Abnormal Alpha Cell Function in Diabetics

Response to Insulin

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SUMMARY

The extremely high levels of glucagon recently observed in dogs with severe alloxan-induced diabetes decline promptly and precipitously to normal as soon as exogenous insulin is infused. This suggests that the normal response of the pancreatic alpha cell to hyperglycemia requires the presence of circulating insulin. To determine if the relative hyperglucagonemia of human diabetics responds similarly to insulin repletion, the plasma glucagon response of ten adult-type diabetic patients to a large, predominantly carbohydrate meal was determined with and without the simultaneous forty-five-minute intravenous infusion of glucagon-free insulin (0.12 to 0.2 U./kg.). The glucagon response to the carbohydrate meal during prompt and supernormal hyperinsulinemia resulting from the infusion did not differ from that of the control meal, i.e. normal suppression of glucagon by hyperglycemia was not restored by the abundance of circulating insulin.

To determine if still higher plasma levels of insulin would overcome the hyposuppressibility of the diabetic alpha cell to hyperglycemia, 0.6 U. per kilogram per hour of insulin was infused at a constant rate for two hours together with 0.6 gm. per kilogram per hour of glucose to prevent hypoglycemia. Insulin levels of more than 1,200 μU. per milliliter were thus attained. Under these conditions, plasma glucagon declined from a mean preinfusion level of 97 pg./ml. (SEM $\pm$ 11) to a nadir of 75 pg./ml. (SEM $\pm$ 10) ninety minutes later. This slow, modest, statistically significant ($p < 0.01$) decline differed strikingly from the response of eight nondiabetic patients given intravenous glucose alone; in these subjects, at a comparable level of hyperglycemia, glucagon declined from a mean fasting level of 90 pg./ml. (SEM $\pm$ 8) to 57 pg./ml. (SEM $\pm$ 8) within thirty minutes, despite an insulin rise to only 46 μU./ml.

It was concluded that in human diabetics the acute restoration of plasma insulin, even to supernormal levels, does not promptly restore to normal the alpha cell responsiveness to hyperglycemia. Simple insulin lack may not, therefore, adequately explain the alpha cell abnormality in human diabetes. DIABETES *21*:301-07, May, 1972.

Recent studies from this laboratory have reported that in patients with inherited diabetes mellitus the plasma level of pancreatic glucagon is, at all times, increased relative to the levels expected on the basis of the prevailing level of hyperglycemia.[1,2] This state of relative hyperglucagonemia has been interpreted as representing hyposuppressibility of the diabetic alpha cell to hyperglycemia, which in nondiabetics promptly and profoundly suppresses glucagon secretion. Subsequently, it was reported that experimental insulin lack, induced in dogs by alloxan, also is accompanied by marked hyperglucagonemia, which is promptly corrected by modest amounts of insulin.[3] The latter findings strongly imply, first, that normal alpha cell suppression by hyperglycemia requires the presence of insulin, and, second, that the hyperglucagonemia of experimental diabetes is the direct or indirect consequence of insulin deficiency.

Extrapolation of these findings to human diabetes leads one to wonder if the relative hyperglucagonemia of this disease could be the simple consequence of insulin lack. For this reason, studies were designed in genetically diabetic patients to determine if the suppressibility of their alpha cells to glucose could be restored to normal by repletion of insulin lack through the administration of exogenous insulin.

METHODS

Ten adult-type diabetic subjects were recruited from the Parkland Memorial Hospital Diabetes Clinic and from the wards of the Dallas Veterans Hospital. All but two were receiving tolbutamide at the time of the study. One patient was being treated with insulin and one

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with diet alone. Each patient received three tests. In the first test the patients consumed a meal consisting of bread, spaghetti, rice, potatoes, and corn and containing 1 gm. of carbohydrate per kg. of body weight. In the second test, at a later date, the patients were fed an identical meal, but an intravenous infusion of 0.12 or 0.2 U. per kg. of glucagon-free crystalline insulin* was begun at the start of the meal and continued for a period of forty-five minutes. In the third test 0.6 gm. per kg. of glucose per hour as a 15 per cent solution and 0.6 U. per kg. per hour of the glucagon-free crystalline insulin* were infused for three hours. Informed consent was obtained.

Frequent blood samples were obtained through indwelling plastic catheters in the antecubital vein before and during the experimental procedures. Blood specimens were quickly transferred to chilled tubes containing 12 mg. EDTA and 0.8 ml. of Trasylol (500 Kallikrein Inhibition Units per ml. of blood) and centrifuged promptly at 4° C. Plasma was separated promptly and stored at —20° C. until the time of hormone assay not more than eight weeks later.

Glucagon was assayed by a recent modification[4] of the previously described radioimmunoassay[5] using antiserum G-58, which is highly specific for pancreatic glucagon. Insulin was measured by the Herbert modification[6] of the radioimmunoassay of Yalow and Berson.[7] Plasma glucose was measured by the Hoffman method[8] using the Technicon AutoAnalyzer.

RESULTS

The effect of insulin repletion on alpha cell response to a carbohydrate meal

In order to determine if the impaired suppressibility of the diabetic alpha cells by the hyperglycemia induced by a carbohydrate meal was a consequence of insufficient insulin, ten adult-type diabetics were fed 1 gm. per kg. of carbohydrate and a concomitant infusion of crystalline insulin. The levels of glucagon measured before and for 180 min. after the start of the meal were compared with glucagon levels of an identical meal unaccompanied by exogenous insulin administration. The individual results of plasma glucose, glucagon, and insulin measurements are displayed in table 1 and the mean values are depicted in figure 1. The mean glucagon did not fall below the baseline value during the first hour after the meal despite plasma glucose levels averaging as high as 298 mg./100 ml., the lowest value being 129 pg./ml. (SEM ± 20). In contrast nondiabetic subjects were reported previously to exhibit a decline in glucagon from a fasting level of 126 (SEM ± 15) to 93 pg./ml. (SEM ± 14) during the first hour,[2] with peak glucose levels averaging only 137 mg./100 ml. The maximal decline in this diabetic group during the first hour after the meal, calculated in each patient by subtracting the lowest glucagon level below the baseline (if glucagon did not fall below the baseline, a zero value was assigned) from the mean of the three baseline values, averaged 10.7 pg./ml. (SEM ± 4.6) This was significantly less ($p < 0.05$) than the 37.9 (SEM ± 5.6) mean maximal decline observed in a group of eleven nondiabetic controls, in each of whom a substantial decline, ranging from 20 to 80 pg./ml. occurred during the first hour.[2] The mean insulin rose to a peak of only 39 μU./ml. (SEM ± 9) in the diabetics, compared to a peak of 136 (SEM ± 19) in the nondiabetic controls.

To determine if repletion of insulin would correct the subnormal alpha cell response to ingested glucose, each diabetic subject was given the same carbohydrate meal at a later date, but this time an intravenous infusion of 0.12 to 0.2 U. of insulin per kg. of body weight was begun at the start of the meal and continued for forty-five minutes. As shown in figure 1, the infusion of insulin resulted in an immediate increase in mean plasma insulin to a peak of 367 μU./ml. (SEM ± 49) within forty-five minutes, almost ten times the mean maximal level of 39 μU./ml. (SEM ± 7) attained during the meal without insulin.* Despite the abundance of insulin, the normal suppressibility of plasma glucagon to the carbohydrate meal was not restored. The mean glucagon level did not decline at all during the first hour and the nadir at ninety minutes averaged 105 pg./ml. (SEM ± 15), which was not significantly below the baseline or below the 120 min. nadir of 107 pg./ml. (SEM ± 20) observed during the control meal without insulin. The maximal decline for each patient during the first hour, calculated as described above, averaged 18.0 pg./ml. (SEM ± 5.0), not significantly different from the maximal decline of 10.7 pg./ml. observed in diabetics without insulin. The differences in each patient between the decline with and without insulin averaged 7.3 pg./ml. (SEM ± 6.9) and was not statis-

*Actrapid, ten times recrystallized pork insulin, kindly donated by Dr. Joseph Schlichtkrull, Novo Terapeutisk Laboratories, Copenhagen, Denmark.

*Three of the ten patients had insulin antibodies, so that insulin was measured in only seven patients.

TABLE 1

Effect of the intravenous infusion of crystalline insulin upon the glucagon response to a carbohydrate meal

	CHO Meal									CHO Meal Insulin infusion								
Minutes:	—60	—30	0	30	45	60	90	120	180	—60	—30	0	30	45	60	90	120	180
Glucose (mg./100 ml.)	168	165	172	197	237	252	268	254	223	183	177	171	143	116	106	89	91	110
Insulin (μU./ml.)	25	20	20	65	53	53	48	53	33	35	25	22	227	350	225	50	35	22
Glucagon (pg./ml.)	210	210	220	230	240	210	240	230	250	140	140	100	100	90	100	80	90	100
Glucose	378	322	332	360	370	388	460	416	396	316	332	332	310	270	232	262	290	320
Insulin	18	15	15	27	25	23	25	23	23	15	13	17	400	400	103	50	28	20
Glucagon	120	110	130	140	110	130	90	110	130	120	120	130	140	150	150	170	220	100
Glucose	182	171	161	206	240	265	249	224	179	189	181	174	225	230	241	216	201	166
Insulin	7	7	5	12	10	18	13	10	10	10	8	7	150	195	48	18	13	7
Glucagon	90	140	110	90	80	90	60	70	60	90	100	50	110	80	50	60	90	80
Glucose	268	265	261	291	—	306	320	346	304	238	235	230	189	185	171	170	200	242
Insulin	20	22	18	21	—	25	35	22	24	15	10	13	535	512	110	48	25	17
Glucagon	60	60	20	—	—	30	20	30	40	70	90	90	110	70	60	80	100	100
Glucose	292	296	286	246	—	360	338	328	300	320	316	321	288	284	236	195	208	264
Insulin	7	5	7	13	—	13	12	10	7	7	7	8	175	350	42	17	8	8
Glucagon	30	80	90	110	—	100	100	50	60	120	130	120	130	120	130	110	110	120
Glucose	154	152	148	200	231	241	244	224	192	184	177	173	177	162	158	142	246	208
Insulin	27	27	30	38	43	47	47	50	40	33	33	30	170	205	155	59	40	45
Glucagon	90	80	100	100	100	140	100	60	90	110	180	120	110	100	110	100	100	90
Glucose	252	250	241	255	278	300	314	310	292	205	202	204	196	193	163	147	145	145
Glucagon	220	250	210	270	280	270	250	220	220	250	250	260	260	220	260	220	260	260
Glucose	180	185	193	205	260	264	268	250	214	186	196	189	210	238	260	263	249	217
Glucagon	120	120	150	130	120	100	120	120	110	120	130	110	130	140	140	80	110	190
Glucose	273	277	277	288	292	328	332	312	296	272	272	276	288	224	308	244	220	268
Glucagon	80	90	80	90	100	90	70	80	80	110	90	110	90	100	110	80	90	70
Glucose	184	185	175	220	255	273	276	248	209	141	154	148	182	168	150	126	150	147
Insulin	30	33	37	55	66	63	57	55	45	27	32	27	575	560	161	52	40	37
Glucagon	90	120	130	130	150	130	100	100	100	60	70	60	70	70	60	70	80	80
Glucose Mean	233	228	225	255	270	298	317	291	261	223	224	221	221	213	203	190	196	209
SEM	21	18	19	19	15	15	19	19	20	18	19	19	17	17	19	20	19	20
Insulin Mean	19	18	19	33	39	35	38	30	26	10	18	18	319	367	121	42	27	22
SEM	3	4	4	7	9	7	9	7	5	4	4	3	64	49	23	6	4	5
Glucagon Mean	111	126	124	143	147	129	115	107	114	122	130	115	125	114	117	105	125	119
SEM	18	18	18	20	24	20	22	20	21	14	16	17	16	14	13	15	19	18

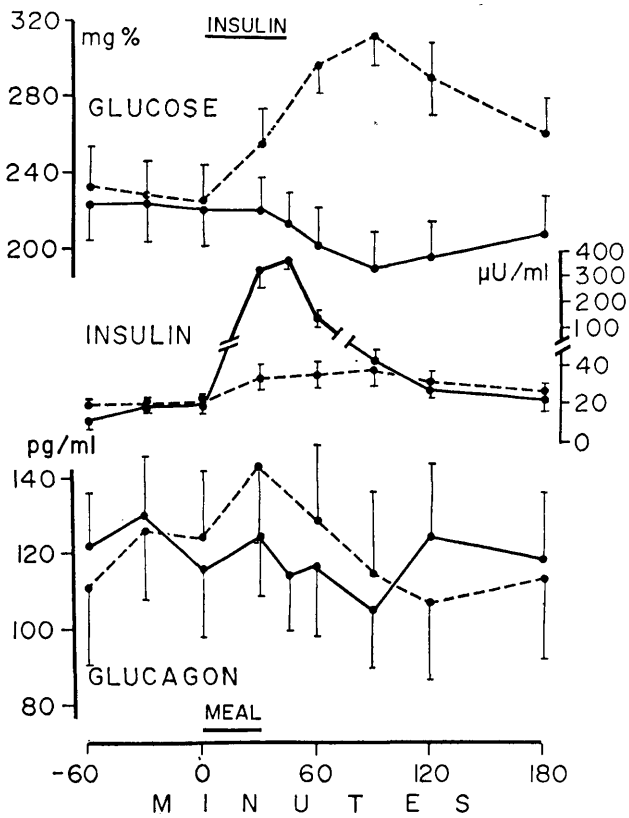

FIG. 1. Effect of a carbohydrate meal on glucagon with (solid line) and without (broken line) an insulin infusion in ten adult-onset diabetics. (Mean ± SEM)

tically significant. Mean plasma glucose did not increase during the meal and declined to a nadir of 190 mg./100 ml. at ninety minutes.

The "within patient baseline variability" in the diabetic group (SEM of differences of each baseline value from the mean of the three baseline values) was ± 15.3 pg./ml.; thus the declines observed in diabetics in the first hour after a carbohydrate meal both without and with insulin are well within the range of baseline variability, in contrast to those of nondiabetic controls.

Effect of large amounts of insulin and glucose on glucagon levels in diabetics

Although correction of the deficient insulin response to a carbohydrate meal failed to restore the alpha cell response of diabetics to normal, it seemed possible that extremely high plasma levels of insulin maintained for a longer period of time might do so. For this reason, the effect upon glucagon concentration of 0.6 U. of glucagon-free crystalline insulin per kg. of body weight per hour infused together with 0.6 gm. of glucose per kg. of body weight per hour for 180 min. was determined. The results are shown in figure 2 and table 2.

A gradual decline in plasma glucagon from a preinfusion level of 97 pg./ml. (SEM ± 11) to a nadir of 75 pg./ml. (SEM ± 10) was observed ninety minutes after the start of the infusion. However, this decline, which was highly significant ($p < 0.01$) at ninety minutes and slightly significant ($p < 0.05$) at 60, 120, and 180 min., occurred at plasma insulin levels which averaged over 1,200 μU./ml. This is more than twenty-five times the mean peak insulin level of 45 μU./ml. observed previously[1] in eight normal subjects, in whom hyperglycemia comparable to that of the diabetics was produced by a thirty-minute intravenous glucose infusion at a rate of 1.2 gm. per kg. of body weight per hour (figure 2); yet, in contrast to the slow and somewhat reduced decline of the diabetics, the nondiabetics exhibited a prompt and statistically highly significant ($p < 0.01$) decline in plasma glucagon from a mean preinfusion level of 90 pg./ml. (SEM ± 8) to a level of 57 pg./ml. (SEM ± 8) within thirty minutes.

The mean maximal glucagon decline of the diabetics, calculated by subtracting the lowest value during the 180-min. infusion from the mean of the three baseline values, averaged 34.0 pg./ml. (SEM ± 6.5), and a measurable decline occurred in all but two patients (no. 3 and no. 8). The mean maximal decline during the first sixty minutes of the infusion, the period in which glucagon levels of nondiabetics decline during the infusion of glucose alone, averaged 28.3 pg./ml. (SEM ± 7.2) and a measurable decline during that time was observed in all but three of the ten patients (nos. 3, 4 and 8).

DISCUSSION

The foregoing results reveal that in genetically diabetic human subjects, the normal suppressibility of the alpha cell to hyperglycemia is not restored, at least not promptly, by raising the plasma insulin concentration to normal or supernormal levels. When insulin was infused during the ingestion of a carbohydrate meal, thus providing a prompt and supernormal rise in plasma insulin persisting for at least sixty minutes, neither the mean glucagon levels nor the mean of individual maximal declines within the first hour differed significantly from those exhibited by these patients following the same meal without benefit of exogenous insulin. Only when plasma insulin was increased to twenty-five times the maximal normal peak levels by infusing an insulin-glucose mixture for three hours did the plasma glucagon

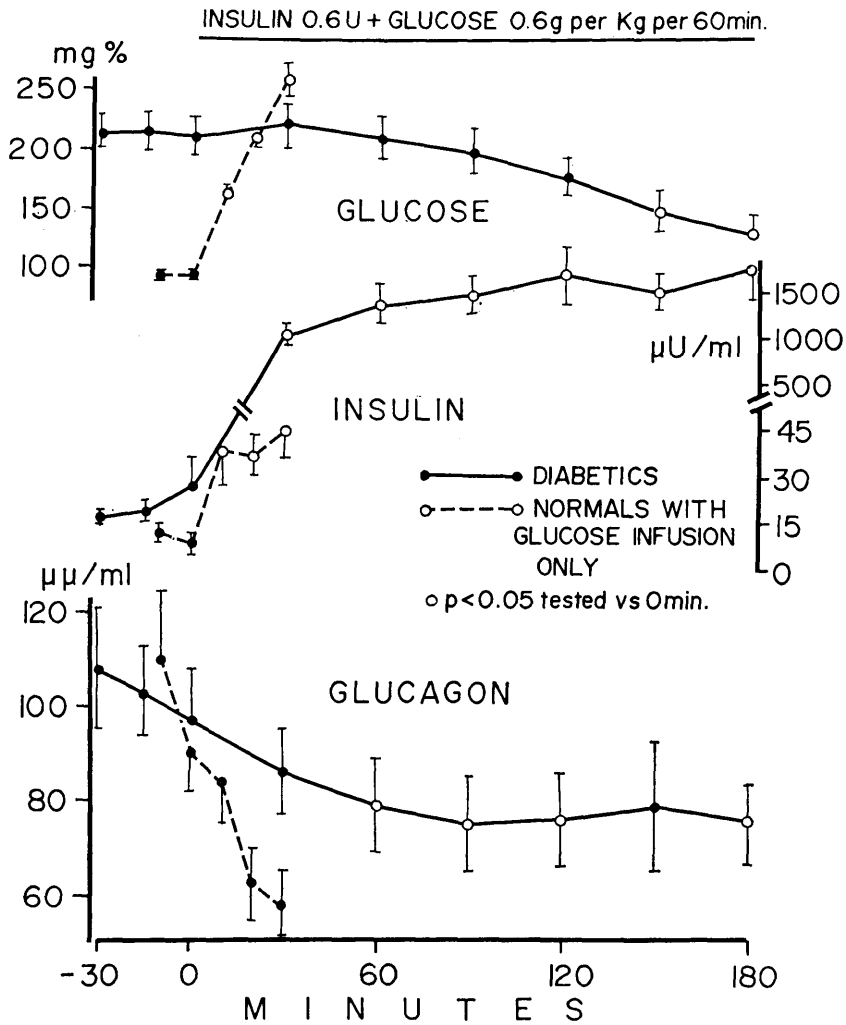

FIGURE 2

Effect of insulin-glucose infusion on plasma glucagon in ten adult-onset diabetics. (Mean ± SEM)

decline. However, the reduction in plasma glucagon thus achieved was not as great nor as rapid as that which occurs in normal subjects when comparable hyperglycemia is induced by a glucose infusion, and plasma insulin concentration rises to a level of only 45 μU./ml., about 1/25 of the levels artificially achieved here in the diabetics.[1] It is also in sharp contrast to the experiments in alloxan diabetic dogs in whom marked hyperglucagonemia can be promptly and dramatically corrected by infusing insulin so as to raise the insulin level from 0 to 40 μU./ml.[3]

Clearly, then, in both the nondiabetic human and in the alloxan diabetic dog glucagon secretion is suppressed far more readily by hyperglycemia in the presence of modest increments of plasma insulin than is that of the genetically diabetic human, even at enormously elevated plasma insulin levels. One is, therefore, confronted with an apparent contradiction. On the one hand, the experiments in alloxan diabetic animals reveal that hyperglycemic suppression of glucagon secretion cannot occur in the absence of insulin, which suggests that the alpha cell requires insulin to sense and respond appropriately to an extracellular surplus of glucose. On the other hand, while the human alpha cell may be no less insulin-dependent than that of lower mammals, the hyperglycemia-responsiveness in genetic human diabetes is not promptly restored by insulin. This suggests that it is not the simple consequence of insulin lack in the plasma; and the fact that the alpha cells of mild, adult-type diabetics, in whom the intrapancreatic insulin content may be near normal, are no less unresponsive to hyperglycemia than those of juvenile-type diabetics, in whom pancreatic insulin is virtually absent, tends to rule out simple insulin lack within the islets of Langerhans as the explanation for the difference.

The cause of the alpha cell abnormality in human

TABLE 2

The effect of intravenous infusion of large amounts of insulin (0.6 U./kg.) and glucose (0.6 gm./kg.) on plasma glucagon in diabetic patients

Patient No.					Insulin + Glucose infusion					
	Minutes:	—30	—15	0	30	60	90	120	150	180
1	Glucose (mg./100 ml.)	162	159	157	171	143	132	116	103	99
	Insulin (μU./ml.)	25	25	20	1,000	1,360	1,500	1,460	1,540	1,660
	Glucagon (pg./ml.)	100	80	80	60	40	60	60	40	60
2	Glucose (mg./100 ml.)	267	270	279	292	268	238	200	177	160
	Insulin (μU./ml.)	11	15	83	1,400	2,100	1,700	2,460	2,500	3,200
	Glucagon (pg./ml.)	100	90	100	80	100	70	60	80	70
3	Glucose (mg./100 ml.)	169	172	174	227	230	223	216	211	193
	Insulin (μU./ml.)	7	7	7	620	670	850	900	970	930
	Glucagon (pg./ml.)	60	100	100	100	100	80	120	100	100
4	Glucose (mg./100 ml.)	264	285	245	128	262	248	205	194	175
	Insulin (μU.ml.)	18	25	20	1,000	1,230	1,500	3,000	1,900	3,150
	Glucagon (pg./ml.)	90	90	60	80	80	60	70	60	70
5	Glucose (mg./100 ml.)	289	285	285	261	156	116 *	195	147	123
	Insulin (μU./ml.)	11	11	10	800	1,000	1,150	880	950	1,150
	Glucagon (pg./ml.)	120	100	90	60	50	50	50	60	50
6	Glucose (mg./100 ml.)	206	208	205	248	209	202	189	194	148
	Insulin (μU./ml.)	22	18	22	720	850	900	1,000	1,130	950
	Glucagon (pg./ml.)	100	90	90	70	40	40	40	140	70
7	Glucose (mg./100 ml.)	191	180	178	264	287	291	273	—	—
	Glucagon (pg./ml.)	190	170	160	120	130	110	110	—	—
8	Glucose (mg./100 ml.)	162	159	150	239	262	283	169	—	—
	Glucagon pg./ml.)	60	60	50	70	60	60	70		
9	Glucose (mg./100 ml.)	253	255	256	180	118	109	88	75	70
	Glucagon (pg./ml.)	180	150	160	150	130	150	130	130	130
10	Glucose (mg./100 ml.)	166	167	167	208	165	143	126	88	66
	Insulin (μU./ml.)	20	85	23	1,540	2,240	2,540	2,060	1,500	1,200
	Glucagon (pg./ml.)	80	100	80	70	60	70	80	20	60
	Glucose Mean	213	214	210	222	210	199	178	149	129
	SEM	15	16	16	15	18	21	16	18	
							N.S.	N.S.	p<0.05	p<0.01
	Insulin Mean	16	18	26	1,011	1,350	1,449	1,680	1,499	1,749
	SEM	2	3	9	121	212	202	295	196	351
	N=7									
	Glucagon Mean	108	103	97	86	79	75	76	79	76
	SEM	13	10	11	9	10	10	10	14	9
					N.S.	p<0.05	p<0.01	p<0.05	N.S.	p<0.05
									N=8	

*Received 30 gm. of glucose intravenously for hypoglycemic symptoms.

diabetes cannot now be satisfactorily explained, but several possibilities may be considered. The genetically diabetic human has considerably thicker muscle capillary basement membranes[9] than either the nondiabetic human or the experimentally diabetic dog. One might postulate that basement membrane thickening of the pancreatic capillaries is also present, as recently suggested by Siperstein,* and imposes a diffusion barrier restricting the movement of insulin or of glucose to the alpha cell without impeding the release of glucagon. The exorbitant insulin levels required to achieve sluggish glucagon suppression would then be explained. However, since no barrier to overall insulin disappearance from the extracellular space has, as yet, been identified in human diabetics, a localized intrapancreatic barrier would have to be postulated to defend this theory.

Second, a circulating insulin antagonist, as yet unidentified, could oppose insulin action and thus account for the apparent unresponsiveness of the diabetic alpha cell to the circulating insulin. However, since alpha cell unresponsiveness was encountered in patients who, on clinical grounds, were deemed quite sensitive to insulin, such a factor would have to be selectively more antago-

*Siperstein, M.D. Unpublished observations.

nistic to alpha cells than to other target cells of insulin.

A third explanation relates to the duration of hyperglycemia which, of course, was present for only moments in the human nondiabetics, a few days in the alloxan diabetic dogs, and years in the human diabetics. It is possible that the years of chronic hyperglycemia in the latter group somehow raised the threshold level of alpha cell responsiveness to hyperglycemia far above the highest glucose concentrations observed in diabetics. (It is unlikely that the long-term use of oral hypoglycemic agents in eight of the ten patients of this study was responsible for the hyporesponsiveness of the alpha cells, since similar findings have been observed in juvenile-type diabetics who had never received such drugs.[2])

A final possibility is that, like the beta cell, the alpha cell is afflicted with some primary genetic defect which impairs its response to glucose and perhaps to other fuels. Since an abundance of glucose is required to "turn off" glucagon release,[10] just as it is required to "turn on" insulin release, either a primary metabolic defect within the alpha cell or an extracellular barrier to substrate diffusion, common to all islet cells, might explain the observations cited.

Whatever its cause, the presence of inappropriately high and hyposuppressible levels of circulating glucagon, shown recently to be biologically active,[11] may have important clinical implications. Despite their marked hyperglycemia, severely decompensated diabetics may have striking hyperglucagonemia[12] and, in severe ketoacidosis, levels up to ten times normal have been reported.[1] Since, on a molar basis, glucagon is at least four times as potent as insulin in terms of hepatic action,[13] the insulin resistance so common in severe ketoacidosis could well be a consequence of hyperglucagonemia. According to Assan et al.,[12] glucagon levels fall after several hours of insulin treatment, and, when the ketoacidosis clears and the insulin requirements return to maintenance levels, glucagon has returned to the normal range. The slow reduction of plasma glucagon in such patients, despite the enormous doses of insulin, is reminiscent of the slow decline observed in the high-dose insulin infusion experiments depicted in figure 2, and may be an important factor in the insulin resistance which characterizes the initial period of therapy.

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