Characteristics of Growth Hormone Secretion in Clinically Stable Diabetes

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SUMMARY

Although the secretion of growth hormone (GH) is widely accepted as being increased in diabetes mellitus, this conclusion appeared to us to be the result of bias in case selection. Newly diagnosed and hospitalized diabetics cannot be assumed as representative of the clinically stable diabetic state. In view of the postulated importance of growth hormone to the vascular complications of diabetes, we re-examined this problem in 58 clinically stable diabetics and 38 age-matched controls. GH was measured in serum samples collected hourly during a 24-h period of normal activity.

Control and clinically stable diabetic subjects of normal weight had similar mean serum GH concentrations for 24 h. In subjects of normal weight, values were 4.4 ± 0.6 ng/ml for women of the control group and 4.0 ± 0.8 ng/ml for diabetic women (P = NS) and values in men were 2.4 ± 0.3 ng/ml in controls and 2.1 ± 0.3 ng/ml in diabetic men (P = NS). Obese controls and obese diabetics had similar, but significantly lower values than nonobese subjects.

When hyperglycemia (125 mg to 175 mg/dl) was induced in 12 control subjects for 24 h, glycemia was obtained comparable to that seen in a select group of nine diabetics. Mean serum concentrations of GH were significantly less in the glucose-infused controls than in both euglycemic controls and diabetics.

These data do not support an absolute increase of circulating GH in stable diabetics; GH levels appear to be normal in this group but inappropriate for the level of glycemia. DIABETES 28:308–312, April 1979.

Growth hormone may be related to the long-term complications of the diabetic state, in particular to microvascular disease.[1–4] Although characterization of its secretion in diabetes is important, such characterization in the past has actually yielded extremely variable results.[5–10] Any investigation of growth hormone secretion is susceptible to multiple sources of bias. Diabetics, in particular, who are newly diagnosed, hospitalized, and acutely ill subjects, cannot be assumed comparable to diabetics functioning in everyday life. The studies of growth hormone reported in this paper were designed to examine the physiologic patterns of growth hormone secretion in diabetics who were clinically stable.

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METHODS

In order to avoid bias in case-control selection, diabetic and normal control subjects for this study were selected from patients that required routine histories and physicals. None were acutely ill or hospitalized; all were functioning well in society. A total of 58 diabetics between the ages of 18 and 68 and 38 controls of a comparable age span were studied. Subsequent histories indicated that 18 patients had classic juvenile-onset diabetes. This diagnosis was based on onset of diabetes (a) before age 10 in 10 patients and (b) before age 20 in four additional patients. Four diabetics with onset of their disease between ages 30 and 40 were placed in the juvenile-onset category on the basis of onset with ketoacidosis, demonstrable episodes of acidosis that followed the initial diagnosis, and a firm history to indicate an absolute medical need for exogenous insulin that began and persisted after the initial discovery of the diabetic state. Forty diabetics with adult-onset diabetes were identified. Subjects placed in this category met the following qualifications: (a) they were not hospitalized for ketoacidosis in the first three years of their disease and (b) they required no insulin for management for periods varying from 3 to 17 yr after the initial diagnosis of diabetes. At the time of the study, however, 28 of the latter subjects were receiving exogenous insulin treatment.

Each patient was admitted, after giving informed consent, to the Clinical Research Center at the University of Florida. Patients were admitted on the afternoon or evening before the 24-h study period. On the day of the study, an indwelling catheter or scalp vein needle was inserted at 0800

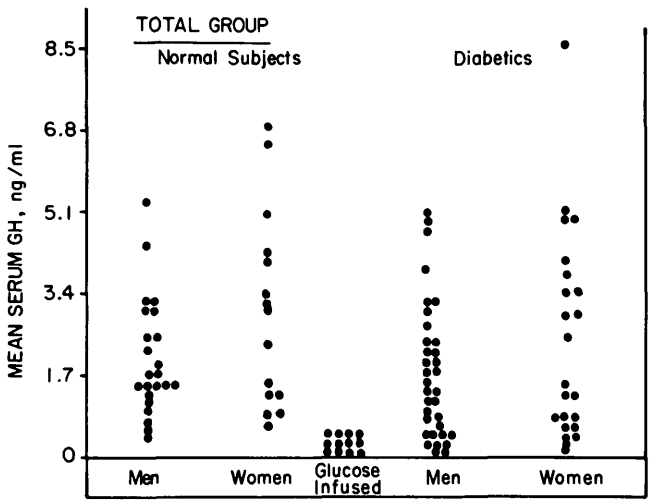


FIGURE 1. The mean serum concentrations of GH during 24 h are shown for 38 normal and 58 diabetic men and women. The mean value for each individual was obtained from samples collected hourly. Glucose-infused controls received $D_{10}W$ and $D_{20}W$ for 24 h.

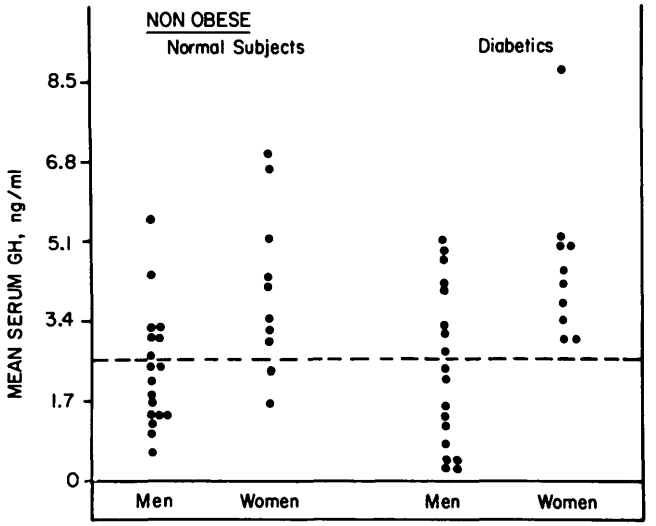


FIGURE 2. Subjects ≤ 20% of ideal body weight are excluded from the comparison initially made in Figure 1. The broken line at 2.5 ng/ml is made arbitrarily to emphasize the similarity of nonobese diabetics and nonobese controls and the sex variation within each group.

and the first serum sample was collected. The scalp vein needle was placed at the wrist; antecubital veins were not used unless absolutely necessary in order to provide maximum mobility to the subject. Two diabetics and three controls required a second venipuncture for adequate sampling. Food intake and activity during the 24-h period were at the discretion of each patient; all subjects, however, were encouraged to maintain a level of activity and a diet comparable to that observed during their usual life routine. Bedrest was not permitted from 1000 to 2000 h, unless this formed a normal part of the patient's daily routine.

Twelve of the control subjects (eight of normal weight and four obese) were admitted on a second occasion, and a similar study was performed, in which hyperglycemia was maintained at between 125 and 175 mg/dl. Hyperglycemia was attained by means of a continuous infusion of $D_{20}W$ and $D_{10}W$ (dextrose 20 g/dl and 10 g/dl, respectively, in water).

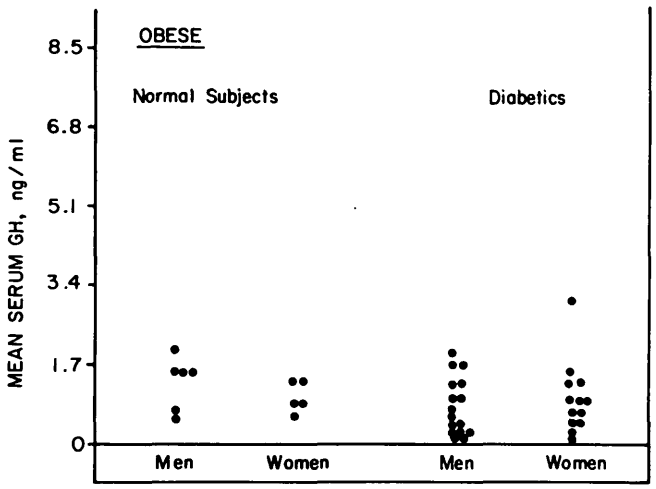
Blood samples were collected hourly and centrifuged; the serum was then stored at −20°C until analysis. Serum glucose was measured by a glucose oxidase method, with the variation between duplicate samples not exceeding 1.5 mg/dl; serum free fatty acids were determined by a colorimetric technique.[12,13] Serum growth hormone was measured by a single antibody technique using Wilhelmi growth hormone HS2019G as a standard and a charcoal separatory technique.[14,15] Samples were assayed in duplicate with an appropriate control for each sample. Recovery studies indicated an ability to detect 0.25 ng/ml in 100% of recovery experiments and 0.15 ng/ml in 85% of such studies. In sera totally devoid of GH, 7.0% gave readings between 0.10 and 0.25 ng/ml and none exceeded 0.25 ng/ml. The intra-assay coefficients of variability were 10.2% at 0 to 0.5 ng/ml, 8.6% at 1 to 2.5 ng/ml, 4.2% at 2.5 to 5 ng/ml, and 4.0% at 5 to 12.0 ng/ml. More importantly, in each assay, sera from a similar number of control and diabetic subjects were measured, and the same 10 internal sera standards were used for the final adjustment of each curve. Normal and diabetic groups were compared by means of Student's *t* test. Rank analysis was applied to individual data.

RESULTS

Figure 1 shows the mean serum concentration of growth hormone over 24 h for each individual—normal and diabetic. Mean serum concentrations of growth hormone are also given for the 12 normal subjects infused with glucose.

Rank analysis of the individual data shown in Figure 1 failed to show a significant difference of growth hormone secretion in diabetics as compared with controls; however, since there was a higher incidence of obesity (20% or greater than ideal body weight) in the diabetics than in normal controls, comparisons were made also in subjects of similar weight. Ideal body weight was defined from the Metropolitan Life tables utilizing the appropriate frame value. Figures 2 and 3 show the individual data expressed for nonobese and obese subjects, respectively. When

FIGURE 3. Mean serum concentrations of GH over 24 hours are shown for obese controls and obese diabetics only. No significant difference existed between controls and diabetics, and no sex variation could be detected.



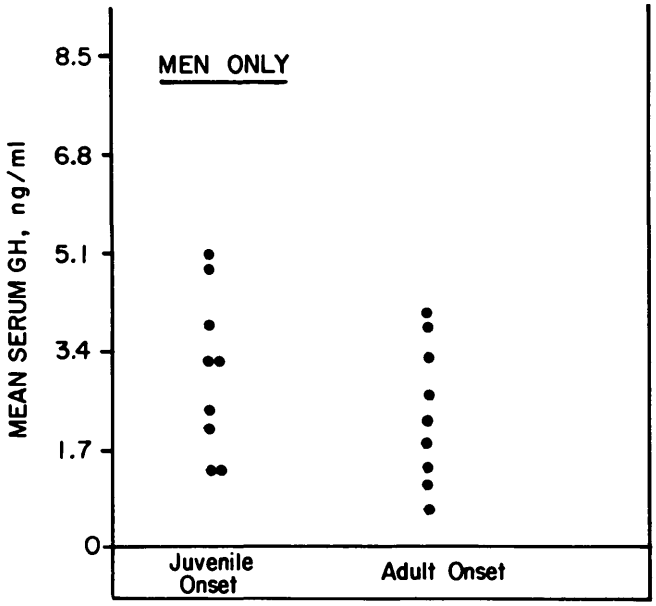


FIGURE 4. Mean serum concentrations of GH over 24 h are shown for nonobese adult-onset and juvenile-onset diabetics. The comparison involved only male subjects. No significant difference occurred between controls and diabetics.

only nonobese subjects were compared, mean serum concentrations of growth hormone over 24 h were similar in diabetics and controls. There was also a significant difference of growth hormone between women and men—both normal and diabetic—which was not evident in the initial comparison. Because of the high incidence of obesity in the diabetic women with both adult-onset and juvenile-onset types of diabetes, a comparison was not possible within this group. In men, no significant difference in growth hormone levels was noted in subjects with juvenile-onset and adult-onset types of diabetes (Figure 4).

Mean data, calculated from the individual results illustrated in Figures 1–4, likewise indicated the similarity of diabetics and normal controls. The mean serum growth hormone concentrations for all controls and all diabetics irrespective of weight were, respectively, 2.1 ± 0.3 ng/ml and 1.6 ± 0.3 ng/ml ($\bar{X}$ ± SEM) (P = NS). The mean values for all control and diabetic women were, respectively, 3.2 ± 0.5 ng/ml and 2.1 ± 0.5. In subjects of normal weight, values were 4.4 ± 0.6 ng/ml for women of the control group and 4.1 ± 0.8 ng/ml for diabetic women (P = NS); values in men were 2.4 ± 0.3 ng/ml in controls and 2.1 ± 0.3 ng/ml in diabetic subjects (P = NS).

In the 12 normal subjects (eight of normal weight and four obese) whose growth hormone concentration was monitored for 24 h while they were hyperglycemic, the concentration in serum never exceeded 1.5 ng/ml; the mean serum concentration of growth hormone in this group was less than 0.6 ng/ml, significantly less than both normal and diabetic values ($P < 0.01$). The mean serum glucose concentration in the infused controls was 148 ± 12 mg/dl.

GLUCOSE AND GROWTH HORMONE SECRETION

An attempt was made to correlate changes of serum glucose concentration with periodic increases of growth hormone concentration in serum. In control and diabetic subjects, 50% of all concentrations of growth hormone greater than 5 ng/ml occurred between 0800 and 2200. In the period 2200 to 0800, in which fluctuations of serum glucose were spontaneous and not related to food ingestion, no correlation existed between changes of serum glucose and growth hormone. These data were analyzed by the chi square test.

In control subjects receiving a glucose infusion, serum glucose fluctuated between 125 and 175 mg/dl. Figure 5 shows three representative control subjects who were made hyperglycemic by a glucose infusion. The remainder of the control subjects studied in this manner showed a similar pattern. In all cases, growth hormone remained suppressed despite fluctuations in blood sugar. The relationship between serum growth hormone concentrations and overall glycemia is compared in Figure 6.

In the normal individuals, the mean increase of glucose over the fasting value for 24 h was 5.76 ± 1.32 mg/dl. For diabetics with fasting serum glucose greater than 120 mg/dl, the mean increase was 105.4 ± 13.6 mg/dl; for diabetics with fasting serum glucose less than or equal to 120 mg/dl, the mean increase was 48.1 ± 14.2 mg/dl. A mean increase of glucose similar to the latter group of diabetics was obtained in controls infused with an intravenous infusion of glucose. However, unlike the latter diabetics, growth hormone secretion in the controls was totally suppressed by this level of hyperglycemia (Figure 6).

DISCUSSION

Although growth hormone is thought to contribute to the pathogenesis of diabetic microvascular disease, its secretion in clinically stable diabetics has not been characterized adequately. Previous studies of growth hormone secretion in diabetics have either examined secretion after nonphysiologic stimuli or selected patients not typical of the general diabetic population.

Garlaschi could find no difference of growth hormone secretion in 22 diabetics and 19 controls after maximal exercise, whereas Vigneri found increased growth hormone in serum in poorly controlled diabetics and normal values when more adequate control was established.[5,8] Hansen reported growth hormone secretion increased in diabetics both after severe exercise and during a typical 24-h period. The latter patients, however, were newly diagnosed and not restudied after a clear-cut establishment of a stable state.[6,7,9,10] The time required for the clear-cut establishment of a clinically stable state after diagnosis has not been established, and it is doubtful if this can be determined merely by assessing glucose control. It appeared to us, in reviewing such data, that elevated concentrations of growth hormone in diabetics probably reflected simple, associated stress or illness, since these occur in nondiabetics as well.[16,17]

To avoid the pitfalls in the previous approaches of characterizing growth hormone secretion in diabetics, we gave close attention to the selection process for both patients and controls. The cases were selected from the most natural group available—diabetics functioning in society in their usual state of control. Controls were chosen in the same manner. No prior attempt was made

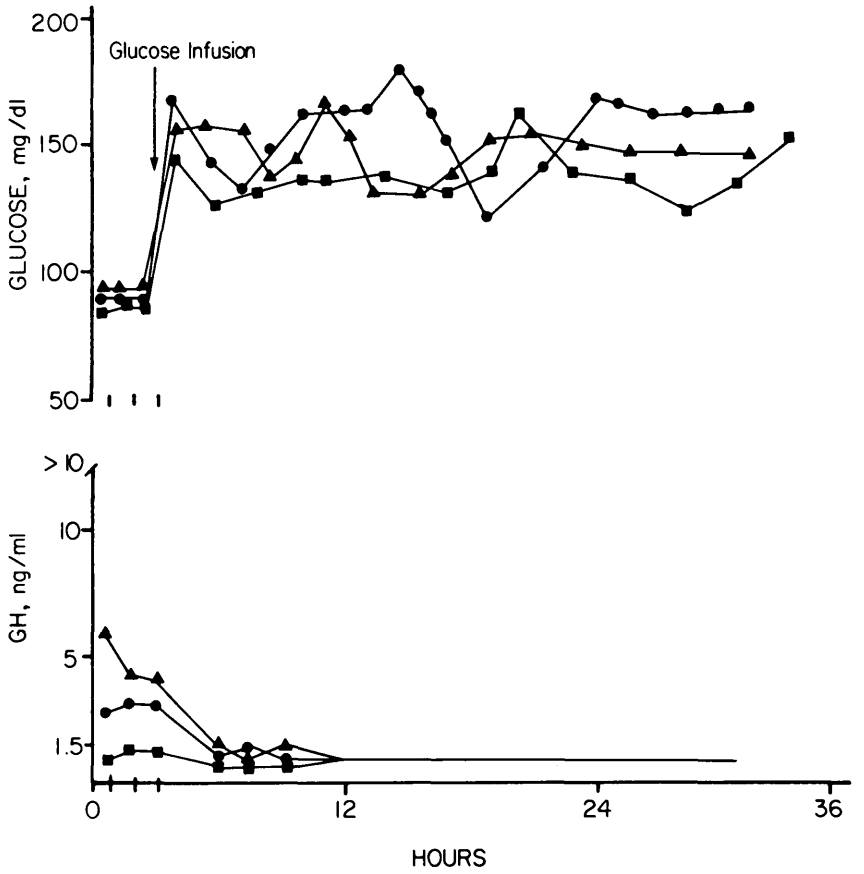


FIGURE 5. The glucose and GH concentrations in serum at hourly intervals are shown for three representative controls who received $D_{10}W$ and $D_{20}W$ for 24 h. Additional controls were similar.

to screen for degree of obesity. No subject was acutely ill, and, during the study period, conditions were maintained as close as possible to the individual's usual routine. With the sample population chosen as indicated, the following conclusions seem reasonable:

(a) In nonobese diabetics and controls, mean serum levels of growth hormone are virtually identical, and a prominent variation in GH between men and women is noted in both groups.

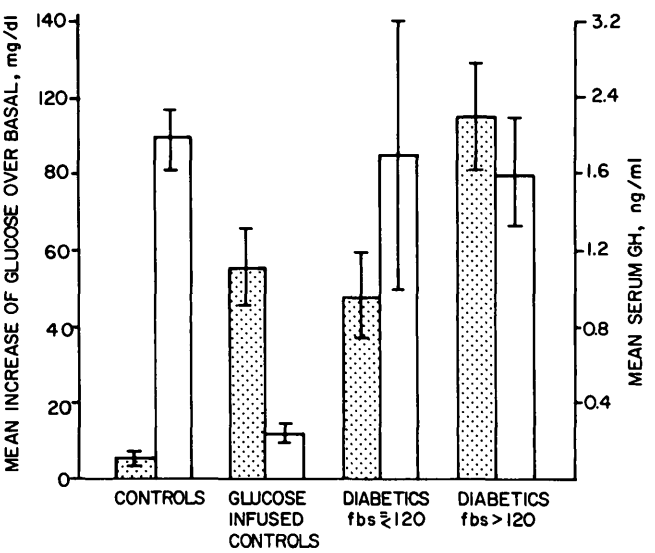
(b) Obese diabetics and obese controls have similar, but significantly lower, serum concentrations of growth hormone over 24 h than do subjects of normal weight. Obesity exerts a more profound influence over growth hormone secretion than does the difference between sexes.

(c) There appears to be no difference in the levels of growth hormone between juvenile-onset and adult-onset diabetics; however, because of obesity in diabetic women, this analysis could be made only for men.

(d) The mean serum growth hormone concentration is greater in diabetics than in control subjects who have a similar level of hyperglycemia induced by glucose infusion. This inappropriateness of growth hormone concentration to the level of glycemia is consistent with a resetting of the mechanism controlling growth hormone secretion, a situation analogous to that reported for glucagon in diabetics.[18] Adaptations to hyperglycemia, however, probably require more than 24 h, and more chronic experiments would be required to validate this point.

(e) Growth hormone levels in the diabetic are not maintained by periodic decreases of blood glucose acting as a stimulus for the secretion of growth hormone. Although such a relationship has been suggested,[19] no increase of growth hormone concentration exceeding 1.5 ng/ml was recorded in 12 control subjects whose hyperglycemia fluctuated between 125 and 175 mg/dl (see Figure 5). In diabetics, no consistent relationship existed between

FIGURE 6. The mean increase of glucose over basal is shown by the shaded bars and the mean serum GH concentration over 24 h by the solid bars. SEM are indicated. Note the similarity of glucose values in glucose-infused controls and diabetics with FBS ≷ 120 mg/dl.



growth hormone concentrations in serum and prior changes of serum glucose concentration.

We believe these data may bear on the manner in which growth hormone could contribute to the complications of diabetes; at the very least, they do provide for some clarity in a hitherto confused area of facts and speculation. For example, explanations for the development of diabetic complications based on hypersecretion of growth hormone are, actually, unsupportable by clinical data on close examination. Diabetic complications are rare in acromegalics. If, however, the more critical factor is an inappropriate relationship between growth hormone and hyperglycemia, then the latter observation becomes rational, i.e. frank diabetes with elevated fasting blood glucoses is uncommon in acromegalics.

There are several possible mechanisms by which an inappropriate relationship between GH and glycemia could contribute to diabetic complications. There is, for example, evidence that aberrations exist in several structural and circulating glycoproteins in diabetics. This has been described for proteins of the capillary basement membrane and it is reported for circulating glycoproteins, some of which, like haptoglobin, contribute substantially to blood viscosity.[20,21] It is conceivable that growth hormone secretion, when unsuppressed in a chronic hyperglycemic state, contributes to glycoprotein abnormalities by the dual mechanism of maintaining protein synthesis at a high rate and inducing preferential shunting of glucose through metabolic pathways favoring hexose incorporation into glycoprotein. We are not aware of definitive data on the first alternative, but data from Winegrad would strongly support the latter possibility.[22]

Lastly, we believe these studies underscore the importance of proper recognition of aberrations of the clinically stable diabetic state as contrasted with sporadic abnormalities occurring in diabetics. It is reasonable to postulate that the long-term complications of diabetes will relate to consistent metabolic abnormalities of the stable diabetic rather than to aberrations that occur only sporadically.

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REFERENCES

[1] Lundbaek, K., Malmros, R., and Anderson, H. C.: Hypophysectomy for diabetic angiopathy. *In* Symposium on the Treatment of Diabetic Retinopathy. Goldberg, M. F., and Fine, S. L., Eds. Washington, DC, Public Health Service Publ. No. 1890, 1969, p. 291.

[2] Merimee, T. J., Fineberg, S. E., and McKusick, V. A.: Diabetes mellitus and sexual ateliotic dwarfism. A comparative study. J. Clin. Invest. *49*:1096, 1970.

[3] Lundbaek, K.: Growth hormone's role in diabetic microangiopathy. Diabetes *25* (Suppl. 2):845, 1976.

[4] Ledet, T.: Growth hormone stimulating the growth of arteriol medial cells in vitro: absence of effect of insulin. Diabetes *25*:1101, 1976.

[5] Garlaschi, C., Natale, B., Guercio, M. J., Carcamo, A., Garantini, L., and Chiumello, G.: Effect of exercise on secretion of growth hormone, glucagon and cortisol in obese and diabetic children. Diabetes *24*:758, 1976.

[6] Johansen, K., and Hansen, A. P.: High 24 hour levels of serum growth hormone in juvenile diabetics. Br. Med. J. *2*:356, 1969.

[7] Kjeldsen, H., Hansen, A. P., and Lundbaek, K.: Twenty-four-hour serum growth hormone levels in maturity-onset diabetics. Diabetes *24*:977, 1975.

[8] Vignerni, R., Squatrito, S., Pezzino, V., Filetti, S., Branca, S., and Polosa, P.: Growth hormone levels in diabetes. Diabetes *25*:167, 1976.

[9] Hansen, A. P., and Johansen, K.: Diurnal pattern of blood glucose, serum FFA, insulin, glucagon and growth hormone in normals and juvenile diabetics. Diabetologia 6:27, 1970.

[10] Hansen, A. P.: Normalization of growth hormone hyperresponse to exercise in juvenile diabetics after normalization of blood sugar. J. Clin. Invest. *50*:1806, 1971.

[11] Horwitz, R. I., and Feinstein, A. R.: New methods of sampling and analysis to remove bias in case control research: no association found for estrogens and endometrial cancer. Clin. Res. *25*:459A, 1977.

[12] Huggot, A. St. G., and Nixon, D. A.: Use of glucose oxidase, perioxidase and O-dianisidine in determination of blood and urinary glucose. Lancet *2*:368, 1957.

[13] Trout, D. L., Estes, E. H., Jr., and Friedberg, S. J.: Titration of free fatty acids of plasma: a study of current methods and a new modification. J. Lipid Res. *1*:199, 1959.

[14] Glick, S. M., Roth, J., Yalow, R. S., and Berson, S. A.: Immunoassay of growth hormone in plasma. Nature (London) *199*:784, 1963.

[15] Herbert, V., Lau, K., Gottlieb, C. W., and Bleicher, S. J.: Coated charcoal immunoassay of insulin. J. Clin. Endocrinol. Metab. *25*: 1375, 1965.

[16] Glick, S. M., Roth, J., Yalow, R. S., and Berson, S. A.: The regulation of growth hormone secretion. Recent Prog. Horm. Res. *21*: 241, 1965.

[17] Merimee, T. J., and Rabin, D.: A survey of growth hormone secretion and action. Metab. Clin. Exp. *22*:1235, 1973.

[18] Horwitz, D. H., Zeidler, A., Gonen, B., Rodman, D., Langer, B., Jaspan, J. B., and Rubenstein, A. H.: Diabetic control by the artificial pancreas. Comparison of glucose and glucagon levels with those achieved by traditional insulin therapy. Clin. Res. *24*:527A, 1976.

[19] Luft, R., Cerasi, E., Madison, L. L., et al.: Effect of a small decrease in blood glucose on plasma growth hormone and urinary excretion of catecholamines in man. Lancet *2*:254, 1966.

[20] McMillan, D. E.: Plasma protein changes, blood viscosity, and diabetic microangiopathy. Diabetes *25* (Suppl. 2):858, 1976.

[21] Beisswenger, P. J., and Spiro, R. G.: Studies on the human glomerular basement membrane: composition, nature of the carbohydrate units, and chemical changes in diabetes mellitus. Diabetes *22*:180, 1973.

[22] Winegrad, A. I., and Borden, C. L.: L-xylulose metabolism in diabetes mellitus. N. Engl. J. Med. *274*:298, 1966.