tus of diabetes in our country (1). The bulletin gave detailed information on various treatments, diets, physical training, life-style, and education of people with diabetes.

We have limited access to oral hypoglycemic agents from Western pharmaceutical companies. The crystalline insulin that was used is made by Pharmachim of Sofia or Actrapid (Novo, Denmark). Insulins for sustained action are Pharmachim Lente (Bulgaria), Novo Lente–Monotard (Denmark). Patients allergic to insulin medication are treated with monocomponent and human insulin from Novo (Monotard, Actrapid, Protaphan, Actraphan 30/70).

Blood glucose profiles, glucosuria, and in some centers testing of HbA_1 and dosage are used for regular monitoring of patients with diabetes. We do not have enough testing material for the self-monitoring of glycosuria and blood glucose. Both individual and group teaching methods are used; Sofia's Diabetes Centre and the Higher Medical School in Varna offer well-organized educational programs of each variety. Diabetic patients learn about various kinds of therapy, therapeutic diets, side effects of insulin therapy, the significance of physical exercise, related medical and social problems, and other aspects of diabetes care. One year ago, the Association of Diabetic Patients was formed. This year, they have begun publishing a newsletter, *Diabetes*.

A considerable amount of research and development at endocrinology clinics is being devoted to diabetes. Several conventions in Bulgaria dedicated to diabetes brought international participation. Five of the country's clinics have Biostators, which are being used for both research and therapeuty. Several Master's and doctoral theses have focused on diabetes. There are also a few monographs issued in Bulgaria on the topic. At the Institute of Endocrinology, the Medical Academy, and the Institute of Internal Medicine there are some short-term postgraduate courses for physicians exploring the problems of diabetes.

References

1. Gavrailov M, Koev D, Ovtcharova N, Staikov T, Tannev V, Todorov K: The fight against socially significant diseases—diabetes mellitus. *Ministry of Public Health Bulletin 8–9, 1988*
2. Staikov T: *Incidence and Characteristics of Diabetes Mellitus in Bulgaria*. MSc thesis. Institute for Endocrinology, Geriatrics and Gerontology at the Medical Academy of Sofia, Bulgaria, 1972
3. Staikov T: *Incidence, Characteristics and Prognostics of Endocrine Diseases in Bulgaria and Organization of Prevention and Treatment*. PhD thesis. Institute for Endocrinology, Geriatrics and Gerontology at the Medical Academy of Sofia, Bulgaria, 1983
4. Andreiev D: *Diabetes*, Sofia, Bulgaria, Medicina Physcultura, 1978 (monogr.)

Fluctuations in Plasma Proinsulin in an Insulinoma

Implications for Screening Test

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A characteristic feature of many patients with insulin-producing tumors is an elevated concentration of circulating proinsulin (1–4). The measurement of serum proinsulin has been simplified by the development of sensitive radioimmunoassays (RIA; 5–8). It has been suggested that serum proinsulin measurement by RIA may be a useful adjunct in the diagnosis of patients with hypoglycemia resulting from insulin-producing tumors (8,9). We report a patient study in which elevated proinsulin concentrations appeared to be a variable and intermittent phenomenon. The results suggest that evaluation of plasma proinsulin concentrations by size exclusion chromatography and expressing the results as percentage of total insulin immunoreactivity may be misleading and that specific RIA is the preferable approach.

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A 62-yr-old man presented with a clinical and biochemical picture of an insulinoma had a single 1.5-cm diam mass in the tail of the pancreas at surgery, which was resected and consisted of a β-cell tumor on histology. Symptoms resolved after surgery. Blood samples for this study were obtained in the fasting state at different time points over a 3-mo period before surgery.

SIZE-EXCLUSION CHROMATOGRAPHY— Two milliliters of plasma was concentrated twofold in a Savant speedvac concentrator and then eluted through a Sephadex G50 column (1 × 50 cm) with a 3 M acetic acid buffer or Biogel P30 column with a phosphate buffer. Recovery of insulin and proinsulin after chromatography with this methodology was 73–102%. The entire plasma sample was used so that when the specific RIA for proinsulin became available at a later date, identical samples could not be processed by both methods.

RIAS— After separation by size-exclusion chromatography, the eluate was subjected to a previously described RIA for insulin (10). Detection limit was 0.02 pmol/ml. Intra- and interassay coefficients of variation were 6.5 and 8.0%, respectively. Proinsulin had a 60% cross-reactivity in the insulin RIA. RIA for C-peptide was carried out with a previously described method (10), with antiserum generously provided by Bruce Frank (Lilly). The detection limit in this assay was 0.025 pmol/ml. Proinsulin had 50% cross-reactivity in the C-peptide assay. Intra- and interassay coefficients of variation were 6.9 and 8.2%, respectively. Proinsulin was measured with a highly specific RIA (11), with <0.01% cross-reactivity with insulin or C-peptide. The lower limit of sensitivity of this assay was 5 fmol/ml. Intra- and interassay coefficients of variation were 8.0 and 9.0%, respectively.

In this patient, the diagnosis of an insulinoma was based on the finding of hyperinsulinemia and elevated plasma C-peptide concentrations in the presence of low plasma glucose concentrations (Table 1). However, plasma proinsulin levels were not consistently elevated. Size-exclusion chromatography of plasma demonstrated a variable relationship of insulin to proinsulin in three fasting plasma samples, taken at different times (Table 1). In sample 1, almost all of the insulin immunoreactivity eluted as a peak coinciding with the proinsulin marker. In marked contrast, sample 2 consisted predominantly of a peak coeluting with insulin. Sample 3 had an intermediate, but elevated percent of proinsulin. However, with the proinsulin RIA in four other fasting samples obtained consecutively over a 1-h period (samples 4–7), elevated concentrations were uniformly observed (Table 1).

The apparent variability in proinsulin concentrations is particularly obvious when expressed as a percentage of total insulin immunoreactivity. The likely reason for this is the fluctuation observed in plasma insulin levels, which varied dramatically, despite similar glucose concentrations in the different samples. Sample 2, evaluated by chromatography, had elevated serum insulin concentrations, despite the fact that the patient was fasting. Sample 7 also demonstrated an increase of insulin concentrations without apparent explanation because the patient was under observation and known to be fasting and resting in bed at the time. Plasma C-peptide measured in the same blood samples was elevated in each case, confirming an increase in β-cell secretion. However, proinsulin was not correspondingly elevated in sample 7 (and presumably also in sample 2, although not measured by RIA). Therefore, when expressed as a percentage of total insulin immunoreactivity, based on its known cross-reactivity in the insulin assay, proinsulin was within the normal range in sample 7.

This dissociation between fasting serum insulin and proinsulin has not, to our knowledge, been previously described. It is unlikely to be related to methodological problems because insulin levels were well correlated with elevated C-peptide concentrations ($r = 0.96$, $P < 0.001$, $n = 7$). The mechanism for the sudden increase in fasting insulin concentrations that occurred in samples 2 and 7 is unclear but may reflect erratic (12) or episodic (13) secre-

Table 1—*Variable relationship of insulin to proinsulin*

Sample	Glucose (mM)	Insulin (pmol/ml)	C-Peptide (pmol/ml)	Proinsulin (fmol/ml)	%
1	2.4	0.14	1.2		95
2	2.4	1.21	4.0		5
3	3.6	0.42	2.3		32
4	4.1	0.10	0.44	105	67
5	3.5	0.09	0.36	103	68
6	3.6	0.11	0.44	94	54
7	3.4	0.62	2.80	128	13
Normal fasted	3.9–6.1	0.04–0.11	0.54 ± 0.05*	9.7 ± 1.2*	<25

Samples 1–3 were processed by size-exclusion chromatography. Samples 4–7 were obtained consecutively in the fasting state within a period of 1 h and measured in the proinsulin radioimmunoassay. %, Percentage of total insulin immunoreactivity. In samples 4–7, this was estimated with known cross-reactivity of proinsulin in the insulin radioimmunoassay.
*Means ± SE.

tions that have been previously demonstrated in these tumors (12,13).

This case is instructive in that it demonstrates that insulin concentrations may fluctuate independently of proinsulin in a patient with a proven insulinoma. Thus, caution should be used in interpreting results of the chromatographic approach; proinsulin levels may appear to be normal if expressed as percentage of total immunoreactivity. We suggest that specific RIA for proinsulin be the preferred approach for screening of insulinomas. Multiple samples in the fasting state will further elucidate whether proinsulin, like insulin, is also secreted in fluctuant fashion.

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References

1. Gutman RA, Lazarus MR, Penhos JC, Fajans S, Recant L: Circulating proinsulin-like material in patients with functioning insulinomas. *N Engl J Med* 284: 1003–1008, 1971
2. Melani F, Ryan WG, Rubenstein AH, Steiner DF: Proinsulin secreted by a pancreatic beta cell adenoma. *N Engl J Med* 283:713–16, 1970
3. Sherman BM, Pek S, Fajans S, Floyd JC, Conn JW: Plasma proinsulin in patients with islet cell tumors. *J Clin Endocrinol Metab* 35:271–76, 1972
4. Alsever RN, Roberts JP, Gerber JG, Mako ME, Rubenstein AH: Insulinoma with low circulating insulin levels: the diagnostic value of proinsulin measurements. *Ann Intern Med* 82:347–51, 1975
5. Heding LG: Specific and direct radioimmunoassay for human proinsulin in serum. *Diabetologia* 13:467–72, 1977
6. Deacon CF, Conlon JM: Measurement of circulating human proinsulin using proinsulin-specific antiserum. *Diabetes* 34: 491–97, 1985
7. Ward WK, Paquette TL, Frank BH, Porte D: A sensitive radioimmunoassay for human proinsulin, with sequential use of antisera to C-peptide and insulin. *Clin Chem* 32:728–33, 1986
8. Cohen RM, Given BD, Liciano-Paixao J, Provow SA, Rue PA, Frank BH, Root MA, Polonsky KS, Tager HS, Rubenstein AH: Proinsulin radioimmunoassay in the evaluation of insulinomas and familial hyperproinsulinemia. *Metabolism* 35: 1137–46, 1986
9. Cohen RM, Camus F: Update on insulinomas or the case of the missing (pro) insulinoma. *Diabetes Care* 11:506–507, 1988
10. Ipp E, Sinai Y, Forster B, Nesher R, Cerasi E: A glucose reduction challenge in the differential diagnosis of fasting hypoglycemia—a two center study. *J Clin Endocrinol Metab* 70:711–17, 1990
11. Bowsher RR, Apathy JM, Ferguson AL, Frank BH: An improved radioimmunoassay for proinsulin in human serum and plasma (Abstract). *Diabetes* 36:94A, 1987
12. Villaume C, Beck B, Dollett J-M, Pointel J-P, Drouin P, Debry G: 28 hour profiles of blood glucose, plasma immunoreactive insulin in four insulinomas. *Ann Endocrinol* (Paris) 45:155–60, 1984
13. Chou HF, Ipp E, Bowsher RR, Berman N, Ezrin C, Griffiths S: Sustained, pulsatile insulin secretion from adenomatous human β-cells. *Diabetes* 40:1453–58, 1991

Do You Really Get a Diet Soft Drink When You Order One?

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Our patients with diabetes mellitus periodically complain that the "diet" soft drinks they receive at restaurants, particularly fast-food chains, may actually contain sugar. The calories in a typical "regular" cola soft drink, ~160 kcal for a 12 oz drink, are derived exclusively from simple sugars (1). The consumption of a regular soft drink can thus have a substantial impact on the blood glucose of an individual with diabetes. For many years, we have used a simple and reliable method to assess the presence of sugar in soft drinks. Tes-tape (Lilly, Indianapolis, IN), a glucose oxidase– and peroxidase-impregnated urine test strip, changes color from yellow to green in the presence of very small

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