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### Research Article

The development of a glucagon radioimmunoassay with a relatively high degree of specificity for pancreatic glucagon made possible studies of alpha cell function in healthy nondiabetic subjects and in patients with diabetes mellitus. In the former group mean fasting plasma glucagon averaged 108  $\mu\text{g/ml}$  (SEM  $\pm 10$ ). In 12 juvenile-type diabetics fasting glucagon averaged 110 ( $\pm 9$ ) and in 33 adult-type diabetics the average was 114 ( $\pm 8$ ). The diabetic averages did not differ significantly from the nondiabetic subjects; however, when hyperglycemia was induced by glucose infusion in the nondiabetic subjects so as to simulate the fasting hyperglycemia of the diabetics, mean glucagon fell to 57  $\mu\text{g}$  ( $\pm 8$ ), which was significantly below the diabetic mean.

In 28 healthy subjects the infusion of arginine elicited a rise in glucagon of at least 100  $\mu\text{g/ml}$  with a peak level averaging 331  $\mu\text{g/ml}$  ( $\pm 22$ ) at 40 min. This response to arginine was diminished but not abolished during hyperglycemia induced by simultaneous glucose infusion. In everyone of 45 diabetic subjects tested the infusion of arginine elicited a rise in glucagon of at least 140  $\mu\text{g/ml}$  to levels significantly greater than in nondiabetics. The peak glucagon level in juvenile-type diabetics averaged 458  $\mu\text{g/ml}$  (SEM  $\pm 36$ ) and in adult-type diabetics averaged 452  $\mu\text{g/ml}$  (SEM  $\pm 38$ ). The glucagon response to arginine was unrelated to duration of diabetes, to body weight, [...]

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# Studies of Pancreatic Alpha Cell Function in Normal and Diabetic Subjects

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**ABSTRACT** The development of a glucagon radioimmunoassay with a relatively high degree of specificity for pancreatic glucagon made possible studies of alpha cell function in healthy nondiabetic subjects and in patients with diabetes mellitus. In the former group mean fasting plasma glucagon averaged 108  $\mu\text{g/ml}$  (SEM  $\pm 10$ ). In 12 juvenile-type diabetics fasting glucagon averaged 110 ( $\pm 9$ ) and in 33 adult-type diabetics the average was 114 ( $\pm 8$ ). The diabetic averages did not differ significantly from the nondiabetic subjects; however, when hyperglycemia was induced by glucose infusion in the nondiabetic subjects so as to simulate the fasting hyperglycemia of the diabetics, mean glucagon fell to 57  $\mu\text{g}$  ( $\pm 8$ ), which was significantly below the diabetic mean.

In 28 healthy subjects the infusion of arginine elicited a rise in glucagon of at least 100  $\mu\text{g/ml}$  with a peak level averaging 331  $\mu\text{g/ml}$  ( $\pm 22$ ) at 40 min. This response to arginine was diminished but not abolished during hyperglycemia induced by simultaneous glucose infusion. In everyone of 45 diabetic subjects tested the infusion of arginine elicited a rise in glucagon of at least 140  $\mu\text{g/ml}$  to levels significantly greater than in nondiabetics. The peak glucagon level in juvenile-type diabetics averaged 458  $\mu\text{g/ml}$  (SEM  $\pm 36$ ) and in adult-type diabetics averaged 452  $\mu\text{g/ml}$  (SEM  $\pm 38$ ). The glucagon response to arginine was unrelated to duration of diabetes, to body weight, type of diabetic treatment, or to other known factors. Marked hyperresponsiveness of glucagon to arginine infusion was observed in two patients with advanced Kimmelsteil-Wilson disease. Glucagon levels were markedly elevated in certain

patients with severe diabetic ketoacidosis before treatment with insulin.

The findings suggest that alpha cell function is inappropriately increased in diabetes mellitus and could play a significant role in the diabetic syndrome.

## INTRODUCTION

Despite the availability of relatively sensitive radioimmunoassays for glucagon for almost a decade (1-4), valid measurements of pancreatic glucagon in human plasma have only recently become possible (5-7), and human alpha cell function has remained virtually unexplored. This is the consequence of technical problems which have plagued the radioimmunoassay, the most serious and intractable of which has been the problem of its specificity for pancreatic glucagon. It is now recognized that most antisera to pancreatic glucagon cross-react with so-called "glucagon-like immunoreactivity" (GLI), polypeptide substances in the upper gastrointestinal tract. Inasmuch as plasma GLI may comprise more than 90% of the total plasma "glucagon" measured with such antisera, it is extremely doubtful that earlier efforts to measure plasma levels of pancreatic glucagon in man (3, 8, 9) are quantitatively valid.

The chance discovery of a highly sensitive antiserum with an unprecedented degree of specificity for pancreatic glucagon (10) has made possible preliminary studies of pancreatic alpha cell function in man (5, 6). The following report describes more extensive evaluation of alpha cell function in healthy nondiabetic and diabetic subjects during varying circumstances of nutrient availability.

*Subjects.* The healthy nondiabetic subjects in these studies were recruited from the medical student body, the house staff, hospital employees and their friends, and from among nondiabetic ambulatory Dallas VA Hos-

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pital patients without serious current acute illnesses. The latter group consisted of five patients convalescing from minor surgery who were considered to be in good general health at the time of testing. Although results of glucagon assays in the five patients were significantly lower than the remainder of the control group ( $P < 0.01$ ), they were combined with the other controls and will be considered as a single group. Their ages ranged from 20 to 52 yr and averaged 26 yr. Their weights ranged from 56 to 87 kg and averaged 72 kg. None were regarded as obese.

The diabetic subjects in this study were either inpatients at the Dallas VA Hospital, or were outpatients of Parkland Memorial Hospital or the Dallas VA Hospital. They were regarded as genetic diabetics because of a negative history of pancreatic disease. The ages of the juvenile-type diabetic group averaged 36 yr, with a range from 11 to 58 yr, and the weights averaged 63 kg with a range from 38 to 79 kg. The adult-type diabetic group averaged 52 yr of age with a range from 30 to 67 yr; weights averaged 83 kg with a range from 49 to 135.

## METHODS

*Arginine infusion test.* All subjects were tested between the hours of 8 and 10 a.m. after an overnight fast. Diabetic patients were asked to omit their medication, whether insulin or an oral agent, on the morning of their test. An indwelling needle was placed in an antecubital vein for ease of blood sampling and its patency was maintained by local instillation of heparin.

Each experiment began with a 30 min baseline period, during which blood samples were obtained at 10-min intervals. L-Arginine monochloride<sup>1</sup> was infused in the antecubital vein as a 5% glucose-free solution at a constant rate of 468 mg/kg body weight for 40 min. During the infusion period bloods were drawn at 5-min intervals for the first 10 min and at 10-min intervals thereafter. 8 ml of blood was drawn in a dry disposable plastic syringe. The blood was placed promptly in chilled tubes containing 12 mg of EDTA ( $\text{Na}_2$ ) and 4000 or 8000 U of Trasylol<sup>2</sup> in a volume of 0.8 ml. Blood specimens were immediately centrifuged at 4°C and the plasma removed and frozen at -20°C until the time of assay not more than 5 wk later.

*Analytical methods.* Insulin was measured by a modification (11) of the Yalow-Berson radioimmunoassay (12). Plasma glucose was measured by the Hoffman method (13) using the Technicon AutoAnalyzer.

Glucagon was measured in duplicate by a modification (6) of the previously described radioimmunoassay (14). Antiserum obtained from rabbit G-58 was used for the measurement of all specimens. This antiserum cross-reacts very weakly with acid-alcohol extracts of gastrointestinal tissues; for example, a solution of jejunal extract, which reads 80  $\mu\text{g}$  of glucagon-like immunoreactivity per ml using antiserum 78 J and 18  $\mu\text{g}/\text{ml}$  with antiserum G-128, the one used to obtain most of the physiologic data published

from this laboratory since 1963, reads only 2  $\mu\text{g}/\text{ml}$  with antiserum G-58 (10). Furthermore, antiserum G-58 gives zero readings in plasma from dogs in which the glucagon-secreting portions of the pancreas have been resected, the first antiserum demonstrated to do so; in addition to greater specificity, i.e. diminished cross-reactivity with GLI, this antiserum is not influenced by gamma globulin or by other unidentified plasma factors which inhibit the reaction of glucagon-<sup>125</sup>I with certain antisera (15). In the assay 0.1 ml of the G-58 antiserum (1:9000) was added to 20  $\mu\text{g}$  of glucagon-<sup>125</sup>I (specific activity 775-950 mCi/mg) in 0.1 ml volume, 500 U of Trasylol in 0.05 ml, and 0.05 ml of either the undiluted plasma sample or the crystalline glucagon beef-pork glucagon standard. All solutions were made up in 0.2 M glycine buffer containing 0.25% human albumin (Cutter Laboratories). The reaction mixture was incubated at 4°C for 4 days. Separation of free and bound glucagon-<sup>125</sup>I was carried out either by chromatography on Toyo paper using the previously described method (14) or by a modification (6) of the charcoal method of Herbert, Lau, Gottlieb, and Bleicher (11), which was found to give values identical with the paper method for unknown plasma samples. In a typical standard curve 50  $\mu\text{g}$  of crystalline glucagon causes a 9% fall in glucagon-<sup>125</sup>I bound to antibody. The standard deviation of the difference of each replicate from the mean of replicates was  $\pm 6.8 \mu\text{g}/\text{ml}$ , so that a change of 40  $\mu\text{g}/\text{ml}$  or greater can be regarded as authentic with 95% confidence.

## RESULTS

### Nondiabetic subjects

*Fasting glucagon levels.* The mean fasting glucagon level of 28 healthy nondiabetic subjects was 108  $\mu\text{g}/\text{ml}$  ( $\text{SEM} \pm 10$ ). The range was from 50 to 210  $\mu\text{g}/\text{ml}$ . The plasma glucose concentration of this group averaged 87 mg/100 ml ( $\text{SEM} \pm 1$ ). The glucagon concentration of the single female member of the normal group was 80  $\mu\text{g}/\text{ml}$ . No correlation between glucose and glucagon concentration or between glucagon and insulin levels was observed.

To determine the degree of moment-to-moment change in glucagon concentration in the fasting state, in 25 of the nondiabetic subjects four blood samples were obtained within an hour and the standard deviation of the difference of these glucagon determinations from their mean was calculated. The value was  $\pm 13.2 \mu\text{g}/\text{ml}$ , not far above the  $\pm 6.8 \mu\text{g}/\text{ml}$  standard deviation of the difference of replicates of a single blood specimen from their mean. This indicates a considerable degree of "within-hour" stability of plasma glucagon levels in normal fasting subjects.

The day-to-day variation in glucagon concentration was examined in a group of nine healthy males. The glucagon level averaged 126  $\mu\text{g}/\text{ml}$  on each of 2 consecutive days and the mean difference between the days was 15  $\mu\text{g}/\text{ml}$  with a maximum difference of 34  $\mu\text{g}/\text{ml}$ .

*Effect of glucose infusion.* Studies in dogs have demonstrated that hyperglycemia profoundly suppresses

<sup>1</sup> R-Gene, Cutter Laboratories, Berkeley, Calif.

<sup>2</sup> Donated through the kindness of Dr. Frank G. Falco of Delbay Pharmaceuticals, Inc., Bloomfield, N. J.

pancreatic glucagon secretion (16, 17). To determine if a similar effect occurs in man, 5% glucose was infused intravenously in eight healthy subjects at a constant rate of 1.5 g/min for 30 min, raising plasma glucose concentration progressively to a peak of 260 mg/100 ml (SEM  $\pm$ 15) at the end of the infusion. The mean glucagon level declined from a pre-infusion level of 90  $\mu$ g/ml (SEM  $\pm$ 8) to a nadir of 57  $\mu$ g/ml (SEM  $\pm$ 8). This value differed significantly from the pre-infusion value ( $P < 0.01$ ). The mean values are shown in Fig. 1 and in Table I.

*Glucagon response to arginine infusion.* Studies in dogs (18) and in man (9, 19) indicate that increased blood levels of some amino acids constitute a powerful stimulus to pancreatic glucagon secretion; although the results in humans were obtained with antisera which were not specific for pancreatic glucagon, they have been confirmed (6) with the more specific antiserum employed here.

A total of 27 healthy males and one female have now been studied. The infusion of 468 mg/kg of arginine at a constant rate for 40 min was associated in every subject with a significant rise in plasma glucagon within the first 5 min of the infusion. The mean glucagon level rose from 108  $\mu$ g/ml (SEM  $\pm$ 10) to a peak of 331  $\mu$ g/ml (SEM  $\pm$ 22) at 40 min, the time at which the infusion was discontinued. Mean glucagon concentration approached the baseline level within the next 30 min. The mean maximal rise was 239  $\mu$ g/ml (SEM  $\pm$ 19). Every subject exhibited a maximal rise of at least 100  $\mu$ g/ml.

Insulin also rose promptly in all subjects from 8.5  $\mu$ U/ml (SEM  $\pm$ 10) to a peak of 34  $\mu$ U/ml (SEM  $\pm$ 4) at 30 and 40 min. The mean glucose level rose from 87 (SEM  $\pm$ 1) to 99 mg/100 ml (SEM  $\pm$ 2). The mean results are depicted in Fig. 2 and in Table II.

*Effect of induced hyperglycemia upon glucagon response to arginine.* Hyperglycemia induced in dogs by glucose infusion suppresses completely the usually brisk

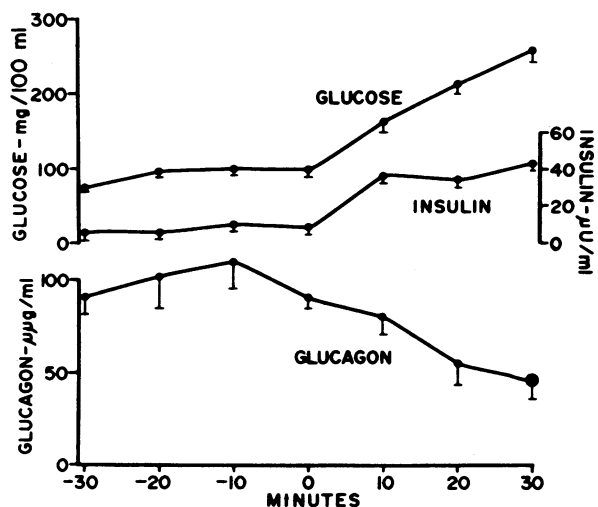


FIGURE 1 The effect of glucose infusion (115 g/min) on plasma glucagon (mean  $\pm$ SEM) in healthy subjects ( $\bullet$  = statistically significant difference from zero time).

hyperglucagonemic response to hyperaminoacidemia (18, 20). To determine if arginine-induced hyperglucagonemia in man could be similarly suppressed, 10% glucose was infused intravenously at a rate of 15 ml/min in a group of seven healthy subjects. 30–40 min later, at which time plasma glucose averaged 286 mg/100 ml (SEM  $\pm$ 21) and glucagon 53  $\mu$ g/ml (SEM  $\pm$ 4), arginine infusion was begun. The mean glucagon level rose to 190  $\mu$ g/ml (SEM  $\pm$ 41) 20 min later, which is significantly less than the rise to 304  $\mu$ g/ml (SEM  $\pm$ 23) observed at 20 min in normoglycemic subjects. The mean values are shown in Fig. 3 and in Table III. It would appear that in normal subjects the glucagon rise in response to this dose of arginine is only slightly reduced by induced hyperglycemia, and that the levels attained are at all times less than in normoglycemic subjects.

TABLE I  
Effect of Glucose Infusion on Plasma Glucagon in Nondiabetic Subjects

	Minutes . . . -10	0	Glucose 1.5 g/min				
			5	10	20	25	30
Glucose mean	90	90	130	162	210	250	260
$\pm$ SEM	2	2	5	7	10	15	15
Glucagon mean	110	90	92	84	62	64	57
$\pm$ SEM	15	8	8	9	8	8	8
$P$ (vs. time 0) =			NS	NS	NS	<0.05	<0.01
Insulin mean	11	8	27	38	36	47	45
$\pm$ SEM	3	3	7	11	7	11	10

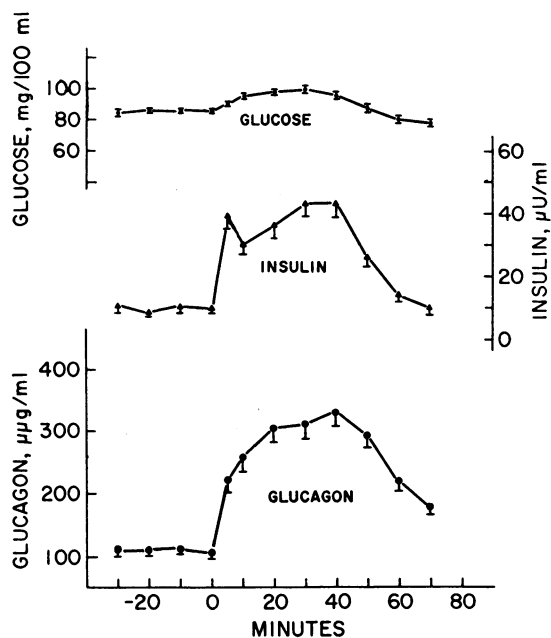


FIGURE 2 The effect of arginine infusion (11.7 mg/kg per min) on plasma glucagon and insulin in 28 healthy subjects.

### Diabetic subjects

**Fasting glucagon levels.** Among 12 patients classified as insulin dependent, ketoacidosis-prone, juvenile-type diabetics the fasting glucagon averaged 118  $\mu\text{g/ml}$  (SEM  $\pm 9$ ) with a range of 70–200  $\mu\text{g/ml}$ . The mean fasting plasma glucose level in this group was 198 mg/100 ml (SEM  $\pm 25$ ) with a range from 66 to 385 mg/100 ml.

Among 33 adult-type, insulin-independent diabetics the average was 114  $\mu\text{g/ml}$  (SEM  $\pm 8$ ) and the range from 10 to 240  $\mu\text{g/ml}$ . Glucose averaged 177 mg/100 ml (SEM  $\pm 10$ ).

The mean fasting glucagon level of the diabetic groups did not differ significantly from that of non-diabetic subjects nor from each other (Fig. 4). Within-

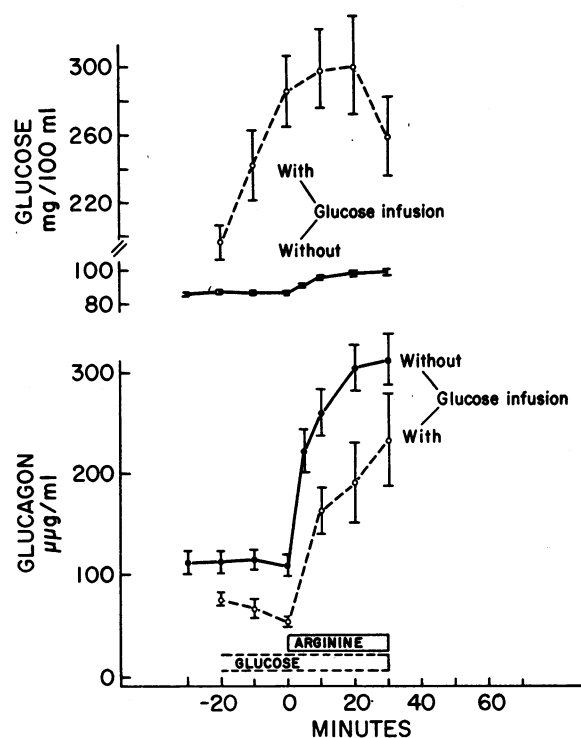


FIGURE 3 The effect of glucose infusion (1.5 g/min) on the arginine-induced glucagon response of healthy subjects.

hour variation was estimated by calculating the standard deviation from their mean of four glucagon values within an hour. The value was  $\pm 6.5 \mu\text{g/ml}$  in the juvenile-type diabetic group and  $\pm 8.4 \mu\text{g/ml}$  among the adult-type diabetics.

**Relationship of therapy to fasting glucagon level.** 21 of the adult-type diabetics had been receiving sulfonylurea therapy until the morning of the test; their fasting glucagon levels averaged 114  $\mu\text{g/ml}$  (SEM  $\pm 9$ ) and ranged from 10 to 210  $\mu\text{g/ml}$ . Nine patients had been receiving insulin therapy until the morning of their test; the glucagon levels of this group averaged 112

TABLE II  
Effect of Arginine Infusion on Plasma Glucagon of Nondiabetic Subjects

Minutes . . .	Arginine infusion 460/mg per kg per 40 min											
	-30	-20	-10	0	5	10	20	30	40	50	60	70
Glucose mean	86	87	87	87	91	96	98	99	95	88	81	79
$\pm$ SEM	1	1	1	1	1	1	2	2	2	2	2	2
Glucagon mean	112	112	115	108	222	260	304	312	331	297	223	183
$\pm$ SEM	11	11	11	10	21	24	23	25	22	22	19	16
Insulin mean	10	8	10	8	36	30	36	43	43	26	14	10
$\pm$ SEM	1	1	1	2	3	3	4	4	4	3	2	2

TABLE III  
Effect of Induced Hyperglycemia on Glucagon Response to Arginine Infusion in Nondiabetic Subjects

	Glucose infusion (1.5 g/min)					
	Minutes... -20	-10	0	Arginine infusion (460/mg per kg per 40 min)		
				10	20	30
Glucose mean	197	242	286	298	301	259
±SEM	14	21	21	24	29	23
Glucagon mean	75	67	53	163	190	233
±SEM	8	10	4	23	41	46
Insulin mean	33	39	44	>100	>100	>100
±SEM	7	10	13			

μg/ml (SEM ±19) and ranged from 40 to 240 μg/ml. Three subjects were treated with diet alone; their fasting glucagon levels were 100, 180, and 90 μg/ml. No relationship between the form of diabetic therapy and the fasting glucagon concentration was evident.

Relationship between glucose and glucagon concentration in diabetic and nondiabetic subjects

The lack of a significant difference in the fasting glucagon level of nondiabetic and diabetic subjects was unexpected in view of the fact that hyperglycemia, which effectively suppressed glucagon in the nondiabetic group, was present in the majority of the diabetics in this series. Since a "normal" glucagon level in the face of hyperglycemia might, in fact, represent a state of relative hyperglucagonemia, glucagon concentration

of diabetics and nondiabetics with similar glucose levels were compared.

In Fig. 5 the glucagon values in diabetic and nondiabetic subjects are plotted as a function of glucose concentration. Seven of the diabetics had fasting glucose levels below 110 mg/100 ml because of aggressive therapy with insulin or oral antihyperglycemic agents; the mean glucagon level of the seven normoglycemic diabetics averaged 149 μg/ml (SEM ±16), but did not differ significantly (0.05 < P < 0.1) from the 108 μg/ml average of the 28 nondiabetics. Three of these seven diabetics were of the juvenile type (Fig. 5 A).

The glucagon level of 19 diabetics with fasting hyperglycemia was compared with that of nine nondiabetics in whom hyperglycemia in excess of 200

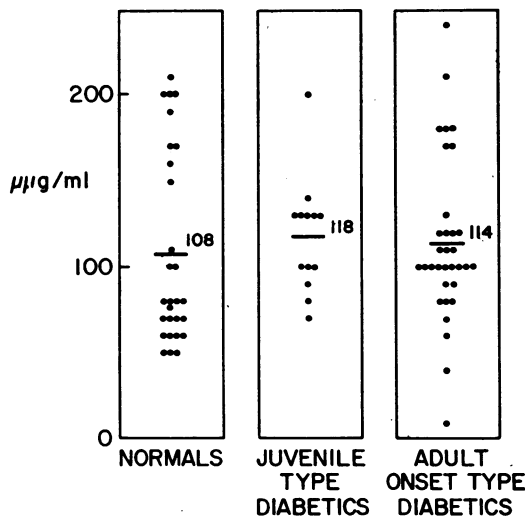


FIGURE 4 Comparison of fasting glucagon levels on non-diabetic and diabetic subjects.

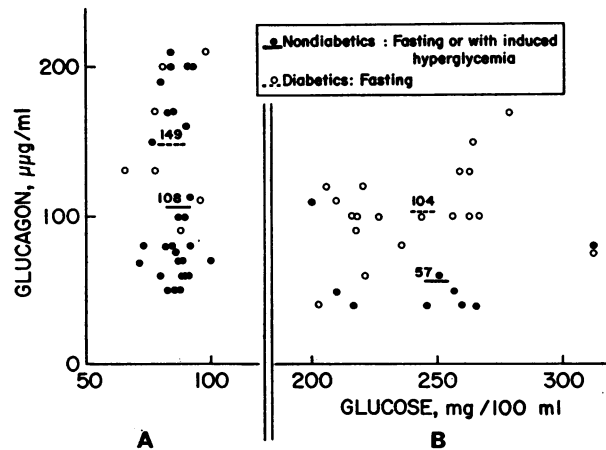


FIGURE 5 Glucose-glucagon relationships in diabetic and nondiabetic subjects. A. Comparison of glucagon levels of normoglycemic diabetics and nondiabetics. B. Comparison of glucagon levels of diabetics with fasting hyperglycemia over 200 mg/100 ml with nondiabetics in whom comparable hyperglycemia had been induced by glucose infusion.

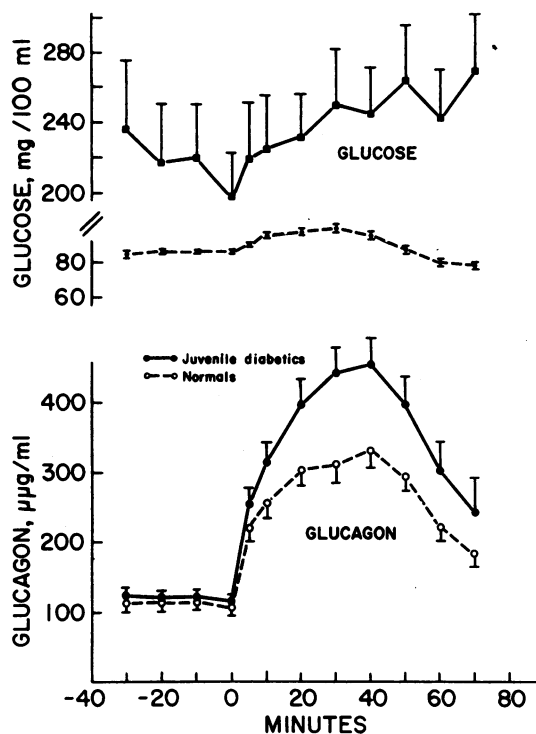


FIGURE 6 The effect of arginine infusion (11.7 mg/kg per min) on plasma glucagon in 12 juvenile-type diabetics.

mg/100 ml had been induced by infusing glucose at a rate of 1.5 g/min. In the hyperglycemic nondiabetic group glucose levels at the end of the 30-min glucose infusion ranged from 201 to 346 mg/100 ml and averaged 260 mg/100 ml (SEM  $\pm 15$ ). Mean minimal glucagon level within this time was 57  $\mu\text{g/ml}$  (SEM  $\pm 8$ ). This was significantly lower ( $P < 0.001$ ) than the 104  $\mu\text{g/ml}$  (SEM  $\pm 7$ ) average glucagon value of the diabetics whose plasma glucose levels were in the same range. 7 of these 19 diabetics were of the juvenile type; their mean fasting glucagon was 103  $\mu\text{g/ml}$  (SEM  $\pm 6$ ) and ranged from 80 to 130  $\mu\text{g/ml}$ ; the 12 adult-type diabetics in this group had a mean glucagon level of 105

$\mu\text{g/ml}$  (SEM  $\pm 10$ ) with a range from 40 to 170  $\mu\text{g/ml}$ . These results are depicted in Fig. 5 B.

Many of the diabetics had received an insulin injection on the day before the test. It seemed possible that contamination of the insulin with glucagon might have contributed to the glucagon levels of some patients. However, since lente insulin was assayed to contain less than 1  $\mu\text{g}$  of glucagon per unit of insulin, it seemed unlikely that exogenous glucagon significantly contributed to the plasma glucagon level more than 24 hr after its administration. Furthermore, as indicated above, the mean fasting glucagon level of insulin-treated patients was not significantly higher than that of sulfonyleurea-treated diabetics.

#### Glucagon response to arginine infusion in juvenile-type diabetics

The response to the arginine infusion was tested in a group of 12 insulin-dependent, juvenile-type diabetics. All 12 exhibited a prompt rise in glucagon concentration within 10 min of the start of the arginine infusion. The mean glucagon level rose from a baseline value of 118  $\mu\text{g/ml}$  (SEM  $\pm 9$ ) to a peak of 458  $\mu\text{g/ml}$  (SEM  $\pm 36$ ) at 40 min and declined when the infusion was stopped. The mean plasma glucagon level in this group was significantly higher than that of nondiabetic subjects at 20 ( $P < 0.05$ ), 30 ( $P < 0.01$ ), 40 ( $P < 0.01$ ), and 50 min ( $P < 0.02$ ). The mean maximal glucagon rise in this group was 362  $\mu\text{g/ml}$  (SEM  $\pm 33$ ), significantly greater ( $P < 0.01$ ) than the 239  $\mu\text{g/ml}$  (SEM  $\pm 19$ ) rise of the nondiabetic group.

The mean glucose level rose from 198 mg/100 ml (SEM  $\pm 25$ ) at the start of the infusion to 246 mg/100 ml (SEM  $\pm 26$ ) at 40 min and continued upward to a peak value of 271 mg/100 ml (SEM  $\pm 32$ ) 30 min after termination of the infusion. This rise in glucose was considerably greater than that of nondiabetics. Insulin could not be measured in this group because of insulin antibodies resulting from insulin treatment. The mean results are depicted in Fig. 6, and in Table IV.

TABLE IV  
Effect of Arginine Infusion on Plasma Glucagon of Juvenile-Type Diabetics

Minutes . . . .	-30	-20	-10	0	Arginine infusion 460/mg per kg per 40 min							
					5	10	20	30	40	50	60	70
Glucose mean	237	218	221	198	220	226	232	251	246	265	243	271
$\pm$ SEM	39	22	39	25	32	30	25	31	26	31	28	32
Glucagon mean	126	123	123	118	258	316	400	444	458	400	305	246
$\pm$ SEM	10	9	9	9	24	28	35	37	36	39	41	48

A statistically significant relationship between the rise in glucagon and the rise in glucose was not observed in this group.

### Glucagon response to arginine infusion in adult-type diabetics

The glucagon response to arginine infusion was tested in 33 adult-type diabetics. All 33 exhibited a prompt rise in plasma glucagon beginning within 10 min of the start of the infusion. Mean glucagon rose from 114  $\mu\text{g}/\text{ml}$  (SEM  $\pm 8$ ) at zero time to a peak of 452  $\mu\text{g}/\text{ml}$  (SEM  $\pm 38$ ) at 30 min and returned gradually towards basal levels when the infusion was stopped. The glucagon levels were significantly greater than those of the nondiabetic group at 20 ( $P < 0.02$ ), 30 ( $P < 0.01$ ), and 40 min ( $P < 0.05$ ). The mean of maximal increments was 371 (SEM  $\pm 36$ ), significantly greater than 239  $\mu\text{g}/\text{ml}$  (SEM  $\pm 19$ ) rise of nondiabetic subjects ( $P < 0.01$ ). Mean glucose concentration rose from a pre-infusion level of 177 mg/100 ml (SEM  $\pm 10$ ) to a peak value of 196 mg/100 ml (SEM  $\pm 11$ ), not significantly greater than in normals. Insulin, which averaged 18  $\mu\text{U}/\text{ml}$  (SEM  $\pm 3$ ) at the start of the infusion, rose to 66  $\mu\text{U}/\text{ml}$  (SEM  $\pm 12$ ) at 40 min and then declined toward the baseline values upon termination of the infusion. A statistically significant relationship between the glucagon rise and either the glucose or the insulin increment was not observed.

The mean values are depicted in Fig. 7 and in Table V. The maximal arginine-induced glucagon rise of non-diabetics, juvenile-type diabetics, and adult-type diabetics are compared in Fig. 8.

### Relationship of therapy to arginine-induced glucagon increment in adult-type diabetics

The nine adult-type diabetic patients receiving insulin therapy exhibited a mean maximal rise in plasma glucagon of 264  $\mu\text{g}/\text{ml}$  (SEM  $\pm 40$ ), while that of the 21 patients treated with a sulfonylurea was 381  $\mu\text{g}/\text{ml}$

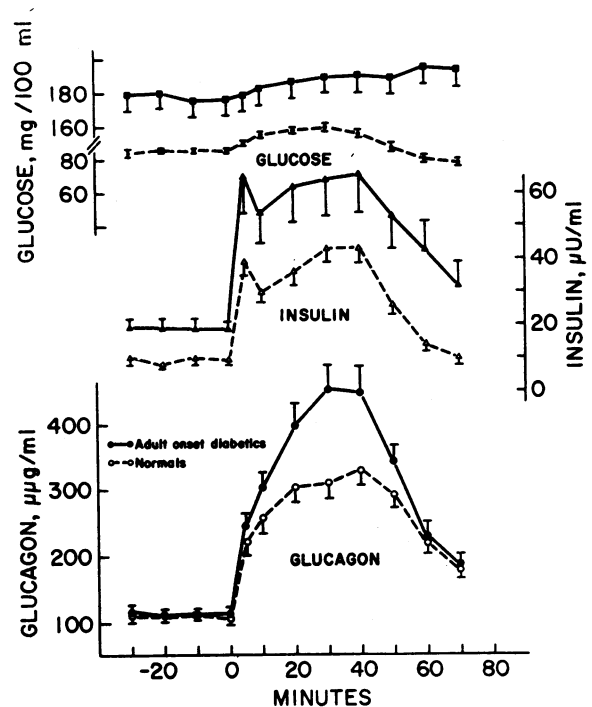


FIGURE 7 The effect of arginine infusion (11.7 mg/kg per min) on plasma glucose and insulin in 33 adult-type diabetics.

(SEM  $\pm 35$ ). The difference between these groups was not statistically significant ( $0.05 < P < 0.1$ ). The three patients treated with diet alone rose 280, 430, and 1140  $\mu\text{g}/\text{ml}$ , respectively.

### Relationship of obesity to the fasting glucagon level and the maximal arginine-induced glucagon increment in adult-type diabetics

In contrast to the well-documented elevation in insulin levels observed in obesity (21) both in the fasting state and after stimulation of insulin secretion, which was again confirmed in these patients, glucagon levels

TABLE V  
Effect of Arginine Infusion on Plasma Glucagon of Adult Onset-Type Diabetics

Minutes...	-30	-20	-10	0	Arginine, 460/mg per kg per min							
					5	10	20	30	40	50	60	70
Glucose mean	179	181	176	177	179	183	187	190	191	190	196	194
±SEM	10	10	10	10	10	10	10	10	11	11	11	11
Glucagon mean	118	113	113	114	245	305	400	452	450	342	232	189
±SEM	8	7	7	8	18	21	31	38	38	23	18	14
Insulin mean	19	19	19	18	65	54	62	64	66	54	43	32
±SEM	3	3	3	3	11	9	11	11	12	11	8	7



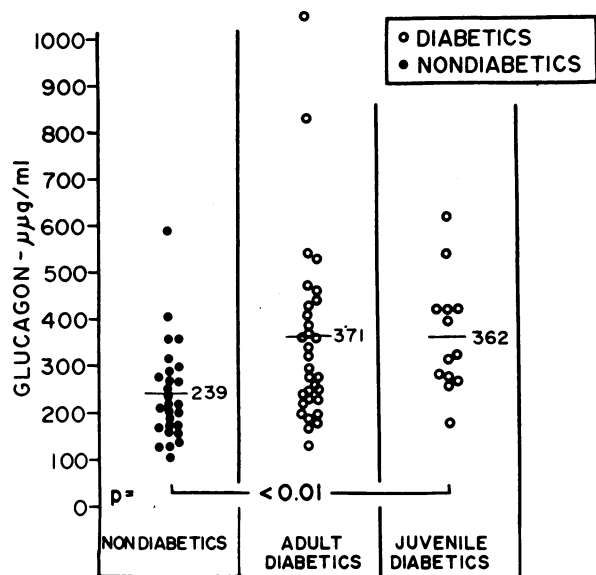


FIGURE 8 Comparison of the mean maximal arginine-induced increments of plasma glucagon in nondiabetic and diabetic subjects.

appeared not to be influenced by obesity. No correlation between body weight and either the fasting glucagon level or the maximal glucagon increment during the infusion of arginine was observed. The mean fasting glucagon levels and the maximal arginine-induced glucagon increments of the 10 most obese of the adult-type diabetics, whose weight averaged 111 kg (90–135 kg) were compared with those of the 10 leanest of the group, whose weight averaged 59 kg (49–67 kg). The obese patients averaged in the fasting state 136  $\mu\text{g/ml}$  (SEM  $\pm 13$ ) and exhibited a mean maximal arginine-induced glucagon rise of 408  $\mu\text{g/ml}$  (SEM  $\pm 86$ ), while in the lean group fasting glucagon levels and maximal arginine-induced glucagon rises averaged 102  $\mu\text{g/ml}$  (SEM  $\pm 14$ ) and 371  $\mu\text{g/ml}$  (SEM  $\pm 65$ ), respectively. These values did not differ significantly. However, the fasting insulin level and the maximal insulin rise during arginine infusion were significantly greater in the obese group at the 2% and 5% level, respectively, and a significant correlation between body weight and fasting insulin ( $r = 0.58$ ;  $P < 0.01$ ) and arginine-induced insulin rise ( $r = 0.652$ ;  $P < 0.01$ ) was observed.

#### Relationship of the arginine-induced glucagon response of juvenile-type and adult-type diabetics to duration of diabetes

In true ketoacidosis-prone, juvenile-type diabetes beta cells are presumed to be either absent or incapable of producing insulin in significant quantity. The brisk glucagon response to arginine exhibited by every juve-

nile type diabetic indicates that alpha cells retain their secretory function long after total loss of beta cell function. To determine if a gradual loss of alpha cell function occurs with time, the relationship between the maximal glucagon rise of each juvenile-type diabetic and duration of diabetes was analyzed. A statistically significant relationship between glucagon response and duration of diabetes was not evident.

Table VI shows the maximal arginine-induced glucagon increment and the duration of diabetes in all 12 juvenile-type diabetics. It is noteworthy that the patient with diabetes of 21 yr duration exhibited as brisk a rise in glucagon as the patient with diabetes of less than 1 yr duration. It must be concluded that diabetes of this type probably represents an isolated beta cell deficit without diffuse involvement of all cellular components of the islets of Langerhans.

Similar results were obtained in the 33 adult-type diabetics; a statistically significant correlation between maximal arginine-induced glucagon increment and the estimated duration of their diabetes was not observed.

#### Arginine-induced glucagon response in Kimmelsteil-Wilson disease

The striking reduction in insulin requirements observed to occur in many patients with advanced Kimmelsteil-Wilson disease has not been satisfactorily explained. Diminished glucagon secretion late in the course of diabetes seemed to be a possible mechanism worth considering. Two insulin-sensitive diabetic patients, in whom a reduction in insulin requirement had been attributed to Kimmelsteil-Wilson disease, received an arginine infusion test. In one glucagon rose from a 210  $\mu\text{g/ml}$  fasting level to a peak of 650  $\mu\text{g/ml}$  at

TABLE VI  
Duration of Juvenile-Type Diabetes and Alpha Cell Responsiveness

Patient No.	Duration of diabetes yr	Maximal glucagon increment during arginine infusion $\mu\text{g/ml}$
5	21	280
10	20	540
12	14	620
6	13	420
4	9	270
3	8	400
8	7	260
9	2	310
2	2	276
1	1	180
13	1	420
7	<1	310

TABLE VII  
Plasma Glucagon in Diabetic Ketoacidosis

Patient No.	Values on admission			Initial insulin units until acetone small* at 1:1	Before treatment	Glucagon				
	Plasma glucose	CO <sub>2</sub> content	Acetone "small" at			Hours after				
						2	4	6	>24	
	mg/100 ml	mEq/liter				μg/ml				
1†	558	<4	1:1	850	>2000	1440	1120	1500		
2	789	<4	1:8	800	260	120	90	120		
3	1190	<6	1:16	750	430					
4	420	<4	1:16	650	285					20
5	735	5.5	1:32	600	300	130	180	210		
6	865	<10	1:8	600	580					130
7	720	7	1:4	570	610					
8	580	10	1:16	425	230					130
Mean of severe cases					587					
±SEM					195					
9	400	10	1:4	250	165					100
10	426	15	1:2	175	190					190
11	505	18	1:4	40	225					190
12	411	25	1:2	30	160					
Mean of mild cases					185					
±SEM					13					

\* "Small" refers to a slight lavender color appearing 2 min after plasma is applied to a powdered Acetest Reagent Tablet (Ames Company, Elkhart, Ind.).

† This patient had an elevated serum amylase and hyperglucagonemia may have been secondary to acute pancreatitis.

40 min. In the other patient glucagon rose from 130 to 750 μg/ml. Clearly no deficiency in glucagon existed in these two subjects.

#### Glucagon levels in diabetic ketoacidosis

Glucagon was measured in eight patients with relatively severe ketoacidosis. Their CO<sub>2</sub> was below 10 mEq/liter and their insulin requirements before their serum acetone had receded to "small" at 1:1 dilution ranged from 425 to 850 U. Before treatment, glucagon averaged 587 μg/ml (SEM ±195), ranging from 230 to more than 2000 μg/ml. With insulin treatment glucagon declined towards normal (Table VII).

In four milder ketoacidotic patients, requiring 250 U of insulin or less during the first 6 hr of therapy (patients 8-12 in Table VII) glucagon averaged 185 μg/ml (SEM ±13) and ranged from 165 to 225 μg/ml.

#### DISCUSSION

The glucagon assay system used in the foregoing studies appears to measure primarily pancreatic glucagon and little, if any, cross-reacting GLI of gastrointestinal origin. This statement is based on the fact that zero values were obtained in this system in plasma from a patient

with extensive calcific pancreatitis and pancreatic insufficiency (6) and in plasma from partially depancreatized animals (20). The glucagon levels of the 28 normal fasting subjects in this study averaged 108 μg/ml or about 10% of the values obtained with assay systems employing less specific antisera, and, to the best of our knowledge, are the lowest thus far reported. Since there appears to be no discernible immunologic difference between human and beef-pork glucagon, it seems likely that the values reported here are extremely close to the true values for pancreatic glucagon. With respect to sensitivity and precision, this assay system compares favorably with those previously reported. It seems fair to conclude that it is capable of providing valid measurements of glucagon in peripheral venous plasma without the technical problems that have plagued this technique in the past.

The study reveals that in normal human subjects intravenous administration of glucose suppresses glucagon secretion and that the infusion of arginine stimulates its secretion. Both of these effects, but particularly the effect of arginine, appear to be remarkably consistent in all subjects tested; a rise of at least 100 μg/ml has been observed during the infusion of 11.7 mg/kg per min of arginine in every one of 75 nondiabetic and dia-

betic individuals studied thus far. The only exceptions have been patients with chronic pancreatitis reported elsewhere (6). Failure of the plasma glucagon to rise 100  $\mu\text{g}/\text{ml}$  or more during the infusion test may, therefore, signify a loss of functioning alpha cell-containing pancreatic tissue. It is possible that this procedure or a modification thereof might provide a clinically useful test of pancreatic function.

Perhaps the most interesting finding of this study is the apparent demonstration of alpha cell hyperfunction in diabetics. The notion that diabetes mellitus might, at least in part, be a consequence of excessive glucagon secretion is not a new one. Rodriguez-Candela reported in 1947 that alloxan diabetes of dogs with ligated pancreatic ducts could be ameliorated by removal of the pancreatic remnant, suggesting that the presence of alpha cells contributed to the severity of the diabetes (22). In 1953 Ferner claimed that the ratio of alpha cells to beta cells was greater in certain diabetic patients than in nondiabetic subjects (23). However, the present study provides the first direct evidence of increased glucagon levels in patients with diabetes mellitus.

A state of relative hyperglucagonemia may be inferred from the fact that the mean fasting level of glucagon of both juvenile-type and adult-type diabetic groups does not differ significantly from that of the nondiabetic group, despite hyperglycemia which in nondiabetic subjects readily suppresses the glucagon level to subnormal values. It is reasonable to suppose that the inappropriately high glucagon, *as well as* the inappropriately low insulin levels, relative to the prevailing glucose level, are influencing the net hepatic glucose balance and increasing the severity of hyperglycemia, and the amount of insulin required to reduce hepatic glucose production to normal.

An unequivocal absolute increase in alpha cell function was demonstrated in diabetic subjects by means of the arginine infusion test. The mean glucagon level of diabetics rose within 10 min to a height which in nondiabetics was not attained before 40 min of infusion. The glucagon concentrations attained and the mean of maximal increments was significantly greater in both juvenile-type and adult-type diabetics than in nondiabetics. In 13 of the 33 patients with adult-type diabetes a rise to more than 500  $\mu\text{g}/\text{ml}$  was observed; in two such patients glucagon rose to above 1000  $\mu\text{g}/\text{ml}$ . In 5 of 12 juvenile-type diabetics plasma glucagon rose above 500  $\mu\text{g}/\text{ml}$ . Only one of 28 nondiabetics exhibited a rise above 500  $\mu\text{g}/\text{ml}$ .

Hyperresponsiveness of glucagon secretion to hyperaminoacidemia should increase hepatic glucose production and unfavorably influence blood glucose control unless a parallel release of insulin were to nullify this influence. This could explain the more marked hyper-

glycemic response to arginine noted in the juvenile-type diabetics, in whom no insulin release could have occurred, than in nondiabetics and adult-type diabetics, in whom a brisk rise in insulin accompanied the hyperglucagonemia. The hyperglycemic response to arginine averaged 12 mg/100 ml in the two latter groups, as compared to the 53 mg/100 ml in the juvenile-type diabetic group. Obviously, however, other factors are equally plausible as causes of hyperglycemia during the experimental period in juvenile-type diabetics.

The demonstration of an arginine-induced glucagon rise in patients with diabetes of long duration is noteworthy; in the juvenile-type diabetic group it indicates that alpha cells continue to function many years after beta cell function has been completely lost. This favors an isolated beta cell lesion in diabetes mellitus and speaks against a diffuse lesion involving all cellular components of the islets of Langerhans. The vigorous glucagon response to arginine observed in the two cases of Kimmelsteil-Wilson's disease suggests that even in the presence of extensive microangiopathic involvement of many tissues, the secretory function of the alpha cells is not diminished. Since hyalinization of the islets is said to occur in approximately 40% of diabetics (24), the failure to encounter a single case of glucagon deficiency in the 45 diabetics studied is surprising.

The mechanism of the relative hyperglucagonemia encountered in diabetics in the fasting state and the absolute hyperresponsiveness of glucagon secretion observed during arginine infusion is, of course, uncertain. There is little reason to suspect primary alpha cell hyperfunction, although no evidence now available can exclude this.

If the hypersecretion of glucagon in diabetes is a secondary phenomenon, several possible factors warrant consideration. Of these, obesity cannot be implicated on the basis of this study. However, diabetic capillary microangiopathy (25), which may well involve capillaries throughout the body, could conceivably interfere with the accession of certain key substrates to the islet cells. For example, a reduced rate of glucose entry through thickened capillaries of the islets of Langerhans would, at a given arterial glucose concentration, account for both reduced insulin secretion (hypostimulation) and increased glucagon secretion (hyposuppression). However, in the absence of evidence that basement membrane thickening slows the transfer rate across capillaries this hypothesis is tenuous.

A more attractive hypothesis, which can be supported in part with data already available, would ascribe diabetic hyposuppressibility of glucagon secretion by glucose to defective glucose penetration of alpha cells because of insulin lack. Hypoglycemia is well established as a stimulus to glucagon secretion, whether

induced by insulin (26, 17) phlorizin (26), or starvation (7). Hyperglycemia suppresses glucagon secretion (16, 17) when insulin is available, but seems not to suppress when insulin is lacking, as if glucose penetration of the alpha cell were insulin requiring. Preliminary studies in severely alloxan-diabetic dogs<sup>3</sup> and in the severely ketoacidotic patients reported here show remarkable elevations in plasma glucagon despite the extreme hyperglycemia; the administration of insulin reduces hyperglucagonemia and hyperglycemia simultaneously. However, the insulin lack hypothesis fails to explain the hyperglucagonemia of adult-type diabetics, in whom the insulin levels were greater than normal.

The demonstration of strikingly elevated plasma glucagon levels in certain patients with severe diabetic ketoacidosis may be of some clinical importance. Similar observations have been reported by Assan, Rosselin, and Dérot (27). The return of glucagon towards normal appears to coincide with the recession of the insulin resistance, which characterizes the early hours of therapy in severe ketoacidosis, and with the return towards normal of the glycemia and ketonemia. As stated before, high concentrations of a hormone, which accelerates the very processes in liver and, perhaps, fat tissue that insulin suppresses, may well contribute to the high insulin requirements and to the clinical configuration of the syndrome.

A final point concerns the report by Samols, Tyler and Mialhe, that tolbutamide suppresses glucagon secretion in human subjects and may exert its therapeutic effect, in part at least, through a reduction in glucagon secretion (28). In the present study, however, the sulfonylurea-treated diabetics exhibited even more marked elevations in glucagon than the insulin-treated patients; while it is conceivable that their values represent a reduction from even higher levels prior to therapy, these data offer little support to this proposal.

It is concluded that (a) fasting glucagon levels of nondiabetic and diabetic subjects average slightly over 100  $\mu\text{g/ml}$ ; (b) in nondiabetic subjects glucagon secretion is diminished by induced hyperglycemia to levels significantly below that of diabetics with comparable degrees of spontaneous hyperglycemia; (c) glucagon secretion is uniformly stimulated by arginine infusion in nondiabetic subjects, (d) in diabetic subjects without a history of pancreatitis the glucagon response to arginine is markedly exaggerated despite the hyperglycemia, and (e) in severe diabetic ketoacidosis glucagon levels may be extraordinarily high before treatment.

<sup>3</sup> W. A. Müller, G. Faloona, and R. H. Unger. Unpublished observations.

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