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Arginine
diabetes mellitus
glucagon
growth hormone
insulin
tolbutamide

Endogenous Insulin and Growth Hormone Response in Children with Newly Diagnosed Diabetes Mellitus

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Extract

Forty-five children with newly diagnosed diabetes mellitus were evaluated in terms of endogenous release of insulin and growth hormone using glucose, tolbutamide, arginine and glucagon as stimuli. The mean concentration of insulin in the plasma obtained from fasting diabetic children was found to be 10.0 μU/ml, a value significantly below the mean level of 15.3 μU/ml in the control group. Essentially no increase in the concentration of insulin in plasma resulted from stimulation with the above agents. The mean concentration of growth hormone in the plasma of fasting diabetics was found to be 3.8 mμg/ml, a value higher than but not significantly different from the value of 2.5 mμg/ml in the contrast patients. Following the intravenous infusion of arginine, however, the mean concentration of growth hormone in the plasma of diabetic children rose to 20.8 mμg/ml, a level significantly higher than the mean peak value of 7.4 mμg/ml in the nondiabetics. Although no change in the concentration of growth hormone followed glucagon injection in the nondiabetic children, a prompt, significant elevation to 9.4 mμg/ml occurred in the diabetics. Glucagon administration has not previously been reported to stimulate the release of growth hormone.

Speculation

Insulin deficiency alone will not adequately explain the variability in clinical symptomatology and metabolic derangement seen in children with newly diagnosed diabetes mellitus. These data suggest that growth hormone may play an important role. The finding that glucagon administration stimulates release of growth hormone in the juvenile diabetic may indicate the presence of a previously unrecognized interrelation between glucagon-growth hormone and insulin.

Introduction

Although the basic etiology of diabetes mellitus remains obscure, the disease as it occurs in childhood is regularly associated with a deficiency in the production of pancreatic insulin. The relation between maturity-onset diabetes and insulin production is less clear. Concentrations of insulin in plasma in excess of that seen in normal individuals have been observed in many maturity-onset diabetics. These elevated levels, however, may be inappropriately low for the degree of hyperglycemia. An impairment of efficiency in the timing of the release of insulin is apparent in many of these patients [15, 19, 39].

The insulin requirements during the initial months of clinical diabetes in the child may be extremely va-

riable, with a general tendency toward decreasing insulin needs. In a small number of children, the insulin requirement may actually fall to zero and remain there for weeks or months. The phase of diminishing insulin requirement is usually followed by a phase of slowly increasing need until a relatively stable state is established one to three years after the onset of clinical symptomatology. On the basis of these clinical features, it has been assumed that endogenous production of insulin is not completely exhausted for many months. KLEIN *et al.* [20] studied the response of the concentration of glucose in blood following oral administration of tolbutamide to children who had had diabetes mellitus for varying periods of time. Results of this study appeared to confirm the clinical impression that the child with newly acquired diabetes mellitus could produce insulin and that the loss of this capability was a function of the duration of the disease.

The primary purpose of the present report is to reevaluate this question using several newly available techniques. Endogenous release of insulin in the normal individual may be stimulated by a variety of agents, including glucose [2, 42], tolbutamide [34, 43], arginine [13, 24] and glucagon [3, 35, 39]. It is possible that each of these four compounds may produce stimulation of the pancreatic beta cell by basically different mechanisms. Measurement of the concentration of glucose in blood and insulin in plasma following administration of each of these agents should provide accurate information on the functional capacity of the pancreatic islet tissue. An additional aim of this study is to define the responsiveness of the anterior pituitary to these same stimuli in an attempt to determine whether growth hormone may play any role in the pathology of carbohydrate tolerance characteristic of these children.

Patient Population

Diabetics. During the 12-month period of the present study, there were 146 hospital admissions for diabetes mellitus at the Children's Hospital of Pittsburgh. Of this group of patients, 70 were children with newly discovered diabetes who had not previously been treated with insulin. The studies carried out on 45 of these children are the substance of this report. For obvious reasons children with extreme ketoacidosis and coma at the time of initial hospital admission could not be studied and were not investigated in the present program.

Control patients. We have not had the opportunity to study entirely normal children with all of the tolerance tests conducted on our diabetic patients. Consequently, a contrast group rather than a normal control group will be used for comparison with the diabetic patients. The results of all tolerance tests on nondiabetic children performed in our clinic during the 12-month period of the study are included. One hundred and three children have been studied with one or more of the four types of tolerance tests, as indicated below. The diagnoses have included: metabolic and nonmetabolic renal disease, renal rickets, nonmetabolic bone disease, thyroid and parathyroid disorders, obesity, failure to thrive, adrenal hyperplasia, hypoglycemia, genetic dwarfism and hypopituitarism. Also studied were a number of patients in whom there was no evidence of organic disease.

Materials and Methods

A series of tolerance tests was administered in an attempt to assess the functional capacity of the pancreatic beta cells: agents included glucose, tolbutamide, arginine and glucagon. The dosage of each test substance and routes of administration were as follows:

A. Oral glucose tolerance test: 1.75 g/kg of body weight. Maximum dose: 100 g.
B. Intravenous tolbutamide tolerance test: 20 mg/kg of body weight. Maximum dose: 1 g.
C. Intravenous arginine infusion: 0.5 g/kg of body weight administered over a 30-minute period. Maximum dose: 30 g.
D. Subcutaneous glucagon tolerance test: 50 μg/kg of body weight. Maximum dose: 1 mg.

All tolerance tests were carried out after the subjects had fasted overnight. The patients remained at bedrest during the course of the study. Heparinized venous specimens were obtained from indwelling venous catheters at 'zero' time and at standard intervals following administration of the test substance. In most cases, two of the four studies were carried out on each patient. In an occasional child, all four tolerance tests were completed. At least one test-free day preceded each day of testing.

None of the diabetic patients had been exposed to exogenous insulin prior to hospitalization. In the majority of these children, it was possible to defer insulin therapy until the completion of all studies. However, in those children who had significant symptomatology or in whom the concentration of CO_2 in blood was below 18 mEq/l at the time of admission, treatment with regular insulin was initiated and continued for two or three days until the patient's status stabilized. Regular insulin was then discontinued. In no case was a long-acting insulin preparation administered to any patient prior to the initiation of studies, and in no case was regular insulin administered closer than 12 hours prior to the beginning of a tolerance test. Insulin antibodies were not detected in the plasma of any patient.

Table I. Comparison of levels of electrolytes in serum of normal and diabetic children

	N	Sodium mEq/l	Potassium mEq/l	Chloride mEq/l	CO_2 mEq/l	pH	PCO_2 mm Hg
Controls	39	146.3±0.47	4.5±0.06	103.4±0.56	24.4±0.28	(7.36–7.44)[1]	(34–46)[1]
Diabetics	45	137.6±1.05	3.9±0.12	101.2±1.13	19.7±1.36	7.29±0.23	37.5±1.93

Values are means ± standard error of the mean.

[1] Astrup normal range. (SIGGAARD-ANDERSEN, O.: Blood acid base alignment nomogram. Scand. J. clin. Lab. Invest. *15:* 211 [1963]).

Basal insulin levels were no higher in the few patients who received regular insulin than in those who did not, indicating that 12 hours was a sufficiently long period of time to clear exogenous insulin from the circulation.

The concentration of growth hormone and insulin in plasma was determined by a modification of the radioimmunoassay charcoal-dextran technique of HERBERT [17]. The reliability and reproducibility of this technique in our laboratory are indicated by the results obtained on standard reference specimens of plasma included in every growth hormone and insulin assay. Two plasma reference specimens have been used for growth hormone standardization: a specimen obtained from a normal adult, after fasting, with a level of 3.1±0.73 mμg/ml and a specimen from an acromegalic patient with a level of 40.7±4.9 mμg/ml (mean± standard deviation). The reference specimens used in each insulin assay were obtained from normal adults following glucose administration. On repeated analyses, these specimens have measured 109±8 and 156± 10 μU/ml (mean±standard deviation), respectively.

The concentration of glucose in whole blood was determined using the glucose oxidase method. The concentration of electrolytes and gases in blood was determined by standard microtechniques used in the Clinical Chemistry Laboratory of the Childrens' Hospital of Pittsburgh or the Core Laboratory of the Pediatric Clinical Research Unit.

In the tables, data for glucose represent values in whole blood while those for insulin and growth hormone were obtained on plasma samples.

Results

Of the 45 diabetic children studied, 27 were males and 18 were females. The average age at the time of admission was 9.5 years, with an age range from 1 to 14 years. All patients had glycosuria at the time of admission and most had slightly to strongly positive tests for acetonuria. The mean concentration of bicarbonate in blood in the diabetic patients was 19.7 mEq/l. Eleven children had concentrations of bicarbonate in blood below 18 mEq/l. In table I, the concentration of electrolytes and gases in blood at the time of admission of the diabetic children is compared with that of a population of normal children of comparable age [7].

In table II the values are presented for the mean concentration for glucose in whole blood and insulin and growth hormone in plasma following an overnight fast in the diabetic patients and in the contrast group. As all 'zero' time values for each tolerance test are included, the numbers of observations exceed the numbers of individual patients in each category. The mean concentration of insulin in plasma for the diabetic patients is 10.0 μU/ml, a value significantly less than that of 15.3 μU/ml found in the nondiabetic patients. The slightly higher mean concentration of growth hormone in plasma in the diabetic population (3.8 mμg/ml) is not significantly different from that of the contrast group (2.5 mμg/ml).

A comparison of the response of the diabetic and nondiabetic patients to oral glucose administration is presented in table III. The responses of the contrast

Table II. Fasting data: Nondiabetic and diabetic children

	Values in blood			
	Nondiabetic		Diabetic	
	N	Mean±SE	N	Mean±SE
Glucose mg %	158	76±2.6	97	270±13[3]
Insulin μU/ml	79	15.3±1.1	81	10.0±0.6[2]
Growth hormone mμg/ml	58	2.5±1.0	76	3.8±0.9

[2] Diabetics significantly different from controls at $p < 0.01$.

[3] Diabetics significantly different from controls at $p < 0.001$.

Table III. Response of nondiabetic and diabetic children to oral glucose tolerance test

		N	Values in blood at indicated hours after ingestion of glucose						
			0	0.5 h	1 h	2 h	3 h	4 h	5 h
Non-diabetic	Glucose mg %	38	82 ±2.5	142 ±5.6	126 ±5.0	104 ±3.7	78 ±3.2	69 ±3.0	74 ±3.6
	Insulin μU/ml	25	17.0 ±2.4	94.8 ±26.7	82.2 ±21.8	67 ±24.4	27.9 ±7.4	16.7 ±5.8	13.2 ±3.2
	Growth hormone mμg/ml	18	1.7 ±0.3	2.2 ±0.5	4.3 ±1.6	6.0 ±2.3	5.2 ±1.4	6.0 ±2.0	3.5 ±1.6
Diabetic	Glucose mg %	32	248[3] ±19	376[3] ±23	430[3] ±23	428[3] ±22	349[3] ±19	311[3] ±20	257[3] ±19
	Insulin μU/ml	28	11.2[1] ±1.0	10.8[2] ±0.9	10.8[2] ±0.9	13.9[1] ±0.9	10.5[1] ±0.9	13.9 ±1.0	15.1 ±1.0
	Growth hormone mμg/ml	25	3.7[1] ±0.7	3.5 ±0.7	3.2 ±0.4	3.5 ±1.1	4.1 ±1.2	3.1 ±1.5	3.0 ±1.9

Values are means ± standard error of the mean.

[1] Diabetics significantly different from controls at $p < 0.05$.
[2] Diabetics significantly different from controls at $p < 0.01$.
[3] Diabetics significantly different from controls at $p < 0.001$.

Table IV. Response of nondiabetic and diabetic children to intravenous injection of tolbutamide

		N	Values in blood at indicated minutes after injection of tolbutamide							
			0	5	15	30	45	60	90	120
Non-diabetic	Glucose mg %	21	75 ±3.3	74 ±3.5	57 ±4.2	47 ±3.9	53 ±4.1	55 ±3.2	60 ±3.6	62 ±3.3
	Insulin μU/ml	14	17.4 ±2.0	115.8 ±25.2	73.6 ±16.7	36.0 ±7.5	31.0 ±7.0	24.9 ±4.5	18.4 ±2.3	17.2 ±1.9
	Growth hormone mμg/ml	11	2.6 ±0.7	3.2 ±0.8	3.0 ±0.6	4.9 ±1.4	6.0 ±1.3	6.8 ±1.2	3.8 ±0.8	2.1 ±0.3
Diabetic	Glucose mg %	34	264[3] ±18	260[3] ±17	264[3] ±19	255[3] ±19	252[3] ±20	248[3] ±21	238[3] ±21	224[3] ±20
	Insulin μU/ml	25	11.0[2] ±1.0	14.4[2] ±1.9	13.7[2] ±1.7	12.7[2] ±1.3	12.3[2] ±1.2	12.4[2] ±1.2	12.3[1] ±1.0	12.8[2] ±1.2
	Growth hormone mμg/ml	25	3.5 ±0.7	4.0 ±0.6	4.1 ±0.8	4.1 ±0.7	4.4 ±0.8	3.4[1] ±0.6	2.1 ±0.4	2.7 ±0.5

Values are means ± standard error of the mean.

[1] Diabetics significantly different from controls at $p < 0.05$.
[2] Diabetics significantly different from controls at $p < 0.01$.
[3] Diabetics significantly different from controls at $p < 0.001$.

Table V. Response of nondiabetic and diabetic children to intravenous injection of arginine

		N	Values in blood at indicated minutes after starting infusion of arginine							
			0	30	45	60	75	90	120	150
Non-diabetic	Glucose mg %	44	74 ±3	82 ±4	78 ±6	79 ±4	79 ±5	71 ±4	74 ±3	72 ±5
	Insulin μU/ml	28	13.7 ±1.3	40.7 ±7.0	31.3 ±5.2	21.7 ±3.7	17.9 ±2.9	12.9 ±1.1	12.7 ±1.1	12.8 ±1.2
	Growth hormone mμg/ml	22	3.2 ±0.7	5.9 ±1.1	6.6 ±1.3	7.4 ±1.6	7.0 ±1.4	3.8 ±0.7	2.6 ±0.4	2.5 ±0.3
Diabetic	Glucose mg %	16	289[3] ±13	282[3] ±14	285[3] ±14	276[3] ±13	273[3] ±23	266[3] ±14	259[3] ±15	253[3] ±17
	Insulin μU/ml	13	8.0[2] ±0.6	9.9[2] ±1.2	11.8[2] ±0.8	9.9[2] ±0.9	8.8[2] ±0.7	9.0[2] ±0.6	8.6[2] ±0.6	8.8[2] ±0.7
	Growth hormone mμg/ml	14	3.2 ±0.8	4.6 ±1.3	6.1 ±1.4	11.5 ±3.4	20.8[1] ±5.2	11.3[1] ±3.2	4.1 ±0.6	2.1 ±0.3

Values are means ± standard error of the mean.

[1] Diabetics significantly different from controls at $p < 0.05$.
[2] Diabetics significantly different from controls at $p < 0.01$.
[3] Diabetics significantly different from controls at $p < 0.001$.

Table VI. Response of nondiabetic and diabetic children to glucagon stimulation

		N	Values in blood at indicated minutes after subcutaneous injection of glucagon							
			0	10	20	30	45	60	90	120
Non-diabetic	Glucose mg %	15	70 ±5	106 ±8	124 ±10	129 ±14	111 ±10	96 ±7	74 ±6	75 ±6
	Insulin μU/ml	13	15.3 ±1.6	35.6 ±11.9	33.7 ±10.9	31.7 ±9.3	28.2 ±11.4	24.3 ±10.9	14.6 ±3.4	11.5 ±1.6
	Growth hormone mμg/ml	8	3.1 ±0.9	3.8 ±1.3	3.0 ±1.0	2.4 ±0.8	2.3 ±0.5	3.2 ±0.9	2.2 ±1.2	3.3 ±1.4
Diabetic	Glucose mg %	15	301[3] ±23	328[3] ±21	330[3] ±19	320[3] ±19	314[3] ±20	307[3] ±21	293[3] ±23	286[3] ±25
	Insulin μU/ml	12	8.5[2] ±0.7	10.7[1] ±0.9	8.3[1] ±0.9	8.3 ±1.0	8.3 ±0.8	8.5 ±0.8	8.3 ±1.1	8.3 ±1.0
	Growth hormone mμg/ml	12	4.1 ±0.8	9.4[1] ±2.2	8.0[1] ±1.8	7.6[1] ±1.9	6.6[1] ±1.4	4.8 ±0.9	3.2 ±0.4	4.2 ±1.2

Values are means ± standard error of the mean.

[1] Diabetics significantly different from controls at $p < 0.05$.
[2] Diabetics significantly different from controls at $p < 0.01$.
[3] Diabetics significantly different from controls at $p < 0.001$.

group, in terms of changes in the concentration of glucose in whole blood and insulin and growth hormone in plasma, are quite comparable to those reported in the literature for normal adults and children [1, 2, 8, 11, 18, 27, 30, 42]. Although the level of hyperglycemia is far greater in the diabetic patients, the concentration of glucose declined in the diabetics between two and five hours, and by five hours the mean concentration of glucose returned to the fasting level. This 'glucose disappearance' (utilization and/or renal loss) was accomplished with essentially no change in the concentration of insulin. The concentration of growth hormone in plasma remained unchanged in the diabetic patients following the administration of glucose, in contrast to the increase in concentration of growth hormone associated with the decline in glucose concentration in the nondiabetic subjects.

The results of intravenous tolbutamide administration are presented in table IV. In the nondiabetic group, tolbutamide stimulated a prompt six-fold increase in the concentration of insulin followed by a fall in glucose concentration reaching a nadir at 30 minutes. This was followed by a reactive increase in the concentration of growth hormone which reached a peak at 60 minutes. These responses are not appreciably different from those reported by other investigators for normal children and adults [4, 6, 16, 34, 36, 37, 40, 41, 43]. In the diabetics, there was a slow, continuous decline in the concentration of glucose in blood over the two-hour period of observation. This was unassociated with a change in the concentration of insulin. In the absence of a hypoglycemic effect of tolbutamide, no increase in the concentration of growth hormone occurred in the diabetic patients.

The results of the intravenous arginine infusion are presented in table V. In the nondiabetics, arginine administration was associated with minimal change in the concentration of glucose. There was, however, an appreciable and prompt increase in the concentration of insulin and a modest, slower increase in the concentration of growth hormone. These results are similar to the observations reported for normal subjects, but the magnitude of the changes in concentration of growth hormone are somewhat less than those which others have reported [4, 6, 16, 34, 36, 37, 40, 41, 43]. In the diabetic, infusion of arginine and of tolbutamide is associated with a continuous, gradual decline in the concentration of glucose. When the glucose values are expressed as change from 'zero' time rather than the absolute concentration of glucose, the rate of glucose disappearance in the diabetic is entirely comparable following tolbutamide and arginine, while quite dissimilar effects are produced by these two agents in the nondiabetic. Arginine administration does not result in an increase in the concentration of insulin in the diabetic; however, a very considerable increase in the concentration of growth hormone follows arginine administration in these patients. The concentrations of growth hormone reached at 75 and 90 minutes post-infusion are significantly higher in the diabetic than in the contrast group.

The results of glucagon administration are presented in table VI. A prompt increase in the concentration of glucose followed when glucagon was administered to the nondiabetic group, with an absolute increase of 59 mg % at 30 minutes. There was a two-fold increase in the concentration of insulin at 10 minutes with a slow return to basal values by 90 minutes. There was no increase in the concentration of growth hormone noted in the nondiabetic subjects. These results are similar to those reported for normal subjects [13, 35]. Glucagon is about one-half as effective in the diabetic as in the nondiabetic, using as a criterion the increase in the concentration of glucose from the 'zero' time value. There was no increase in the concentration of insulin following glucagon administration to diabetic patients. Surprisingly, there was a prompt increase in the concentration of growth hormone to 9.4 mμg/ml at 10 minutes, followed by a gradual decline to basal values by 90 minutes.

Discussion

Our study documents the absence of any significant endogenous release of insulin following the administration of glucose, tolbutamide, arginine and glucagon in a group of children with newly diagnosed diabetes mellitus. Similar results following administration of tolbutamide to juvenile diabetics have recently been reported by others [5, 28]. A number of intriguing questions arise from these observations. A basic question is whether insulin deficiency alone accounts for the development of diabetes mellitus in the child. One might also ask whether other factors may be responsible, not for the basic etiology of diabetes mellitus but for further deterioration of carbohydrate tolerance and the development of ketoacidosis in these patients. Factors which have been previously implicated include growth hormone, ACTH, hypothalamic or pituitary lipid mobilizing substances, the adrenal cortical hormones, catecholamines and glucagon.

A possible etiological relation between endogenous growth hormone production and the development of diabetes mellitus has been debated for more than 30 years. The induction of diabetes mellitus in laboratory animals following chronic growth hormone administration, the development of diabetes mellitus in patients with acromegaly, amelioration of diabetes and diabetic retinopathy following hypophysectomy, and reexacer-

bation of ketosis-prone diabetes in the posthypophysectomy diabetic given human growth hormone suggest the probability of some causal relation between growth hormone release and the breakdown in carbohydrate homeostasis. The relation between growth hormone and insulin in the control of normal intermediary metabolism, as well as its possible significance in the development of diabetes mellitus, has been extensively reviewed in recent years [9, 10, 14, 21, 22, 32]. No definitive conclusions have been reached. Recent studies by POWELL *et al.* [29] on diabetic retinopathy suggest that growth hormone is not a causative factor in the progression of this complication. MERIMEE *et al.* [26] reported marked impairment in both endogenous release of insulin and growth hormone following infusion of arginine in five patients with maturity-onset diabetes mellitus. FINE *et al.* [12] reported increased release of growth hormone following insulin-induced hypoglycemia and arginine infusion in insulin-dependent diabetics but diminished response in maturity-onset diabetics.

Insulin in low concentration was detected in the plasma of every diabetic child studied. The effect of this small, basal concentration of insulin on glucose metabolism is not clear. Several of these children were asymptomatic and nonacidotic at the time glycosuria was initially noted. The concentrations of insulin in the plasma of these children were no higher than that in the patients with more severe symptomatology and ketoacidosis. The maintenance of a certain degree of glucose metabolism is suggested by the response to oral glucose administration in the diabetic children. Despite the marked hyperglycemia induced following administration of glucose, this 'excess glucose' is cleared from the extracellular fluid with five hours. Undoubtedly, an appreciable amount of the glucose load is excreted in the urine. It is probable, however, that a significant portion of the administered load is metabolized.

The mechanism of decline in glucose concentration following administration of tolbutamide to the child with newly diagnosed diabetes mellitus is not clear. The present study, as well as those of others [5, 28], indicates that this is probably not insulin-mediated. DEBELLE *et al.* [5] suggested that the fall in glucose results from a direct effect of tolbutamide on hepatic release of glucose. This appears to us to be an unlikely explanation. In our patients, the decline in the concentration of glucose in blood is essentially identical following administration of tolbutamide and arginine. As there is no evidence to suggest that arginine blocks hepatic release of glucose, it would appear more likely that the observed change in glucose concentration is independent of a pharmacologic effect of the administered agents and results from a combination of basal endogenous metabolism of glucose and renal loss. Studies comparing the effect of saline infusions with tolbutamide or arginine infusions on the concentration of glucose in blood and urine are currently in progress in our laboratory in an attempt to further clarify the mechanisms of glucose decline.

A primary thesis suggested by the results of this study is that growth hormone may play an intimate role in the etiology of diabetes mellitus or contribute significantly to the deterioration of carbohydrate homeostasis in patients with a genetic predisposition toward inadequate insulin production. The mean basal concentration of growth hormone in the fasted diabetic patients is higher than but not significantly different from that of the contrast group. The basis for suggesting that growth hormone may be involved in the etiology of diabetes rests on two observations: (a) glucagon administration is followed by an increase in the concentration of growth hormone; and (b) arginine infusion stimulates an appreciably greater increase in the concentration of growth hormone in the diabetic than in contrast patients.

The response of the diabetic to the administration of glucagon is one of the most interesting and unexpected findings in this study. There is a prompt and statistically significant increase in the concentration of growth hormone in plasma at 10 minutes following administration of glucagon. The concentration of growth hormone remains significantly elevated above the 'zero' time value for the diabetic and the normal control values from 10 minutes through 45 minutes postinjection. There have been no previous reports on the effect of glucagon on growth hormone regulation. With the exception of its stimulatory effect on insulin secretion, glucagon has not been previously demonstrated to play a role in hormonal feedback mechanisms. The physiological significance of this response in diabetic patients is not clear.

Although infusion of arginine resulted in a significantly greater elevation of growth hormone in the diabetic patients than in the nondiabetic patients, the value of this observation rests heavily on the relative normality of our contrast subjects. The peak concentrations of growth hormone obtained in these patients following tolbutamide-induced hypoglycemia (6.8 mμg/ml), insulin-induced hypoglycemia (10.8 mμg/ml) and arginine infusion (7.4 mμg/ml) are somewhat lower than those which others have reported. ROOT *et al.* [33] have recently reported that the peak concentration of growth hormone was 12.8 mμg/ml following arginine infusion in 19 normal children. In contrast, MERIMEE *et al.* [24], in studying the response of normal adults, report that females gave a peak response of 27 mμg/ml, while males attained a maximum plasma concentration of growth hormone of only 8.0 mμg/ml

following arginine infusion. The increased levels seen in our diabetic children do not result from exaggerated responses in adolescent females. Few adolescent girls were included in the study and their response to arginine infusion was not found to be greater than that of our adolescent male diabetics.

Although it is probable that peak concentrations of growth hormone would have been somewhat higher if a group of entirely normal controls had been used, it is unlikely that this would have changed the conclusions of the study.

Our results suggest the possible existence of a glucagon-growth hormone-insulin interrelation in the child with diabetes mellitus. Study of the concentration of each of these hormones in the plasma of the child with prediabetes, as well as overt diabetes, is in progress in our laboratory.

Conclusions

Studies directed toward the evaluation of glucose metabolism and endogenous release of insulin and growth hormone have been carried out on 45 children with newly diagnosed diabetes mellitus and compared with results of the same type of studies carried out on a group of nondiabetic children. At basal times, insulin levels were significantly decreased in the diabetics; essentially no increase in the concentration of insulin in plasma resulted from stimulation with glucose, tolbutamide, arginine or glucagon. The basal concentration of growth hormone is not significantly different between the two groups but arginine infusion induced a significantly greater increase in the concentration of growth hormone in the diabetic than in the nondiabetic patients. Unexpectedly, glucagon administration resulted in a prompt, statistically significant increase in the concentration of growth hormone in the diabetic but not in the contrast patients. The significance of the increased release of growth hormone following arginine and the stimulation of the release of growth hormone following glucagon is not clear. The possibility that growth hormone may play a basic role in the development of diabetes mellitus is suggested from these observations.

References and Notes

1. Burkeholder, J.N.; Pickens, J.M. and Womack, W.N.: Oral glucose tolerance test in siblings of children with diabetes mellitus. Diabetes *16:* 156 (1967).
2. Crockford, P.M.; Harbeck, R.J. and Williams, R.H.: Influence of age on intravenous glucose tolerance and serum immunoreactive insulin. Lancet *i:* 465 (1966).
3. Crockford, P.M.; Porte, D., Jr.; Wood, F.C., Jr., and Williams, R.H.: Effect of glucagon on serum insulin, plasma glucose and free fatty acids in man. Metabolism *15:* 114 (1966).
4. Cunningham, G.C., Jr.: Tolbutamide tolerance in hypoglycemic children. Amer.J.Dis.Child. *107:* 417 (1964).
5. DeBelle, R.; Belmonte, M.M. and Colle, E.: Effect of intravenous tolbutamide in juvenile diabetes mellitus. Diabetes *16:* 215 (1967).
6. DiGeorge, A.M. and Chiowanich, P.: The intravenous tolbutamide response test in infants and children. A preliminary report. Diabetes, Suppl. *11:* 135 (1962).
7. Drash, A.: Clinical laboratory evaluation; in Human growth: Body composition, cell growth, energy and intelligence (ed. Cheek, D.B.) (Lea and Febiger, Philadelphia, Pa. 1967).
8. Duncan, G.G.: Diseases of metabolism, 5th ed., p.921 (Saunders, Philadelphia, Pa. 1964).
9. Editorial: Diabetes and the pituitary gland. New Engl.J.Med. *263:* 407 (1960).
10. Editorial: Growth hormone and diabetes. New Engl.J.Med. *275:* 961 (1966).
11. Fajans, S.S.: Diagnostic tests for diabetes mellitus; in Diabetes (ed. Williams, R.H.), p.397 (Hoeber, New York 1960).
12. Fine, P.H.; Burday, S.Z. and Schalch, D.S.: Growth hormone secretion in normal and diabetic subjects: relationship to blood glucose levels. Clin. Res. *14:* 477 (1966).
13. Floyd, J.C., Jr.; Fajans, S.S.; Conn, J.W.; Knopf, R.F. and Rull, J.: Stimulation of insulin secretion by amino acids. J.clin.Invest. *45:* 1487 (1966).
14. Greenberg, E.: Growth hormone and diabetes mellitus. Diabetes *14:* 43 (1965).
15. Grodsky, G.M.; Karam, J.H.; Pavlatos, F.C. and Forsham, P.H.: Serum-insulin response to glucose in prediabetic subjects. Lancet *i:* 290 (1965).
16. Gurol, F.: The use of the intravenous tolbutamide test. Mich.Med. *65:* 720 (1966).
17. Herbert, V.; Lau, K.S.; Gottlieb, C.W. and Bleicher, S.J.: Coated charcoal immunoassay of insulin. J.clin.Endocrin. *25:* 1375 (1965).
18. Hunter, W.M.; Clarke, B.F. and Duncan, J.P.: Plasma growth hormone after an overnight fast and following glucose loading in healthy and diabetic subjects. Metabolism *15:* 596 (1966).
19. Karam, J.D.; Grodsky, G.M.; Pavlatos, F.C. and Forsham, P.H.: Critical factors in excessive serum-insulin response to glucose. Lancet *i:* 286 (1965).

20. KLEIN, R.; MARKS, F. and MIRSKY, I.A.: Insulin-producing capacity of the pancreas of children with diabetes mellitus. Pediatrics *22:* 289 (1958).
21. LEVINE, R. and LUFT, R.: The relation between the growth and diabetogenic effects of the so-called growth hormone of the anterior pituitary. Diabetes *13:* 651 (1964).
22. LUFT, R.: Human growth hormone and the control of blood glucose concentration. Israel med.J. *1:* 1266 (1965).
23. MACGILLIVRAY, M.D.; ACETO, T., Jr., and FROHMAN, L.A.: Growth hormone responsiveness to stimulation by insulin hypoglycemia, arginine and piromen in normal and growth deficient children. Program of the 77th Annual Meeting of the American Pediatric Society. Abstract, p. 10 (1967).
24. MERIMEE, T.J.; BURGESS, J.A. and RABINOWITZ, D.: Sex-determined variation in serum insulin and growth hormone response to amino acid stimulation. J. clin. Endocrin. *26:* 791 (1966).
25. MERIMEE, T.J.; LILLIGRAP, D.A. and RABINOWITZ, D.: Effect of arginine on serum-levels of human growth-hormone. Lancet *ii:* 668 (1965).
26. MERIMEE, T.J.; BURGESS, J.A. and RABINOWITZ, D.: Arginine infusion in maturity-onset diabetes mellitus. Lancet *i:* 1300 (1966).
27. PICKENS, J.M.; BURKEHOLDER, J.N. and WOMACK, W.N.: Oral glucose tolerance test in normal children. Diabetes *16:* 11 (1967).
28. PILDES, R.S.; PARKER, M.L.; CHAO, K.L. and CORNBLATH, M.: Growth-onset diabetes mellitus: a deficiency in insulin. Program of the 27th Annual Meeting of the American Diabetes Association. Abstract, p. 36 (1967).
29. POWELL, E.D.U.; FRANTZ, A.G.; RABKIN, M.T. and FIELD, R.A.: Growth hormone in relation to diabetic retinopathy. New Engl.J.Med. *275:* 922 (1966).
30. RABINOWITZ, D.; MERIMEE, T.J.; MAFFEZZOLI, R. and BURGESS, J.A.: Patterns of hormonal release after glucose, protein, and glucose plus protein. Lancet *ii:* 454 (1966).
31. RABINOWITZ, D.; MERIMEE, T.J.; BURGESS, J.A. and RIGGS, L.: Growth hormone and insulin release after arginine: Indifference to hyperglycemia and epinephrine. J. clin. Endocrin. *26:* 1170 (1966).
32. RABINOWITZ, D.; MERIMEE, T.J. and BURGESS, J.A.: Growth hormone-insulin interaction. Diabetes *15:* 905 (1966).
33. ROOT, A.W.; SAENZ-RODRIGUEZ, C.; BONGIOVANNI, A.M. and EBERLEIN, W.R.: The response of growth hormone, insulin and glucose to arginine in children. Program of the 77th Annual Meeting of the American Pediatric Society. Abstract, p. 7 (1967).
34. SAMOLS, E. and MARKS, V.: Insulin assays in insulinomas. Brit. med. J. *i:* 507 (1963).
35. SAMOLS, E.; MARRI, G. and MARKS, V.: Promotion of insulin secretion by glucagon. Lancet *ii:* 415 (1965).
36. SCHNEIDER, V.H. und COLOMBO, J.P.: Der Tolbutamidtest im Kindersalter. Helv. paediat. Acta *4:* 344 (1964).
37. SCHOTLAND, M.A.; KAPLAN, S.L. and GRUMBACH, M.D.: The tolbutamide tolerance test in the evaluation of childhood hypoglycemia. Pediatrics *39:* 939 (1967).
38. SELTZER, H.A.; ALLEN, E.W.; HERRON, A.L., Jr., and BRENNAN, M.T.: Insulin secretion in response to glycemic stimulus: relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. J. clin. Invest. *46:* 323 (1967).
39. SIMPSON, R.G.; BENEDETTI, A.; GRODSKY, G.M.; KARAM, J.H. and FORSHAM, P.H.: Stimulation of insulin release by glucagon in noninsulin-dependent diabetes. Metabolism *15:* 1046 (1966).
40. UNGER, R.H. and MADISON, L.L.: Intravenous tolbutamide test; in Diabetes mellitus: Diagnosis and treatment (ed. DANOWSKI, T.D.) (American Diabetes Association, New York, N.Y. 1964).
41. VOLL, A.: The tolbutamide tolerance test. Acta med. scand. *117:* 90 (1965).
42. WELBORN, T.A.; RUBENSTEIN, A.H.; HASLAM, R. and FRASER, R.: Normal insulin response to glucose. Lancet *i:* 280 (1966).
43. YALOW, R.S.; BLACK, H.; VELLAZON, M.J. and BERSON, S.A.: Comparison of plasma insulin levels following administration of tolbutamide and glucose. Diabetes *9:* 356 (1960).
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