactive glucagon; IRG^{3500}, 3500-dalton IRG; NPH, neutral protamine Hagedorn (insulin).

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secreting islet cell tumors (glucagonoma) have severely depressed blood amino acid levels and advanced muscle wasting (10, 11). However, this aspect of glucagon action has been relatively neglected and the evidence remains incomplete. Most of the previous work was done with pharmacological doses of glucagon and the significance of these studies for human physiology can be seriously questioned (12). Sherwin et al. (13) infused glucagon into normal overnight-fasted volunteers at a rate of 3 ng/kg per min, which raised plasma glucagon concentrations from about 100 pg/ml to slightly above 300 pg/ml. They observed no consistent changes in amino acid concentrations except for a 7% decrease in serine concentration during the 150–180-min infusion period. Doubling of the infusion rate, however, resulted in a 10–19% reduction in glycine, serine, and threonine concentrations. Effects of infusions of physiologic doses of glucagon on plasma amino acids have also been studied in obese subjects after prolonged fasting (14). Results in this group can be expected to be different because obesity (15) as well as fasting (16) have effects of their own on amino acid metabolism. The effects of glucagon deficiency on amino acids have never been studied. It appears, therefore, that the evidence for a physiologic role of glucagon in amino acid metabolism could be strengthened considerably if it could be demonstrated that chronic glucagon deficiency has effects that are opposite to those seen during chronic glucagon excess (glucagonoma).

This study was, therefore, designed to answer the following questions: (*a*) Are pancreatectomized patients glucagon deficient, and if so, to what extent? (*b*) Do these patients have abnormal amino acid profiles, and if so, can these abnormalities be related to glucagon deficiency?

METHODS

Patients. Clinical data on the nine pancreatectomized patients (three males, six females) studied are listed in Table I. All underwent total pancreatectomy, duodenectomy, gastrectomy, and splenectomy. In addition, three had partial jejunostomy, five had cholecystectomy, and three had vagotomy. The reasons for these procedures were: adenocarcinoma in six patients, recurrent pancreatitis with intractable pain, insulinoma, and suspected vipoma in one patient each. Preoperatively,

TABLE I
Clinical Data of Nine Pancreatectomized Patients

Patients	Sex	Age	Percent ideal weight	Reason for Px	Operation	Studied after Px	FBS day of study	Insulin treatment			
									a.m.	Noon	p.m.
		yr				*mo*	*mg/dl*			*U*	
E.G.	F	43	73	Suspected vipoma	Px, Dx, Ax, Sx, cholecystojejunostomy	4	70	NPH	20	—	10
V.B.	F	59	77	Adeno Ca	Px, Dx, Ax, Partial Jx, Sx	7	120	NPH Regular	15 5	— 10	— 15
L.D.	F	63	93	Adeno Ca	Px, Dx, Ax, Sx	5	57	Lente	12	—	—
C.C.	F	39	92	Adeno Ca	Px, Dx, Ax, Prox. Jx, Sx, Cx, Vagotomy	6	200	Lente Regular	7 6	— —	6 5
R.B.	M	47	120	Adeno Ca	Px, Dx, Ax, Sx, Cx	6	119	NPH Regular	30 10	— —	— —
M.C.	F	59	78	Adeno Ca	Px, Dx, Ax, Prox. Jx, Cx, Sx	7	100	NPH	15	—	—
C.W.	M	45	112	Adeno Ca	Px, Dx, Ax, Cx, Sx, Vagotomy	2	188	NPH Regular	25 10	— —	— —
A.H.	M	30	90	Insulinoma	Px, Dx, Ax, Sx	108	292	Lente Semi-lente	21 10	— —	— —
E.D.	F	49	77	Pancreatitis	Px, Dx, Ax, Vagotomy, Cx, Sx	5	130	NPH	20	—	—

Abbreviations used in this table: Ax, antrectomy; Cx, cholecystectomy; Dx, duodenectomy; Jx, jejunectomy; Px, pancreatectomy; Sx, splenectomy.

none of the patients had diabetes or any other endocrinopathy. Postoperatively, the patients were managed with low-fat diets, which were free of concentrated carbohydrates, insulin, and pancreatic enzyme replacements. A detailed breakdown of the consumed diet was obtained in four patients. They consumed between 1,780 and 4,260 cal/d consisting of carbohydrates (42–49%), protein (21–28%), and fat (27–34%). All patients received their last insulin dose 24 h before the collection of blood for glucagon and amino acid determination, except for patient V.B. who received 15 U of regular insulin 16 h before blood sampling. None of the patients had clinical signs of dumping or steatorrhea, although, 72-h fecal fat excretion was increased to 76 and 95 g, respectively, in the two patients where it was determined. Weights of all patients had been stable for at least 1 mo at the time of study.

Controls. Plasma amino acid profiles were determined in 20 healthy subjects (12 males, 8 females; mean age, 31 yr; range, 20–45 yr) and in 5 patients with gastrectomy (4 males, 1 female; mean age, 53 yr; range, 33–66 yr). Three of these patients had total gastrectomies because of gastric carcinoma and two had partial gastrectomies because of peptic ulcer disease. Normal plasma IRG values were determined in 10 healthy subjects (8 males, 2 females; mean age, 33 yr; range, 21–46 yr). Normal molecular IRG distribution patterns were obtained in plasma of five healthy subjects (four males, one female; mean age, 38 yr; range, 28–46 yr).

Chemical analyses. Blood from nine pancreatectomized patients was collected into ice cold tubes that containing EDTA plus Trasylol (500 kallikrein inhibitory U/ml; FBA Pharmaceuticals, Inc., New York) after an overnight fast and before administration of insulin. Plasma was separated and stored at −20°C until assayed for glucose (17), amino acids, and glucagon. Amino acids were measured with an amino acid AutoAnalyzer (Phoenix Precision Instrument Div., Gardiner, N. Y.) (18). Analyses were performed on 0.5 ml samples of plasma, that had been deproteinized with 4.5% sulfosalicylic acid. Norleucine (0.25 μmol per sample) was used as internal standard. Glutamine and glutamate were not determined because of their instability in frozen plasma. Asparagine could not be measured with this technique because it eluted from the column together with glutamine. Two different antisera, were used to measure IRG (19). The 30-K antiserum was obtained from Dr. R. H. Unger, Dallas, Texas. This antiserum recognizes the COOH-terminal portion of the glucagon molecule. The RCS-5 antiserum was obtained from Dr. S. R. Bloom, London. This antiserum also reacts with the COOH-terminal end of the molecule. Both antisera have been reported to have minimal cross-reactivity with human gut extracts (19, 20). For the assay, 0.25 ml of plasma or 0.9 ml of column eluate were added to 0.75 ml or 0.1 ml of assay buffer, respectively. Sensitivity with the 30-K antiserum was 20 pg/ml of plasma and 6 pg/ml of column eluate. Using the RCS-5 antiserum, sensitivity was between 5 and 10 pg/ml of plasma and 2–3 pg/ml of column eluate.

Gel filtration. Sufficient plasma was available for fractionation from six of the nine patients. 5-ml samples of postabsorptive plasma were lyophilized, redissolved in 3 ml of borate buffer (0.05 M, pH 8.0) and gel filtrated over Sephadex G-50 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) fine columns (1.5 × 90 cm) as described (11). 2-ml fractions were collected, of which 0.9-ml aliquots were used for glucagon assays using the 30-K and the RCS-5 antisera. The void volumes from the Sephadex G-50 columns of three pancreatectomized patients (V.B., E.G., C.C.) were individually pooled, lyophilized, redissolved in 3 ml of borate buffer, and gel filtrated over Sephadex G-200 columns (1.5 × 90 cm). Equilibration, elution buffer, collection, and assay of eluates were as described for the Sephadex G-50 fractionations. The flow rate was 6 ml/h. The following column markers were used: fibrinogen ($330 \cdot 10^3$), catalase ($200 \cdot 10^3$), lactate dehydrogenase ($140 \cdot 10^3$), malate dehydrogenase ($70 \cdot 10^3$), and ^{125}Iodine (salt peak marker).

Glucagon infusions. Two patients (E.G. and V.B.) received glucagon (beef-pork, Eli Lilly & Co., Indianapolis, Ind.) by continuous intravenous infusion at rates of 6.25 and 8.0 μg/h for 24 and 22 h, respectively. During the glucagon infusion E.G. received two injections of neutral protamine Hagedorn insulin (20 U before breakfast and 10 U before dinner). V.B. received regular insulin by continuous infusion (2.4 U/h between 8 a.m. and 8 p.m., and 1.2 U/h between 8 p.m. and 6 a.m.). E.G. consumed 1,720 cal (carbohydrates, 196 g; protein, 177 g; fat, 52 g), whereas V.B. consumed 2,134 cal (carbohydrates, 196 g; protein, 112 g; fat, 75 g). The same diets and insulin regimens were given during the 24-h period preceding the glucagon infusion, which served as control period.

Arginine and glucose infusions. E.G. and V.B. also received arginine (30 g/30 min) by intravenous infusion as described (11). Glucose (0.5 g/kg body wt) was given by continuous intravenous infusion for 20 min to patient E.G. Patient V.B. received the same dose as intravenous bolus within 3 min. Results of intravenous arginine and glucose testing done on patients A.H., E.B., and E.D. have been published (8).

RESULTS

Basal plasma IRG (Table II). With the 30-K antiserum, mean basal IRG concentration of nine pancreatectomized patients was 286±101 pg/ml, not significantly different from the 213±46 pg/ml value obtained in 10 normal controls. 1 of 10 controls and 3 of 9 pancreatectomized patients had basal IRG concentrations far exceeding the normal range (50–270 pg/ml in our laboratory). Excluding these very high values, mean IRG was 102±15 pg/ml in pancreatectomized patients and 169±10 pg/ml in normal controls. The difference again was not statistically significant. With the RCS-5 antiserum mean basal IRG concentrations was 44±8 pg/ml in the nine pancreatectomized patients and 70 ±10 pg/ml in the 10 controls ($P > 0.05$).

TABLE II
Fasting Plasma IRG (pg/ml) in Pancreatectomized Patients and in Normal Subjects

Pancreatectomy patients	30-K	RCS-5	Normal subjects	30-K	RCS-5
E.G.	161	42	I.T.	215	25
V.B.	692	45	V.T.	619	66
L.D.	103	56	G.B.	168	55
C.C.	880	79	M.C.	157	56
R.B.	95	40	R.G.	126	34
M.C.	125	<40*	E.P.	157	64
C.W.	55	24	R.M.	154	72
A.H.	385	73	I.C.	215	111
E.D.	75	40	J.G.	157	83
			T.R.	169	130
Mean±SEM	286±101	44±8		213±46	70±10

* For calculation this value was considered equal to zero.

Molecular species of IRG in pancreatectomized patients (Fig. 1, Table III). Sufficient plasma was available from six of the nine pancreatectomized patients to allow identification of molecular IRG species by fractionation over Sephadex G-50 columns and assay of the eluates with both antisera. Using the 30-K antiserum an average of 80.8±9% of total immunoreactivity eluted within the void volume, whereas 18.3±9% eluted in the area of the glucagon marker. Mean plasma 3,500-dalton IRG (IRG^{3500}) was calculated to be 18±7 pg/ml. No IRG^{3500}, however, was detected in the plasma of patient C.C. and E.G. By comparison 75.8±5% of total IRG from five normal controls eluted within the void volume, whereas 24.2±5% eluted in the area of the glucagon marker. Mean IRG^{3500} in normal controls was 49±9 pg/ml, which was significantly greater than that in pancreatectomized patients ($P < 0.05$).

Using the RCS-5 antiserum, all IRG was found in the IRG^{3500} area in all five patients tested (patient R.B.'s samples were lost and could not be assayed with this antiserum). Small amounts of IRG^{3500} were detected in patients C.C. and E.G. with the RCS-5 but with not the 30-K antiserum, probably because of the greater sensitivity of the RCS-5 antiserum. Mean plasma IRG^{3500} was calculated to be 30±6 pg/ml. Recoveries of plasma IRG was poor in four of the five patients (range, 22–41%), whereas recoveries of labeled and unlabeled glucagon on the same column were between 85–95%.

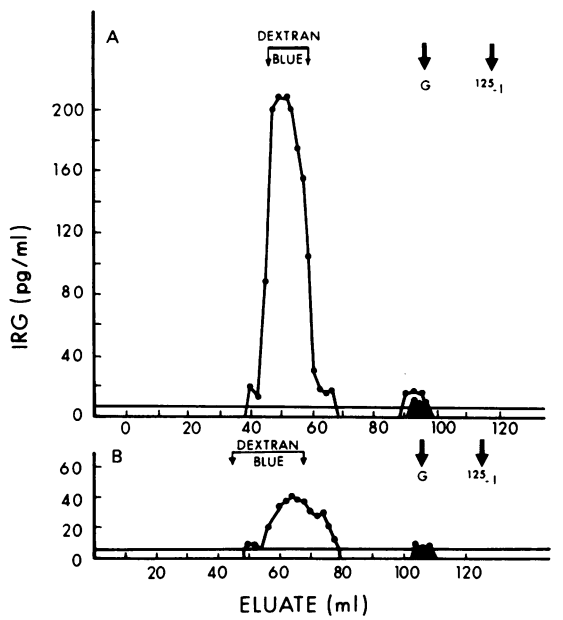

FIGURE 1 Sephadex G-50 gel fractionation in plasma. Data from patient V.B. (A) and from E.G. (B) are shown. The shaded areas depict results obtained with antiserum RCS-5, the non-shaded areas those obtained with antiserum 30-K. The line above the abscissa denotes the limit of detectability of IRG. The arrows denote column markers (G, glucagon; ^{125}I, the salt peak marker). (A) Patient VB: IRG loaded, 3050 pg; IRG recovered, 2837 pg (93%). (B) Patient EG: IRG loaded, 805 pg; IRG recovered, 700 pg (87%).

Fig. 1 illustrates characteristic elution patterns of two patients. In patient V.B. (A) 30-K detected 97% of total recovered IRG within the void volume and 3% in the IRG^{3500} region. Recovery was 93%. RCS-5 measured IRG only in the 3,500-dalton region. Recovery was 31%. In patient E.G. (Fig. 1B) 30-K detected all recovered IRG within or just outside the void volume (recovery, 87%). RCS-5 again measured IRG exclusively in the 3,500-dalton region with poor recovery (24%). The void volume IRG of three pancreatectomized patients and three normal controls were rechromatographed on Sephadex G-200 (Fig. 2). The elution patterns of plasma from pancreatectomized patients and from normal controls were comparable. The large majority of IRG (83% in pancreatectomized patients and 91% in normal controls) eluted with the catalase marker and, thus, had an apparent mol wt of ~200,000. This fraction was probably identical with big plasma glucagon, first described by Valverde et al. (21). Its nature and biological activity are unknown. A second smaller peak (17% in pancreatectomized patients and 9% in normal controls) eluted with the salt marker and, thus, had an apparent mol wt of <5,000. This fraction could have been IRG^{3500}, IRG^{2000}, a salt effect, or a combination of these three.

Plasma IRG after arginine and glucose (Fig. 3). Fig. 3 shows data on patients E.B. and V.B. Intravenous infusion of arginine or glucose produced no consistent changes in IRG concentration with the 30-K or RCS-5 antibodies in either patient. Three of the remaining seven patients had previously been studied by Werner et al. (8). They found that in response to intravenous arginine, IRG (30-K) rose in patient A.H. but did not rise in patients E.D. and R.B. In addition, patients A.H. and R.H. received oral glucose and both responded with a rise in IRG (8).

Postabsorptive amino acid levels (Table IV). Concentrations of 19 amino acids were measured in plasma of nine pancreatectomized patients after an overnight fast and before insulin administration. Compared to values from 20 adult normal controls mean concentrations of the following amino acids were significantly elevated: serine, glycine, alanine, arginine, threonine, citrulline, α-aminobutyric acid, and tyrosine. Compared to values obtained from five patients with gastrectomy, the same amino acids were significantly elevated with exception of glycine and tyrosine, where the differences failed to achieve statistical significance. Abnormal elevations were seen most commonly in plasma concentrations of serine and arginine. Both exceeded the normal range in eight of nine patients. Alanine and glycine were elevated in seven of nine and threonine in six of nine patients.

Fig. 4 illustrates the day-to-day variation of the elevated amino acids in two patients (E.G. and V.B.), whose

TABLE III

Molecular IRG Species in Pancreatectomized Patients and Normal Subjects

	30-K						RCS-5					
	Plasma IRG	Recovery*	IRG void volume		IRG^{3500}		Plasma IRG	Recovery	IRG void volume		IRG^{3500}	
	pg/ml	%	*pg/ml*	%	*pg/ml*	%	*pg/ml*	%	*pg/ml*	%	*pg/ml*	%
Pancreatectomized patients												
E.G.	161	87	140	100	0	0	42	24	0	0	10	100
V.B.	610	93	550	97	17	3	45	31	0	0	14	100
L.D.	108	106	79	69	35	31	63	41	0	0	26	100
R.B.	105	62	27	42	38	58						
C.C.	860	78	637	95‡	0	0	45	22	0	0	10	100
E.D.	75	122	75	82	17	18	43	109	0	0	39	100
Mean±SEM	320	91.3	251	80.8	18	18.3	48	45.4	0	0	20	100
	136	8.6	110	9.1	7	9.4	4	16.2			6	
Normal subjects												
R.M.	191	85	107	66	55	34						
I.C.	181	100	123	68	58	32						
G.B.	168	83	92	72	47	28						
V.T.	622	79	435	91	56	9						
E.P.	95	100	78	82	17	18						
Mean±SEM	251.4	89.4	167.0	75.8	48.6§	24.2						
	94.2	4.4	67.4	4.7	8.5	4.7						

* Recovery (%) of IRG loaded.

‡ The remaining 5% eluted between the void volume and the ^{125}I-glucagon marker.

§ IRG^{3500} patients vs. normal subjects, $P < 0.05$.

plasma was obtained on three consecutive days after an overnight fast and before insulin administration. It can be seen that concentrations of alanine, glycine, serine, arginine, threonine, ornithine, tyrosine, and citrulline were repeatedly elevated in one or both patients.

Effect of glucagon infusion on glucagon, amino acid, and glucose concentrations (Fig. 5). In patients E.G. and V.B. physiologic glucagon increments were produced by infusion of exogenous glucagon. During this infusion, IRG concentrations (antiserum RCS-5) rose about 100 pg/ml from basal concentrations of 40 pg/ml to plateau concentrations of between 115 and 165 pg/ml in both patients (Fig. 5A). Patients E.G.'s fasting as well as postprandial blood glucose concentrations (Fig. 5B) were within normal limits as were the corresponding values for the day preceding the glucagon infusion, which served as a control period. Patient V.B.'s preinfusion fasting blood glucose was 125 mg/dl. Post-lunch and post-dinner values were elevated (>300 mg/dl), but returned to normal values during the postabsorptive period. A comparable blood glucose pattern had been observed the day preceding the glucagon infusion. Fig. 5C depicts changes of those amino acids that had been consistently elevated. During the first 16 h of glucagon infusion, concentrations of alanine, serine, threonine, arginine, and citrulline declined while concentrations of tyrosine, proline, and ornithine rose in both patients. The mean of these eight amino acids did not change significantly during this period. Between 16 and 22 h, all eight amino acids decreased sharply in both patients. Mean decreases at the end of the glucagon infusion were 32 and 48% for E.G. and V.B., respectively. Most of the other amino acids were initially normal concentrations also declined but to a lesser extent (10 and 32% for E.G. and V.B., respectively; data not shown).

DISCUSSION

Glucagon deficiency. IRG concentration in plasma has been shown to increase after pancreatectomy in dogs (22) and glucagon indistinguishable from pancreatic glucagon has been extracted from canine stomach and upper intestinal tissues (23, 24). There is, however, conflicting evidence as to whether glucagon disappears completely from the blood of totally pancreatectomized humans. Muller et al. (5) found that between 75 and 90 pg/ml of IRG (30-K) remained in plasma of two totally pancreatectomized patients. Between 15 and 35 pg/ml of this could be bound to charcoal and, therefore, was suspected to be "true glucagon". Werner et al. (8) found between 48 and 189 pg/ml of IRG (30-K) in plasma from six pancreatectomized patients. After acetone

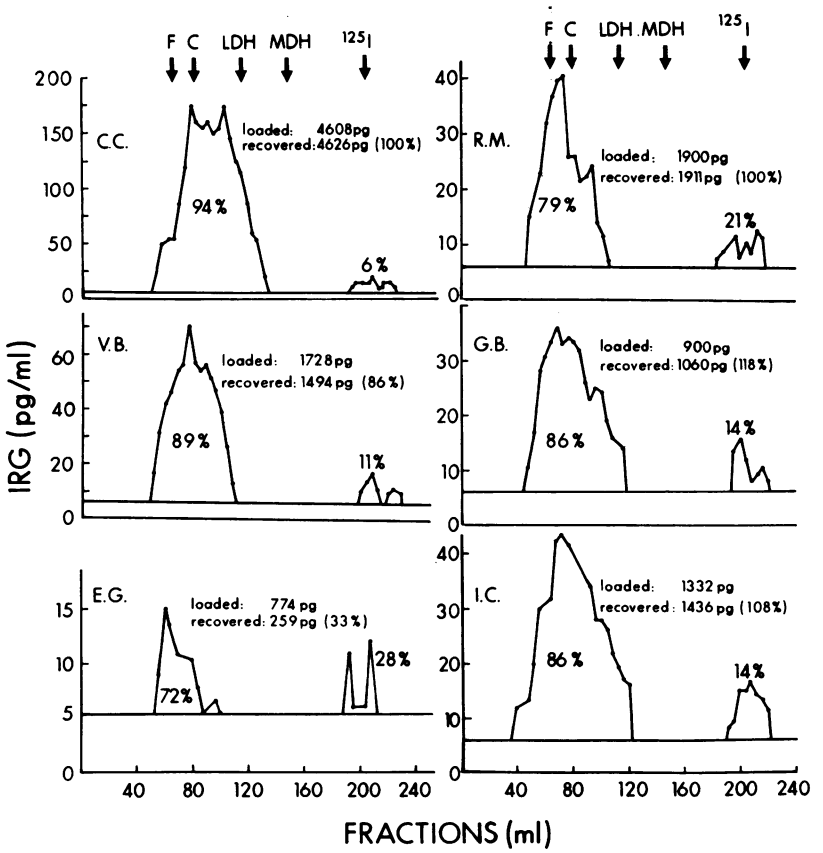

FIGURE 2 Sephadex G-200 fractionation of void volume IRG. Plasma from patients C.C., V.B., E.G., and from three normal controls (R.M., G.B., I.C.) was fractionated over Sephadex G-50. The void volumes were pooled individually, lyophilized, and rechromatographed over Sephadex G-200. IRG was measured in the column eluates with the 30-K antiserum. The line above the abscissa denotes the limit of detectability of IRG. The arrows denote column markers (F, fibrinogen, C, catalase, LDH, lactate dehydrogenase, MDH, malate dehydrogenase).

extraction, which presumably eliminated the large molecular weight IRG species, these values decreased to between 35 and 60 pg/ml (mean, 46 pg/ml). Barnes and Bloom, however, using their RCS-5 antiserum found no IRG in plasma of five pancreatectomized patients (4). The differences in results between their and other studies may be explainable, at least in part, by differences in methodology. Barnes and Bloom ran each patient's standard curve with plasma rendered "glucagon free" by pretreatment with an immunoabsorbent. However, it is likely that this plasma still contained IRG because the immunoabsorbent was equilibrated with antiserum that reacted with large molecular weight IRG weakly or not at all (see below). Thus, by adding the "stripped plasma" they may have created an arbitrary 0 standard, which led to underestimation of plasma IRG. In agreement with Muller et al. (5) and Werner et al., (8) we found IRG (30-K) in plasma of all of our nine pancreatectomized patients. Our mean IRG value was higher than theirs largely because of very high IRG concentrations in three patients (C.C., A.H., and E.G.). Fractionation over Sephadex G-50 of plasma from two of the three (C.C. and E.G.) revealed that 95 and 100% of their IRG, respectively, consisted of large molecular weight material (Fig. 1 and Table III). The cause and significance of these excessive IRG concentrations, also present in 1 of 10 controls, remains obscure. In contrast to Barnes and Bloom, we detected IRG in eight of nine pancreatectomized patients by using their antiserum (RCS-5).

Our gel filtration data revealed that neither of the two antisera accurately measured "authentic" glucagon (IRG^{3500}) in plasma. When 30-K was used, most of the IRG in pancreatectomized patients (81%) and in normal subjects (76%) eluted within the void volume. Rechromatography over Sephadex G-200 (Fig. 2) showed that this IRG had an apparent molecular weight of ~200,000. This IRG species is probably identical with big plasma glucagon, first described by Valverde et al. (21). Its exact nature and bioactivity are unknown. Only 18% of IRG (range, 0–58%) in pancreatectomized patients and 24% of IRG (range, 9–34%) in normal subjects

TABLE IV

Fasting Plasma Amino Acid Profile in Pancreatectomized Patients

	E.G.	V.B.	L.D.	C.C.	R.B.	M.C.	C.W.	A.H.	E.D.	Mean±SEM (n = 9)	P pancreatectomized vs. normal	Normal controls (n = 20)	Gastrectomized patients (n = 5)	P pancreatectomized vs. gastrectomized
Taurine	51	103	47	52	39	37	81	34	66	57.7 7.6		46 2	112 25	
Threonine	212	62	171	344	434	179	220	127	208	217.4 37.1	<0.05	129 7	103 12	<0.025
Serine	237	292	210	393	235	245	179	149	140	231.1 25.9	<0.001	106 5	114 8	<0.005
Proline	158	688	243	326	203	232	203	211	181	217.7 54.4		188 17	172 30	
Citrulline	77	89	36	53	56	44	15	41	65	52.9 7.4	<0.025	30 3	34 4	<0.025
Glycine	590	599	325	388	344	359	279	214	343	382.0 43.5	<0.005	222 12	271 34	
Alanine	668	1,350	563	828	533	571	315	424	532	642.7 100.4	<0.01	332 22	381 51	<0.05
α-Amino-butyrate	14	18	23	87	40	36	47	35	23	35.9 7.3	<0.05	20 2	18 3	<0.05
Valine	207	218	199	278	228	175	184	239	188	212.9 10.7		226 11	204 20	
Methionine	21	25	25	34	21	23	23	19	5	21.8 2.5		26 1	31 3	
Isoleucine	67	56	58	72	66	75	78	57	47	64.0 3.4		64 3	71 12	
Leucine	—	94	120	126	119	117	120	117	96	113.6 4.2		127 5	128 19	
Tyrosine	38	75	90	90	89	68	96	54	45	71.7 7.2	<0.05	53 2	52 10	
Phenyl-alanine	32	38	49	44	48	60	49	47	40	45.2 2.7		53 2	65 8	
Ornithine	174	184	55	129	91	87	64	40	153	108.6 17.8		70 8	90 9	
Lysine	206	129	284	325	263	208	316	170	211	234.7 22.2		184 10	190 13	
Histidine	57	90	71	83	84	78	59	66	66	72.7 3.9		83 4	76 9	
Tryptophan	27	35	28	61	46	28	30	43	43	37.9 3.8		39 4	33 6	
Arginine	87	144	153	292	311	228	117	133	198	184.8 26.1	<0.005	74 6	108 11	<0.05

eluted in the 3,500-dalton area, which presently is the only glucagon species with proven bioactivity (25). Thus, most of the IRG measured with the 30-K antiserum in plasma consisted of a large molecular weight fraction, the bioactivity of which is uncertain.

When the RCS-5 antiserum was used, all IRG eluted in the 3,500-dalton region, provided lyophilized plasma was employed for fractionation. However, column recoveries were low (45%). There are two possible explanations for the low recovery rate. Firstly, recoveries

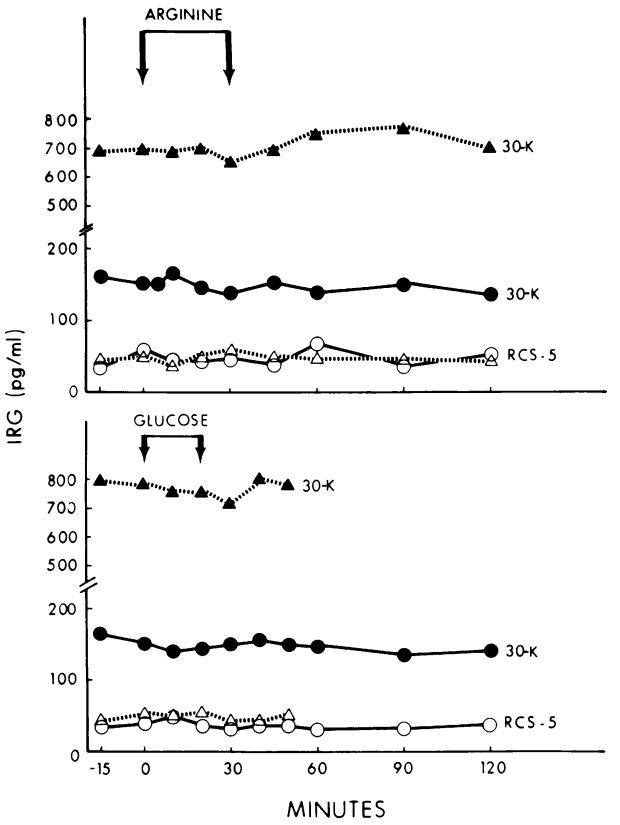

FIGURE 3 Glucagon after intravenous arginine and glucose. Data from patient E.G. are shown with solid lines; those from patient V.B. with broken lines. Solid circles indicate use of antiserum 30-K, open circles use of antiserum RCS-5.

of plasma IRG with 30-K (Table III), ^{125}I-glucagon, and crystalline glucagon, were all satisfactory (>85%) on the same column. In addition, the amount of IRG^{3500} recovered with both 30-K and RCS-5 antisera was comparable (18 vs. 20 pg/ml). This indicated that IRG was not lost during passage through the column and that the poor recoveries may have been the result of falsely high IRG measurements in unfractionated plasma. A likely mechanism for this could have been nonspecific interactions between plasma proteins and the RCS-5 antiserum. Secondly, the RCS-5 antiserum measured IRG^{3500} specifically only if lyophilized plasma was used for fractionation. When native plasma was fractionated, the antibody detected void volume IRG, albeit in much smaller amounts than the 30-K antiserum (data not shown). As a result, RCS-5, when used with native plasma, probably measured a small amount of large molecular weight IRG and, in addition, may have overestimated IRG concentration because of nonspecific protein interaction.

Gel fractionation revealed unequivocally that there was IRG^{3500} present in plasma of all six pancreatectomized patients that were studied. In two of the six (C.C. and E.G.) IRG^{3500} was not found with the 30-K but was detected with the RCS-5 antiserum, which was more sensitive. Our results are comparable to those reported by Botha et al. (7) who fractionated plasma of one pancreatectomized patient and found large molecular weight IRG and IRG^{3500}. These findings indicated that pancreatectomized patients are not aglucagonemic, as postulated by Barnes and Bloom (4) and, therefore, cannot serve as model for diabetes without glucagon. They also demonstrated that both antisera are not specific for pancreatic glucagon and that the commonly used designation "pancreatic glucagon specific" antiserum is a misnomer. The source for the extrapancreatic glucagon was probably the fundic mucosa, most of which was not removed during hemigastrectomy.

Mean IRG^{3500} (30-K) was significantly smaller in pancreatectomized patients than in normal controls (18 ± 7 vs. 49 ± 9, $P < 0.05$). There was, however, a wide range of basal IRG^{3500} values in these patients (from 10 to 38 pg/ml), which probably reflected varying amounts of remaining upper intestinal tissue. For instance, the lowest IRG concentrations were found in three patients (C.C., V.B., and E.G.) who had lost part of their jejunum in addition to total duodenectomy, pancreatectomy, and partial gastrectomy.

Most of the patients studied by us and others (4–7) showed little or no IRG responses to stimulation with arginine. Similar data have been reported in pancreatectomized dogs, where it has been shown that arginine stimulated release of IRG only if the animals were severely insulin deficient (26, 27). Thus, our data, although obtained from a relatively small number of patients, suggested, that the pancreatectomized patients were partially glucagon deficient as result of reduced basal IRG concentrations associated with absent or subnormal IRG responses to stimulation. Moreover, because glucagon concentrations are higher in the portal than in the peripheral circulation, glucagon deficiency in these patients must have been most pronounced at the liver, which is the major target organ for glucagon.

Hyperaminoacidemia. In this study it was found that total pancreatectomy was associated with chronic hyperaminoacidemia in all nine patients studied. Postabsorptive plasma concentrations of serine, alanine, arginine, glycine, threonine, citrulline, α-aminobutyric acid, and tyrosine were significantly elevated compared to normal controls (Table IV). The most prominent and consistent elevations were seen in plasma levels of serine, alanine, arginine, and glycine. Recognized causes for hyperaminoacidemia such as obesity (4), and diurnal variation of amino acid levels (27), could be ruled out in these patients. All, except one patient, were nonobese and the pattern of amino acids observed were totally unlike that seen in obesity (4). Blood samples for amino acid determinations were drawn from patients and controls at the same time of the day which rules out diurnal variation as a cause. The elevated fasting blood glucose concentrations (130–292 mg/dl) in four

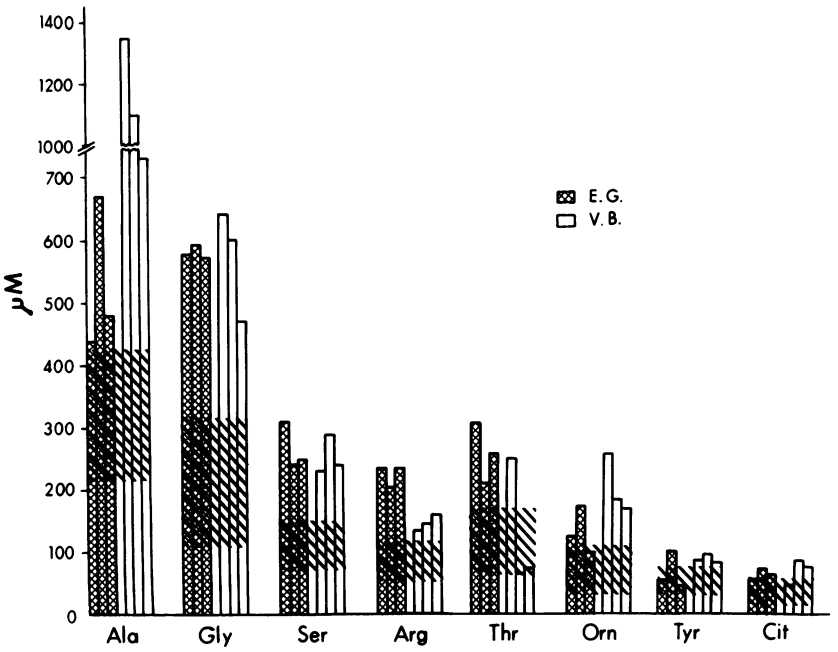

FIGURE 4 Amino acids in two pancreatectomized patients. Shown are plasma concentrations of peripheral venous amino acids in patients E.G. (open bars) and V.B. (crosshatched bars) determined on three consecutive days. Blood was drawn after an overnight fast and before insulin administration. The shaded areas represent the normal range as determined in 20 normal subjects.

patients suggested that these patients were insulin deficient at the time of the study and that this may have altered amino acid levels. However, insulin deficiency characteristically results in elevations of the branched-chained amino acids (leucine, isoleucine, and valine) and diminished concentrations of some glycogenic amino acids, particularly of alanine (28, 12) as result of increased splanchnic uptake of these amino acids (29). This pattern was not present in any of our patients. In fact, eight of nine had markedly elevated alanine concentrations and branched-chain amino acids were within normal limits in all nine.

Four of nine patients were undernourished (<90% of ideal weight). The causes included postprandial discomfort caused by abdominal adhesions, subclinical maldigestion despite replacement therapy with pancreatic enzymes and recurrent and/or metastatic malignancy. However, amino acid profiles observed in our patients bore little resemblance to those found in various states of malnutrition. For instance, Smith et al. (30) have reported that patients with chronic severe protein-calorie malnutrition showed severe depression of all essential amino acids. Of the nonessential amino acids α-aminobutyric acid, one-half cystine, tyrosine, histine, and arginine were all depressed, whereas the remaining amino acids were unchanged except for glycine, which was elevated. A similar pattern was found in normal subjects after 7–10 d of protein-free diet (31). All essential amino acids declined, whereas glycine and alanine rose. Felig et al. (16), upon studying obese healthy subjects, reported that 5–6 wk of total starvation led to a generalized decline in plasma amino acids, in which the fall of alanine was most prominent. Adibi (32) also observed a fall in alanine in normal subjects after 2 wk of total starvation. Moreover, our underweight, normal, and overweight pancreatectomized patients all demonstrated the same type of amino acid abnormalities, which makes it unlikely that these changes were caused by malnutrition.

On the other hand, our pancreatectomized patients were partially glucagon deficient. This may be expected to lead to hyperaminoacidemia, inasmuch as the opposite condition, glucagon hypersecretion, in patients with glucagonoma invariably results in severe hypoaminoacidemia (10, 11). Similarly, infusion of supraphysiologic doses of glucagon has been shown to result in decreases of many amino acids with alanine showing the greatest decline (33), and infusion of physiological doses of glucagon (4.2 μg/h × 24 h) into 4–6 wk fasted subjects resulted in small decreases in alanine, serine, proline, threonine, ornithine, tyrosine, and one-half cystine (14). The observation in this study that small glucagon increments normalized previously elevated amino acid concentrations in two patients are compatible with, but do not prove a cause and effect relationship between, glucagon deficiency and hyperaminoacidemia. It cannot be excluded that the glucagon infusion produced mild portal hyperglycagonemia, which in turn

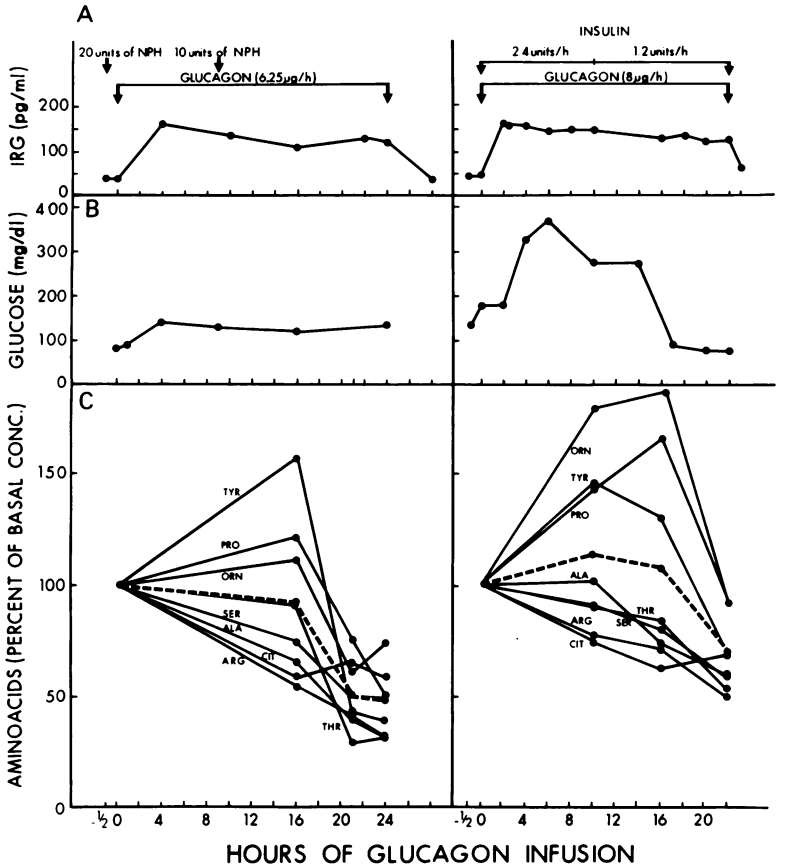

FIGURE 5 Effect of glucagon infusion on glucagon, glucose, and amino acid levels. Shown are plasma IRG (A), plasma glucose (B), and individual (solid lines) and mean (broken lines) amino acid concentrations in patients E.G. (left) and V.B. (right) during infusion of glucagon (6.25 μg/h × 24 h for E.C. and 8.0 μg/h × 22 h for V.B.). Amino acids are expressed as percentage of the preinfusion (8 a.m.) concentrations.

may have been responsible for the fall in plasma amino acid concentrations. Our recent observation, however, that acute selective glucagon deficiency, produced in normal subjects by infusion of somatostatin plus insulin, led to significant increases in plasma concentrations of glycine, alanine, arginine, glutamine, and lysine within 8 h (34) further strengthened the concept that glucagon deficiency produces hyperaminoacidemia.

Our studies provide no information on how glucagon deficiency leads to hyperaminoacidemia. Experiments showing that glucagon infusion stimulated (35) and glucagon deficiency inhibited gluconeogenesis from alanine, (36) suggested alteration in the rate of gluconeogenesis from amino acids as the mechanism by which glucagon can control blood amino acids levels. In support of this thesis are our findings that (*a*) the most prominent elevations occurred in plasma concentration of those amino acids (alanine, glycine, serine, threonine) that are most avidly extracted by the splanchnic bed (37) and (*b*) that acute selective glucagon deficiency in normal subjects significantly decreased urinary urea excretion (34). Lastly, it is noteworthy that those amino acids that are potent stimulators of glucagon release, (particularly alanine, and arginine) are also most affected by its metabolic actions. This suggests the existence of a feedback system between glucagon and certain glycogenic amino acids.

ADDENDUM

While this manuscript was under review, Muller et al. (1979. *J. Clin. Invest.* **63**: 820–827.) published data showing significant elevations of plasma alanine, serine, ornithine, and arginine in five patients with duodenopancreatectomy. In eight duodenectomized, pancreatectomized patients basal IRG (30-K) was 162±68 pg/ml and did not change during the arginine infusion period. Bio-Gel P-30 column chromatography revealed that virtually all IRG had a mol wt of >3,500. Their failure to detect IRG^{3500} in all but one patient seems clearly related to the relative insensitivity of their assay system. Muller et al. measured IRG in 0.2 ml of eluate with a sensitivity of 20 pg/ml of eluate, whereas we used 0.9 ml of eluate with a sensitivity of 6 pg/ml of eluate. Our data indicate that IRG^{3500} concentrations in pancreatectomized patients are too small to be detected by an assay system with a sensitivity of 20 pg/ml.

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