The Role of Aminogenic Glucagon Secretion in Blood Glucose Homeostasis

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ABSTRACT Hyperaminoacidemia is a powerful stimulus of pancreatic glucagon secretion. These studies were designed to elucidate the role of aminogenic hyperglucagonemia in glucoregulation. Conscious dogs with previously implanted indwelling venous catheters were employed. The results support the view that a role of glucagon is to limit blood glucose decline during hyperaminoacidemia.

First, a significant negative correlation between the area of glucagon increment during the 1st 20 min of a 10 amino acid infusion and the maximum fall in glucose concentration was observed. Second, when endogenous glucagon secretion was suppressed by means of a continuous glucose infusion, hyperaminoacidemia induced a maximal glucose decline which averaged 35 mg/100 ml, differing significantly from mean maximal fall of 3 mg/100 ml, which normally occurs in the presence of endogenous hyperglucagonemia. Third, when, during hyperglycemic suppression of endogenous glucagon secretion, 50 mμg of exogenous glucagon/min was infused via the mesenteric vein with the amino acids, the fall in glucose was reduced to an average of 5 mg/100 ml. Similarly when pancreozymin, administered during the combined infusion of glucose and amino acids, overcame glucose suppression of endogenous glucagon secretion, plasma glucose did not fall.

Similar results were obtained when aminogenic hyperglucagonemia was prevented by other means. Hyperlipacidemia, induced by infusing a triglyceride emulsion and giving heparin injections, also suppressed aminogenic hyperglucagonemia in two of four experiments; in these two dogs glucose fell 15 and 11 mg/100 ml. In a final group of experiments, the canine pancreas was resected except for the uncinate process, which is virtually devoid of α-cells. In two dogs, in which this procedure resulted in zero portal venous glucagon levels, the administration of amino acids and/or pancreozymin resulted in a glucose decline of 14 and 16 mg/100 ml, despite the reduced β-cell population resulting from the subtotal pancreotectomy.

It thus appears that the secretion of pancreatic glucagon during hyperaminoacidemia in association with insulin secretion, serves to limit the decline of glucose concentration.

Received for publication 8 August 1968 and in revised form 24 October 1968.

INTRODUCTION

Recent studies indicate that glucagon, the glycogenolytic, gluconeogenic hormone secreted by the pancreas in response to glucose need (1, 2,[1]), is also stimulated by hyperaminoacidemia (3–5). Of the three known stimuli of pancreatic glucagon secretion, hypoglycemia, pancreozymin administration, and hyperaminoacidemia, only the latter would, in the strictest sense, appear to constitute a regularly occurring physiologic event. Therefore, it may well be that an understanding of the function of aminogenic hyperglucagonemia would provide knowledge of glucagon's most important physiologic function.

As has been suggested previously (5), the purpose of aminogenic hyperglucagonemia may be to prevent a decline in blood glucose concentration secondary to aminogenic insulin secretion during carbohydrate-free protein meals. This point of view is supported by the following observations: first, during hyperaminoacidemia glucagon secretion increases virtually in parallel to insulin secretion, yet, despite the hyperinsulinemia glu-

[1] Buchanan, K., J. E. Vance, K. Dinstl, and R. H. Williams. 1969. Effect of blood glucose on glucagon secretion in anesthetized dogs. *Diabetes.* **18:** 11.

cose concentration declines only slightly (5); and second, infusion of glucose prevents aminogenic hyperglucagonemia, suggesting that it is superfluous when exogenous glucose is available (5).

The present study was designed to elucidate the role of aminogenic hyperglucagonemia in blood glucose homeostasis by determining the effect of suppression and restoration of hyperglucagonemia upon the plasma glucose response to hyperaminoacidemia.

Inasmuch as a permanent glucagon deficiency state could not be induced pharmacologically by the alpha-cytotoxic agents cobaltous chloride (6) or neutral red,[2] other means were used in an attempt to prevent aminogenic hyperglucagonemia. Hyperglycemia (1, 2) and hyperlipacidemia (7) have both been reported to inhibit glucagon secretion and were, therefore, employed to bring about physiologic suppression. The uncinate process of the canine pancreas has been shown by Bencosme and Liepa (8) to be almost devoid of alpha cells and glucagon, and selective resection of all other regions of the pancreas was, therefore, carried out to produce a chronic state of glucagon deficiency.

METHODS

Experimental preparations

Triply catheterized dogs. A triply catheterized dog preparation was employed in most of the experiments to permit sampling from the pancreaticoduodenal vein and the inferior vena cava in fully conscious dogs, and to make possible the infusion of solutions via the mesenteric vein. As has been pointed out previously (5), measurement of glucagon in the pancreatic venous effluent minimizes certain problems of sensitivity and specificity that have plagued attempts to measure glucagon in the peripheral blood.

2 or more days before each experiment, a dog was anesthetized with pentobarbital and the abdomen was opened by midline incision under sterile conditions. A small glass T cannula connected to Teflon tubing was inserted into the superior pancreaticoduodenal vein at a distance of about 3 cm from its junction with the portal vein. The tubing was fixed at the duodenum with a suture. A second Teflon catheter was inserted through the left jugular vein and was fixed in place with its opening reposing in the inferior vena cava between the heart and the hepatic vein. A third catheter, this one of polyethylene, was threaded through a small mesenteric venous radicle into a major mesenteric vein. All catheters were exteriorized, sutured in place, and heavily bandaged with tape. The pancreaticoduodenal vein catheter was perfused continuously until the time of the experiment with diluted heparin-saline solution so as to deliver 100 units of heparin in a volume of 20 ml/hr.

After surgery each animal received an intramuscular injection of 600,000 U of penicillin daily. Dogs were fed on a Purina Chow diet. Recovery from the surgical procedure was uneventful in the majority of dogs. Only dogs that appeared to be in good health were employed for experiments. The appearance of either diarrhea, loss of appetite, weight loss in excess of 1.5 kg, a white blood count above 30,000 (normal 10,000–20,000), or elevation of body temperature above 104°F (normal 101°F) was regarded as grounds for disqualification. Dogs with a hematocrit of less than 35% were transfused on the day before the experiment. After completion of a series of experiments each dog was sacrificed and the position of the catheters and the patency of veins were examined. Dogs in which the pancreaticoduodenal vein was not patent were excluded from the study.

"Uncinate dogs." In an effort to create in dogs a chronic state of relative glucagon deficiency the alpha cell–bearing segments of the pancreas were resected and an attempt made to preserve a viable and functioning uncinate process. Through a midline incision, the tail of the pancreas was removed and the uncinate process was ligated at a distance of about 3 cm from the opening of the pancreatic duct and separated from the head of the pancreas. The superior pancreaticoduodenal artery and vein and their upper duodenal branches were separated by blunt dissection from the pancreas, and an attempt made to resect all of the pancreas except at least half of the uncinate process. A polyethylene catheter was threaded through a mesenteric radicle into the portal vein and was sutured in place with its opening reposing close to the liver. A jugular vein catheter was inserted as described above. Whenever possible, the uncinate process of each "uncinate dog" was collected immediately after its death, stored at −15°C until the time of extraction by the Kenny technic (9), and assayed subsequently for glucagon and insulin.

Experimental procedures

All experiments were conducted after an overnight fast. In a fully conscious state, dogs were suspended in a cradle sling with their extremities touching the floor throughout the experimental period. Solutions of glucose, amino acids,[3] or triglycerides[4] were infused either into a crural vein or into the portal vein at a constant rate using an infusion pump.

Blood specimens were collected in syringes rinsed with a 10% solution of ethylenediamine tetraacetic acid (EDTA). Plasma was separated immediately and stored at −15 to −20°C until the time of assay. Plasma glucose concentration was measured by the ferricyanide method of Hoffman (11), using the Technicon AutoAnalyzer. Plasma amino nitrogen level was determined by the method of Frame, Russell, and Wilhelmi (12). Plasma free fatty acids were measured by the Mosinger's modification (13) of the method of Dole. Insulin was determined by the radioimmunoassay of Yalow and Berson (14), and glucagon by the previously described radioimmunoassay as recently modified (15).

In most experiments, glucagon was assayed with anti-glucagon antiserum No. G-128, which reacts with both gastrointestinal glucagon-like immunoreactivity and with pancreatic glucagon. However, in all but one of the Lipomul infusion experiments, and in all of the "uncinate dog" experiments, glucagon was assayed with a relatively specific anti-glucagon antiserum, No. G-58, which cross-reacts only very slightly with gastrointestinal glucagon-like immunoreactivity (16).

[2] Ohneda, A., A. M. Eisentraut, and R. H. Unger. Unpublished observations.

[3] The 10 amino acid mixture used was identical in composition to that employed by Floyd et al. (10). At the concentration encountered in plasma the amino acid mixture did not affect the radioimmunoassay for glucagon or insulin.

[4] The triglyceride emulsion used was glucose-free Lipomul kindly donated by Dr. Keith Borden, Upjohn Co., Kalamazoo, Michigan.

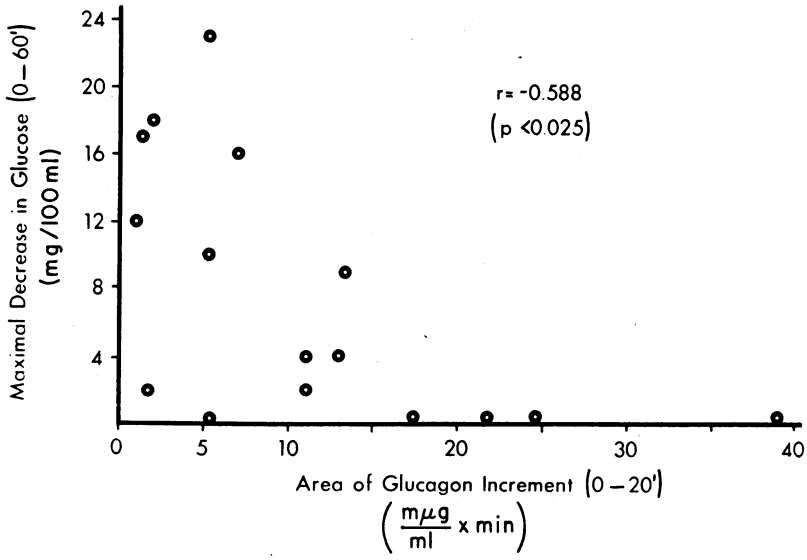

FIGURE 1 Relationship between the maximal plasma glucose decline during a 60 min amino acid infusion and area of rise in pancreaticoduodenal vein glucagon concentration during the 1st 20 min of the infusion.

RESULTS

Correlation between glucagon rise and decline in glucose during hyperaminoacidemia. It was recently reported that during hyperaminoacidemia in dogs mean concentration of pancreaticoduodenal vein insulin rises 375 μU/ml, the mean pancreaticoduodenal vein glucagon rises 1.6 mμg/ml, and the mean inferior vena caval plasma glucose declines only 3 mg/100 ml (5). If the hyperglucagonemia is responsible for restricting the magnitude of the fall in glucose concentration, a negative correlation between the increase in glucagon secretion and the decrease in plasma glucose might well exist. To determine this, the previously published data were analyzed. As shown in Fig. 1, a statistically significant negative correlation ($r = -0.588$; $P < 0.025$) was observed between maximal glucose fall during the hour after the start of the infusion and the area of glucagon increment during the 1st 20 min.

Variation in the insulin response to hyperaminoacidemia could also account for variations in glucose change. The relationship between the area of insulin increment of the 1st 20 min and maximal fall in glucose within 60 min of the start of the infusion was, therefore, examined. No statistically significant relationship between insulin increment and glucose decline ($r = 0.106$; $P > 0.5$) was observed (Fig. 2).

These findings are compatible with the possibility that glucagon secretion limited the fall in plasma glucose concentration.

Effect of hyperglycemic suppression of aminogenic hyperglucagonemia upon the glucose response to hyperaminoacidemia. If, as the above correlation suggests, increased glucagon secretion was responsible for restricting the magnitude of the decline of glucose during hyperaminoacidemia, then prevention of hyperglucagonemia would result in a larger decline in glucose concentration. Because of earlier studies indicating that hyperglycemia prevents aminogenic hyperglucagonemia (5), a 15% glucose solution was infused at a rate of 200 or 300 mg/min to each of a group of 10 dogs in order to maintain the plasma glucose concentration above 142 mg/100 ml, a level found to inhibit glucagon secretion in most dogs. 60 min or more after the start of

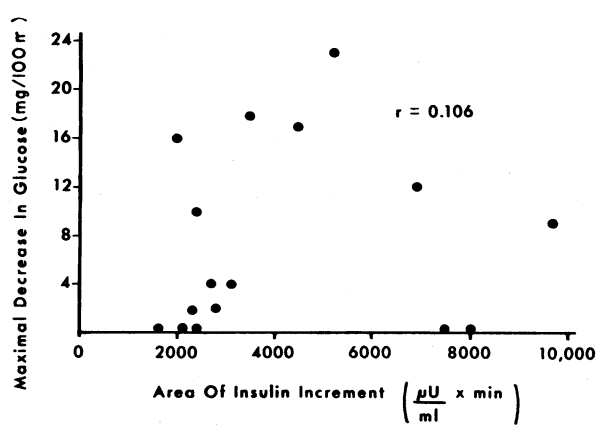

FIGURE 2 Relationship between the maximal plasma glucose decline during a 60 min amino acid infusion and area of rise in pancreaticoduodenal vein insulin concentration during the 1st 20 min of the infusion.

TABLE I

Effect of Glucose Infusion upon Amino Acid–Induced Changes in Glucagon and Glucose

Infusion	Mean maximal glucagon rise (±SEM) above fasting	Mean maximal glucose fall (±SEM)
	mμg/ml	*mg/100 ml*
Amino acid only (six dogs)	1.3 ±0.57	3.0 ±1.9
Amino acid and glucose (10 dogs)	0	35 ±3.3
P value		<0.001

the glucose infusion, when the glucose concentration seemed to have reached a relatively stable plateau, the amino acid infusion was begun at a rate of 100 mg/min, raising the mean plasma amino nitrogen level to 6.3 mg/100 ml. Whereas in the previously reported normoglycemic dogs, hyperaminoacidemia of this magnitude was associated with a 1.2 mμg/ml rise in mean pancreaticoduodenal vein glucagon above the fasting level and a maximal fall in mean glucose of only 3 mg/100 ml (SEM ±1.9) (5), in these hyperglycemic dogs the mean glucagon level remained well below the fasting value and the maximal glucose decline averaged 35 mg/100 ml (SEM ±3.3), a statistically significant difference ($P < 0.001$) (Table I). The results of the experiments in the hyperglycemic suppression group are summarized in Table II and the mean values are depicted in Fig. 3. The results are compatible with the notion that suppression of aminogenic hyperglucagonemia enhances the magnitude of the cataglycemia.[5]

It seemed possible that the decline in glucose concentration noted during the simultaneous infusion of glucose and amino acids was simply the consequence of prolonged infusion of glucose, and would have occurred in the absence of hyperaminoacidemia. To exclude this possibility, glucose was infused alone at the same rate (200 or 300 mg/min) in each dog 1 day or more before its amino acid experiment. Mean glucose concentration did not decline from the level prevailing 90–150 min after the start of the glucose infusion, the time at which the amino acid infusion would have been started. This indicates that the fall in plasma glucose observed during hyperaminoacidemia was not a consequence of the prolonged glucose infusion itself but of the concomitant hyperaminoacidemia.

The greater decline in plasma glucose observed in the hyperglycemic animals during hyperaminoacidemia could also be consequence of the significantly greater hyperinsulinemia induced by the simultaneous infusion of two insulin-stimulating substrates, amino acids and glucose. The maximal mean insulin level was 1334 (SEM

[5] Defined as any fall in glucose concentration, whether or not it falls to a hypoglycemic level.

TABLE II

Effect of Hyperglycemic Inhibition of Aminogenic Hyperglucagonemia upon Glucose Response to Hyperaminoacidemia

			Time before amino acid infusion (min)			Time after start of amino acid infusion (min)					
						Glucose infusion					
	Control period					Amino acid infusion					
Measurement	−10	0	−30	−15	0	5	10	20	30	45	60
Mean ±SEM*											
Glucose‡ (*mg/100 ml*)	110.1	111.2	170.9	169.1	169.8	171.3	169.3	159.4	148.9	140.7	136.0
±SEM	3.9	3.8	7.6	6.4	5.1	5.9	4.9	6.9	6.9	5.0	3.4
Amino N‡ (*mg/100 ml*)	4.7	4.5	3.8	3.2	3.7	5.0	5.6	5.8	6.1	6.1	6.3
±SEM	0.3	0.3	0.3	0.6	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Insulin§ (*μU/ml*)	119.3	110.9	632.7	710.2	888.1	1168	1079	981.0	1334	848.6	1076
±SEM	30.0	28.8	152.6	208.0	150.3	207.1	129.2	208.3	199.5	196.8	211.7
Glucagon§ (*mμg/ml*)	3.0	2.9	1.8	1.6	1.4	1.7	1.6	1.6	1.6	1.6	1.6
±SEM	0.38	0.41	0.13	0.14	0.15	0.10	0.16	0.16	0.12	0.21	0.25
P value	NS		<0.02	<0.02	<0.01	<0.025	<0.01	<0.01	<0.01	<0.01	<0.01

* 10 experiments were conducted, only the mean values are tabulated.

‡ All glucose and amino nitrogen determinations were made on inferior vena caval plasma samples.

§ All hormone assays were made on pancreaticoduodenal vein plasma.

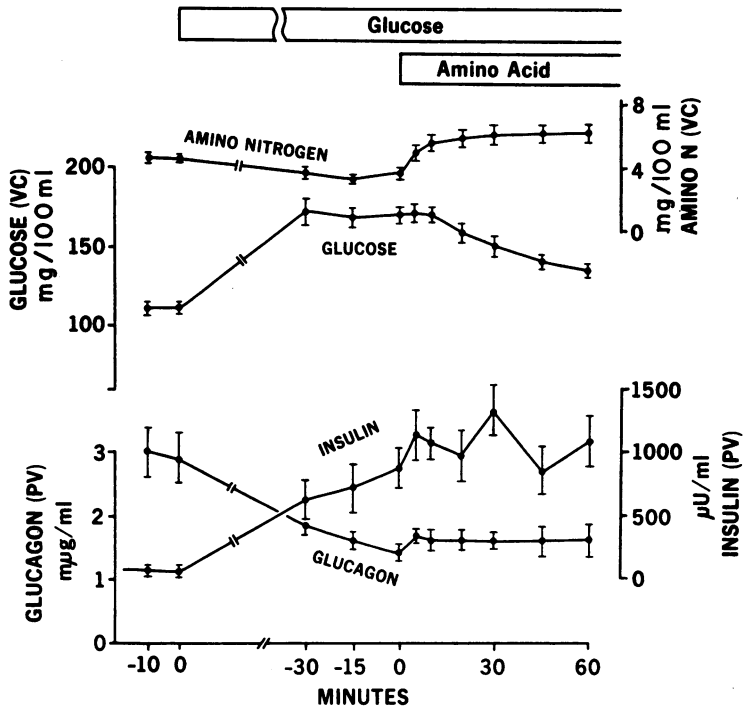

FIGURE 3 The effect of hyperglycemic suppression of aminogenic hyperglucagonemia upon the mean level of vena caval (VC) glucose.

TABLE III

Effect of Exogenous Glucagon upon Glucose Response to Hyperaminoacidemia during Hyperglycemic Suppression of Endogenous Glucagon

		Time before amino acid and glucagon infusion (min)		Time after amino acid and glucagon infusion (min)								
				Glucose infusion (300 mg/min)								
							Amino acid infusion (100 mg/min)					
		Control period					Glucagon injection (100 mμg) and infusion (50 mμg/min)					
Measurement, Source		−10	0	−30	−15	0	1	3	6	10	15	20	30
Mean ±SEM													
Glucose (*mg/100 ml*)	IVC‡	103.3	102.4	173.6	165.1	161.6	160.2	161.4	162.6	160.5	166.3	161.4	160.3
±SEM		1.3	1.9	14.3	9.8	9.7	9.6	9.0	9.0	9.0	9.0	9.0	7.0
Amino N (*mg/100 ml*)	IVC	4.5	4.3					4.0	5.0	5.0		5.0	5.5
±SEM		0.2	0.2					0.2	0.3	0.3		0.4	0.4
Insulin (*μU/ml*)	PV§	109.6	80.4	454.5	729.2	396.6	1172	602.0	674.0	669.0	732.0	701.0	1253
±SEM		44.3	29.2	242.8	304.8	141.4	259.	190	182	136	235	194	343
Glucagon (*mμg/ml*)			1.08	1.1		1.08	1.2	1.1				1.1	
±SEM	IVC		0.2	0.2		0.2	0.16	0.23				0.24	
Glucagon (*mμg/ml*)			2.2			1.30	1.8	1.6	1.5			1.8	
±SEM	PV		0.25			0.25	0.5	0.24	0.30			0.6	

* Six experiments were conducted; only the mean values are presented.

‡ IVC, inferior vena cava.

§ PV, pancreaticoduodenal vein.

±199.5) μU/ml in the latter experiments, as compared to a level of 296 (SEM ±104.4) μU/ml during amino acid infusion alone. However, despite the greater hyperinsulinemia, no statistically significant quantitative relationship between the rise in insulin secretion and the fall in plasma glucose was found in the hyperglycemic suppression experiments ($r = 0.246$; $P < 0.5$). This suggests that the greater insulin secretion may not have been the sole determining factor responsible for the greater amino acid–induced decline in plasma glucose.

Effect of exogenous glucagon upon glucose response to hyperaminoacidemia during hyperglycemic suppression of endogenous glucagon. If the greater fall in glucose concentration induced by amino acids during hyperglycemia was a consequence of suppression of aminogenic hyperglucagonemia, then administration of exogenous glucagon in a quantity no greater than that normally secreted during hyperaminoacidemia should prevent or reduce the glucose decline. Accordingly, six dogs were studied in a fashion identical to the foregoing suppression experiments except that crystalline beef-pork glucagon [6] was infused endoportally simultaneously with the amino acid infusion. After an initial priming dose of 100 mμg, glucagon was infused through the mesenteric vein catheter at a rate of 50 mμg/minute for 30 min in four of the dogs and for a full 60 min in two dogs. The rate of glucagon administration in these experiments would appear to have been considerably less than the rate at which endogenous glucagon is secreted in response to hyperaminoacidemia, inasmuch as the mean glucagon concentration in the inferior vena cava barely changed, rising only 0.12 mμg/ml, as compared to a rise of 0.4 mμg/ml in vena caval endogenous glucagon reported in hyperaminoacidemia (6).

The results of these experiments are summarized in Table III and in Fig. 4. Glucose concentration declined an average of only 5 mg during the 1st 30 min of the glucagon infusion even though pancreaticoduodenal vein insulin rose more than 600 μU/ml to a maximal mean level of 1253 μU/ml.

In four of the six experiments, the glucagon infusion was discontinued after 30 min, after which the glucose level fell an average of 39 mg/100 ml below the zero time level. In two of the dogs (Nos. 384 and 385) the glucagon infusion was continued for a full 60 min, and in the final 30 min the glucose concentration declined to 35 and 48 mg/100 ml below the initial value.[7] The

[6] Kindly donated by Dr. John Galloway, Eli Lilly Company, Indianapolis, Ind.

[7] After the glucagon infusion was stopped in dog Nos. 384 and 385, a further decline in glucose concentration of 11 and 12 mg/100 ml, respectively, was observed.. The post glucagon

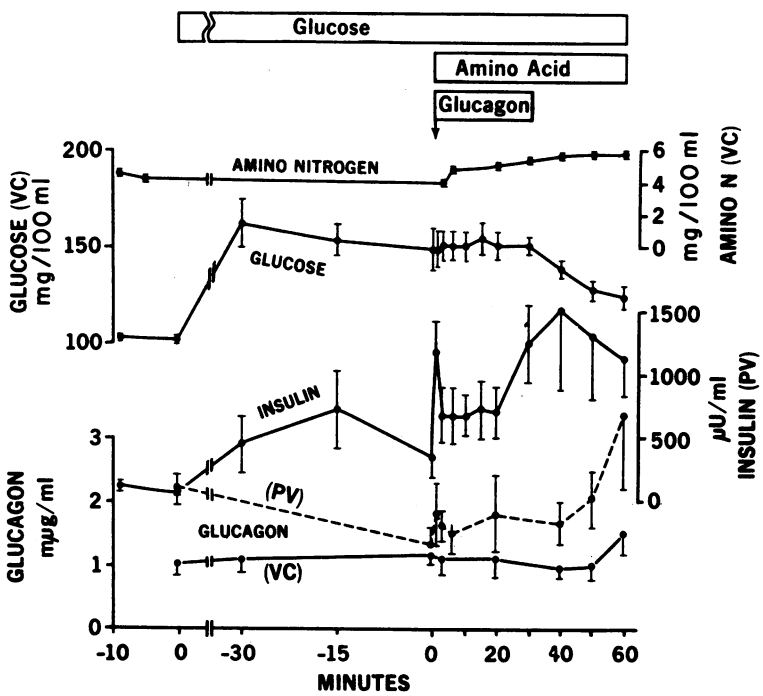

FIGURE 4 The effect of exogenous glucagon (100 mμg stat and 50 mμg/min) upon the mean level of vena caval (VC) glucose during hyperglycemic suppression of aminogenic hyperglucagonemia. See footnote 5.

TABLE IV

Effect of Exogenous Glucagon upon Maximal Glucose Fall during Hyperglycemic Suppression of Aminogenic Hyperglucagonemia

Dog No.	Maximal glucose fall (0–30 min)	Insulin rise (mean of increments) (0–30 min)
	mg/100 ml	
Control dogs (no glucagon)		
402	26	1022
403	18	348
288	24	682
301	0	75
303	22	383
304	8	550
391	52	0*
395	6	560
405	11	120
406	48	660
Mean ±SEM	21.5 ±5.4	488 ±99
Glucagon-treated dogs (100 mμg stat and 50 mμg/min)		
402	0	434
403	0	387
381	0	595
383	8	193
384	14	226
382	8	915
Mean ±SEM	5.0 ±2.4	458 ±109
P value	<0.05	*NS*

* Dog 391 has been omitted from the calculations because of lack of insulin rise above the high baseline level.

striking decline in glucose in the last 30 min may be related to the enormous rise in insulin unaccompanied by an adequate rise in glucagon.

In Table IV the maximal blood glucose decline during the 1st 30 min of amino acid infusion in the six hyperglycemic-hyperaminoacidemic dogs given exogenous glucagon is compared with that of the 10 hyperglycemic-hyperaminoacidemic dogs of Table II, in which no glucagon was administered. The difference is barely significant ($P < 0.05$), and suggests that exogenous glucagon may have restricted the aminogenic decline in plasma glucose for 30 min. However, a comparison of the mean insulin levels of the glucagon-treated group (Fig. 4) with that of the untreated "control" group (Fig. 3) reveals it to be higher in the latter, and this, rather than glucagon lack, could be interpreted as being the cause of the greater decline in plasma glucose. Yet the absolute insulin level reflects the response to the sum of the aminogenic and glycemogenic stimuli, whereas the increment of insulin concentration above the level at "zero time" represents only the amino acid–induced component of the insulin response, and it is this which is most germane to the amino acid–induced glucose response. The mean insulin increment during 30 min of amino acid infusion was calculated for each glucagon-treated dog of Table III and each untreated "control" dog of Table II, and the values recorded in Table IV. The average mean increment of the glucagon-treated group was 456 μU/ml (SEM ±109), while in the untreated "control" group it was 488 μU/ml (SEM ±99). This lack of a statistically significant difference tends to point away from insulin response as the primary cause of the difference in the glucose response of the groups.

Effect of restoring aminogenic hyperglucagonemia by overcoming suppression of endogenous glucagon secretion. Pancreozymin has recently been demonstrated to be a potent stimulus of both insulin (17, 18) and glucagon secretion (18) and it seemed possible that it might overcome the suppressive effect of hyperglycemia upon glucagon secretion. Pancreozymin was, therefore, infused at a rate of 8 U/min for 20 min in a group of four dogs during hyperglycemic suppression of aminogenic hyperglucagonemia. In two of the experiments, one of which is illustrated in Fig. 5 A, such a "breakthrough" was achieved. In this experiment the infusion of pancreozymin was accompanied by a 2.8 mμg/ml rise in pancreaticoduodenal vein glucagon; despite the associated outpouring of insulin, plasma glucose did not fall, and in fact, rose. In the other experiment glucagon rose 1.9 mμg/ml and glucose fluctuated but never declined more than 8 mg/100 ml. However in two other experiments, one of which is shown in Fig. 5 B, pancreozymin elicited a rise of only 0.7 and 0.8 mμg/ml in pancreaticoduodenal glucagon; in these the associated massive increase in insulin secretion was accompanied by a 35 and 17 mg/100 ml fall in plasma glucose, respectively.

These findings provide further evidence that glucagon secretion can limit the decline in plasma glucose concentration during stimulation of insulin secretion by betacytotropins other than glucose.

Effect of hyperlipacidemic suppression of aminogenic hyperglucagonemia upon plasma glucose response to hyperaminoacidemia. To exclude the possibility that at the high glucose level required to suppress aminogenic hyperglucagonemia a given quantity of insulin effects a greater reduction in glucose concentration than at a normoglycemic level, at which counterregulatory defenses would undoubtedly be more active, an attempt was made

data are not included in Table III, but have been incorporated in Fig. 4 by omitting from these two experiments (dog Nos. 384 and 385) the data of the last 30 min of glucagon infusion and substituting data obtained after stopping the glucagon infusion, thus making the entire group more homogeneous.

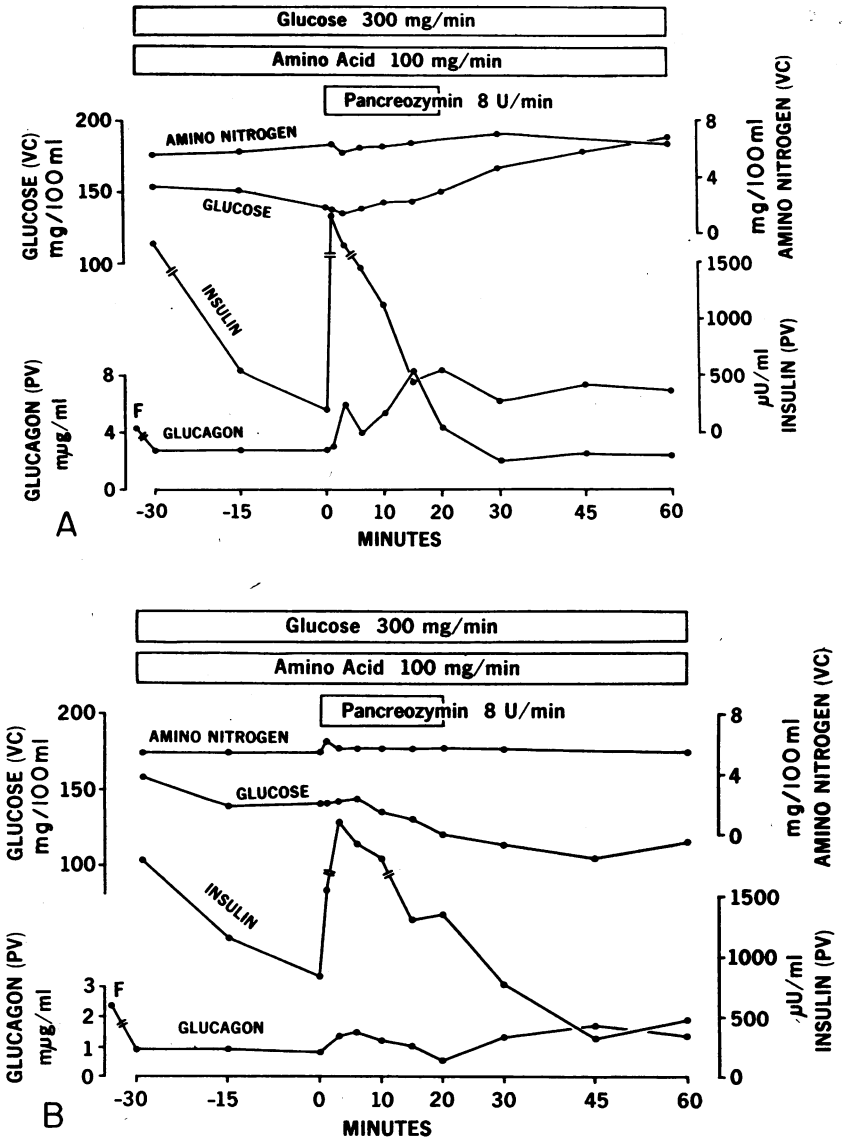

FIGURE 5 A The effect of pancreozymin-induced hyperglucagonemia upon the plasma glucose during hyperglycemic suppression of aminogenic hyperglucagonemia. B. The effect of hyperglycemic inhibition of pancreozymin-induced hyperglucagonemia upon the plasma glucose.

to suppress aminogenic glucagon secretion without hyperglycemia by inducing hyperlipacidemia, recently reported to inhibit glucagon secretion (*7*).

An intravenous infusion of Lipomul, a triglyceride emulsion, was administered to four dogs at a rate of 1 ml/min and injections of heparin were given intravenously in a dose of 3000 U before and 2000 U at 30 and 90 min after the start of Lipomul infusion. Mean free fatty acid concentration rose from 638 to 3570 μEq/liter with maximum levels ranging from 1800 to 6750 μEq/liter. The infusion of the amino acid mixture at a rate of 100 mg/min was begun 60 min after the start of the Lipomul infusion. The results of these experiments are recorded in Fig. 6. In all but one of these experiments glucagon was assayed with antiserum G-58 which reacts only with pancreatic glucagon.

In only two of the dogs did hyperlipacidemia seem to prevent an aminogenic rise in glucagon above the fasting level. In these two (Fig. 6 A and 6 B,[8]), plasma glucose declined 15 and 11 mg/100 ml, respectively. In a third

[8] Assayed with antiserum G-128, which cross-reacts with extracts of gastrointestinal tissue.

dog, (Fig. 6 C) glucagon rose during the Lipomul infusion to 2.3 mμg/ml, and 10 min after the amino acid infusion was begun, it rose further to a short-lived peak of 3.8 mμg/ml; during this abrupt rise and for 20 min thereafter, glucose changed little, but when the glucagon level returned to the 2.2 mμg/ml range, unaccompanied by a concomitant decline in insulin, glucose concentration fell 19 mg/100 ml. The subsequent hyperglycemia occurred after a second rise in glucagon which was unaccompanied by an insulin rise. The other dog (Fig. 6 D)

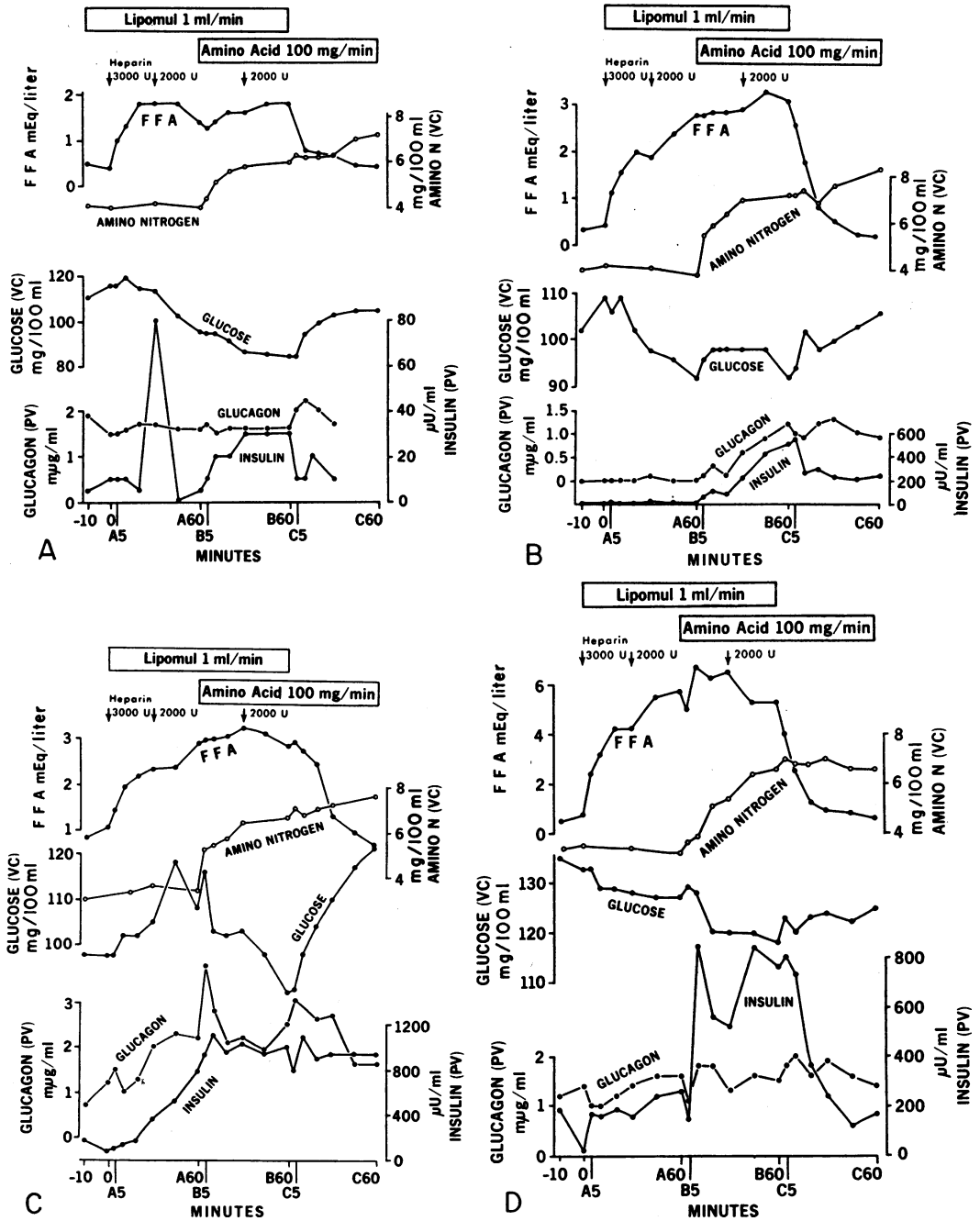

FIGURE 6 The effects of hyperlipacidemia upon the pancreaticoduodenal vein glucagon, insulin, and vena cava glucose responses during hyperaminoacidemia.

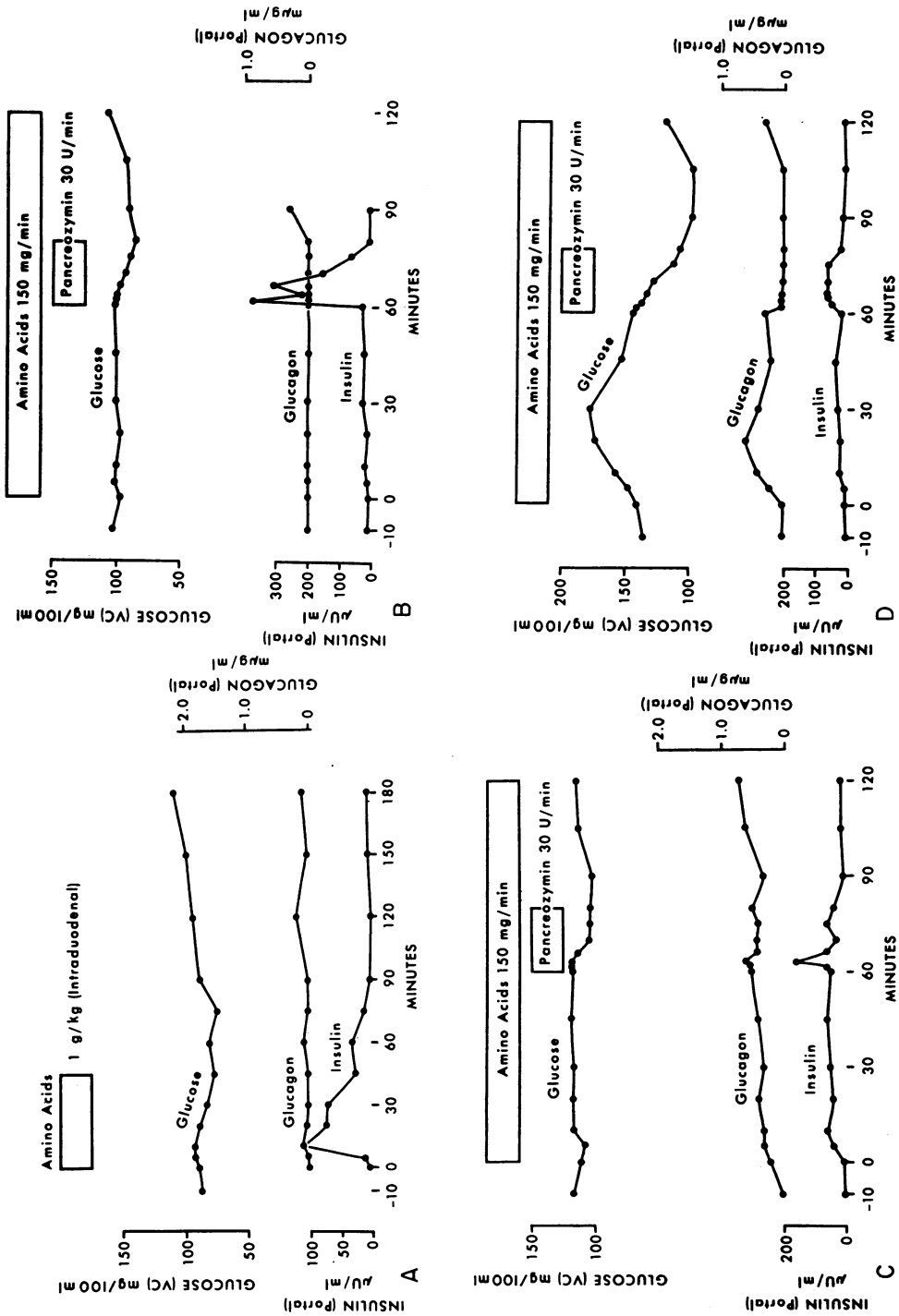

FIGURE 7 The effects of administration of amino acids intraduodenally (A) and intravenously (B, C, and D) upon portal vein insulin and glucagon and vena caval glucose in "uncinate dogs."

exhibited a rise in glucagon during the amino acid infusion and plasma glucose did not fall below the level at the start of the amino acid infusion.

The effect of resection of alpha cell–bearing pancreas upon response to hyperaminoacidemia. Bencosme and Liepa have reported that the alpha cells are sparse in the uncinate process and that its glucagon content is low (8). Measurement of acid-alcohol extracts of pancreas obtained from four intact dogs immediately after sacrifice revealed a total glucagon content of 0–0.54 μg and an insulin content of 1.0–3.9 U in the uncinate process, as compared to a total pancreatic content of 48–144 μg of glucagon and from 14–33 U of insulin. Thus, in these dogs the uncinate process contained 5–25% of the total insulin but only 1% or less of the total glucagon. It was hoped, therefore, that the surgical removal of all pancreatic tissue except the uncinate process would yield a dog with a greater deficiency of glucagon than insulin. In such a dog hyperaminoacidemia might stimulate secretion of insulin to a relatively greater degree than glucagon, and the importance of the glucagon response in preventing a fall in glucose could then be evaluated.

Four "uncinate dogs" survived the surgery and endured the postoperative period without morbidity. All but one had a fasting plasma glucose level below 120 mg/100 ml at the time of the experiment 3 or more days postoperatively. In these dogs glucagon was assayed in portal vein plasma using antiserum G-58, which is relatively specific for pancreatic glucagon. Two of the four dogs had zero values for glucagon in portal vein plasma and were regarded as glucagon deficient. One such dog (Fig. 7 A). was given 1 g of amino acid mixture/kg of body weight intraduodenally over a 45 min period and, despite hyperaminoacidemia, portal vein glucagon failed to rise above zero. However, the portal vein insulin rose to 100 μU/ml and glucose fell 14 mg/100 ml. The extract of the uncinate process of this dog contained a total of 0.33 μg of glucagon, but insulin was not measured. In the second dog (Fig. 7 B) portal vein glucagon failed to rise above zero during an intravenous infusion of amino acids at a rate of 150 mg/min; the portal vein insulin rose only slightly to 30 μU/ml and plasma glucose did not fall. However, when an infusion of pancreozymin at a rate of 30 U/minute was added, the insulin rose briskly to 376 μU/ml, the glucagon remained at zero, and glucose fell 16 mg/100 ml. The extract of the uncinate process of this dog contained a total of 0.6 μg of glucagon and 2U of insulin.

In a third dog (Fig. 7 C) both glucagon and insulin rose in parallel during the amino acid infusion and plasma glucose remained constant. When an infusion of pancreozymin was added, a further small rise in insulin took place with little or no rise in glucagon, and glucose fell 14 mg/100 ml. The extract of this dog's pancreatic remnant contained 17.8 μg of glucagon and 3.1 U of insulin. The fourth dog (Fig. 7 D) was overtly diabetic with fasting hyperglycemia and the amino acid infusion caused only a slight rise in insulin; however, a substantial rise in glucagon occurred and this was accompanied by a parallel rise in plasma glucose concentration. When the hyperglucagonemia began to wane after 30 min, the hyperglycemia declined. The administration of pancreozymin was associated with a modest rise in insulin, but, inexplicably, glucagon fell to zero at this point, whereupon a more rapid decline in plasma glucose occurred. The pancreatic remnant of this dog contained 6.2 μg of glucagon and 1.3 U of insulin.

These findings are in keeping with the premise that normoglycemia requires an appropriate secretory relationship between insulin and glucagon. When glucagon secretion is disproportionately low, glucose declines; when insulin is reduced in relation to glucagon, glucose rises.

DISCUSSION

The foregoing results suggest that the prompt increase in pancreatic glucagon secretion that normally occurs during hyperaminoacidemia in concert with increased insulin secretion serves to limit the decrement in glucose concentration. This conclusion is supported by the demonstration of an inverse relationship between the magnitude of the cataglycemia that occurs during the 1st 60 min of hyperaminoacidemia and the amount of glucagon released during the 1st 20 min, and by the lack of a quantitative relationship between the cataglycemia and the amount of insulin released. Furthermore, when the pancreaticoduodenal vein glucagon concentrations were prevented from rising above the fasting level, either by hyperglycemia, by hyperlipacidemia, or by resection of the glucagon-secreting areas of the pancreas, the magnitude of the glucose fall during hyperaminoacidemia increased. Finally, restoration of hyperglucagonemia, by infusing exogenous glucagon, even at a physiologically suboptimal rate, reduced the cataglycemia during the 1st 30 min of hyperglycemic inhibition of the aminogenic glucagon response; similarly, restoration of hyperglucagonemia by means of pancreozymin-induced "breakthrough" of the hyperglycemic suppression prevented the cataglycemia.

These findings suggest that, were it not for aminogenic hyperglucagonemia, a carbohydrate-free protein meal, as is commonly consumed by carnivores, would result in an important reduction of blood glucose as a consequence of an uncompensated increase in glucose utilization secondary to aminogenic insulin secretion. However, glucagon allows for proper compensation by increasing hepatic glucose production and thus replacing the glucose postulated to enter insulin-sensitive tis-

sues in the company of amino acids.[9] The fact that glucose infusion so readily inhibits aminogenic hyperglucagonemia at glucose concentrations above 142 mg/100 ml (5) lends additional credence to this concept of glucagon's role.

It may be that the entry of glucose into cells postulated to occur during hyperaminoacidemia is more than merely a passive and inadvertent event without physiologic meaning; Wool and Krahl have found that in the diaphragms of rats fasted 48 hr glucose availability is essential to incorporation of amino acids into protein (19), the so-called "protein-sparing" effect of glucose.

Although this concept of the role of aminogenic hyperglucagonemia seems well supported in a qualitative sense by the results of this study, these data do not provide quantitative evidence of its physiologic importance. For example, one can argue with some justification that the mean glucose fall of 35 mg/100 ml, which occurred during suppression of aminogenic hyperglucagonemia for 1 hr, hardly constitutes a serious threat, even if it had begun at a normal fasting glucose concentration. Moreover, this decline occurred during hyperglycemia, a state in which other defenses against hypoglycemia might be obtunded, thereby permitting a larger decline in glucose concentration than would have occurred at normal glucose levels. In both the hyperlipacidemic suppression experiments and in the uncinate dog experiments, the initial glucose levels were normal and cataglycemia did not exceed 31 mg/100 ml and averaged considerably less. If the fall in glucose concentration prevented by aminogenic hyperglucagonemia is no larger than this, it is difficult to regard it as an extremely important physiologic protective device. Furthermore, as shown in Table III, the protective effect of exogenous glucagon during hyperglycemic suppression seems to wane after the 1st 30 min of a 60 min glucagon infusion, and by the end of the infusion the plasma glucose has declined as much as in the control experiments, in which no exogenous glucagon had been administered.

However, none of the foregoing arguments necessarily precludes the possibility that the anti-cataglycemic effect of aminogenic hyperglucagonemia is of greater quantitative importance than these data indicate, since each argument can be effectively countered. As for the relatively small glucose decline during suppression of glucagon secretion, it is likely that suppression was incomplete during hyperglycemia; in fact, when hyperaminoacidemia was induced, the mean glucagon concentration rose 0.3 mμg/ml above the nadir (Table II). Furthermore in a realistic physiologic setting hyperaminoacidemia occurs only after the ingestion of protein, in which an associated secretion of protein-responsive enteric hormones augment the insulin and glucagon response to hyperaminoacidemia (5,[10]). If, however, the augmented insulin response were unaccompanied by a concomitant augmentation of the glucagon response, cataglycemia would be exaggerated, as was the case in the experiment depicted in Fig. 7 B. With respect to the modest degree of cataglycemia observed in the hyperlipacidemic suppression experiments, one can find refuge in the fact that free fatty acids appear to be a weak suppressant of glucagon secretion, and that the free fatty acids themselves would tend to oppose the glucose decline through the so-called "glucose–fatty acid cycle" (20). As for the relatively unimportant glucose decline induced by hyperaminoacidemia in the relatively glucagon-deficient "uncinate dogs," the associated reduction in insulin-secreting tissue easily accounts for this. Finally, the failure of the 60 min endoportal infusion of glucagon to prevent cataglycemia during the final 30 min can be ascribed to the low dose of glucagon employed and to a failure of the glucagon concentration to rise terminally in proportion to the rising insulin concentration.

These counterarguments neutralize the array of evidence against the quantitative importance of the blood glucose reduction which aminogenic hyperglucagonemia prevents. Obviously, however, ultimate proof of the existence of such a reduction and accurate assessment of its magnitude must await either experiments in totally glucagon-deficient animals or quantitative studies of glucose turnover during hyperaminoacidemia.

Although at least one counterregulatory hormone in addition to glucagon, growth hormone, is stimulated by hyperaminoacidemia (21), the rise occurs 40 min after the start of the hyperaminoacidemia (22) and is probably too late to play an early role in the defense against hypoglycemia. Furthermore, according to Rabinowitz et al. aminogenic hypersomatotropinemia is not inhibited by hyperglycemia (23), which suggests that its function relates primarily to the disposition of the amino acids. Best, Catt, and Burger report a lack of such inhibition, however (24).

Since hyperaminoacidemia is perhaps the only physiologic potential cause of insulinogenic hypoglycemia, and since euglycemia requires a well coordinated secretory response of glucagon and insulin, it is not unlikely that disorders of glucoregulation will now be found to result from faulty alpha cell function or a loss of bihormonal coordination. Protein-induced hypoglycemia would seem to be the clinical expression *par excellence* of a pure

[9] The assumption that glucose turnover must be increased during hyperaminoacidemia is based on the fact that glucose concentration remains essentially unchanged during a major rise in both insulin and glucagon secretion.

[10] Dupré, J., J. D. Curtis, R. H. Unger, R. W. Waddell, and J. C. Beck. 1969. Effects of secretin, pancreozymin, or gastrin on insulin and glucagon secretion in response to administration of glucose or arginine in man. *J. Clin. Invest.* **48:** 745.

glucagon deficiency. It seems entirely possible that the syndrome of idiopathic infantile hypoglycemia, currently attributed to an excessive insulin response to certain amino acids, represents partial or complete glucagon deficiency, a view first proposed by McQuarrie et al. on the basis of the histologic observation of absent alpha cells (25).

ACKNOWLEDGMENTS

This work was supported by U. S. Public Health Service Grant AM-02700-09, Hoechst Pharmaceutical Company, Cincinnati, Ohio, Upjohn Co., Kalamazoo, Mich., and Pfizer Laboratories, New York. The generosity of Mrs. Florence Latz contributed importantly to this study.

REFERENCES

1. Unger, R. H., A. M. Eisentraut, M. S. McCall, and L. L. Madison. 1962. Measurements of endogenous glucagon in plasma and the influence of blood glucose concentration upon its secretion. *J. Clin. Invest.* **41**: 682.
2. Ohneda, A., E. Parada, A. M. Eisentraut, and R. H. Unger. 1969. Control of pancreatic glucagon secretion by glucose. *Diabetes.* **18**: 1.
3. Assan, R., G. Rosselin, and J. Dolias. 1967. Effets sur la glucagonemie des perfusions et ingestions d'acides aminés. *Journées Ann. Diabètologie Hôtel Dieu.* Éditions Médicales Flammarion. **7**: 25.
4. Fajans, S. S., J. C. Floyd, Jr., R. F. Knopf, and J. W. Conn. 1967. Effects of amino acids and proteins on insulin secretion in man. *Recent Progr. Hormone Res.* **23**: 617.
5. Ohneda, A., E. Parada, A. M. Eisentraut, and R. H. Unger. Characterization of response of circulating glucagon to intraduodenal and intravenous administration of amino acids. *J. Clin. Invest.* **47**: 2305.
6. Lochner, J. de V., A. M. Eisentraut, and R. H. Unger. 1964. The effects of $CoCl_2$ on glucagon levels in plasma and pancreas of the rat. *Metab. Clin. Exp.* **13**: 868.
7. Madison, L. L., W. A. Seyffert, Jr., R. H. Unger, and B. Barker. 1968. Effect of plasma free fatty acids on plasma glucagon and serum insulin concentrations. *Metab. Clin. Exp.* **17**: 301.
8. Bencosme, S. A., and E. Liepa. 1955. Regional differences of the pancreatic islet. *Endocrinology.* **57**: 588.
9. Kenny, J. A. 1955. Extractable glucagon of the human pancreas. *J. Clin. Endocrinol. Metab.* **15**: 1089.
10. Floyd, J. C., Jr., S. S. Fajans, J. W. Conn, R. F. Knopf, and J. Rull. 1966. Insulin secretion in response to protein ingestion. *J. Clin. Invest.* **45**: 1479.
11. Hoffman, W. S. 1937. A rapid photoelectric method for the determination of glucose in blood and urine. *J. Biol. Chem.* **120**: 51.
12. Frame, E. G., J. A. Russell, and A. E. Wilhelmi. 1943. The colorimetric estimation of amino nitrogen in blood. *J. Biol. Chem.* **149**: 255.
13. Mosinger, F. 1965. Photometric adaptation of Dole's microdetermination of free fatty acids. *J. Lipid Res.* **6**: 157.
14. Yalow, R. S., and S. A. Berson. 1960. Immunoassay of endogenous plasma insulin in man. *J. Clin. Invest.* **39**: 1157.
15. Unger, R. H., and A. M. Eisentraut. 1967. Hormones in blood. **1**: 83.
16. Eisentraut, A. M., A. Ohneda, E. Parada, and R. H. Unger. 1968. Immunologic discrimination between pancreactic glucagon and enteric glucagon-like immunoreactivity (GLI) in tissues and plasma. *Diabetes.* **17** (Suppl. 1): 321.
17. Meade, R. C., H. A. Kneubuhler, W. J. Schulte, and J. J. Barboriak. 1967. Stimulation of insulin secretion by pancreozymin. *Diabetes.* **16**: 141.
18. Unger, R. H., H. Ketterer, J. Dupré, and A. M. Eisentraut. 1967. The effects of secretin, pancreozymin, and gastrin on insulin and glucagon secretion in anesthetized dogs. *J. Clin. Invest.* **46**: 630.
19. Wool, I. G., and M. E. Krahl. 1959. Incorporation of C_{14} histidine into protein of isolated diaphragm. Interaction of fasting, glucose and insulin. *Amer. J. Physiol.* **197**: 367.
20. Randle, P. J., P. B. Garland, C. N. Hales, and E. A. Newsholme. 1963. The glucose fatty acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet.* **1**: 785.
21. Merimée, T. J., D. A. Lillicrap, and D. Rabinowitz. 1965. Effect of arginine on serum levels of human growth hormone. *Lancet.* **2**: 668.
22. Knopf, R. F., J. W. Conn, S. S. Fajans, J. C. Floyd, E. M. Guntsche, and J. A. Rull. 1965. Plasma growth hormone response to intravenous administration of amino acids. *J. Clin. Endocrinol. Metab.* **25**: 1140.
23. Rabinowitz, D., T. J. Merimée, J. A. Burgess, and L. Riggs. 1966. Growth hormone and insulin release after arginine: indifference to hyperglycemia and epinephrine. *J. Clin. Endocrinol. Metab.* **26**: 1170.
24. Best, J., K. J. Catt, and H. G. Burger. 1968. Nonspecificity of arginine infusion as a test for growth-hormones secretion. *Lancet.* **2**: 124.
25. McQuarrie, I., E. T. Bell, B. Zimmermann, and W. S. Wright. 1950. Deficiency of alpha cells of pancreas as possible etiological factor in familial hypoglycemosis. *Fed. Proc.* **9**: 337.