Plasma Insulin Activity after Glucose

An Index of Insulogenic Reserve in Normal and Diabetic Man

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Time will finally tell whether the net deficiency of insulin activity characterizing human diabetes mellitus is fundamentally due to decreased secretion of the hormone by failing beta cells, or to increased peripheral destruction of insulin produced in normal amounts. Until then, however, preponderant evidence indicates that the functional abnormality is deterioration of a normal quantitative relationship between the blood glucose concentration and the magnitude of insulin secretion.

Anderson[1] and Brown and his associates[2] showed that pancreatic perfusion with glucose caused acute and sustained elevation of insulin secretion, as evidenced by peripheral hypoglycemia in test animals. In confirmation, Metz[3] recently found rapid and prolonged rise of insulin activity in pancreatic venous blood during peripheral infusion of glucose into dogs. Continuous hyperglycemia, however, has experimentally induced degranulation of beta cells,[4] "hydropic degeneration" (glycogen infiltration) of the islets,[2,5] and complete exhaustion of the betacytotropic mechanism resulting in permanent diabetes.[5] The clinical counterparts of these observations were elucidated independently by Bornstein[6,7] and Vallance-Owen.[8,9] These investigators share major credit for establishing that fasting blood of both normal men and elderly diabetics contains significant insulin activity, which increases after glucose feeding, whereas juvenile diabetics have no circulating insulin activity either before or after a glucose load.

The present study was designed to correlate the insulin-secreting capacity of normal subjects and diabetic patients with clinical criteria of carbohydrate tolerance. It was found that diabetics of increasing severity revealed diminishing insulogenic reserve not only by progressively lower titers of fasting plasma insulin activity, but also by proportionately smaller increments of circulating insulin activity in response to a glycemic stimulus.

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MATERIALS AND METHODS

Subjects and patients were all males, who were classified into four groups (table 1). Twelve metabolically normal subjects served as controls. Nine elderly diabetic patients had moderately elevated fasting blood sugars which subsequently were easily controlled by diet alone, or diet and daily tolbutamide (tolbutamide responders). Six other elderly diabetics had higher fasting blood sugars which showed only minimal or transient depression on later combined diet and tolbutamide therapy (tolbutamide nonresponders). Five juvenile diabetics promptly developed severe ketonuria when exogenous insulin was reduced. The mean age of respective groups was 41, 54, 52 and 38 years. Only two patients were obese. No elderly patient received insulin within a week before the test. One patient with juvenile diabetes (MER) tolerated complete withdrawal of insulin, but the others required periodic small injections of regular insulin daily to prevent development of frank ketoacidosis. No insulin was given later than noon of the day before the test. All individuals consumed 300 gm. of carbohydrate and maintenance calories for three days prior to testing.

After an overnight fast, 5 ml. of oxalated blood was drawn for blood glucose, and 50 ml. of heparinized blood for estimation of plasma insulin activity. The patient then swallowed a solution containing 100 gm. of glucose, and repeat specimens were obtained one hour later. Blood glucose was determined in duplicate by the Somogyi-Nelson method.[10] Immediately after obtaining heparinized blood, plasma was separated by centrifugation and stored at 7° C. Specimens were assayed within a week of procurement, and paired fasting and one-hour samples were always run simultaneously. Plasma insulin activity was assayed by determining the glucose uptake from 2 ml. of undiluted plasma by isolated rat hemidiaphragm, adhering strictly to the technic described by Vallance-Owen.[8] Male Wistar rats, weighing between 100 and 130 gm., were used after food was withheld for eighteen to twenty-four hours. Glucose concentration of plasma or buffered salt solution (GBSS)[11] was 300 mg. per 100 ml. Samples of plasma, GBSS alone, or GBSS containing 10, 100, or 1,000

TABLE 1

Increment of blood glucose concentration in normal subjects and diabetic patients one hour after swallowing 100 gm. of glucose.

Patient	Age	Weight (lb.)	Blood glucose (Somogyi—mg. per cent) Fasting	One hour after glucose	Increment (Δ-Gluc.)
		NORMAL SUBJECTS			
DUN	25	145	71	95	24
ENI	37	114	70	145	75
SEL	40	165	91	159	68
PAR	61	128	77	146	69
BEL	43	161	88	128	40
HEN*	39	183	88	163	75
EDM	27	156	76	118	42
SLE	52	144	85	109	24
WAT	34	135	66	120	54
EWI	64	162	80	169	89
DOC	40	168	71	115	44
MOO	32	167	73	118	45
Mean	41	152	78	132	54
±S.E.M.†	± 4	± 6	± 2	± 7	± 6
	ADULT-DIABETIC-TOLBUTAMIDE-RESPONDERS				
SHA	58	140	169	321	152
MUE	54	172	128	241	113
SHE	47	142	195	352	157
GIL	67	147	104	266	162
THO	67	163	172	307	135
SMI	64	162	119	252	133
JOH	46	159	181	386	205
UTZ*	41	253	101	237	136
COO	46	122	120	231	111
Mean	54	162	143	288	145
±S.E.M.	± 3	± 12	± 12	± 19	± 10
	ADULT-DIABETIC-TOLBUTAMIDE-NONRESPONDERS				
SHA	65	156	320	460	140
BRO	59	150	187	267	80
NEI	43	155	288	441	153
RUT	35	167	276	392	116
RIC	67	162	217	335	118
GRE	40	149	243	350	107
Mean	52	157	255	374	119
±S.E.M.	± 6	± 3	± 20	± 29	± 10
	JUVENILE DIABETICS				
MAJ	45	146	298	481	183
MAN	38	157	388	592	204
SCO	25	159	367	452	85
MER	39	156	430	639	209
JAC	42	137	474	607	133
Mean	38	151	391	554	163
±S.E.M.	± 3	± 4	± 30	± 37	± 24

* Patient was obese. † Standard error of the mean.

micro-units of insulin* per ml. of GBSS, were incubated in a Warburg apparatus for ninety minutes at 37.2° C. under an atmosphere of 95 per cent O_2 and 5 per cent CO_2. Residual glucose was determined by the iodometric method of King.[12] Diaphragms were dried for two hours at 110° C., cooled and weighed. Glucose uptake was expressed as micrograms of glucose per 10 mg. of dried diaphragm. "Net glucose uptake" signified total glucose uptake from a sample minus baseline glucose uptake by diaphragm incubated in GBSS alone. Glucose uptake values were converted into micro-units of "effective insulin concentration"† per ml. of plasma

*Five times recrystallized zinc insulin was kindly supplied by Dr. W. R. Kirtley, Eli Lilly and Company, Indianapolis, Indiana.

†(See column 2.) Vallance-Owen[8,13] has emphasized that this method does not measure total insulin content of plasma, but estimates ". . . plasma-insulin activity, or the effective plasma-insulin concentration, i.e., the sum of insulin and its synergists, if any, on the one hand and its antagonists on the other."[13]

from the dose-response curve shown in figure 1. A few glucose uptakes exceeded the equivalent for an insulin concentration of 1,000 micro-units per ml. of GBSS (table 2), but were included as the latter value.

TABLE 2

Plasma insulin activity* before and after ingestion of 100 gm. of glucose

	(A) Mean net glucose uptake (micrograms/10 mg. diaphragm)			(B) Mean effective plasma insulin concentration (micro-units/ml. plasma)		
Patient	Fasting	One hour after glucose	Increment (Δ-G.U.)	Fasting	One hour after glucose	Increment (Δ-P.I.)
			NORMAL SUBJECTS			
DUN	189	252	63	240	980	740
ENI	153	184	31	107	215	108
SEL	145	279	134	89	>1,000†	911
PAR	130	250	120	62	940	878
BEL	130	240	110	62	750	688
HEN	123	299	176	53	>1,000	947
EDM	96	359	263	27	>1,000	973
SLE	78	147	69	18	93	75
WAT	74	157	83	16	115	99
EWI	70	187	117	15	230	215
DOC	63	136	73	12	71	59
MOO	56	220	164	11	480	469
Mean	109	226	117	59	573	514
±S.E.M.	± 12	± 19	± 18	± 19	±118	±110
"p"			<0.001			<0.001
			ADULT-DIABETIC-TOLBUTAMIDE-RESPONDERS			
SHA	202	257	55	320	1,000	680
MUE	189	245	56	240	830	590
SHE	168	335	167	150	>1,000	850
GIL	158	228	70	120	570	450
THO	115	208	93	43	365	322
SMI	76	119	43	17	48	31
JOH	63	161	98	12	130	118
UTZ	61	150	89	12	100	88
COO	59	107	48	11	36	25
Mean	121	201	80	103	453	350
±S.E.M.	± 18	± 25	± 13	± 38	±136	±103
"p"			<0.02			<0.025
			ADULT-DIABETIC-TOLBUTAMIDE-NONRESPONDERS			
SHA	160	205	45	125	340	215
BRO	112	123	11	40	53	13
NEI	76	97	21	17	28	11
RUT	72	87	15	15	22	7
RIC	51	48	— 3	<10‡	<10	0
GRE	37	43	6	<10	<10	0
Mean	85	101	16	36	77	41
±S.E.M.	± 18	± 24	± 7	± 18	±53	± 35
"p"			<0.7			<0.5
			JUVENILE DIABETICS			
MAJ	23	71	48§	<10	15	<10
MAN	— 2	—15	0	0	0	0
SCO	—33	4	4	0	0	0
MER	—44	—61	0	0	0	0
JAC	—82	0	0	0	0	0
Mean	—28	0	10	1	3	2
±S.E.M.	± 18	± 21	± 9	± 2	± 3	± 2
"p"			<0.7			<0.6

* Insulin activity expressed both as (A) directly measured net glucose uptake, and as (B) conversion value for effective insulin concentration derived from dose-response curve in figure 1.

† Maximal conversion value was limited to "1,000 micro-units insulin per ml." when net glucose uptake from plasma exceeded glucose uptake for this concentration of insulin in GBSS (figure 2).

‡ Insulin equivalents between 0 and 10 micro-units per ml. were calculated as the latter figure.

§ Only positive increments (values above zero) were used in calculating the average for this group.

RESULTS

Dose-Response Curve (*figure 1*): On a semilogarithmic plot, an almost straight line related mean glucose uptakes for insulin concentrations spanning the physiological range of plasma insulin activity. Respective net glucose uptakes (mean ± S.E.M.) for insulin concentrations of 10, 100, and 1,000 micro-units per ml. of GBSS were 54 ± 14 micrograms per 10 mg. diaphragm; 150 ± 17 micrograms per 10 mg. diaphragm; and 253 ± 22 micrograms per 10 mg. diaphragm.

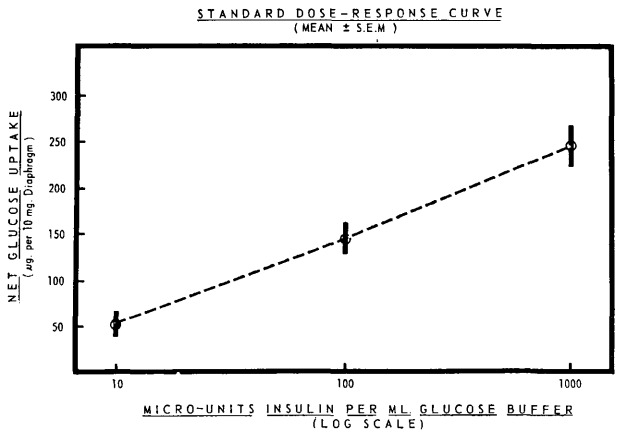

FIG. 1. Virtually a straight line relates net glucose uptakes from logarithmic dilutions of insulin in glucose-buffered-salt-solution (GBSS). Net uptakes from plasma were converted into "micro-units of effective insulin concentration per ml. of plasma" from this dose-response curve.

Fasting Blood Sugar and Increment One Hour After Oral Glucose (*table 1*). Decreased carbohydrate tolerance in successive groups was first manifested by stepwise elevation of fasting blood sugar levels. In normal subjects fasting glucose was 78 ± 2 mg. per 100 ml. (mean ± S.E.M.); in adult-diabetic-tolbutamide-responders it was 143 ± 12 mg. per 100 ml.; in adult-diabetic-tolbutamide-nonresponders it was 255 ± 20 mg. per 100 ml.; and in juvenile diabetics it was 391 ± 30 mg. per 100 ml.

One hour after the oral glucose load, the absolute increase in blood sugar concentration was two or three times higher in diabetic patients than in the control group. The mean "△-blood-glucose" (one-hour value minus fasting value) in normal subjects was 54 ± 6 mg. per 100 ml.; in adult-tolbutamide-responders it was 145 ± 10 mg. per 100 ml.; in adult-tolbutamide-nonresponders it was 119 ± 10 mg. per 100 ml.; and in juvenile diabetics it was 163 ± 24 mg. per 100 ml.

Plasma Insulin Activity Before and After Glucose, Expressed as Net Glucose Uptake (*table 2 and figure 2*). Control subjects and both groups of adult diabetics exhibited insulin activity in fasting plasma, while juvenile diabetics showed none. The mean fasting value for mild adult diabetics was actually slightly higher than the normal mean, and the corresponding level in more severe adult diabetics was below normal, but neither variation was statistically significant. Enhancement of insulin activity after glucose, however, was progressively subnormal in all three diabetic groups. In normal subjects fasting plasma insulin activity (mean ± S.E.M.) was 109 ± 12 micrograms of net glucose uptake per 10 mg. of diaphragm, and one hour after glucose it was 226 ± 19 micrograms per 10 mg. ($p<0.001$). In tolbutamide-responders the fasting level of 121 ± 18 micrograms per 10 mg. had increased an hour later to 201 ± 25 micrograms per 10 mg. ($p<0.02$). Tolbutamide-nonresponders had a fasting value of 85 ± 18 micrograms per 10 mg., which increased minimally to 101 ± 24 micrograms per 10 mg. ($p<0.7$) one hour after glucose. Glucose uptake from fasting plasma of juvenile diabetics was less than from GBSS alone, resulting in a negative value of —28 ± 18 micrograms per 10 mg.; one hour after glucose it was 0 ± 21 micrograms per 10 mg. The mean "△-glucose-uptake" (one-hour value minus fasting value) for respective groups was: normals, 117 ± 18 micrograms glucose per 10 mg. diaphragm; tolbutamide-responders, 80 ± 13 micrograms per 10 mg.; tolbutamide-nonresponders, 16 ± 7 micrograms per 10 mg.; and juveniles, 10 ± 9 micrograms per 10 mg.

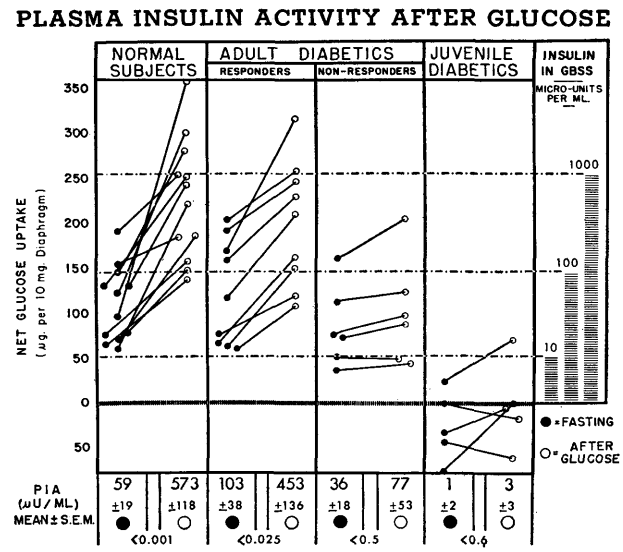

FIG. 2. Solid circles denote fasting plasma insulin activity; open circles are corresponding levels one hour after glucose. Values plotted as net glucose uptakes from plasma. Dose-response curve shown by columns at right is correlated via dashed horizontal lines with glucose uptakes from plasma. At bottom, mean values are in micro-units effective insulin content per ml. plasma, derived from dose-response curve.

Plasma Insulin Activity Before and After Glucose, Expressed as Effective Insulin Concentration (table 2 and figure 2). Intra-group and inter-group relationships were similar to those when insulin activity was measured as net glucose uptake, despite limitation of maximal conversion values to 1,000 micro-units per ml. of plasma for net glucose uptakes which were actually higher. In normal subjects plasma insulin activity (mean ± S.E.M., micro-units of effective insulin concentration per ml. of plasma) increased tenfold after glucose, from 59 ± 19 micro-units per ml. fasting to 573 ± 118 micro-units per ml. one hour later ($p<0.001$). Tolbutamide-responders showed a fourfold rise, from 103 ± 38 micro-units per ml. fasting to 453 ± 136 micro-units per ml. at one hour ($p<0.025$). Respective values in tolbutamide-nonresponders were 36 ± 18 micro-units per ml. fasting, and 77 ± 53 micro-units per ml. after glucose ($p<0.5$); and in juvenile diabetics they were 1 ± 2 micro-units per ml. fasting, and 3 ± 3 micro-units per ml. after glucose. The mean "△-plasma-insulin" (one-hour value minus fasting value) was 514 ± 110 micro-units of insulin per ml. of plasma in normal subjects; 350 ± 103 micro-units per ml. in tolbutamide-responders; 41 ± 35 micro-units per ml. in tolbutamide-nonresponders; and 2 ± 2 micro-units per ml. in juveniles.

Index of Insulogenic Reserve (table 3 and figure 3). The "insulogenic index" related the amount of increased plasma insulin activity after glucose to the magnitude of the insulogenic stimulus itself. It was calculated by dividing the post-glucose increment of insulin activity (△-glucose-uptake or △-plasma-insulin) by the increment of blood glucose concentration (△-blood-glucose). Figure 3 illustrates that insulin-secreting reserve fell sharply

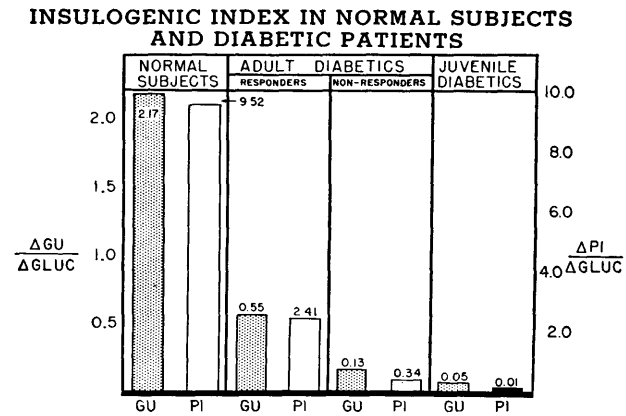

FIG. 3. Dotted columns (GU) show insulogenic index when increment of plasma insulin activity after glucose is in micrograms net glucose uptake per 10 mg. diaphragm (△GU). Open columns (PI) depict index when enhanced insulin activity is expressed as micro-units insulin per ml. plasma (△PI). Blood sugar increment after oral glucose is "△GLUC."

across the spectrum of carbohydrate tolerance, especially when clinical status changed from normal to mildly diabetic. With insulin activity expressed as the more accurate net glucose uptake per 10 mg. diaphragm, the insulogenic index was 2.17 for normal subjects, 0.55 for adult-tolbutamide-responders, 0.13 for adult-tolbutamide-nonresponders, and 0.05 for juvenile diabetics. Relative values were almost identical when increased insulin activity was recorded in more meaningful terms as micro-units of effective insulin content per ml. of plasma, respective indexes being 9.52 for normal subjects (100 per cent); 2.41 for tolbutamide-responders (25 per cent); 0.34 for tolbutamide-nonresponders (4 per cent); and 0.01 for juvenile patients (0.1 per cent).

TABLE 3

Index of insulogenic reserve*

Clinical status	Increment† of plasma insulin activity: Glucose uptake (Δ GU) micrograms per 10 mg. diaphragm	Increment† of plasma insulin activity: Plasma insulin concentration (Δ PI) micro-units per ml. plasma	Increment† of blood glucose (Δ Gluc.) mg. per 100 ml.	Insulogenic indexes: Δ GU / Δ Gluc.	Insulogenic indexes: Δ PI / Δ Gluc.	Per cent of normal
Normal subjects	117‡	514‡	54‡	2.17	9.52	100
Adult diabetics Tolbutamide-responders	80	350	145	0.55	2.41	25
Tolbutamide-non-responders	16	41	119	0.13	0.34	4
Juvenile diabetics	10	2	163	0.05	0.01	0.1

* Increment of plasma insulin activity per mg. per cent rise of blood sugar after oral glucose load.
† Increment = one-hour value minus fasting value.
‡ Mean values.

DISCUSSION

The present data do not help localize the initial metabolic block which upsets normal pegging of insulin secretory rate to blood glucose concentration. However, the sequence of events in these patients is better explained by assuming primary disruption of beta-cell function than by invoking peripheral insulin antagonists, among which insulinase[14] comes foremost to mind.

Subsequent to Metz' demonstration that normal islets respond rapidly and quantitatively to a rising blood sugar,[3] the authors have also found a tenfold rise in pancreatic vein insulin activity within eight minutes after starting to infuse 50 per cent glucose at 3 ml. per minute into a femoral vein.[15] Rapid release of adequate insulin is clearly an important property of intact islets and reasonably accounts for the low peak on the normal glucose tolerance curve.

Evidence that the first step in functional deterioration of the beta cell is *delayed rate of response* to a rising blood sugar level is suggested by the shape of the glucose tolerance curve in even milder diabetics than those included in the present study. Seltzer, Fajans and Conn[16] described patients with symptomatic post-alimentary hypoglycemia as an early manifestation of diabetes mellitus. Their glucose tolerance test was characterized by normal fasting blood sugar, an early hyperglycemic plateau, and a late drop to hypoglycemic levels. The syndrome was thought to reflect sluggish initial release of insulin by islets which ultimately responded to the prolonged hyperglycemia with excessive insulogenesis. When diabetes progressed in these individuals, an upward shift of the glucose tolerance curve eliminated postprandial hypoglycemia at the cost of imposing fasting hyperglycemia, and the patient advanced into the present "tolbutamide-responsive-adult-diabetic" classification. In early diabetes, therefore, initial blunting of the normal swift response to a rising blood sugar level seems to activate a self-perpetuating cycle of (a) first, intermittent, postprandial hyperglycemia; which causes (b) prolonged, excessive stimulation of beta cells; which (c) progressively expends insulin-secreting reserve; until finally (d) *fasting* hyperglycemia supervenes, signifying round-the-clock flogging of an already weakened insulogenic mechanism.

In the present study, the mild fasting hyperglycemia of tolbutamide-responsive adult diabetics was associated with slightly above-normal levels of fasting plasma insulin activity. This suggestion of partial, albeit inadequate, response to a chronic betacytotropic stimulus was even better demonstrated in Vallance-Owen's group of elderly diabetics,[9] whose mean fasting insulin activity was 193 micro-units of insulin per ml. of plasma compared to 70 micro-units per ml. in normal subjects. In further parallel to our tolbutamide-responders, Vallance-Owen's patients also showed subnormal enhancement of circulating insulin activity after oral glucose, their mean increment being 219 micro-units of insulin per ml. of plasma compared to a rise of 333 micro-units per ml. in normal subjects.

The sequelae of unremitting hyperglycemia have been documented experimentally, and were also evidenced in our two severest diabetic groups. Barron[4] degranulated beta cells of normal dogs by administering glucose continuously by vein for only four to nine days; and Dohan and Lukens[5] produced permanent diabetes in the intact cat by sustaining hyperglycemia for forty days with intraperitoneally injected glucose. In our adult diabetics who did not respond to tolbutamide, severe depletion of insulin-secreting capacity was indicated both by subnormal insulin activity during fasting, and its minimal enhancement after glucose loading despite a twice-normal rise of blood sugar. Finally, with both morphology and function of beta cells obliterated in advanced juvenile diabetes,[17] neither fasting nor postglucose insulin activity was anticipated or encountered.

In contrast to their facile correlation with the thesis of primary beta-cell failure, the present findings do not fit well with Mirsky's concept[18] that ". . . an increase in the rate of destruction of insulin by the liver and other tissues, rather than a decrease in the production of insulin by the pancreas, is responsible for the clinical syndrome in the majority of patients with diabetes mellitus." Insulin is presumably inactivated mainly by the enzyme system, insulinase. If insulin secretion keeps pace with magnitude of glycemic stimulus, however, circulating insulin activity in more hyperglycemic tolbutamide-nonresponsive diabetics should be higher, instead of lower, than in the milder tolbutamide-responsive patients. This conclusion obtains whether the liver or peripheral tissues be favored as the major quantitative site of insulinase activity. The hypothesis of primary peripheral destruction of insulin thus seems to require the corollary that the resulting hyperglycemic state begins to deplete insulogenic reserve fairly early in the clinical course of the disease. In brief, even when one seeks the etiological locus of diabetes mellitus outside the beta cell, one is returned to that cell in short order.

SUMMARY

Plasma insulin activity during fasting and one hour after ingestion of 100 gm. of glucose was determined in twelve normal subjects, nine tolbutamide-responsive

adult diabetics, six tolbutamide-nonresponsive adult diabetics, and five juvenile diabetics. An "insulogenic index" relating enhancement of circulating insulin activity to magnitude of glycemic stimulus, enabled comparison of the insulin-secreting capacity of respective groups.

Average fasting blood glucose levels of diabetic patients were proportional to clinical severity of disease. After glucose loading, increments of blood sugar concentration were two or three times greater in diabetics than in control subjects.

Mean fasting plasma insulin activity was 59 micro-units of effective insulin concentration per ml. of plasma in normal subjects; 103 micro-units per ml. in tolbutamide-responsive adult diabetics; 36 micro-units per ml. in tolbutamide-nonresponsive adult diabetics; and 1 micro-unit per ml. in juvenile diabetics. One hour after glucose, respective values were 573 micro-units per ml. in normals; 453 micro-units per ml. in tolbutamide-responders; 77 micro-units per ml. in tolbutamide-non-responders; and 3 micro-units per ml. in juvenile diabetics.

Compared to an insulogenic index of 100 per cent for normal subjects, corresponding indexes were 25 per cent for tolbutamide-responsive diabetics, 4 per cent for tolbutamide-nonresponsive patients, and 0.1 per cent for the juvenile group.

The data indicate that total insulogenic reserve is sharply reduced in diabetes mellitus even when apparent impairment of carbohydrate tolerance is minimal, that it drops progressively as the clinical condition worsens, and approaches zero in the "total diabetic."

SUMMARIO IN INTERLINGUA

Le Activitate de Insulina in le Plasma post Cargation a Glucosa: Un Indice del Reserva Insulinogene in Humanos Normal e Diabetic

Le activitate de insulina in le plasma esseva determinate in stato jejun e un hora post le ingestion de 100 g de glucosa in dece-duo subjectos normal, novem diabeticos adulte respondente a tolbutamido, sex diabeticos adulte non respondente a tolbutamido, e cinque diabeticos juvenil. Un "indice insulinogene," destinate a establir un relation inter le promotion del activitate de insulina circulante e le magnitude del stimulo glycemic, permitteva un comparation del capacitate insulino-secretori in le varie gruppos de patientes.

Le valores medie del nivellos de glucosa sanguinee in stato jejun esseva proportional al severitate clinic del morbo in le caso del patientes con diabete. Post le cargation con glucosa, le augmentos in le concentration de sucro sanguinee esseva duo o tres vices plus grande in le diabeticos que in le subjectos de controlo.

Le valor medie del activitate de insulina in le plasma in stato jejun esseva 59 micro-unitates de concentration de insulina effective per ml de plasma in subjectos normal, 103 in diabeticos adulte respondente a tolbutamido, 36 in diabeticos adulte non respondente a tolbutamido, e 1 in diabeticos juvenil. Un hora post le administration de glucosa, le cifras correspondente esseva 573 in normales, 453 in responsores a tolbutamido, 77 in non-responsores a tolbutamido, e 3 in diabeticos juvenil.

Basate super le comparation con un indice insulinogene de 100 pro cento in subjectos normal, le correspondente indices esseva 25 pro cento pro diabeticos adulte respondente a tolbutamido, 4 pro cento pro diabeticos adulte non respondente a tolbutamido, e 0,1 pro cento pro diabeticos juvenil.

Le datos indica que le reserva insulinogene total es marcatemente reducite in patientes con diabete mellite, mesmo quando le apparente disturbation del tolerantia pro hydratos de carbon es minimal, que ille reserva declina progressivemente durante que le condition clinic del patiente deveni pejor, e que illo approcha zero in le caso del "diabetico total."

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Of the various theories concerned with protein chemistry, our results supported only the classical peptide hypothesis of Hofmeister and Fischer. The fact that all our results could be explained on this theory added further proof, if any were necessary, of its validity. The results also showed that proteins are definite chemical substances possessing a unique structure in which each position in the chain is occupied by one, and only one, amino acid residue.

Examination of the sequences of the two chains reveals no evidence of periodicity of any kind, nor does there seem to be any basic principle which determines the arrangement of the residues. They seem to be put together in an order that is random, but nevertheless unique and most significant, since on it must depend the important physiological action of the hormone.

BIOLOGICAL ACTIVITY AND CHEMICAL STRUCTURE

As yet little is known about the relationship of the physiological action of insulin to its chemical structure. One approach to this problem was to study the insulins from different animal species. Since all insulins show the same activity, it could be concluded that differences would be found only in parts of the molecule that were not important for activity.

All the results given above were obtained on insulin from cattle. When insulins from four other species were studied by essentially the same methods, it was found that the whole of the B chain was identical in all species, and the only differences that were found in the three amino acids contained within the disulfide ring of the A chain, which in the cattle are

Ala-Ser-Val

and in the other species are as follows:

Pig, Thr-Ser-Ileu
Sheep, Ala-Gly-Val
Horse, Thr-Gly-Ileu
Whale, Thr-Ser-Ileu

These results suggest that the exact structure of the residues in this position is not important for biological activity, but it does not follow that the whole of the rest of the molecule is important.

The determination of the structure of insulin clearly opens up the way to similar studies on other proteins, and already such studies are going on in a number of laboratories. These studies are aimed at determining the exact chemical structure of the many proteins that go to make up living matter and hence at understanding how these proteins perform their specific functions on which the processes of life depend. One may also hope that studies on proteins may reveal changes that take place in disease, and that our efforts may be of more practical use to humanity.

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