Is There a Delay in the Plasma Insulin Response of Patients with Chemical Diabetes Mellitus?

G. M. Reaven, M.D., S. W. Shen, M.D., A. Silvers, Ph.D., and J. W. Farquhar, M.D., Palo Alto

SUMMARY

We have compared the plasma glucose and immunoreactive insulin responses to oral glucose, intravenous glucose and intravenous tolbutamide of twenty normal subjects with those of eleven patients with chemical diabetes. The two groups were similar in terms of age, sex and degree of adiposity. The insulin response of patients with chemical diabetes was greater at every time interval during all these tests. Thus, we could document no defect in the rapidity of insulin response to these stimuli in patients with chemical diabetes. These results do not support the view that the commonest metabolic abnormality in patients with diabetes is a delay in the insulin response. DIABETES *20*:416-23, June, 1971.

The first study[1] in which plasma immunoreactive insulin was measured suggested that patients with maturity-onset diabetes responded to an oral glucose load with rises in plasma insulin which, although they eventually exceeded normal, were somewhat delayed in onset. Since that time a consensus seems to have developed which holds that this delay in insulin response is the commonest metabolic abnormality in diabetes mellitus, and the evidence for this conclusion has been summarized in a recent editorial.[2] However, as we review the evidence, we are not convinced that a delay of insulin release is found in all patients with diabetes mellitus, particularly in those classified as having chemical diabetes. Indeed, it is not clear to us what is meant by a delay of insulin release. For example, does it mean that diabetic patients have a lower plasma insulin concentration in the early stages of a glucose tolerance test, or simply that patients with diabetes reach a peak level of insulin at a later time? Finally, is the delay (however defined) seen in response to some or to all stimuli of insulin release? In an effort to answer these questions we have studied the insulin response of normal subjects and patients with chemical diabetes to oral glucose, intravenous glucose and to intravenous tolbutamide. The insulin response of patients with chemical diabetes was as prompt as that of normal subjects. Patients with chemical diabetes differed in that their insulin response was more sustained and quantitatively greater than normal to all stimuli.

From the Department of Medicine, Stanford University, and the Veterans Administration Hospital, Palo Alto, California.

Request for reprints should be sent to Gerald M. Reaven, M.D., Veterans Administration Hospital, Palo Alto, California 94306.

METHODS

I. *Subjects*

Patients were selected on the basis of their oral glucose tolerance in order to compare patients with normal glucose tolerance with those classified as having chemical diabetes mellitus on the basis of the criteria of Fajans and Conn.[3] Thirty-one patients were studied, twenty with normal oral glucose tolerance and eleven with chemical diabetes. Some clinical characteristics of the patient groups are seen in table 1, and it is apparent that they are reasonably comparable in terms of age, sex and adiposity. Kilograms of body fat were calculated by the method of Steinkamp and coworkers[4,5] which they found superior to other estimates of adiposity based on measures such as single skinfold thickness, relative weight, or ponderal index. Multiple series of anthropometric measurements were made by one observer, whose repeat data agreed within a $\pm$ 5 per cent coefficient of variation. From the means of these pooled data we calculated estimates of total body fat. For male subjects these estimates were obtained from a least square analysis of the four variable equation applied by Steinkamp and coworkers to white males, thirty-five to forty-four years. Similarly for female subjects we used the three variable equation of Steinkamp et al. for white females, thirty-five to forty-four years. This method has been previously applied to subjects up to forty-four years of

age, and we have assumed that these equations could also be applied to older subjects. Per cent overweight was calculated from height-weight tables prepared by the Metropolitan Life Insurance Company.

2. *Experimental protocol*

Although hospitalized, patients remained ambulatory throughout the study. Patients were maintained on an isocaloric liquid formula diet, which attempted to approximate the average American diet.[6] On the fourth day of hospitalization an oral glucose tolerance test was performed using 7 oz. of a synthetic carbohydrate beverage.* Two days later the plasma glucose and immunoreactive insulin response to intravenous glucose (0.5 gm./kg.) was determined. The glucose solution (50 per cent) was administered within two minutes, and blood drawn at frequent intervals thereafter for the next two hours. In fourteen patients an intravenous tolbutamide test (1.0 gm. tolbutamide) was performed two days later.

3. *Analytical procedures*

All blood was drawn free-flowing into tubes containing EDTA. Plasma was obtained after separation in a refrigerated centrifuge and frozen quickly in acetone-dry ice. Plasma glucose concentrations were determined in duplicate with an AutoAnalyzer.[7] Plasma insulin concentrations were measured in triplicate by the method of Hales and Randle,[8] using I-125 insulin and insulin-binding reagent obtained from the Radiochemical Centre, Amersham, England. All comparisons between the two groups were carried out by the Mann-Whitney *U*-test.[9] This nonparametric test does not require an assumption that the two populations are normally distributed, and considering the limited number of our patients, we believed this a better choice than the more conventional Student *t*-test.

*Glucola, Ames Company, Elkhart, Indiana.

RESULTS

Mean plasma glucose and insulin response to the oral glucose challenge are seen in figure 1. The fasting levels of glucose and insulin did not significantly differ. The mean plasma glucose concentration of patients with chemical diabetes was significantly higher at every time interval following the administration of oral glucose. The mean plasma insulin response for these patients was also higher at each time point, but the difference was only statistically significant at 120 and 180 minutes.

The results of the intravenous glucose tests are summarized in figure 2. Although mean plasma glucose concentrations in patients with chemical diabetes were higher throughout the test, the differences were not statistically significant by the conservative nonparametric method used. There was no delay in the rate at which insulin appeared in the plasma of patients with chemical diabetes, and the mean response of this group was higher at every time interval. However, as with the differences in glucose concentration, this increase was not statistically significant.

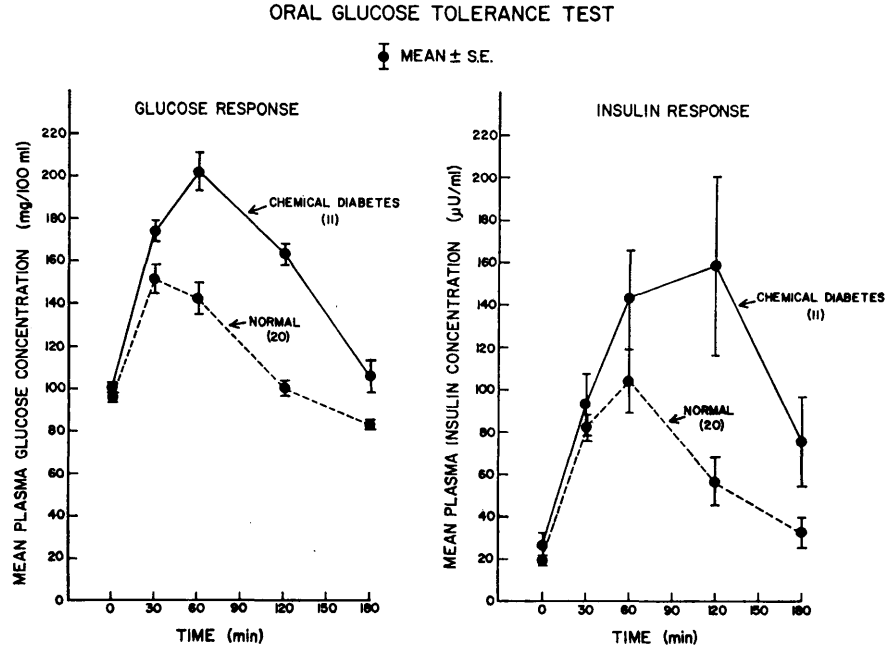

FIG. 1.

Mean plasma glucose and insulin responses to oral glucose.

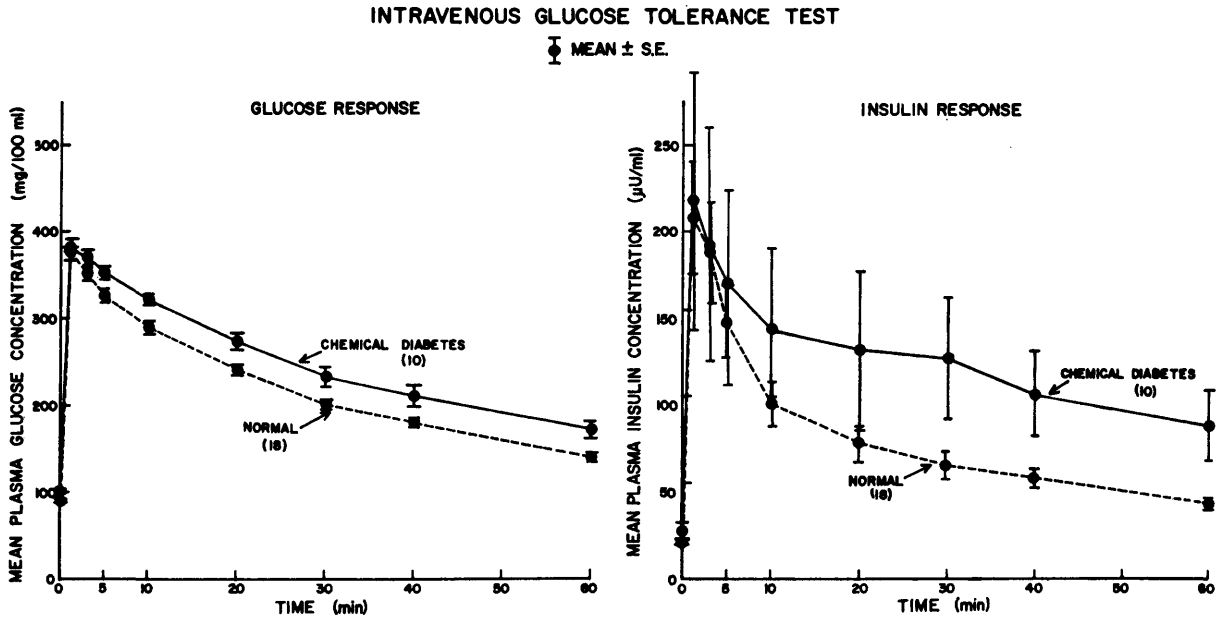

FIG. 2. Mean plasma glucose and insulin responses to intravenous glucose.

Plasma glucose and insulin responses to intravenous tolbutamide tolerance tests are seen in figure 3. The rise in plasma insulin concentration of patients with chemical diabetes was qualitatively as prompt and quantitatively greater than that of the control subjects. In spite of this, the mean plasma glucose concentration was higher at every time interval.

DISCUSSION

The results presented indicate that the plasma insulin concentration of patients with chemical diabetes was equal to or greater than that of the control subjects at every time period during all three tests. Our inability to demonstrate what has been considered to be the commonest metabolic abnormality in diabetes mellitus[2]

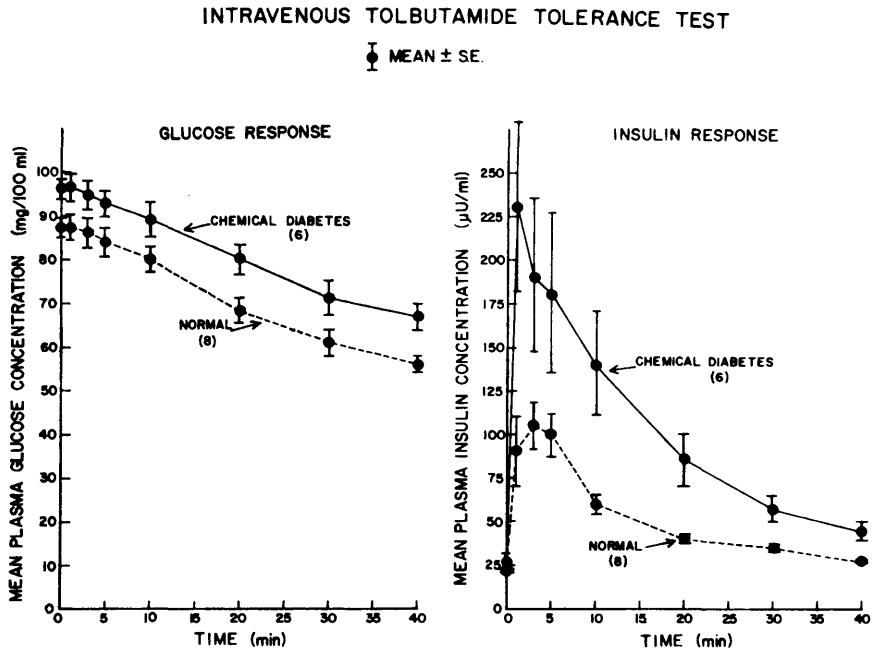

FIG. 3.

Mean plasma glucose and insulin responses to intravenous tolbutamide.

TABLE 1

Clinical characteristics

Group	Number	Age (yrs.) (Mean ± SD)	Height (cm.) (Mean ± SD)	Weight (lb.) (Mean ± SD)	Per cent Overweight (Mean ± SD)	Ponderal Index (Mean ± SD)	Body Fat (kg.) (Mean ± SD)
Normal	20 (14M: 6F)	43 ± 12	171 ± 9	163 ± 34	110 ± 27	12.4 ± .8	22.8 ± 10.7
Chemical diabetes	11 (7M: 4F)	45 ± 11	170 ± 10	178 ± 35	117 ± 22	12.0 ± .6	24.8 ± 7.9
Steinkamp[5] et al.	34 males	35-44	177 ± 7	171 ± 21	109 ± 13	12.6 ± .5	20.7 ± 6.0
	31 females	35-44	164 ± 7	139 ± 29	113 ± 23	12.5 ± .7	23.3 ± 9.5

could arise for several reasons. For example, were our patients not sufficiently hyperglycemic to demonstrate this most basic abnormality? This seems unlikely since several groups have reported a delayed insulin response in prediabetic patients.[10-13] Another alternative is that our two groups differed in ways other than their degree of carbohydrate intolerance. However, this doesn't seem likely in terms of obvious variables known to affect glucose tolerance (table 1). Patients with chemical diabetes appeared to be slightly more obese, but part of this difference is due to the relative preponderance of females in the diabetic group. In any event, the differences between the groups were not significant. Although neither group was lean, their degree of adiposity was not too different from estimates of another series of normal adults.[5] Thus, these physical factors cannot account for our inability to demonstrate a delayed insulin response in patients with chemical diabetes.

Another possibility is that our patients were atypical. In order to consider this alternative we have compared their plasma insulin and glucose responses to those reported by other investigators in which the tests were carried out and reported in a comparable fashion. The results of this comparison for the oral glucose tolerance test are seen in table 2. Our data appear in the first horizontal column, and the plasma glucose and insulin responses of our patients are remarkably similar to those of 124 "lean" patients with impaired glucose tolerance studied by Chiles and Tzagournis.[14] In both instances the insulin response of patients with abnormal oral glucose tolerance was greater than normal at all time intervals. In the next horizontal column we have collated the data from normal subjects and patients with maturity-onset diabetes from the original publication of Yalow and Berson.[1] The mean insulin response of the diabetic group, although higher at all other intervals, is less than normal

TABLE 2

Plasma glucose and insulin response to oral glucose

	Mean plasma glucose concentration (mg./100 ml.)											
	Normal						Diabetic					
Investigator	Pts	0′	30′	60′	120′	180′	Pts	0′	30′	60′	120′	180′
Current	20	95	151	142	100	83	11	99	173	202	163	106
Chiles and Tzagournis[14]	233	89	142	130	100	76	124	96	175	202	161	103
Yalow and Berson*[1]	30	104	152	149	117	—	38	127	210	273	241	—
Seltzer et al.*[17]	21	86	130	116	94	89	10	99	185	231	178	124
Floyd et al.*[18]	17	94	148	145	114	102	18	108	194	224	197	141
	Mean plasma insulin concentration (μU./ml.)											
	Normal						Diabetic					
Investigator	Pts	0′	30′	60′	120′	180′	Pts	0′	30′	60′	120′	180′
Current	20	19	82	104	56	32	11	24	93	143	159	75
Chiles and Tzagournis[14]	233	11	101	111	71	20	124	11	110	155	142	61
Yalow and Berson[1]	30	21	143	139	106	—	38	27	97	156	243	—
Seltzer et al.[17]	21	11	111	122	93	70	10	9	113	195	228	140
Floyd et al.[18]	17	11	113	129	95	65	18	11	59	81	87	57

* These results were originally reported as blood glucose concentration and have been modified for this table, which compares plasma glucose concentration, by adding an increment of 15 per cent.

at thirty minutes. However, the diabetic patients they studied varied from those with abnormal glucose tolerance to patients with unequivocal fasting hyperglycemia, and as a group had significantly higher glucose concentrations than did the patients of the first two studies. Since it has been subsequently[15,16] shown that insulin response decreases with severity of fasting hyperglycemia, it is possible that the lower insulin value at thirty minutes may be a reflection of the greater degree of carbohydrate intolerance. In the next column we have listed plasma glucose and insulin responses of nonobese patients studied by Seltzer et al.,[17] and it can be seen that the plasma insulin response of these patients with mild carbohydrate intolerance was also equal to or greater than normal at all time intervals. In the last horizontal column is similar data from the study of Floyd et al.,[18] in which the plasma insulin response of patients with mild diabetes is lower than normal at all times after receiving oral glucose. Although the insulin response of their normal patients is similar to the other normal groups listed in table 2, their diabetic patients seem quite different. Their insulin levels are much lower in general, and it is the only study in which the response of the diabetic group never exceeds that of normal. The reason for this difference is not obvious. These comparisons indicate that the glucose and insulin responses of patients we studied were comparable to groups studied by a number of other investigators. More specifically, the mean insulin response of both our normal and diabetic subjects was somewhat less than that of "lean"[14] and "nonobese"[17] normal and diabetic subjects, providing further support for the view that our results were not dependent upon the increase in body weight of our patient groups. Thus, these accumulated results indicate that the plasma insulin response to oral glucose of most patients with chemical diabetes is delayed *only* in the sense that it reaches a peak later, and that their insulin response is usually *quantitatively* greater than that of normal subjects at all time intervals after receiving oral glucose.

It was more difficult to find studies of glucose and insulin responses to an acute intravenous glucose challenge which are comparable to ours. In most instances the patient groups differed, or the studies were carried out under different conditions, or the experimental results were presented in a fashion which prevents direct comparison. The only comparable study we could identify was that of Seltzer et al.[17] and even this differed in

TABLE 3

Plasma glucose and insulin response to intravenous glucose

	Glucose concentration (mg./100 ml.)			
	Normal		Diabetic	
Time (min.)	Current (18)†	Seltzer et al.*[17] (21)	Current (10)	Seltzer et al.*[17] (10)
0	88	83	96	99
1	379	—	380	—
3	350	—	371	—
5	325	340	348	338
10	291	302	316	329
20	241	220	268	301
30	200	164	233	240
40	176	—	211	—
60	136	89	172	177
	Insulin concentration (μU./ml.)			
	Normal		Diabetic	
Time (min.)	Current (18)	Seltzer et al.[17] (21)	Current (10)	Seltzer et al.[17] (10)
0	21	10	26	12
1	209	—	218	—
3	191	—	188	—
5	147	142	168	45
10	100	98	143	37
20	78	82	133	42
30	67	56	127	41
40	59	—	104	—
60	43	27	88	47

*These results were originally reported as blood glucose concentration; they have been modified for this table, which compares plasma glucose concentration, by adding an increment of 15 per cent.
†(n) = number of patients.

terms of the rapidity of glucose administration and the times at which glucose and insulin were measured. However, as is seen in table 3, the plasma glucose and insulin responses of normal groups in the two studies were similar. In contrast are the insulin responses of the diabetic groups. The patients we studied had insulin levels higher than normal, whereas the reverse relationship was seen by Seltzer et al. There is no explanation for this discrepancy, and the lack of other comparable studies makes it impossible to know which results are more characteristic. However, there are three somewhat similar studies of insulin response to intravenous glucose of patients with lesser degrees of carbohydrate tolerance. In two of these studies the plasma insulin levels of patients classified as having prediabetes[12] or "steroid-stress" diabetes[19] were equal to or greater than normal. In a third study the insulin response of a group of prediabetic patients was less than that of normal subjects.[13] Thus, some ambiguity still exists as to the quantitative nature of the acute insulin response to intravenous glucose of patients with minimal abnormalities of carbohydrate tolerance.

In table 4 we have listed the plasma glucose and insulin responses following intravenous tolbutamide in our patients, and have also included data from other studies which compare the insulin response of normal subjects to patients with fasting hyperglycemia,[20] and to subjects defined as prediabetic.[13] The insulin response of the various diabetic groups was never less than normal, and a delayed insulin response to tolbutamide does not seem to be characteristic of patients with prediabetes, chemical diabetes, or fasting hyperglycemia. The results of these three studies differ from that of Perley and Kipnis[21] who indicated that the plasma insulin response was lower five minutes after tolbutamide administration in normal as compared to diabetic subjects. The reason for the difference is not apparent, and we have not included their data for comparison as it was not presented in numerical form.

We have discussed these comparisons in detail to point out that there is considerable information indicating that the *quantitative* plasma immunoreactive insulin response to a variety of stimuli in patients with mild abnormalities of glucose intolerance is equal to or greater than normal no matter how early it has been measured. These accumulated results do not rule out the possibility that hyperglycemia in patients with chemical diabetes may be related to a *qualitative* difference in the biological efficacy of what is measured as plasma immunoreactive insulin, and this alternative always exists. However, these combined results do clearly indicate that the conclusion that a delay in insulin release represents the commonest metabolic abnormality of diabetes is entirely based upon judgments as to the *"appropriateness"* of the insulin response. The simplest approach has been to say that the insulin response must be divided by the concomitant glucose concentration, and the quotient, the insulinogenic index, indicates whether the insulin response is adequate or not.[17,21] Although this statement seems to be supported by common sense, there is no experimental data which indicate that plasma insulin concentration is a linear function of the concomitant

TABLE 4

Plasma glucose and insulin responses to intravenous tolbutamide

	Glucose Concentration (mg./100 ml.)						Insulin Concentration (μU./ml.)					
	Normal			Diabetic			Normal			Diabetic		
Time (min.)	1* (8)§	2† (6)	3‡ (13)	1* (6)	2† (6)	3‡ (11)	1* (8)	2† (6)	3‡ (13)	1* (6)	2† (6)	3‡ (11)
0	87	98	87	96	130	87	21	6	20	26	13	21
1	87	—	86	96	—	85	90	—	114	233	—	128
3	86	—	85	95	—	85	106	—	108	190	—	109
5	85	98	83	93	100	84	89	43	104	183	42	94
10	80	83	77	89	110	75	62	31	87	140	38	75
20	68	70	63	80	102	62	42	19	61	86	39	47
30	61	66	50	71	98	49	34	12	41	58	36	32
40	57	65	49	67	92	53	27	10	30	45	22	24
60	63	79	61	67	90	63	24	8	23	38	18	21
90	69	82	71	76	91	69	22	7	21	35	16	19
120	73	—	75	79	—	74	20	—	20	27	—	19

* Current study.
† Varasano-Aharon et al.[20]
‡ Boden et al.[13] Values for plasma glucose have been obtained by adding an increment of 15 per cent to the original figures for blood glucose.
§ (n) = number of patients.

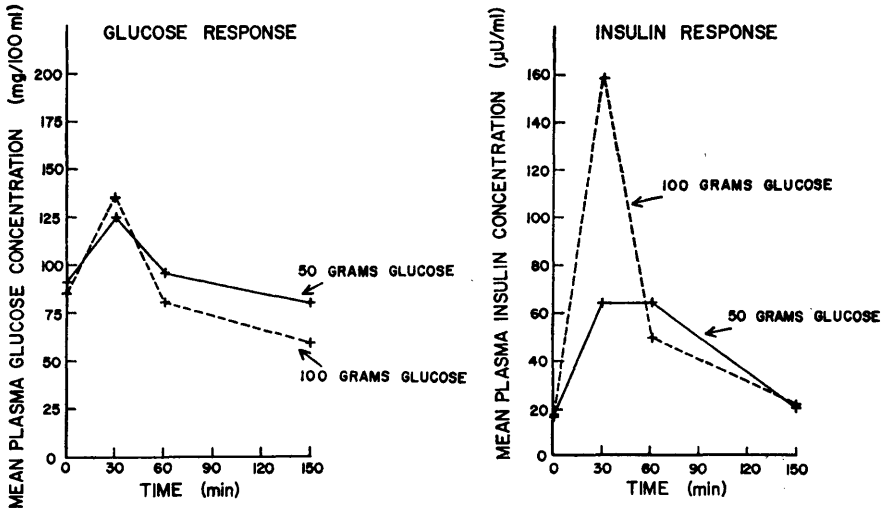

FIG. 4.

The mean plasma glucose and insulin response to a 50 gm. and 100 gm. oral glucose challenge in five normal patients. These figures are derived from the data of Hales and Randle.[22]

plasma glucose concentration. Indeed, the information in figure 4 suggests the contrary. These curves are based on the data of Hales and Randle,[22] in which normal subjects were given 50 and 100 gm. glucose loads on different occasions. It is obvious that the plasma glucose response to these different loads was similar. In contrast is the marked difference in the early insulin response. These subjects had a much greater insulin response at thirty minutes when they received more glucose, and this response was clearly a function of factors other than the concomitant plasma glucose concentration. One of these factors could be the load of glucose administered, and this is relevant to another approach to the question of the *"appropriateness"* of the insulin response. Perley and Kipnis[23] have also attempted to judge this by measuring plasma insulin response to the same glucose concentration achieved by administering different rates of intravenous glucose to normal and diabetic subjects. The plasma insulin response at the same level of plasma glucose was lower in diabetic subjects, and they concluded that patients with diabetes suffer from insulin deficiency. However, it is obvious that although matched for plasma glucose concentration, the two patient groups differed in that normals received a greater glucose load. In terms of the data in figure 4 it is possible that the difference in administered load might be at least partly responsible for the difference in insulin response of the two groups. In this view the lesser insulin response of the patients with diabetes could be a function of the smaller load they received, analogous to the insulin response of the normal patients of Hales and Randle[22] who received 50 gm. of glucose.

A somewhat more sophisticated method of defining *"adequacy"* of insulin response has been employed by Colwell and Lein,[11] by Soeldner et al.[12] and Boden et al.[13] in their studies of prediabetic subjects. Rather than simply dividing insulin by glucose they have computed regression equations relating plasma insulin to plasma glucose concentrations. However, as with the insulinogenic index, these equations are predicated on the assumption that a simple system exists in which glucose is the stimulus and insulin the response, and that the two variables are related in a linear fashion. Obviously, one can also think of insulin as a stimulus and glucose as the response. In these terms the prediabetic patients studied by these three groups can be viewed as being different from normal in that they are more sensitive to insulin, e.g. they dispose of glucose load as efficiently as do normal subjects and need relatively less insulin to do it.

The most sophisticated approach to the question of *"adequacy"* of insulin response has been the introduction by Cerasi[24] of an analogue computer model to interpret the results of a combined acute and sustained intravenous infusion of glucose. On the basis of this model, Cerasi and Luft[25] have concluded that a delay in the initial insulin response to glucose is the basic abnormality in diabetes. However, although their approach is more complicated, they also base their estimate of the insulin response of diabetic and prediabetic subjects on how high they think the insulin *should have gone.*

In an effort to understand the role of insulin in the genesis of hyperglycemia, it may be reasonable to divide the plasma insulin response by the concomitant plasma glucose concentration to produce a numerical index, but it should be made clear that conclusions drawn from such transformations of glucose and insulin data are dependent upon the validity of the transformation. We suggest that validation of the insulinogenic index is

needed before it can be used as evidence for the role of insulin release in the pathogenesis of diabetes. At the present time it seems more important for us to point out that our results are consistent with a large body of previous information which states that the quantitative insulin response of patients with minimal abnormalities of glucose tolerance is as prompt and as great as it is in normal subjects. This conclusion does not negate the possibility that further study may disclose a delay in the insulin response to certain stimuli in patients with chemical diabetes. For example, a delay of insulin release during the first thirty minutes after oral glucose in patients with chemical diabetes is certainly a possibility. However, even if this observation were to be made, it is important to realize that this does not imply causality, and an early delay of insulin release could just as well be considered an effect as it could be the cause of the diabetic state.

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