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Research Article

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Blockade of glucose metabolism was induced in dogs by administration of 2-deoxyglucose or mannoheptulose. A striking rise in glucagon was observed despite accompanying hyperglycemia and hyperinsulinemia, which, in the case of mannoheptulose, was induced by infusing crystalline insulin.

To determine if insulin lack also causes paradoxical hyperglucagonemia, dogs were made severely diabetic by alloxan. Fasting glucagon levels ranged from 3 to 22 times normal despite severe hyperglycemia, and were quickly restored to normal by infusing insulin. Diabetes induced in rats by anti-insulin serum was also associated with significant elevation in plasma glucagon. However, diazoxide-induced insulin lack did not increase glucagon in dogs.

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The Effect of Experimental Insulin Deficiency on Glucagon Secretion

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ABSTRACT Suppression of pancreatic glucagon secretion by hyperglycemia is a characteristic of normal alpha cell function. However, in diabetic subjects, plasma glucagon is normal or high despite hyperglycemia. It seemed possible that the presence of glucose or its metabolites within the alpha cell might be essential for suppression of glucagon secretion, and that in diabetes an intracellular deficiency of glucose secondary to insulin lack might be responsible for the nonsuppressibility. The present study was designed to determine the effect upon glucagon secretion of blockade of glucose metabolism and of experimental insulin deficiency.

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It is concluded that normal suppression of glucagon secretion by hyperglycemia does not occur when glucose metabolism is blocked or when severe insulin deficiency is produced. It is suggested that normal glucose metabolism within the alpha cell may be an insulin-requiring process without which hyperglycemic suppression of glucagon release cannot occur.

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INTRODUCTION

Recent studies from this laboratory suggest that in patients with diabetes mellitus the plasma level of pancreatic glucagon is always high, at least in a relative sense (1, 2). In fasting diabetics, for example, the plasma glucagon concentration is as high as in nondiabetics, despite a level of hyperglycemia, which, if simulated in nondiabetics by means of glucose infusion would suppress glucagon to minimal levels (1). After meals, the glucagon levels of diabetics are also high in relation to the prevailing glucose concentration; a carbohydrate meal fails to induce the prompt decline in plasma glucagon which characterizes the normal response (2). A protein meal induces in diabetics a rise in glucagon as rapid and as brisk as in normoglycemic nondiabetics, despite hyperglycemia, which in nondiabetics would completely abort protein-induced glucagon rise (2). Finally, patients with severe ketoacidosis and marked hyperglycemia frequently exhibit a striking elevation of plasma glucagon (3, 1).

The foregoing findings indicate that in diabetic patients glucagon release is not suppressed by hyperglycemia, which in normal subjects is the most powerful physiologic inhibitor of alpha cell activity (4). The present studies were, therefore, designed in an attempt to gain insight into the mechanism by which this major restraining influence upon glucagon release is rendered ineffective in diabetes.

Because earlier studies had demonstrated that glucagon release was uniformly enhanced by glucose lack, irrespective of cause (4-6), it seemed possible that the hyperglucagonemia of diabetes mellitus might be the consequence of a lack of glucose or its metabolites within the alpha cell, as a consequence of the insulin deficiency. Accordingly, studies were undertaken to assess the effect upon alpha cell function, first, of intracellular blockade of glucose metabolism and, second, of experimental insulin deficiency.

TABLE I
Glucagon Response to 2-Deoxyglucose in Dogs

	Time, min	-30	-20	-10	0	2-Deoxyglucose injection											
						2.5	5	7.5	10	15	20	30	40	60	90	120	
Dog 1	Glucose, (mg/100 ml)	106	125	124	127	—	146	—	170	183	198	207	224	244	225	197	
	Insulin, (μ U/ml)	24	58	95	49	—	144	—	139	124	86	63	82	126	138	143	
	Glucagon, (pg/ml)	860	700	860	860	—	760	—	1700	1560	1360	1500	1300	860	760	560	
Dog 2	Glucose, (mg/100 ml)	99	104	103	103	127	136	141	150	176	197	222	231	225	236	246	
	Insulin, (μ U/ml)	27	30	26	82	240	134	220	158	150	158	185	75	70	70	100	
	Glucagon, (pg/ml)	160	130	150	150	350	770	700	1060	870	990	810	560	350	320	200	
Dog 3	Glucose, (mg/100 ml)	117	115	112	112	131	141	139	155	152	153	162	162	161	169	162	
	Insulin, (μ U/ml)	74	—	40	84	300	146	400	174	110	78	102	74	61	48	48	
	Glucagon, (pg/ml)	40	20	0	0	110	220	300	280	110	30	120	150	70	80	80	
Dog 4	Glucose, (mg/100 ml)	107	103	110	109	128	142	155	160	177	190	185	182	168	172	170	
	Insulin, (μ U/ml)	4	34	176	172	172	300	152	112	110	102	108	58	48	82	90	
	Glucagon, (pg/ml)	160	240	380	220	500	420	960	880	510	600	390	370	240	220	120	
Mean	Glucose, (mg/100 ml)	107	112	112	113	129	142	145	159	172	185	195	200	199	201	194	
	\pm SEM	3	4	4	4	1	2	4	4	6	9	11	14	18	15	16	
	Insulin, (μ U/ml)	32	41	84	97	237	181	257	146	123	106	115	72	76	85	95	
	\pm SEM	13	7	29	23	30	34	60	12	8	16	22	4	15	17	17	
	Glucagon, (pg/ml)	305	273	347	307	320	543	653	980	763	745	705	595	380	345	240	
\pm SEM	162	129	163	164	93	117	157	253	267	246	260	216	147	127	95		

METHODS

Studies in dogs. A T-catheter was implanted by the method of Ohneda, Aguilar-Parada, Eisentraut, and Unger (7) in the superior pancreaticoduodenal vein of anesthetized, adult mongrel dogs weighing between 11 and 25 kg. Catheters were perfused continuously until the time of an experiment with a slow infusion of normal saline containing heparin. Experiments were carried out on the 3rd or 4th postoperative day. After each experiment, the dog was sacrificed and the patency of the T-cannula and the vein was determined. Dogs in which the pancreaticoduodenal vein was not patent were excluded from the study.

Alloxan diabetes was induced in some of the dogs 2 days after the surgery. After a 48 hr fast, these animals were intravenously given 75 mg of alloxan per kg of body weight as a 5% solution, followed by a high carbohydrate meal.

Studies in rats. Anti-insulin diabetes was induced in fed rats by the technique of Wright (8). Under pentobarbital sodium anesthesia (Nembutal, Abbott Laboratories), a polyethylene catheter was inserted into the right jugular vein. 1-ml blood samples were obtained 15 min before and 30, 60, 90, and 120 min after the injection of either guinea pig anti-insulin serum¹ with a neutralizing capacity of 2.5 U insulin per ml, or nonimmune guinea pig serum.

Analytical methods. Blood specimens were obtained in syringes rinsed with a 10% solution of ethylenediaminetetraacetate (EDTA) and collected in tubes containing 0.1 ml of Trasylol² 500 Kallikrein Inactivator Units) per ml blood.

¹ Anti-insulin serum was kindly supplied by Dr. Peter Wright.

² Trasylol was purchased from FBA Pharmaceuticals, Inc., New York.

Plasma was separated immediately and stored at -15 to -20°C for up to 12 wk. Glucose concentration was measured by the ferricyanide method of Hoffman (9) using a Technicon AutoAnalyzer. Insulin was measured by the Herbert modification (10) of the radioimmunoassay of Yalow and Berson (11). Glucagon was assayed by the previously described radioimmunoassay (12) as most recently modified (1). The anti-glucagon serum employed was 30K, which is highly specific for pancreatic glucagon and cross-reacts only very weakly with glucagon-like immunoreactivity of gastrointestinal origin.

RESULTS

Effect of intracellular blockade of glucose metabolism on glucagon secretion

2-Deoxyglucose. 2-Deoxyglucose, a nonmetabolizable analogue of glucose, is phosphorylated competitively with glucose (13) and appears to block glucose metabolism below the level of glucose-6-phosphate by inhibiting the enzyme phosphohexoisomerase (13). To determine the effect of a blockade of glucose metabolism upon glucagon secretion four dogs were given an intravenous injection of 300 mg of 2-deoxyglucose per kg of body weight, and the concentrations of plasma glucagon, insulin, and glucose were determined. The results are shown in Table I and Fig. 1.

Plasma glucose rose promptly in all dogs to a peak averaging 200 mg/100 ml ($\text{SEM} \pm 14$) at 40 min. Insulin also rose in all animals, reaching a peak at 7.5 min which averaged 257 $\mu\text{U/ml}$ ($\text{SEM} \pm 60$). Glucagon rose in all

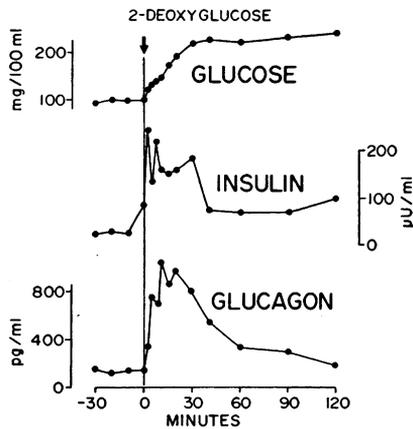


FIGURE 1 Response of glucagon, glucose, and insulin to the injection of 2-deoxyglucose in a conscious dog (dog 2 of Table I).

dogs within 10 min of the injection to a peak averaging 980 pg/ml (SEM \pm 253), returning to preinjection levels 60–100 min later (Table I).

These results are compatible with the thesis that intracellular glucose lack, brought about by interference with glucose metabolism, causes increased secretion of glucagon, despite the presence of high levels of extracellular glucose and insulin, although the unexpected hyperinsulinemia weakens this conclusion.

Mannoheptulose. Mannoheptulose is believed to inhibit insulin secretion (14, 15) by blocking glucose phosphorylation within the beta cell (16), an effect which it presumably exerts on other cells as well. In order to determine if a block of glucose metabolism within the alpha cell at another level would also influence the release of glucagon, 1.2 g of the septose per kilo of body weight

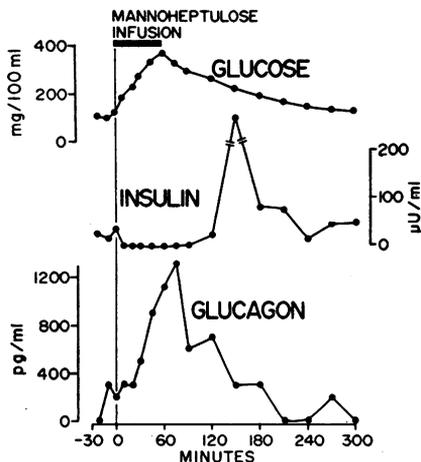


FIGURE 2 Response of glucagon, insulin, and glucose to the infusion of mannoheptulose in a conscious dog (dog 3) of Table II).

was infused for an hour in three dogs (Fig. 2 and Table II). As indicated in Table II, insulin fell promptly to near zero values in all three dogs and glucose rose promptly and progressively to a peak of 401 mg/100 ml at the end of the infusion. The mean glucagon level increased from a preinfusion value of 273 pg/ml to a peak of 1153 pg/ml (SEM \pm 345) 15 min after termination of the infusion and then declined. To determine if insulin lack, rather than a block of glucose metabolism within the alpha cell, was responsible for the increase in glucagon secretion, plasma insulin was restored to 100 μ U/ml or above by infusing insulin with the mannoheptulose (Table III). Glucagon rose at 60 min to 680 pg/ml despite insulin levels of 100 μ U/ml or more. This would suggest that a blockade of glucose metabolism within the alpha cell, rather than the lack of insulin, was the cause of the stimulation of glucagon release.

Effect of insulin lack and insulin repletion on glucagon secretion

Alloxan diabetes. To determine the effect of insulin deficiency upon glucagon secretion, moderate to severe alloxan diabetes with fasting plasma glucose levels in excess of 350 mg/100 ml was produced in four dogs. Despite the hyperglycemia the plasma glucagon was strikingly increased in all, ranging from 1,900 to 14,000 pg/ml, or from about 3 to 22 times normal. Plasma insulin was markedly reduced relative to the hyperglycemia in all but one of the four animals, the one with the lowest glucagon value. The results are recorded in Table IV.

To determine if insulin repletion would correct the extreme hyperglucagonemia of alloxan diabetes, insulin was infused at a rate of from 0.15 to 1.5 U/kg per min in four dogs for 150 min. Within minutes after the start of the infusion, plasma glucagon fell precipitously towards normal despite a declining plasma glucose concentration, which in a nondiabetic dog would tend to increase glucagon secretion (4). These findings, which are recorded in Fig. 3 and Table IV, provide clear evidence that glucagon secretion is greatly increased in insulin deficiency induced by alloxan, despite marked hyperglycemia, and is rapidly suppressed by relatively modest quantities of insulin. It would appear from this that the normal responsiveness of the alpha cells to hyperglycemia requires the presence of insulin.

Diazoxide "diabetes." Diazoxide is believed to suppress directly the release of insulin (17), but is not known to block glucose metabolism. To investigate the effect of diazoxide-induced hypoinsulinemia upon glucagon secretion, two dogs received 50 mg and two dogs 100 mg of diazoxide per kg of body weight as a constant infusion for 120 min. Plasma insulin declined

TABLE II
Glucagon Response to Mannheptulose in Dogs

	Time, min	Mannheptulose infusion																
		-20	-10	0	10	20	30	45	60	75	90	120	150	180	210	240	270	300
Dog 1	Glucose, (mg/100 ml)	114	98	108	151	238	300	366	398	432	398	334	312	264	233	195	181	152
	Insulin, (μ U/ml)	34	17	23	2	2	0	2	2	4	33	70	34	56	85	261	70	33
	Glucagon, (pg/ml)	0	100	0	200	100	260	300	360	360	360	160	—	100	—	0	0	0
Dog 2	Glucose, (mg/100 ml)	108	109	111	188	262	322	382	436	414	388	344	280	225	191	156	144	116
	Insulin, (μ U/ml)	56	54	54	2	2	1	2	2	3	13	59	50	54	339	56	36	91
	Glucagon, (pg/ml)	700	500	620	600	600	1200	1700	1900	1800	1400	1100	1100	600	200	400	0	400
Dog 3	Glucose, (mg/100 ml)	110	106	112	183	230	275	336	370	324	296	264	222	190	166	146	134	122
	Insulin, (μ U/ml)	25	18	35	0	0	0	0	0	0	0	21	337	80	73	11	42	46
	Glucagon, (pg/ml)	0	300	200	300	300	500	900	1100	1300	600	700	300	300	0	0	200	0
MEAN	Glucose, (mg/100 ml)	111	104	110	174	243	299	361	401	390	361	314	271	226	197	166	153	130
	\pm SEM	1	3	1	9	8	11	11	16	27	27	21	22	17	16	12	12	9
	Insulin, (μ U/ml)	38	30	37	1	1	0	1	1	2	15	50	140	63	166	109	49	57
Glucagon, (pg/ml)	\pm SEM	8	10	7	1	1	0	1	1	1	8	12	80	7	71	63	9	14
	Glucagon, (pg/ml)	233	300	273	367	333	653	967	1120	1153	887	653	700	333	100	133	57	133
	\pm SEM	191	94	194	98	119	230	331	363	345	210	222	—	119	—	109	54	109

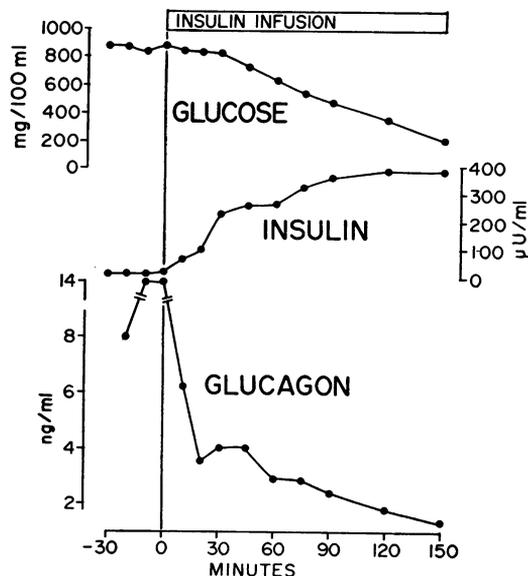


FIGURE 3 Extreme elevation of plasma glucagon and its response to insulin infusion in a dog with severe alloxan diabetes (dog 1 of Table IV).

promptly in all four dogs, the mean insulin concentration averaging less than 5 $\mu\text{U}/\text{ml}$. This was associated with progressive hyperglycemia which averaged more than 300 mg/100 ml at the end of the infusion. Yet, despite the effective suppression of insulin release by the diazoxide, a rise in glucagon concentration was not observed.

Anti-insulin diabetes. To determine the effect of neutralization of circulating insulin upon glucagon secretion a group of five rats was given 1 ml of guinea pig anti-insulin serum by intravenous injection. A control group of nine rats was given 1 ml of nonimmune guinea pig antiserum by the same route. The results are shown in Fig. 4.

The antiserum-treated group developed hyperglycemia, averaging 666 mg/100 ml (SEM ± 31), which was significantly greater than the glucose response of the control group ($P < 0.001$). In this group, plasma glucagon rose from a base line average of 112 pg/ml (SEM ± 20) to a peak of 256 pg/ml (SEM ± 18) at 120 min. Glucagon was

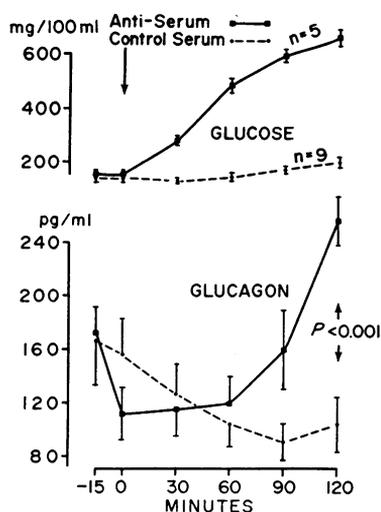


FIGURE 4 The effect of anti-insulin serum on plasma glucose and glucagon in rats.

significantly higher than the level of 104 pg/ml (SEM ± 21) in the control group at this time ($P < 0.001$).

In a group of six other rats the administration of anti-insulin serum induced only a very mild diabetic state with a mean glucose concentration of 131 mg/100 ml (SEM ± 4); in these rats the maximum mean glucagon level did not differ significantly from the controls.

DISCUSSION

The foregoing results indicate that certain forms of severe experimental insulin deficiency are associated with striking elevations in plasma glucagon despite the coexistence of marked hyperglycemia. This was most dramatically exhibited in severe chronic insulin deficiency induced in dogs by alloxan. The fact that the hyperglucagonemia in such animals was corrected in part with remarkable rapidity by the infusion of insulin, and at plasma insulin concentrations of only 32–85 $\mu\text{U}/\text{ml}$, suggests that the “glucose-blindness” of the alpha cell in this form of diabetes, at least, is the consequence of hypoinsulinemia and implies insulin dependence of the alpha cell. The increased levels of glucagon observed during 2-deoxyglucose administration can be interpreted

TABLE III

Glucagon Response to Mannoheptulose during Insulin Infusion in a Dog

Time, min	Infusion of insulin (0.2 U/kg per hr) and mannoheptulose													
	-30	-10	0	10	20	30	45	60	75	90	120	150	180	210
Glucose, (mg/100 ml)	98	104	105	197	262	268	298	295	218	232	204	197	192	185
Insulin, ($\mu\text{U}/\text{ml}$)	15	20	14	112	126	148	170	140	24	16	10	8	12	18
Glucagon, (pg/ml)	120	120	150	218	140	100	500	680	80	300	240	280	280	120

TABLE IV
Glucagon Response to Insulin in Aloxan-Diabetic Dogs

Insulin dosage <i>U/kg per min</i>	Time, min	Insulin infusion											
		-30	-20	-10	0	10	20	30	45	60	75	90	120
Dog 1	Glucose, (mg/100 ml)	880	880	848	888	856	840	736	664	560	508	376	236
	Insulin, (μ U/ml)	7	4	5	4	58	224	254	260	324	356	>400	>400
	Glucagon, (pg/ml)	—	8000	>14000	>14000	6200	3500	4000	2900	2900	2400	1800	1400
Dog 2	Glucose, (mg/100 ml)	396	406	392	404	388	328	280	230	181	148	96	76
	Insulin, (μ U/ml)	14	10	11	8	43	55	60	75	60	50	50	65
	Glucagon, (pg/ml)	4300	3600	3100	3400	2360	1660	1600	1200	1200	1000	1000	1000
Dog 3	Glucose, (mg/100 ml)	440	554	482	488	480	448	400	—	292	240	165	102
	Insulin, (μ U/ml)	10	11	10	9	32	57	70	81	86	85	87	91
	Glucagon, (pg/ml)	2160	2360	2360	2700	1960	1900	1900	1800	1800	1560	1700	1600
Dog 4	Glucose, (mg/100 ml)	549	534	537	528	513	471	435	393	474	390	570	474
	Insulin, (μ U/ml)	42	43	40	38	85	160	176	—	192	248	320	304
	Glucagon, (pg/ml)	1900	1800	2400	1900	810	570	630	—	500	400	—	220
Means	Glucose, (mg/100 ml)	566	594	565	577	513	471	435	393	471	390	570	474
	\pm SEM	95	87	86	93	85	160	176	—	192	248	320	304
	Insulin, (μ U/ml)	18	17	17	15	85	160	176	—	192	248	320	304
Glucagon, (pg/ml)	\pm SEM	7	8	7	7	810	570	630	—	500	400	—	220
	Glucagon, (pg/ml)	3940	5465	5465	5500	3940	5465	5465	5500	5465	5465	5465	5500
	\pm SEM	1216	2468	2468	2468	1216	2468	2468	2468	2468	2468	2468	2468

as evidence that glucose metabolism within the alpha cell is required to suppress glucagon release; if so, one can ascribe the effects of hypoinsulinemia upon glucagon release to insufficient glucose metabolism within the alpha cell, as suggested by Edwards and Taylor (18).

In the case of the more acute forms of insulin deficiency achieved without injury to the beta cells themselves, the effect on glucagon secretion was variable. The administration of diazoxide, which produces hypoinsulinemia by suppressing insulin release (17), failed to change plasma glucagon. Perhaps the hypoinsulinemia was of insufficient duration to deplete the alpha cells of products of glucose metabolism, or perhaps the accumulation of insulin within the beta cells as a consequence of diminished insulin release was sufficient to provide insulin through direct contact with juxtaposed alpha cells, or possibly the drug itself interferes with glucagon release in this situation. In the case of the high glucagon levels induced by the administration of mannoheptulose, which, like diazoxide, inhibits insulin release, a direct blockade by the septose of glucose phosphorylation (16) within the alpha cell is probably responsible for the rise; the results cannot be ascribed to hypoinsulinemia per se, since the concomitant infusion of insulin did not prevent the rise.

On the other hand, hypoinsulinemia, induced by anti-insulin serum, was associated with a significant increase in glucagon at the end of 2 hr. Conceivably, the rise in glucagon induced by this form of hypoinsulinemia may reflect a relative depletion of insulin within the islets of Langerhans induced by the antiserum, in contrast to a pharmacologic over-abundance of tissue insulin produced by diazoxide (19).

In any case, the results in the severely alloxan diabetic dogs indicate that when insulin is deficient both in the plasma and within the islets of Langerhans, alpha cell secretion is not suppressed even by extreme hyperglycemia, possibly because glucose penetration into alpha cells is prevented. The stimulatory effect of 2-deoxyglucose infusion on glucagon secretion suggests that a metabolite of glucose is necessary to suppress the release of glucagon, and it is reasonable, therefore, to ascribe the effect of insulin lack to a deficiency of such metabolites within the alpha cell.

It is of parenthetical teleologic interest that an excess of glucose inhibits the secretion of glucagon, a hormone designed to support vital glucose needs in time of deficit of exogenous glucose, and that a deficit of glucose, whether produced by starvation (4), hypoglycemia (5, 6), or diabetes (1, 2), stimulates its secretion. It would seem that in the basal state the alpha cell is "set" to secrete, just as the beta cell is "set" not to secrete, and that an influx of exogenous glucose will, in a sense, provide energy necessary to "turn off" secretion by the alpha cell

and "turn on" beta cell secretion, a teleologically satisfying arrangement for the two cells designed, respectively, to mobilize stored fuels and to store ingested fuels.

These findings are in harmony with, but do not prove, the previously proposed hypothesis (1, 2) that the relative hyperglucagonemia which characterizes the diabetic state in man is a consequence of insulin lack. Yet simple insulin lack fails to explain certain of the observations in human diabetes. It fails to explain adequately the marked hyperglucagonemia observed in certain adult-type diabetics, in whom plasma insulin is, in an absolute sense, in the normal or low normal range (1, 2). Nor does it account satisfactorily for the high insulin requirements of patients with diabetic ketoacidosis and the hours of therapy required to reduce their marked hyperglucagonemia (3, 1), which contrasts so strikingly with the immediate effect of insulin in severe alloxan diabetes. One is tempted to suggest that if insulin lack is, in fact, the cause of diabetic hyperglucagonemia in man, then some hindrance to insulin-alpha cell interaction must also be postulated.

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