Chemical and Early Overt Diabetes Mellitus in Children

I. Effect of Glucagon on "Insulin Reserve"

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SUMMARY

Glucose disappearance rates and serum immunoreactive insulin levels following the infusion of glucose and glucose with glucagon were studied in eleven normal subjects, forty-eight children classified as chemical diabetics, and seventeen children with the recent onset of overt diabetes in the early recovery phase of the disease.

Children with chemical diabetes had significantly lower glucose disappearance rates than the normal controls and significantly higher disappearance rates than the children with overt diabetes. Compared with the normal controls, the change in glucose disappearance rates due to glucagon was significantly reduced in chemical diabetics and absent in overt diabetics.

Normal subjects and chemical diabetics responded to glucose and glucagon infusion by increasing the serum IRI levels. Chemical diabetics formed a heterogeneous group with respect to the serum IRI response to glucagon. Children classified as "late" chemical diabetics, like the overt diabetics, had decreased serum IRI responses to glucagon.

Children with chemical diabetes who have blood sugar values outside the ninety-seventh and third percentiles of the distribution of normal values during an oral glucose tolerance test are already in an advanced (late) stage of chemical diabetes and are likely to have their disease progress rapidly to overt diabetes. Children with "late" chemical diabetes have glucose disappearance rates less than 1.1 per cent per minute, less than +0.3 change in glucose disappearance rates after glucagon stimulation and an impaired serum IRI response to an intravenous infusion of glucose or glucose and glucagon. Identification of children in earlier stages of chemical diabetes is desirable in order to evaluate the feasibility of altering the progressive loss of insulin reserve. DIABETES *18*:679-85, 1969.

Identification of children in the early chemical stages of diabetes mellitus is desirable in order to evaluate measures designed to alter the progression of the disease. At present oral and intravenous glucose tolerance tests are being used to determine if an early deficiency of insulin secretion exists.[1] In normal subjects insulin release and glucose utilization are stimulated by glucagon.[2-5] Also, glucagon has been shown to release insulin from the isolated pancreas and augment the beta-cytotrophic effects of glucose.[6-8]

This study was undertaken to evaluate the glucagon-modified rapid intravenous glucose tolerance test as a measure of insulin reserve in normal subjects, children with chemical diabetes and children with recent onset of overt diabetes after the disease had been regulated completely with insulin and dietary management.

MATERIAL AND METHODS

Subjects

Forty-eight children classified as having chemical diabetes* were studied. Each subject had two or more abnormal oral glucose tolerance tests separated by at least a two-month time interval. The concentration of blood sugar during oral glucose tolerance tests was compared with values obtained in 200 healthy children without a family history† of diabetes.[9] The criteria for abnormal oral glucose tolerance consisted of:

1. Two or more blood sugar values at or above the eighty-fourth percentile of the distribution of normal values at one-half, one, two, or three hours;

or 2. One or more blood sugar values at one-half or one hour at or above the eighty-fourth percentile with another blood sugar value at or below the

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*In this paper, "chemical diabetes" refers to a state in which there is an asymptomatic abnormality of carbohydrate metabolism detected by oral glucose tolerance tests, and "overt diabetes" refers to the clinical syndrome of hyperglycemia, glycosuria, polydipsia and polyphagia.

†A family history was taken to mean diabetes mellitus in a grandparent, aunt or uncle, or a closer relative.

sixteenth percentile distribution of normal values at two or three hours.

The children were free of infections at the time of the test. Girls were not tested during menstrual periods. Twenty-seven of the forty-eight children had a sibling with overt diabetes. The remaining twenty-one children were studied either because of a history of transient glycosuria usually observed in association with an infection or because an immediate member of the child's family other than a sibling had overt diabetes. There were twenty-four boys and twenty-four girls, ranging in age from five to sixteen years. The heights of all forty-eight children fell within one standard deviation of the mean of the Iowa Growth Charts[10] and weights were normal for their heights. None of the children was obese or undernourished.

Seventeen children with recent onset of overt diabetes were studied during the early recovery phase of their disease, a time when the metabolic abnormality had been controlled completely with dietary supervision and minimal amounts of exogenous insulin. These diabetic children were aglycosuric and normoglycemic at the time of testing. Insulin requirements ranged from 0.05 to 0.10 U. of insulin per kg. per day.

Eleven subjects without a family history of diabetes, five children aged five to twelve years and six young adults aged twenty-two to twenty-five years, served as controls.

Procedures

After an overnight fast, 1 gm. of glucose per kg. body weight of a 50 per cent glucose solution was infused in three to five minutes. Venous blood samples were obtained with the use of an indwelling needle at fasting, at one-minute intervals up to ten minutes, and at ten-minute intervals up to sixty minutes. Twenty-four or forty-eight hours later, the infusion was repeated with 1 mg. glucagon* mixed with glucose. Most of the children experienced transient dizziness and mild nausea during the first two to five minutes of the glucagon infusion.

Serum immunoreactive insulin (IRI) levels were measured during the infusion of glucose, and glucose with glucagon in the eleven normal subjects and in thirty-one of the forty-eight chemical diabetics. Twenty of these thirty-one chemical diabetics had one or more blood sugar values outside of the ninety-seventh percentile and were classified as "late" chemical diabetics. Serum IRI values were interpretable in four of the seventeen children with recent onset of overt diabetes who had no demonstrable binding of exogenous insulin detected by the method of Mitchell and Bradford.[11]

Venous blood sugar concentrations were determined on Somogyi filtrates by the neocuproine copper reduction procedure[12] on an AutoAnalyzer. Glucose disappearance rates (K) were calculated by the method of Hamilton et al.[13] and expressed as per cent per minute decrease of blood sugar. Serum IRI was determined in triplicate by the double antibody method of Morgan and Lazarow.[14] Two serum samples were assayed repeatedly during a three-month period and were included as a control in the insulin assays. The first sample had a mean insulin level of 51.9 $\pm$ 1.66 (S.E.M.) μU./ml. in fifty assays. The second sample had a mean insulin level of 127 $\pm$ 1.88 (S.E.M.) μU./ml. in fifty assays. In our laboratory, the insulin assay is most sensitive from 20 to 250 μU. insulin per ml. More recently, the sensitivity has been increased to detect 5 μU. insulin per ml.

The serum immunoreactive insulin response following the infusion of glucose and glucose with glucagon is expressed as total IRI response in microunits of IRI per milliliter of blood during the first ten minutes and the entire sixty minutes by measuring the area under the IRI curve in replication with a planimeter, using the fasting IRI level as a baseline. This method of expression of IRI secretion has been observed by other workers to average out the normal fluctuations and multiple IRI peaks which follow a glucose load.[15]

Statistical analysis

The serum IRI values and the glucose disappearance rates (K) following the infusion of glucose and glucose with glucagon were skewed in distribution with a few high values relative to the bulk of the values. Accordingly, the nonparametric Wilcoxon rank sum test was used for interpretation of data.[16]

RESULTS

Median glucose disappearance rates following the infusion of glucose are presented in table 1 and figure 1. There were no differences in the glucose disappearance rates observed in children and young adults who served as controls. All the control subjects had disappearance rates that were above 1.1 while all the overt diabetics had disappearance rates less than this figure. Children classified as chemical diabetics had intermediate values. By the Wilcoxon rank sum test,[16] the group with chemical diabetes differed significantly ($p < 0.01$) from both

*Glucagon for injection, U.S.P. Eli Lilly and Company, Indianapolis, Indiana. The insulin concentration was less than 0.05 U. per mg. by mouse convulsion assay and 0.04 to 0.08 U. per mg. by immunoassay.

TABLE 1

Glucose disappearance rates (K) expressed as per cent per minute decrease of blood sugar

Group of subjects	Number of subjects	Median glucose disappearance rate following rapid infusion of glucose		Median glucose disappearance rate following rapid infusion of glucose and glucagon	
Normal controls	11	2.47	$p < 0.01$	2.88	$p < 0.01$
Chemical diabetics	48	1.73		2.06	
Overt diabetics	17	0.63	$p < 0.01$	0.55	$p < 0.01$

*p by Wilcoxon rank sum test.[16]

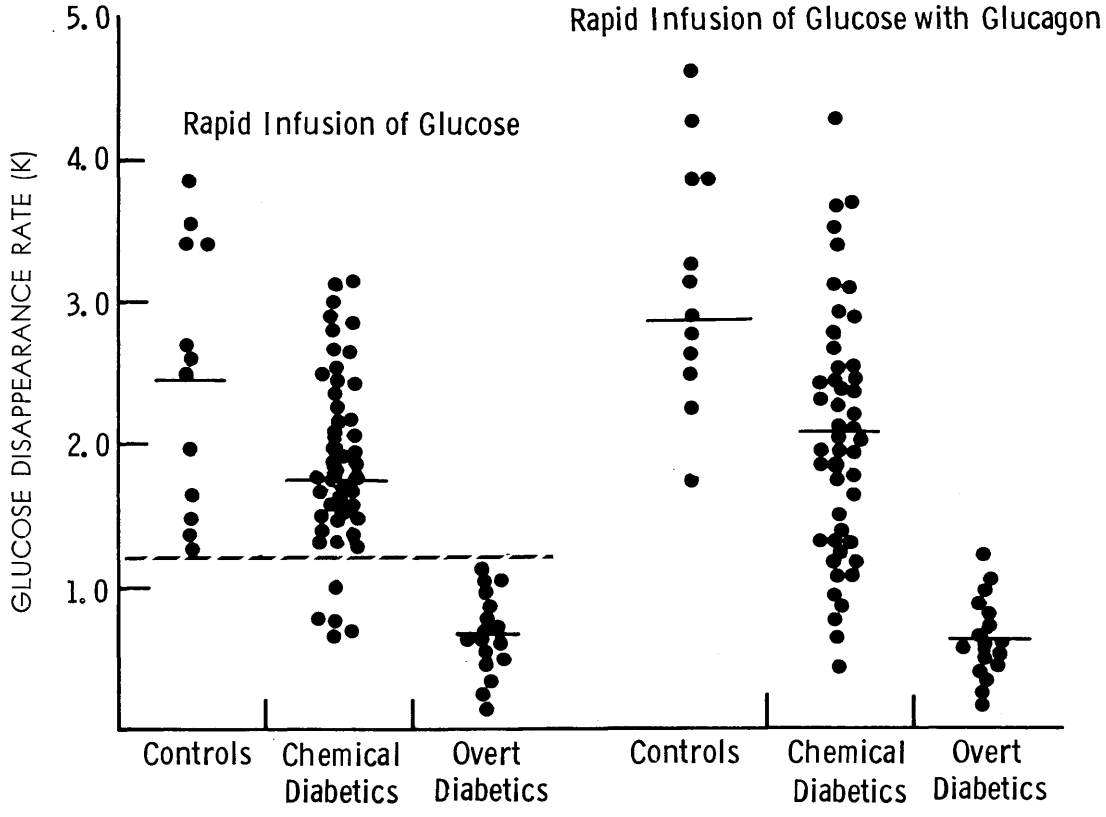

FIG. 1. Glucose disappearance rate (per cent/minute) following rapid infusion of glucose or glucagon and glucose in eleven controls, forty-eight chemical diabetics and seventeen overt diabetics. Median values are indicated by horizontal lines. Dashed line at 1.1 per cent/minute represents cut-off between nondiabetics and overt diabetics.

the control group and the diabetic group (table 1).

The addition of glucagon to the infusion increased the glucose disappearance rate in all the normal subjects, in 44 per cent of the forty-eight children with chemical diabetes and in none of the overt diabetics. The median changes in glucose disappearance rates due to glucagon are presented in table 2 and figure 2. Compared with the control group, the chemical diabetics had a significant response to glucagon ($p < 0.01$). However, this response was about one half the normal response.

The chemical diabetics were arbitrarily divided into two groups based on change in the glucose disappearance rate due to glucagon of less than + 0.3 or + 0.3 or more (figure 3). The median glucose disappearance rates following intravenous infusion of glucose were similar in the two groups. All of the twenty-one children with chemical diabetes, who had an increase in glucose disappearance rate (K) of + 0.3 or more, had K values above 1.1 following rapid intravenous infusion of glucose, and five of the twenty-seven children, who had less than + 0.3 increase in K, had disappearance

TABLE 2

Change in glucose disappearance rate (K) expressed as per cent per minute decrease of blood sugar with glucagon

Group of subjects	Number of subjects	Median change in glucose disappearance rate with glucagon		Significance of change in glucose disappearance rate with glucagon
Normal controls	11	+0.41	$p < 0.02$*	$p < 0.01$†
Chemical diabetics	48	+0.24		$p < 0.01$†
Overt diabetics	17	−0.07	$p < 0.02$*	Not significant

*p by Wilcoxon rank sum test.[16]
†p by Wilcoxon signed rank test.[16]

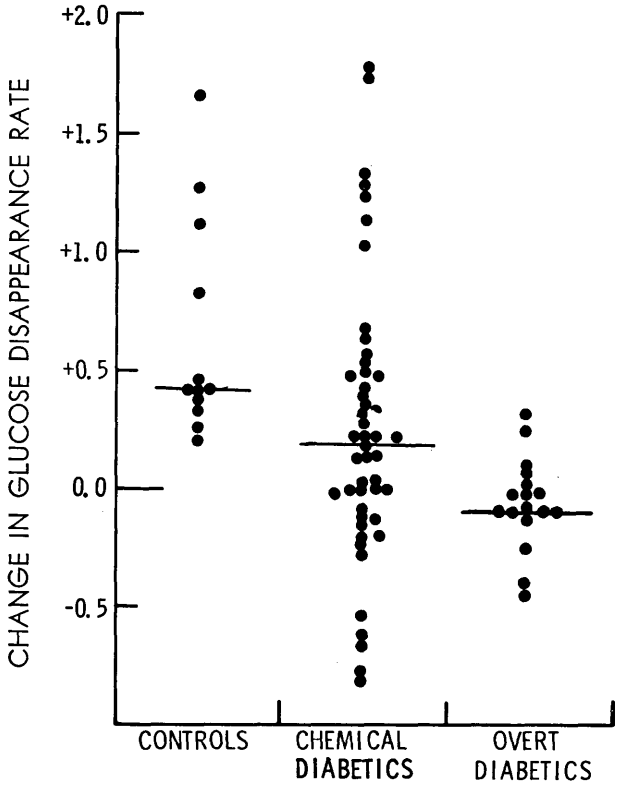

FIG. 2. Change in glucose disappearance rate (per cent/minute) with 1 mg. glucagon and glucose infusion in eleven controls, forty-eight chemical diabetics and seventeen overt diabetics. Median values are indicated by horizontal lines.

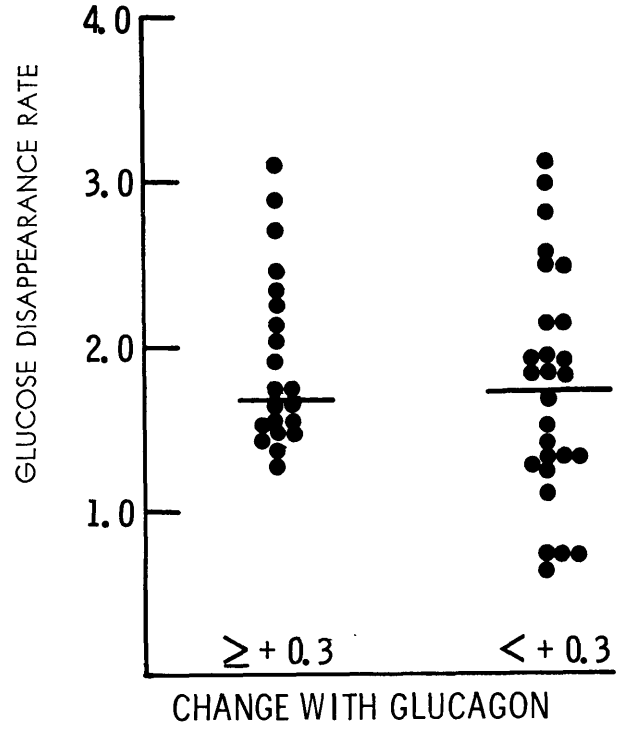

FIG. 3. Glucose disappearance rate (per cent/minute) in twenty-seven chemical diabetics who responded to glucagon (change $\geq +0.3$) and twenty-one chemical diabetics who did not respond (change $< +0.3$). Median values are indicated by horizontal lines.

rates below 1.1. Of the forty-eight chemical diabetics, twenty-four (50 per cent), who had one or more blood sugar values during oral glucose tolerance tests outside the ninety-seventh or third percentile of normal distribution, were classified as "late" chemical diabetics. Eighteen of these twenty-four children with more abnormal oral glucose tolerance tests had K value increases of less than + 0.3 with glucagon (table 3).

The increases in serum IRI during the first ten minutes and during the entire sixty-minute period after glucagon in the normal subjects and the chemical diabetics are recorded in table 4. Chemical diabetics formed a heterogeneous group with respect to IRI response following the infusion of glucose and glucose with glucagon (table 5). Some of the children classified as "early" chemical diabetics, on the basis of less abnormal oral glucose tolerance tests, had increased IRI responses to infusion of glucose and glucose with glucagon. Children classified as "late" chemical diabetics on the basis of oral glucose tolerance tests, like the overt

TABLE 3

Comparison of change in glucose disappearance rate (K) expressed as per cent per minute decrease of blood sugar following rapid infusion of glucose with glucagon in children with chemical diabetes classified according to degree of abnormality in oral glucose tolerance tests

	Change in glucose disappearance rate with glucagon	
	K less than +0.3	K of +0.3 or more
Total number of chemical diabetics in study	27	21
Number of "late" chemical diabetics (one or more blood sugar values outside of ninety-seventh or third percentile of normal distribution)	18	6
Number of "early" chemical diabetics (two or more blood sugar values outside of eighty-fourth or sixteenth percentile of normal distribution	9	15

Chi square test for independence $p < 0.01$.

diabetics, had decreased IRI response to glucagon (table 5).

The peak level of IRI was reached in one to two minutes following the infusion of glucose and between three and five minutes following the infusion of glucose with glucagon. The time for reaching the peak IRI levels was similar in normal subjects, chemical diabetics and overt diabetics.

DISCUSSION

The development of diabetes in children is characterized by a progressive decrease in the capacity of the islets of Langerhans to secrete biologically active insulin when appropriately stimulated. Diabetes becomes overt as islet-cell function is exhausted. The amount of islet-cell function present at any one point in time may be called the *insulin reserve.* Changes in insulin production and secretion are conditioned by other regulatory substances such as glucagon,[2-5] growth hormone,[18] and catecholamines.[19] Direct or indirect evaluation of these control mechanisms is helpful in understanding the changes in insulin reserve. Glucose levels after oral glucose, glucose disappearance rates following rapid infusion of glucose or glucose and glucagon, and blood sugar responses to intravenous tolbutamide are indices of insulin reserve and indirectly measure the amount and rate of release of biologically active insulin.[20]

The increase in serum IRI levels in normal subjects following the rapid intravenous infusion of glucose with glucagon indicates that the combined stimuli of glucose and glucagon evoke an intensive islet-cell response. Children classified as "late" chemical diabetics had more

TABLE 4

Levels of immunoreactive insulin (IRI) following rapid infusion of glucose (1 gm./kg.) and glucose (1 gm./kg.) with glucagon (1 mg.) in normal subjects, chemical diabetics and overt diabetics (median and range)

Group of subjects	Number of subjects	IRI level following infusion of glucose		IRI level following infusion of glucose and glucagon	
		First ten minutes (μU./ml./10 min.)	Entire sixty minutes (μU./ml./60 min.)	First ten minutes (μU./ml./10 min.)	Entire sixty minutes (μU./ml./60 min.)
Normal	11	976	3,440	2,432	8,544
		320-2,752	1,280-6,832	824-5,376	1,824-19,328
		NS	NS	NS*	NS
Chemical diabetics (all)	31	848	3,568	2,128	6,132
		32-2,284	38-11,696	80-4,816	1,200-24,832
		NS	NS	†p = .025	p < .05
Chemical diabetics (late)	20	656	3,220	928	2,736
		32-1,264	38-3,800	480-3,040	1,200-9,968
		NS	p < .05	p < .05	p < .05
Overt diabetics	4	437	960	168	528
		80-992	112-1,408	160-176	496-608

*NS = not significant.
†p by Wilcoxon rank sum test.[16]

TABLE 5

Change in immunoreactive insulin (IRI) level with glucagon during the first ten minutes and during the entire sixty minutes in normal subjects, chemical diabetics and in overt diabetics

Group of subjects	Number of subjects	Change in IRI with glucagon (median and range)* First ten minutes (μU./ml./10 min.)	Change in IRI with glucagon (median and range)* Entire sixty minutes (μU./ml./60 min.)	Significance of change in IRI with glucagon† First ten minutes (μU./ml./10 min.)	Significance of change in IRI with glucagon† Entire sixty minutes (μU./ml./60 min.)
Normal	11	+1,600 −368 to +3,808	+4,128 +563 to +14,128	$p < 0.01$	$p < 0.01$
		NS‡	NS		
Chemical diabetics (all)	31	+1,280 −336 to +3,904	+1,864 −9,579 to +5,250	$p < 0.025$	$p < 0.05$
		$p < 0.05$	NS		
Chemical diabetics (late)	20	+616 +144 to +1,928	+1,360 +1,024 to +6,168	NS	$p < 0.05$
		$p < 0.05$	$p < 0.05$		
Overt diabetics	4	−150 −400 to +196	+334 −976 to +410	NS	NS

*p by Wilcoxon rank sum test.[16]
†p by Wilcoxon signed rank test.[16]
‡NS = not significant.

severe abnormalities in the oral glucose tolerance test. The serum IRI levels in these children, as in the overt diabetics, were low following the rapid intravenous infusion of glucose with glucagon. These observations indicate that, with the progressive deterioration of the oral glucose tolerance, there is a decreasing insulin reserve. Our data confirm the recent evidence presented by Simpson and coworkers[5] who have postulated that, in the course of development of diabetes, there is a change from normal early insulin release to a phase of diminished insulin release and further to a final stage of decompensation with both insulin release and glucose tolerance markedly decreased.

Glucose disappearance rates of less than 1.1 per cent per minute are characteristic of overt diabetes.[21] The lower glucose disappearance rates following the rapid intravenous infusion of glucose in the child with early overt diabetes and the lack of response to glucagon indicate that these children have severe insulin insufficiency. Three of the children in this study with chemical diabetes and with K values less than 1.1 per cent per minute developed overt diabetes under observation. It is our feeling that, unless children with early overt diabetes receive continuous substitution therapy with insulin, their disease progresses rapidly to late overt diabetes with total insulin deficiency. Control of the disease becomes progressively more difficult as insulin reserve is lost. Other investigators have observed poor insulin responses to glucose or tolbutamide in children with a recent onset of overt diabetes.[22]

Forty-three out of forty-eight children classified as chemical diabetics in this study had intravenous glucose disappearance rates of 1.1 per cent per minute or greater, suggesting that this loading test was not as sensitive in estimating the insulin reserve as the oral glucose tolerance test used to define chemical diabetes. A similar lack of correlation between intravenous and oral glucose tolerance tests in the diagnosis of chemical diabetes has been reported.[23]

The change in glucose disappearance rates in response to glucagon was less than + 0.3 in nearly half of the chemical diabetics. Such responses characterize the overt diabetic group and suggest a low insulin reserve. The response to glucagon plus glucose is a more sensitive measure of the insulin reserve than the glucose disappearance rate following rapid intravenous infusion of glucose. When glucose is ingested, insulin secretion is stimulated not only by glucose but also by hormones of the gastrointestinal tract (glucagon, secretin,[24] and pancreozymin[25]). Consequently, an oral glucose load elicits a greater insulin response than an intravenous infusion of glucose.[24]

Similar mechanisms for insulin secretion may be operative both in the oral glucose tolerance test and in

the intravenous test with glucose and glucagon.

The criteria used in this study for the diagnosis of chemical diabetes in children may have allowed the inclusion of some normal children. Nearly half of the subjects with chemical diabetes had, at one point in time, responses to glucagon which were similar to those of overt diabetics. Limiting the definition of chemical diabetes to children with glucose values outside the ninety-seventh and third percentile range during an oral glucose tolerance test is too restrictive since most of the children classified accordingly have lost their ability to respond to glucagon.

In this study only five of the children with chemical diabetes had glucose disappearance rates less than 1.1 per cent per minute and three of these five children became overt diabetics within a period of two to four months; the other two children have had progressive deterioration of glucose tolerance. These observations suggest that overt diabetes is likely to ensue within a few months if a child has blood sugar values outside the ninety-seventh and third percentile of the distribution or normal values and a glucose disappearance rate less than 1.1 per cent per minute following the rapid intravenous infusion of glucose.

Early detection of chemical diabetes in children is necessary in order to evaluate the feasibility of altering the progressive loss of insulin reserve. In order to measure the effect of dietary treatment, tolbutamide, and/or insulin on the insulin reserve of chemical diabetics, it is essential to define the insulin reserve by as many means as possible. It is suggested that efforts be made to alter progression of the disease as soon as the diagnosis of chemical diabetes can be made and before there is further loss of insulin reserve.

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