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Serum Insulin-Like Activity in Genetic and Experimental Diabetes Mellitus

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A modification of the epididymal fat pad assay for insulin-like activity has been applied to serum from normal subjects, untreated diabetic patients, and dogs with experimentally induced diabetes. Diabetic patients exhibited abnormally elevated serum ILA levels before and after glucose loading, and also demonstrated a delay in their ILA response to glucose. Dogs with experimental diabetes did not show an elevation in their ILA values. Genetically determined diabetes is obviously different from simple insulin deficiency diabetes. The data confirm and extend previous observations that a significant degree of hyperinsulinemia exists in the garden variety of mildly diabetic patients. Unusually high levels of insulin-like activity were found in 7 of 27 apparently normal subjects. Their ILA exceeded at most times the mean of those found in the diabetic patients. Two of these subjects later discovered a family history of diabetes. Some of the implications of these observations are discussed.

THE POSSIBILITY THAT DIABETES MELLITUS and hyperinsulinism are pathogenically interrelated encounters fewer objections at the present time than when it was suggested, on the basis of indirect evidence, nearly 20 years ago.[1] Refinements in the assay of insulin have provided methods sensitive enough to measure insulin in human serum. By both biological and immunological technics, elevated levels of insulin and insulin-like activity have been demonstrated in serum of patients with untreated diabetes mellitus.[2-4]

The studies recorded below utilized as the insulin assay a modification of the epidiymal fat technic. They had as their objective the measurement of serum insulin-like activity (ILA) in normal subjects, untreated diabetic patients, and partially and totally pancreatectomized dogs, before and after glucose loading. The results demonstrate clearly that genetically determined diabetes mellitus is different from insulin deficiency diabetes.

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Materials and Methods

Assay Technic

Adipose tissue, incubated in the presence of small concentrations of insulin, has been shown to utilize more glucose, produce more CO_2, and synthesize more lipid than in the basal state.[5] Ditschuneit et al.[6] have developed an assay for insulin-like activity that measures the uptake of glucose, and Renold et al.[7,8] have developed an assay that measures the conversion of 1-C14 glucose to C14-CO_2. The modification described here uses as its parameter of insulin-like activity, the conversion of 1-C14 glucose to C-14 lipid.

Male Wistar rats fed ad libitum and weighing 225 Gm. are stunned by a blow to the head. The epididymal fat pads are removed through an abdominal incision and placed in a bath of Krebs bicarbonate buffer maintained at 37 C. A total of 12 fragments of tissue of similar weight (20–30 mg.) are cut cleanly from the peripheral margins of both pads. Each fragment is placed in 1 of 12 stoppered flasks containing 2 ml. of incubation medium. Two more animals are similarly treated so that each of the 12 flasks contain 3 fragments of adipose tissue, one from each animal. Tissue from 3 more animals are placed in a duplicate series of flasks.

Each incubation flask contains 0.2 μc. of 1-C14 glucose plus d-glucose to a standard concentration of 6.0 mg. in 2 ml. of serum or Krebs bicarbonate buffer. A 2 point insulin dose response curve is determined for each assay using concentrations of 60 μU/ml. and 600 μU/ml. prepared in a concentrated form monthly from dry crystalline insulin.* The latter is dissolved for assay in gelatin-enriched (2 mg./ml.) bicarbonate buffer.

Incubation takes place in a Dubnoff metabolic shaking incubator for 2 hours at 37 C. and 72 oscillations/minute in an atmosphere of 95 per cent O_2-5 per cent CO_2. After incubation the tissue from each flask is removed, rinsed, placed in preweighed bottles and allowed to dry overnight. The bottles are then reweighed and tissue weight determined.

Five ml. of a 2:1 chloroform-methanol solution are added to each bottle and layered with 1 ml. of distilled water. The aqueous supernatent phase is aspirated and discarded. This washing step is repeated.

The chloroform is allowed to evaporate and scintillation counting solution added to each bottle. Counting is done in a liquid scintillation spectrometer. The results are expressed as counts/minute/mg. of tissue and calculated by an IBM 7090 computer. This provides logarithmic transformation of the data, analysis of variance with a calculation of λ for each assay, and an estimation of the potency of unknowns and their 95 per cent confidence limits.

Glucose Tolerance Tests

Oral glucose tolerance tests were performed after an overnight fast and a 3 day preparatory diet containing 300 Gm. of carbohydrate/day. The loading dose of 1.75 Gm. glucose/Kg. ideal body weight was dissolved in 300 to 400 ml. of tap water.

Normal subjects were healthy male and female medical students and graduates, 20–30 years of age, who at the time of the study had no family history of diabetes mellitus or of newborns weighing more than 9 pounds. Diabetic patients were ambulant adults of both sexes ranging in age from 22 to 66 years, with a family history of diabetes and a recent glucose tolerance test diagnostic of the disorder. Five of these patients were close to their ideal body weights, while the others were from 10 to 30 per cent overweight. None had been treated for their diabetes.

Sample Collection

Venous blood was drawn at timed intervals during the glucose tolerance test and blood glucose levels determined, using the autoanalyzer ferricyanide method. Blood for ILA

*Kindly supplied by Dr. W. R. Kirtley of the Lilly Research Laboratories.

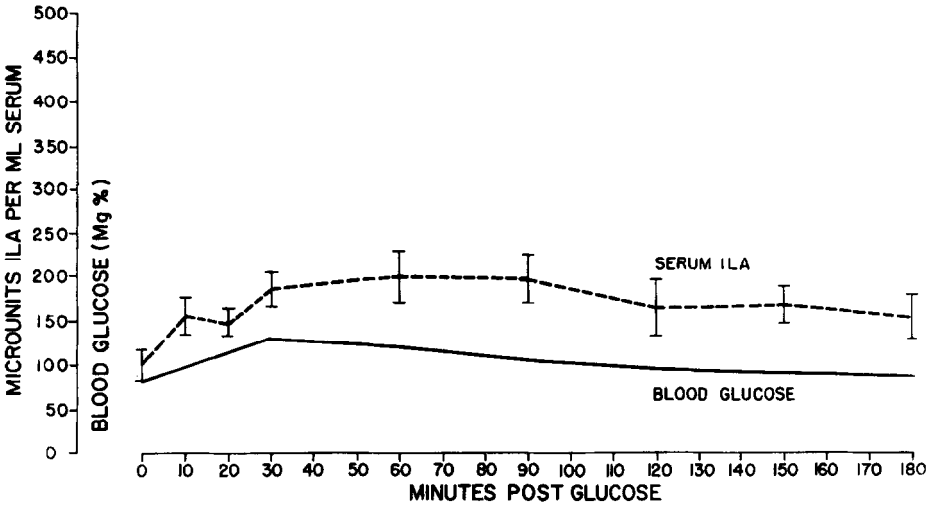

Fig. 1.—Mean insulin-like activity and SEM of undiluted serum from 20 normal subjects during oral glucose loading.

was allowed to clot at room temperature for 30–60 minutes. The serum was separated by centrifugation at 2 C. and stored immediately at −20 C. for 1 to 3 weeks. All assays were done on whole, undiluted serum.

Experimental Diabetes

Mongrel dogs weighing 12–18 Kg. were fed a daily diet of 1 can of dog food and 100 Gm. of glucose. Oral glucose tolerance tests were performed on these animals preoperatively, using 1.75 Gm. glucose/Kg. body weight. After baseline studies, an estimated 5/6th of the pancreas was removed surgically and 1–3 weeks allowed for recovery from the procedure. Pancreatic extract was added to the postoperative diet to maintain gastrointestinal absorption. The aim of the whole procedure was to insure a state of relative insulin insufficiency, manifested by normal fasting blood sugar levels and diabetic responses to glucose loading.

Additional studies were carried out on 5 insulin-dependent dogs.* These animals had been insulin-dependent since pancreatectomy 8–12 months before, and had been maintained in good health. Insulin was withheld for 48 hours preceding each study. All animals developed hyperglycemia and ketosis.

Results

Glucose Loading Tests in Normal Subjects

As demonstrated in figure 1, the mean ILA of undiluted serum in 20 normal subjects rose from a fasting value of 100 μU/ml. to a peak of 200 μU/ml. at 60 minutes, and had not returned to the fasting level at 180 minutes.

Examination of the data in table 1, from which figure 1 was derived, reveals a fairly consistent range of values for any given individual. For most individuals, one observes an increase in ILA within 10 minutes following administration of the glucose load. Many of the subjects exhibited their highest

*We acknowledge the generous assistance of Dr. Piero P. Foa, Detroit, Michigan, in making these preparations available to us for completion of our studies.

Table 1.—*Individual Blood Sugar and Insulin-Like Activity Levels of 20 Normal Subjects*

Serum Concentrations of Glucose and ILA in Normal Subjects (Group 1) during Glucose Loading

	Timed Serum Concentrations															
	Glucose (mg. %)							Insulin-like Activity (μU/ml.)								
Subject	0	30	60	90	120	150	180	−15	0	10	20	30	60	90	120	150	180
	(min.)							(min.)									
Ch	84	130	96	97	96	101	82	**60**	60	**100**	85	85	85	82	75	58	85
Ka	81	127	133	96	111	105	94	**42**	45	45	100	92	80	72	62	88	32
Po	89	160	158	140	80	121	129	**75**	90	**105**	105	88	62	200	175	162	132
Shi	80	140	105	107	127	97	76	55	85	—	90	135	90	140	92	98	60
St	74	106	92	92	95	91	90	85	80	115	130	205	195	—	170	155	140
Ro	63	112	110	99	62	56	55	160	360	220	200	360	300	300	340	380	350
Mo	72	88	90	58	86	96	107	**85**	100	160	160	**155**	300	160	140	85	50
Jo	84	128	135	117	108	87	88	**85**	80	**90**	**205**	190	335	390	—	290	195
Shu	78	162	133	107	104	94	95	100	80	60	40	105	355	400	95	250	175
Fr	82	101	89	86	83	88	89	160	125	300	350	162	580	250	140	305	100
Pa	73	132	73	95	82	89	84		30	60	85	90	70	60	60	60	45
Fa	99	175	184	124	95	91	60		40	70	90	205	205	90	40	70	70
Coh	76	111	92	65	60	73	88		80	210	160	140	160	160	105	150	315
Wa	79	140	124	102	89	84	78		120	160	130	140	170	120	120	105	100
Mul	81	103	118	100	98	63	76		80	—	130	240	70	170	240	120	130
Cr	83	164	177	150	108	110	89		60	80	110	380	150	180	110	165	130
McD	72	114	100	93	68	66	73		130	165	270	130	135	180	170	290	100
Go	80	87	85	86	100	89	83		200	350	225	220	380	—	110	205	460
Ne	86	166	125	133	107	112	75		**60**	**280**	**130**	**350**	**120**	**460**	**220**	**100**	220
Mo	96	165	145	113	96	110	111		95	235	185	250	160	130	650	215	190
Mean	81	131	118	103	93	91	86	86	100	156	149	186	200	197	164	168	154
SEM								**14**	18	22	16	20	30	28	32	21	25

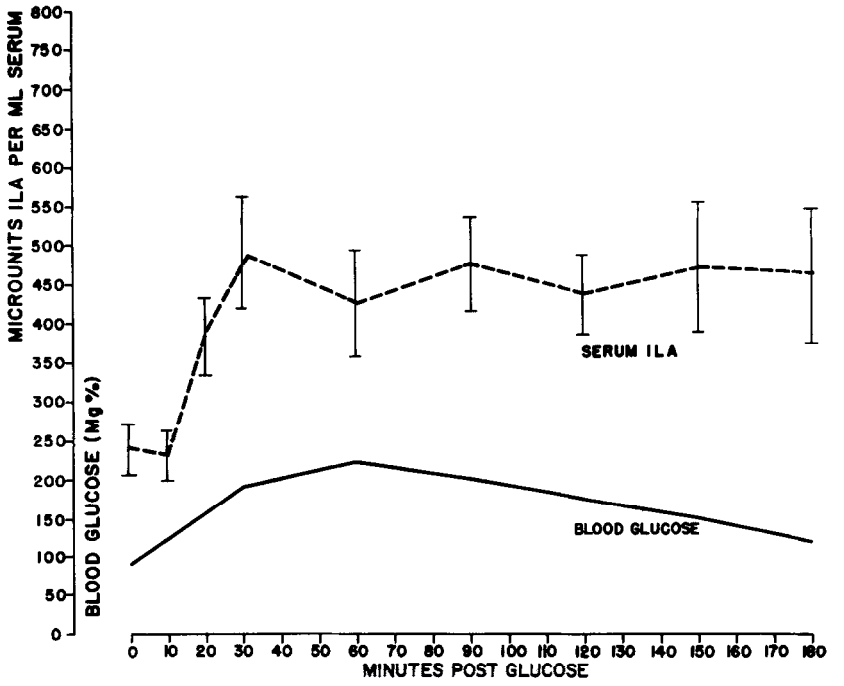

Fig. 2.—Mean insulin-like activity and SEM of undiluted serum from 16 diabetic patients during oral glucose loading.

levels during the first 30 minutes although the peak value for the group was not achieved until 60 minutes. For any given individual ILA does not appear to bear a simple relationship to the blood sugar during the course of a glucose loading test.

Glucose Tolerance Tests in Diabetic Patients

The results obtained in 16 untreated diabetic patients are shown in figure 2. The mean ILA rose from 240 μU/ml. to a peak of 493 μU/ml. at 30 minutes where it remained for the duration of the 3 hour period. The fasting and post-glucose ILA levels of these patients are thus more than double the normal mean. In addition, they differ from normal in that they demonstrate a lag in the response of ILA to a rising blood sugar. Their mean ILA 10 minutes after glucose was not different from their fasting value.

Table 2 demonstrates the mild diminution of carbohydrate tolerance exhibited by this selected group of patients. The tendency for ILA to be maintained within a given range for each individual is also observed. If a relationship between serum ILA and blood sugar exists, it does not appear to be a simple one in this group either.

Glucose Loading Tests in Unusual Normal Subjects

In the course of performing control studies as described above, a significant subgroup of "normal" subjects emerged. These people were unaware of fa-

Table 2.—*Individual Blood Sugar and Insulin-Like Activity Levels of 16 Untreated Diabetic Patients*

Serum Concentrations of Glucose and ILA in Untreated Diabetic Patients during Glucose Loading																
	Timed Serum Concentrations															
	Glucose (mg. %)							Insulin-like Activity (μU/ml.)								
Patient	0	30	60	90	120	150	180	0	10	20	30	60	90	120	150	180
				(min.)								(min.)				
Sm	93	188	200	164	146	144	144	320	—	520	500	380	490	500	570	570
Mun	86	190	196	148	128	86	84	85	255	260	400	250	325	210	225	280
Cad	80	165	—	189	167	141	128	185	550	280	520	—	600	700	>1000	900
Ap	100	274	320	280	110	60	62	415	230	280	>1000	>1000	>1000	640	160	85
Ha	123	234	256	198	230	181	120	100	128	420	260	425	270	117	190	210
Ur	115	156	273	250	—	158	132	350	220	980	>1000	620	700	—	780	560
Al	87	127	166	189	167	159	125	340	430	560	>1000	600	540	430	900	>1000
Ni	84	228	238	162	163	165	96	360	330	460	700	>1000	600	740	340	750
Cr	80	150	162	170	179	97	136	125	130	350	470	270	300	360	120	220
McL	70	195	223	178	198	158	119	400	150	310	430	380	450	430	510	375
Wi	80	218	360	370	300	212	156	200	280	235	200	170	320	350	310	350
Col	81	150	199	142	186	180	176	100	140	310	200	225	150	300	175	225
Re	94	152	157	167	147	158	144	130	140	300	360	325	775	440	>1000	>1000
Lo	102	216	158	140	122	128	111	75	80	205	210	190	180	175	180	165
Ti	105	217	249	279	243	230	—	350	205	530	265	345	430	710	580	—
Di	70	169	190	—	133	—	56	320	190	160	370	225	—	420	—	175
Mean	91	189	223	202	175	150	119	241	231	385	493	427	475	435	469	458
SEM								32	33	50	71	69	61	51	83	85

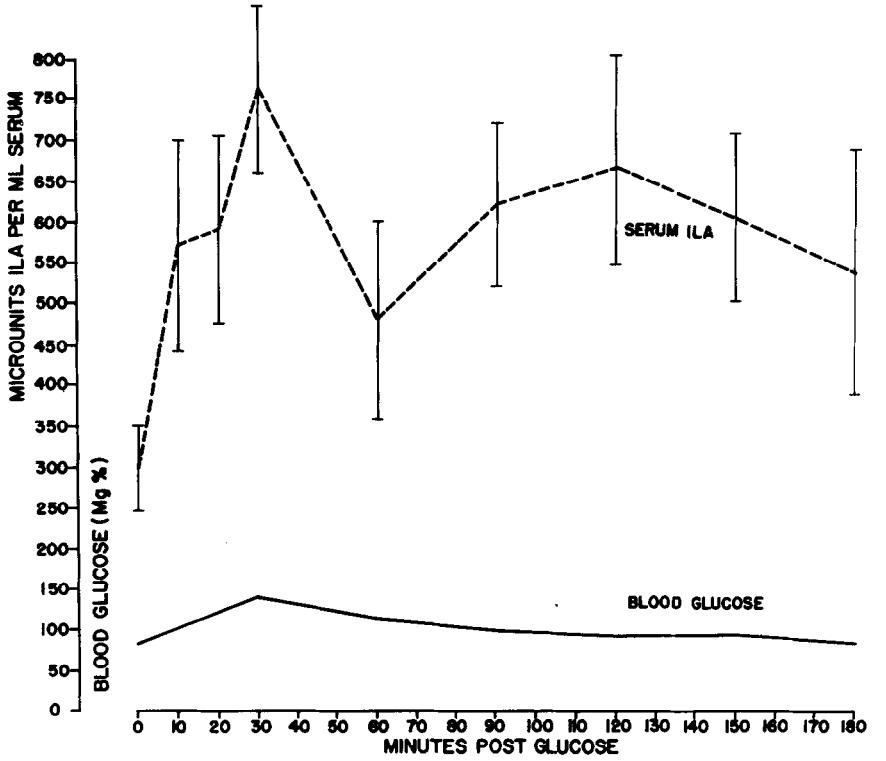

Fig. 3.—Mean insulin-like activity and SEM of undiluted serum from 7 unusual normal subjects during oral glucose loading.

milial diabetes mellitus and demonstrated normal carbohydrate tolerance, but they were found to have high levels of insulin-like activity. The subsequent discovery of diabetes in a grandparent, by 2 of these unrelated subjects, strengthened a decision to consider this group of "normals" separately. Therefore, any "normal" individual who, in the course of his glucose loading test, had 3 or more ILA values *exceeding* the time-comparable mean diabetic values was classified as normal group 2.

The mean fasting ILA in normal group 2 (fig. 3) was 300 μU/ml. It rose to a peak of 763 μU/ml. at 30 minutes and remained in the range of 600 μU/ml. throughout the remainder of the 3 hour period. No difference in carbohydrate tolerance from normal group 1 was distinguishable.

The individual ILA values in this group (table 3), while very high, show patterns of response to glucose loading which are similar to normal group 1. They demonstrate the quick rise and the slow return to basal values. A comparison of the mean levels of ILA in all 3 groups is shown in figure 4.

Glucose Loading Tests in Experimental Diabetes

In a study of the effect of experimental diabetes on serum ILA, 6 dogs underwent partial pancreatectomy after first serving as their own controls. The aim of the procedure was to produce a degree of carbohydrate intolerance

Table 3.—*Individual Blood Sugar and Insulin-Like Activity Levels of Both Groups of Normal Subjects and of the Diabetic Patients during Oral Glucose Loading*

Serum Concentrations of Glucose and ILA in Normal Subjects (Group II) during Glucose Loading																	
	Timed Serum Concentrations																
	Glucose (mg. %)							Insulin-like Activity (μU/ml.)									
Subject	0	30	60	90	120	150	180	−15	0	10	20	30	60	90	120	150	180
	(min.)							(min.)									
Gr	76	123	108	89	99	99	86	235	175	370	350	760	500	480	>1000	540	240
Cam	78	96	102	82	75	88	74	900	600	—	>1000	330	240	>1000	640	650	250
Sc	82	139	93	93	118	100	101	165	215	175	310	860	205	370	540	500	120
Ha	80	145	128	145	98	89	60		290	>1000	630	>1000	340	840	350	160	280
Eg	86	187	125	88	88	117	98		280	900	200	480	250	660	220	>1000	>1000
Sch*	90	144	136	133	82	78	107		260	460	800	910	1000	750	925	520	880
Pe*	92	154	114	70	93	100	68		280	520	850	>1000	840	260	>1000	870	>1000
Mean	83	141	115	100	93	96	85	433	300	571	591	763	482	623	668	606	539
SEM								232	52	130	116	99	120	100	120	103	151

*Family history of diabetes.

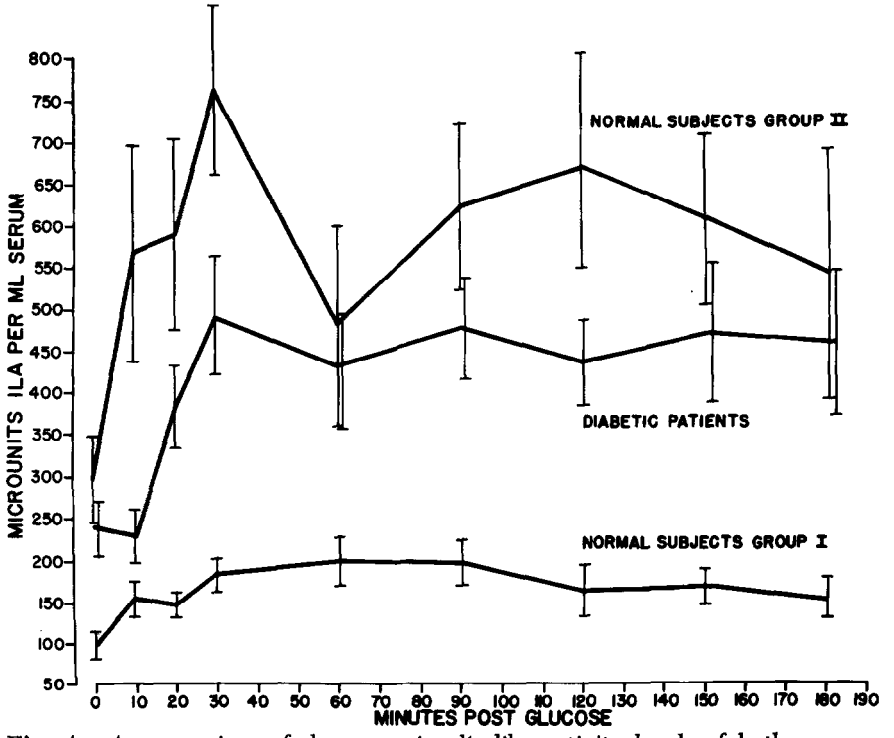

Fig. 4.—A comparison of the mean insulin-like activity levels of both groups of normal subjects and of the diabetic patients during oral glucose loading.

typical of that seen in the human subject with maturity-onset diabetes. Preoperatively, the mean fasting blood sugar was 91 mg. per cent, and 2 hours after oral glucose 104 mg. per cent. Postoperatively, the mean fasting blood sugar was 101 mg. per cent, and 2 hours after oral glucose 184 mg. per cent, a level of carbohydrate intolerance achieved between the first and third postoperative weeks.

Serum from these animals showed a rising ILA after glucose in the normal state (fig. 5) but, unlike the human genetic diabetic, they had falling ILA values after glucose following partial pancreatectomy. Similar results were obtained in 5 ketotic, pancreatectomized animals (fig. 6). Insulin had been withheld 48 hours and the mean fasting blood sugar was 280 mg. per cent. Two hours after glucose, it was 488 mg. per cent. The abrupt and sustained fall in ILA after oral glucose, as the blood sugar rose from 280 to 488 mg. per cent, is of special interest. Injection intravenously of 15 units of regular insulin into one of the animals at the conclusion of the test was associated with a fall in blood sugar from 525 to 147 mg. per cent, and a rise in ILA. It thus appears that changes in insulin activity were measurable in this metabolic state.

Discussion

The Meaning of Serum Insulin-Like Activity

In vitro synthesis of lipid from glucose by adipose tissue is a good index of insulin activity when insulin is added to artificial media.[9] When one

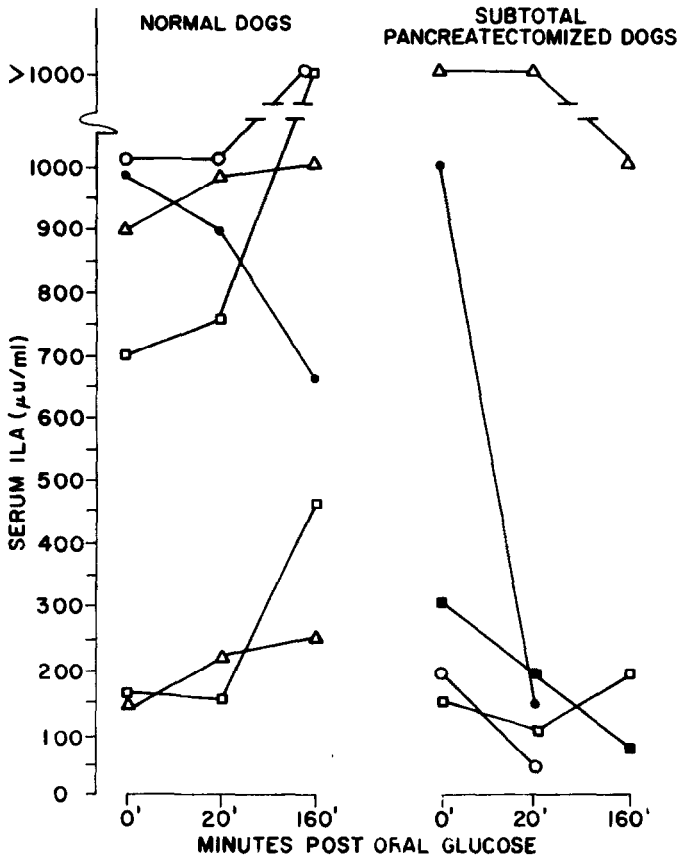

Fig. 5.—Effect of subtotal pancreatectomy on the serum insulin-like activity of 6 dogs before and after glucose loading.

measures ILA of serum, however, factors other than insulin probably contribute to the final result. The biological significance of possible insulin 'inhibitors' and insulin "accelerators" in serum has not yet been elucidated.

On the other hand, it is possible that serum ILA represents the activity of insulin moieties which exist in more than one form. This possibility is supported by demonstrations that serum ILA can be destroyed by insulin inactivators such as cysteine and glutathione,[9,10] and that ILA can be neutralized by insulin-antibodies *after*, but not before, acid alcohol extraction.[10,11]

These latter observations, coupled with the demonstrated responsiveness of serum ILA to glucose loading, justify, for the present, interpretations of changes in serum ILA as representing changes in insulin content of serum.

Serum ILA in Human Diabetes Mellitus

The finding of elevated levels of insulin and of insulin-like activity in the blood of maturity-onset diabetic patients has been reported by investigators using the rat hemidiaphragm,[12] the rat epididymal fat pad,[3,4] and the immunological assay[2] for insulin. The results reported here confirm and extend

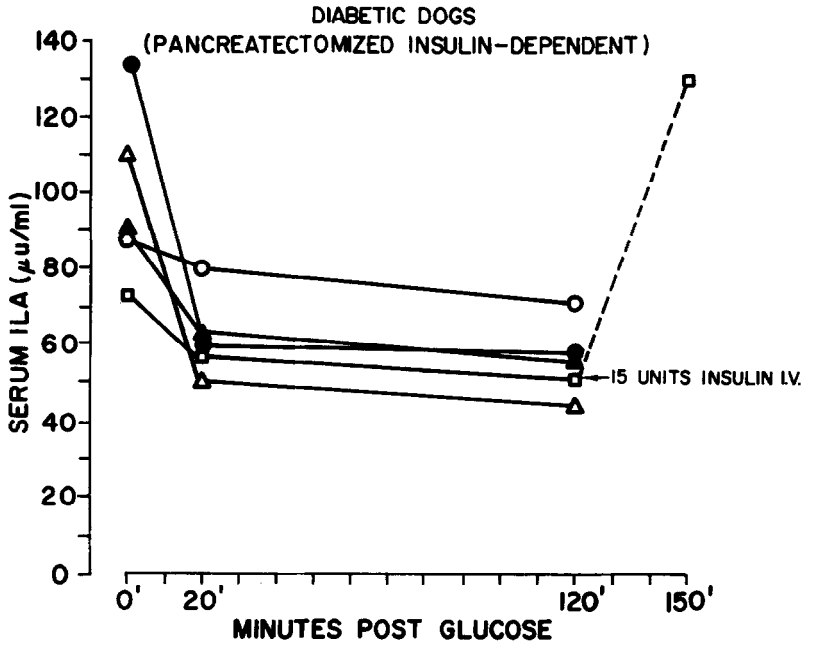

Fig. 6.—Effect of oral glucose loading on the serum insulin-like activity of 5 pancreatectomized dogs in ketoacidosis.

these observations. They make unacceptable the concept that genetic diabetes mellitus is an insulin deficiency state at the time of its onset. The fact that insulin is in excess in the sera of mild diabetics is highly suggestive that this is a secondary, compensatory response to something which antagonizes the action of insulin in the genetic diabetic. With time, the pancreatic capacity may diminish and give way to an absolute deficiency of insulin, as in the older ketosis-prone diabetic patient. Pancreatic tissue from diabetics, when examined within a few weeks of the clinical onset of the disease, has been shown to contain islets which are not only intact but also are hypertrophic and hyperplastic. This appearance is gradually replaced by degeneration and atrophy as the duration of the clinical disease is measured in years.[13] Thus, hyperinsulinemia appears to be a characteristic of mild, genetically determined diabetes mellitus.

A second feature of the ILA pattern in mild diabetes is the lag in response to a rising blood sugar. Ten minutes after ingestion of glucose, the normal group showed a 50 per cent increase in ILA, whereas the diabetic group showed no increase. This finding could be the result of failure to release insulin: (1) from islet tissue, (2) from a circulating inactive form of insulin,[14] or, (3) failure to release some noninsulin component of ILA. Evidence that the normal response to a rising blood sugar is conversion of inactive circulating insulin to an active form[14] is not yet conclusive. Interesting in this respect, however, is the behavior of the totally pancreatectomized dogs after glucose loading. All animals were kept in ketoacidosis with a mean blood glucose

of 280 mg. per cent, 48 hours after insulin. Yet oral glucose was followed by a sustained fall in serum ILA. It is possible that extrapancreatic factors play a role in the ILA response to glucose loading.

Serum ILA in the Unusual 'Normals'

In the course of studying a suitably large sample of normal controls from a population of healthy young adults, an attempt was made to screen out unrecognized diabetic genetic influences by careful inquiry for negative family histories. Despite this precaution, one of the so-called controls demonstrated glucose tolerance tests diagnostic of diabetes mellitus repeatedly. Another subject with an abnormal glucose tolerance curve discovered later that his birth weight exceeded 11 pounds. Neither of these subjects contributed to the data in this paper, but the experience emphasizes the uncertain value of a negative family history.

Two of the group 2 control subjects discovered family histories of diabetes after they had been selected as controls. They were included in the special group because they had normal carbohydrate tolerance and had already separated themselves from the majority of control subjects by exhibiting remarkably high levels of ILA. The subsequent discovery of diabetes in the families of these subjects reinforced our suspicion[15] that unusual phenomena were taking place despite the maintenance of normal carbohydrate tolerance. It has been reported that prediabetics as a group have higher ILA levels than normal subjects.[8] In 4 individuals that we studied (normal carbohydrate tolerance, both parents diabetic), 2 had elevated ILA values and 2 did not. The significance of the increased ILA in group 2 of the normal subjects is not yet clear. Its association with genetic diabetes makes it likely that it may be used, eventually, as an early marker of prediabetes.

Serum ILA in Experimental Diabetes

An important feature of these studies is the failure of the mildly diabetic animal to demonstrate the patterns of ILA observed in the genetically determined diabetes of man. Preoperative ILA values, though showing great variation within the group, were satisfactorily consistent for any given animal. In 5 of the 6, ILA increased after glucose loading. After surgical induction of mild diabetes, a *fall* in serum ILA occurred both before and after glucose loading in 4 of 5 animals. This is opposite to what we observed in the genetic diabetics. The attempt to reproduce in the dog a situation comparable to genetic diabetes of man was a success by blood sugar criteria only.

The concept of dynamic resistance to a diabetogenic influence in the prediabetic period,[15] and the existence of such an influence in early diabetes with secondary hyperinsulinemia receive support from the data presented.

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