glucose tolerance is abnormal by comparison with the control group. Blood glucose levels are significantly different in the twins from those in the controls in the fasting state and after glucose. In only two curves did all the points lie within two standard deviations of normal values. The others showed high values, especially one and two hours after glucose. The two subjects with normal glucose tolerance results were from the only pairs without a family history of diabetes. Serum insulin levels in the fasting state did not differ between the groups. However, insulin levels were lower than normal half an hour and one hour after glucose in the twins.

Discussion

The significance of these abnormal blood glucose levels is increased by the fact that the subjects are young. High blood glucose values after oral glucose are rare in early life (Butterfield, 1964). Despite these high blood glucose levels serum insulin levels are low, particularly after glucose.

There are a number of possible explanations for this reduced response to glucose. It might be that not all the insulin in the blood is being measured. At present there is no conclusive evidence to suggest that immunological methods fail to measure all the available insulin in the blood. When attempts are made to extract insulin from blood proteins by various acid-ethanol methods there does not appear to be any release of insulin previously associated with other proteins (Karam *et al.*, 1963). When higher values for blood insulins have been obtained by bioassay methods their significance is doubtful. It has always been difficult to measure blood insulin levels with precision by the rat diaphragm technique (Taylor and Randle, 1959), and the biological significance of adipose tissue assays for blood insulin is uncertain (Kipnis and Stein, 1964; Rasio *et al.*, 1965).

It might be that insulin was being more rapidly broken down in the twins of diabetics. However, serum insulin levels tend to rise after glucose less than in normal persons, though fasting levels are not different in the two groups. This is not the pattern of response which would be expected if there were a uniformly enhanced rate of insulin destruction throughout the glucose-tolerance test. Neither do these studies give any evidence of antagonism to endogenous insulin, which would lead to raised levels of blood insulin in the presence of a normal glucose tolerance. It seems much more likely that the response of the beta cell to a rise of glucose concentration is less than normal. The diminished glucose tolerance of some of these non-diabetic twins may be a consequence of diminished insulin response to oral glucose.

The clinical significance of these findings is not known. In particular, we do not know whether the abnormalities will progress to frank clinical diabetes. We intend to observe the present group and collect data on other identical twins of diabetics.

Summary

Glucose tolerance and serum insulin levels have been examined in nine apparently normal twins of diabetics and compared with the results in normal people.

Mean glucose tolerance was significantly less than that of normals and serum insulin levels after sugar were also low.

It is suggested that the impairment of glucose tolerance in some of these twins is a consequence of a diminished response of the beta cell to glucose.

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Glucose Tolerance and Serum Insulin in the Unaffected First-degree Relatives of Diabetics

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In the preceding paper the effects of oral glucose on blood glucose and serum insulin levels were examined in the identical twins of known diabetics, and abnormalities of glucose tolerance and serum insulin levels in some of them were shown. It seemed of interest to determine whether these abnormalities were present in other first-degree relatives of diabetic patients. In addition, serum insulin and glucose tolerance were examined in a small group of relatives of diabetic patients who gave clinical evidence of hypoglycaemia.

Methods and Subjects

Blood glucose and serum insulin were measured by methods identical to those in the preceding paper. The results have been compared with the same control series.

Six children of conjugal diabetic couples were studied and studies were made of a family of nine children derived from an additional conjugal pair of diabetics.

Nine other first-degree relatives of diabetics were studied. None of them was previously known to have diabetes. As in the preceding paper, subjects were tested after an overnight fast and after at least three days on a normal carbohydrate diet. All but one of these patients were aged under 45.

Results

Values for Glucose and Insulin in Relatives of Diabetics.—When oral glucose-tolerance tests were performed in the off-

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TABLE I.—*Glucose Tolerance and Serum Insulin in Relatives of Diabetics (not exhibiting hypoglycaemia).*

Case No.	Age and Sex	Relationship to Patient	Blood Glucose (mg./100 ml.)				Serum Insulin (μu./ml.)			
			0 min.	30 min.	60 min.	120 min.	0 min.	30 min.	60 min.	120 min.
1	47 F	Both parents	84	137	128	92	2	16	20	2
2	25 F	,, ,,	70	99	85	63	10	39	33	6
3	26 M	,, ,,	89	158	148	89	10	18	25	21
4	44 M	,, ,,	65	119	117	103	17	72	120	78
5	— F	,, ,,	71		95	67		19	21	5
6	— F	,, ,,	71	157	90	60	5	205	89	
		Mean ± S.E.M.	75 ± 3	134 ± 9	110 ± 11	79 ± 7	10 ± 3	62 ± 31	51 ± 17	22 ± 11
7	30 F	Mother					7	24	30	10
8	25 M	Father					6	28	25	4
9	54 F	Sister	82	137	152	64	28	94	81	34
10	7 F	,,					18			
11	30 F	3 children	83	118	126	130	16	35	144	170
Normals (as in preceding paper)			67 ± 2	98 ± 4	80 ± 3	67 ± 3	21 ± 2	64 ± 5	57 ± 6	31 ± 6

Results are expressed as means ± S.E.M.

spring of conjugal diabetics blood glucose values were somewhat higher than those obtained in the control series (Table I). This is particularly noticeable half to one hour after glucose. By contrast, serum insulin levels in the offspring of the conjugal group were similar to those obtained in the normal series. A similar pattern of insulin and glucose is observable in the other first degree relatives tested. In the instance where the subject was obese (Case 11) rather high serum insulin values were obtained. This result is in line with other observations which suggest that elevated blood insulin levels are characteristic of obesity whether diabetes is present or not (Karam *et al.*, 1963). Studies were also made on nine offspring of a single pair of conjugal diabetics. Values for blood glucose and serum insulin are shown in Table II. One subject (E) was found to be frankly diabetic, and abnormalities of glucose tolerance were discovered in several of the other offspring. Again serum insulins were relatively low, apart from one patient (F) who showed an extreme rise after glucose. The pattern of serum insulin response after glucose is characterized by a normal fasting level and slow rise in insulin, suggestive of a delayed and inadequate response of the beta cells to stimulation by sugars.

TABLE II.—*Serum Insulin and Blood Glucose Concentrations in Nine Children of a Pair of Conjugal Diabetic Patients*

Subject	Age and Sex	Blood Glucose (mg./100 ml.)				Serum Insulin (μu./ml.)			
		0 min.	30 min.	60 min.	120 min.	0 min.	30 min.	60 min.	120 min.
A	58 M	86	194	215	35	11		48	25
B	57 F	89	145	171	123	11	20	36	
C	55 M	80	200	196	129	15	19	28	19
D	55 M	80	162	183	125	9	22	29	22
E	53 F	308	349	377	284	20	25	25	20
F	49 F	92	175	167	38	18	< 250	< 250	35
G	47 M	87	183	206	115	11	24	49	30
H	45 F	72	156	42	66	10	60	45	23
I	41 F	111	201	151	44	10	14	26	15

Blood Insulin and Glucose in Patients with a Clinical History of Hypoglycaemia.—Glucose-tolerance tests were carried out and serum insulin values were measured in four patients with symptoms of hypoglycaemia, all with first-degree relatives with diabetes. Results are shown in Table III. Relatively low blood sugars were obtained two hours after taking glucose, though values at 30 and 60 minutes were high, and serum insulins were usually increased at those times. These values were significantly greater than those in normal people.

Discussion

These results (like those in the preceding paper) suggest that glucose tolerance is often abnormal in the close relatives of diabetic patients. This is so in all the groups tested, but is especially noticeable in the nine children of a single conjugal pair of diabetics. There have so far been few studies of glucose tolerance in large families. Fajans (1964) has drawn attention to some similar results obtained by glucose-tolerance tests in another family study. Thus when 10 apparently healthy children of a diabetic father were tested seven were found to be diabetic.

Despite the abnormal levels of blood glucose recorded in this paper (with the exception of those with hypoglycaemia) blood insulin values have been relatively low. These values confirm those obtained by a number of other workers who have found normal or reduced levels of blood insulin in close relatives of diabetics (Grodsky *et al.*, 1965 ; Colwell and Lein, 1966). The insulin response to intravenous glucose has also been reported to be reduced (Cerasi and Luft, 1963) or normal (Soeldner *et al.*, 1966) in a number of relatives of diabetic patients. There now seems to be general agreement among workers who have used various types of immunological techniques for measuring insulin that blood insulin levels are relatively low in the relatives of diabetics.

A different type of response had earlier been found by Steinke *et al.* (1963), who used an adipose-tissue method to measure insulin in the blood of a large group of the offspring of conjugal diabetics. Increased insulin-like activity was found in the serum of many, though not all, such subjects. The authors were careful to point out that the physiological significance of blood-insulin activity measured in this way is uncertain.

The results shown both in this paper and in the preceding one suggest that in close relatives of diabetics there may be an impairment in the response of the islet cells to glucose. It is at present not known whether this defective response extends to other stimuli for insulin secretion such as amino-acids, glucagon, or sulphonylureas. If it is a defective response only to sugars that is being measured, then the defect could lie either with the phosphorylation of glucose, within the beta cell or in

TABLE III.—*Glucose Tolerance and Serum Insulin in Relatives of Diabetic Patients with Clinical Evidence of Hypoglycaemia*

Patient	Age and Sex	Relationship to Patient with Diabetes	Blood Glucose (mg./100 ml.)				Serum Insulin (μu./ml.)			
			0 min.	30 min.	60 min.	120 min.	0 min.	30 min.	60 min.	120 min.
I	45 M	Father	99	159	213	30	14	21	47	28
II	16 M	,,	74	99	100	72	20	100	205	45
III	45 M	,,	78		154	56	9	124	211	20
IV	26 F	Mother	61	133	117	42	11	148	160	10
		Mean ± S.E.M.	78 ± 6	131 ± 17	146 ± 23	50 ± 9	14 ± 2	98 ± 27	158 ± 38	26 ± 9

its transport across the beta cell membranes. There is at present insufficient evidence to decide which of these steps in glucose metabolism may be rate-limiting for insulin release (Renold *et al.*, 1964).

A reactive type of hypoglycaemia with abnormal glucose-tolerance tests was observed in four subjects who were first-degree relatives of diabetics. In addition, in three of these serum insulin was raised after glucose (Table III). Not all patients who exhibit reactive hypoglycaemia show elevated levels of blood insulin (Yalow and Berson, 1965). Nevertheless increased blood insulin levels may be commoner among the relatives of diabetic patients. Thus Sussman *et al.* (1966) have described the cases of two patients with hypoglycaemia and a strong family history of diabetes in whom serum insulin levels were raised after glucose.

It has been suggested by Seltzer *et al.* (1956) that hypoglycaemia in subjects who are likely to develop diabetes results from a delay in the response of the islet tissue to glucose. In consequence during a glucose-tolerance test blood glucose may rise very rapidly, resulting in an excessive discharge of insulin from the beta cells. There is, however, no evidence of a delay in the response of insulin to glucose in the four subjects whom we have reported here, since the blood insulin values are clearly above normal levels half an hour after glucose (Table III).

Nevertheless in the fasting state blood insulin levels are low despite normal or slightly raised glucose levels. It therefore appears that the islet-cell response to glucose may be defective at low blood glucose levels. At higher blood glucose levels a different type of response is obtained. Serum insulin is then greatly elevated. These results might be explained if, assuming that phosphorylation of sugars was important in insulin release, two different phosphorylating enzymes were present within the beta cell. This has already been shown to be the case in the liver cell (Viñuela *et al.*, 1963 ; Walker and Rao, 1964) where hexokinase regulates phosphorylation at low glucose concentrations and glucokinase phosphorylation at higher levels of glucose. Glucokinase activity is known to vary greatly with the physiological state of animals, and it is possible that an increased activity of this enzyme is associated with the beta cells of these hypoglycaemic subjects. Such an explanation is entirely speculative, and it has to be admitted that glucokinase has not so far been demonstrated in islet tissue with certainty (Renold *et al.*, 1964). A further possible explanation might be that the beta cells of the subjects with hypoglycaemia were especially responsive to a hormonal factor released owing to the presence of sugars in the gut (McIntyre *et al.*, 1964 ; Samols *et al.*, 1955).

Though hypoglycaemia has often been demonstrated as a premonitory feature of diabetes mellitus (Seltzer *et al.*, 1956 ; Baird, 1964) it is not known whether a phase of hyperinsulinism after glucose usually precedes impairment of insulin secretion. Both these types of abnormal response appear to be present in the nine offspring of a pair of conjugal diabetics studied in this paper (Table II). Thus one subject (F) showed high levels of blood insulin which correspond with a low blood sugar, while the rest of the family showed a depressed insulin response to glucose. Families have moreover been described in which the hypoglycaemia precedes actual loss of glucose tolerance (Deuil and Laurent, 1963).

Finally, there is no evidence, from this study, of insulin antagonism in these subjects. Fasting values for serum insulin have been consistently low, often in spite of raised fasting blood glucose levels. The low blood glucose levels frequently encountered two hours after glucose do not suggest that endogenous insulin is ineffective. It seems more likely that the release of insulin by glucose is defective in this series of relatives.

Summary

Glucose-tolerance tests with simultaneous determination of serum insulin have been carried out in normal subjects and in the close relatives of diabetic patients. The groups tested included the offspring of conjugal diabetics as well as other first-degree relatives. In one instance nine children of a pair of conjugal diabetics were studied. Glucose tolerance was often impaired in all the groups tested.

Fasting serum insulin levels were normal. However, after glucose two types of insulin response were observed. In most cases the serum insulin levels were low or normal, despite mildly elevated blood glucose levels. A different type of response was seen in a small number of patients who showed symptoms of hypoglycaemia. In this group blood insulin levels rose from normal fasting levels to unusually high levels, before returning to normal.

It is concluded that the close relatives of diabetic patients often show an inappropriate insulin response to oral glucose, and it is suggested that this may be due to an inherited abnormality of the beta cell associated with insulin release.

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