Specific and Direct Radioimmunoassay for Human Proinsulin in Serum

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Summary. A routine radioimmunoassay for human proinsulin in serum has been developed. The reagents used were: antibodies against the C-peptide part of the proinsulin molecule, human proinsulin as the standard and ^{125}I -labelled synthetic human Tyr-C-peptide as the tracer. The first step in this assay comprises the binding of proinsulin to insulin antibodies covalently coupled to Sepharose (S-AIS). Although bound to the solid-phase S-AIS, the proinsulin retains its second immunogenic site, viz., the C-peptide part of the molecule, accessible. Hence a surplus of C-peptide antibodies is added to the S-AIS-bound proinsulin, and the residual amount of C-peptide antibody is determined by addition of ^{125}I -Tyr-C-peptide. The detection limit is approximately 0.01 pmol/ml. The advantages of this method are: (1) its high specificity (proinsulin is determined as a molecule having both an insulin and a C-peptide moiety), (2) its simplicity and rapid performance, and (3) the low detection limit of the assay. Fasting sera from 24 nondiabetics, 9 maturity-onset diabetics and 10 newly diagnosed insulin requiring diabetics showed the following concentrations of proinsulin: 0.009 ± 0.005 , 0.022 ± 0.23 and 0.010 ± 0.009 pmol/ml (mean $\pm$ SD). One hour after 1.75 g/kg oral glucose, the values increased to 0.052 ± 0.023 , 0.046 ± 0.022 and 0.032 ± 0.022 pmol/ml. The fasting proinsulin constituted 19, 23 and 17% of the IRI, respectively, whereas 1 h post glucose these values changed to 8, 9 and 31% of IRI. Serum from 10 insulin-treated diabetics containing insulin antibodies contained from 0–1.80 pmol/ml, whereas the C-peptide levels in the same patients were 0–0.35 pmol/ml. It is suggested that insulin requiring diabetics hypersecrete proinsulin due to the inability of their B-cell to arrange proinsulin in secretory granules for adequate proinsulin/insulin conversion.

Key words: Radioimmunoassay, human proinsulin, insulin, C-peptide, oral glucose, diabetics.

Proinsulin, the biosynthetic precursor of insulin, was discovered by Steiner et al. [1]. The initial methods of quantitative determination of proinsulin in serum, which also contains insulin and C-peptide, have been based on (i) proinsulin having a higher molecular weight (9000) than insulin (6000), and (ii) its reaction with insulin antibodies. Thus, when serum or an extract of serum was subjected to gel filtration on Sephadex G 50 [2] or Biogel P 30 [3] two peaks of insulin immunoreactivity could be distinguished, one corresponding to insulin and the other one having a higher molecular weight. The latter was referred to either as “big” insulin [2] or the proinsulin-like component [4]. In order to characterize further the high-molecular-weight immunoreactive insulin (IRI) in the fractionated serum, each fraction was analyzed using the human C-peptide assay [3] and C-peptide immunoreactivity was detected in both IRI containing peaks. This strongly indicated that the first peak contained proinsulin, which exerts both insulin and C-peptide immunoreactivity. Furthermore, it was shown that C-peptide eluted in the second peak together with insulin in spite of its low molecular weight (approx. 3000).

The gel filtration procedure is, however, time consuming and has been reported to require at least 60–100 μU [5] or 4–5 ml of serum when the insulin concentration was less than 20 $\mu\text{U}/\text{ml}$ [4]. A new principle of separation was introduced in degrading insulin in serum with an insulin specific protease whilst leaving proinsulin unaltered and detectable with the insulin radioimmunoassay [6].

Fig. 1. Principle of the direct proinsulin radioimmunoassay. Diagram 1 shows the separation procedure using S-AIS, diagram 2 shows the principle of the assay where the S-AIS bound proinsulin is capable of binding antibodies to C-peptide. Ab, insulin antibodies; I, insulin; P-I, proinsulin; C, C-peptide; Ab_C, C-peptide antibodies

The purpose of this paper is to present a new specific and direct radioimmunoassay for proinsulin in serum which allows the assay of a large number of samples simultaneously [8].

Materials and Methods

The principle of the assay is shown in Figure 1. Insulin antibodies covalently coupled to Sepharose are added to serum containing insulin, proinsulin and C-peptide. The solid-phase insulin antibodies bind any molecule containing an insulin moiety, in this case

insulin and proinsulin. The C-peptide remains unbound in the supernatant and is determined by radioimmunoassay without interference from proinsulin [9]. The amount of bound proinsulin is then measured directly by the C-peptide assay since it has retained its ability to bind antibodies to proinsulin.

Human C-peptide in serum was determined according to [9], using anti-proinsulin serum from guinea pig M 1181, ¹²⁵I-labelled synthetic Tyr-C-peptide [10] as the tracer, and synthetic C-peptide [11] as the standard.

The IRI of serum not containing insulin antibodies was determined by direct radioimmunoassay [12]. Serum containing antibodies against insulin was extracted at low pH prior to the determination of IRI [12]. Insulin antibodies raised in guinea pigs and purified by reaction with monocomponent insulin coupled to Sepharose, were coupled to Sepharose 4B (referred to as S-AIS) as described in [9]. The binding capacity of the S-AIS was determined by adding increasing amounts of ¹²⁵I-labelled tracers of porcine insulin, porcine proinsulin and bovine proinsulin. A stock suspension of S-AIS with an estimated binding capacity of 0.1 U/ml was stored in 1 ml portions at -18° C until use [9].

The proinsulin assay procedure was as follows: To 1 ml of serum or standard solution of human proinsulin (0.02–0.2 pmol/ml ~ 0.18–1.8 ng/ml), kindly donated by Professor Arthur Rubenstein of Chicago, was added 100 µl of S-AIS suspension with a binding capacity of 0.01 U/ml. The samples were shaken at 4° C overnight, centrifuged, and 900 µl of the supernatant removed using an Oxford Sampler Model Q. The S-AIS containing bound insulin and proinsulin was then washed twice with 2 ml of phosphate buffer (0.04 mol/l, pH 7.4) containing human albumin (Behringwerke, electrophoretic purity 100%) (60 g/l) and thiomersal (0.2 g/l), in the following referred to as NaFAM, in order to remove the residual C-peptide (top diagram in Figure 1). After each wash, 2.0 ml was pipetted off and discarded, so that 200 µl remained in each tube. After the second wash, 300 µl of NaFAM and 200 µl of anti-proinsulin serum M 1181 (1:700) [9] were added and the tubes shaken at 4° C overnight (bottom diagram in Figure 1). After centrifugation, 2 × 200 µl were pipetted from each supernatant into two glass tubes (100 × 10 mm) and 100 µl of ¹²⁵I-Tyr-C-peptide (0.33 pmol/ml = 1 ng/ml) was added. The mixture was incubated at 4° C for a further 24 h and the antibody-bound ¹²⁵I-Tyr-C-peptide was separated by addition of 1.6 ml 95% (v/v) ethanol, as described for glucagon [13].

The concentrations of insulin, C-peptide and proinsulin are given in pmol/ml. 1 µU of insulin

= 0.0062 pmol/ml (27 U/mg of MC human insulin), 1 pmol/ml of insulin = 6 ng/ml, 1 pmol/ml of C-peptide = 3 ng/ml, and 1 pmol/ml of proinsulin = 9 ng/ml. Due to the shortage of human proinsulin the number of standards as well as the number of recovery experiments were limited to the least necessary.

To extracts of serum from insulin treated diabetics whose IRI ranged 200–2000 μ U/ml, was added 100 μ l of S-AIS with a binding capacity of 0.1 U/ml. Whenever the IRI exceeded 2000 μ U/ml, the extracts were diluted prior to the addition of S-AIS.

Insulin binding to IgG was determined by the immunoelectrophoretic method of Christiansen [39].

Porcine and bovine proinsulin were determined as described in [14]. Recovery of proinsulin after the acid extraction was checked using circa 97% pure porcine and bovine proinsulin [14].

Serum samples from 24 non-diabetics (aged 15–65 years, mean: 37 years, % ideal body weight: 87–134, mean: 108) and 21 newly diagnosed diabetics, fasting and 1 h post 1.75 g/kg oral glucose were obtained from Dr. S. Munkgaard Rasmussen of Hvidøre Hospital (presently of Bispebjerg Hospital, Copenhagen). These diabetics had been divided into two groups: 11 maturity-onset diabetics (aged 24–72 years, mean age: 46 years, % ideal body weight: 85–179, mean: 118) and 10 insulin requiring diabetics (aged 17–70, mean age: 38 years, % ideal body weight: 81–142, mean: 100). None of these patients had ever received insulin and they had no insulin antibodies. Serum samples from 10 juvenile-type diabetics (aged 12–22, mean age: 18 years) treated with conventional insulin for more than one year, all having insulin antibodies, were obtained from Dr. J. Ludvigsson, Department of Paediatrics, University Hospital, Linköping, Sweden, and Dr. Beck Nielsen, Aarhus Amtshospital, Aarhus.

Results

Reactivity of Free and S-AIS-Bound Proinsulin

Figure 2 shows the reaction to antiserum M 1181 of human proinsulin in solution in the C-peptide assay as compared to C-peptide. Three further antisera were tested (M 1017, M 1180 and M 1187), and proinsulin exhibited in all cases much less (30–50%) reactivity than C-peptide. In contrast, S-AIS-bound proinsulin (Fig. 3A) showed approximately the same reactivity as C-peptide with the same antiserum (M 1181).

Fig. 2. Standard curves of proinsulin and C-peptide using antiserum M 1181

Fig. 3. A Standard curves of S-AIS bound proinsulin (Seph-AIS-proinsulin) and C-peptide. B Reproducibility of the 20 S-AIS proinsulin standard curves set up over a period of 12 months

Reproducibility of the Standard Curve, Detection Limit and Sensitivity

Figure 3B shows the reproducibility of the standard curve obtained with M 1181 over a period of 12 months using 6 different batches of 125 I-Tyr-C-peptide. Only those solutions containing 0.05–0.1 and 0.2 pmol/ml were included in the assays, but lately a solution containing 0.02 pmol/ml has also been in use with a view to improving the accuracy in the low range. The detection limit, defined as the smallest quantity of proinsulin that can be distinguished as significantly different from zero (= 2 SD), was

Fig. 4. Standard curves of human insulin and proinsulin in the IRI assay. Antiserum M 8309 was used

slightly below 0.01 pmol/ml; therefore all readings ≥ 0.01 pmol/ml were regarded as positive. In the range of 0–0.1 pmol, the % bound tracer decreased by about 19, corresponding to 1.9% per 0.01 pmol/ml. As two SD in the above-mentioned range were 1% or less, the sensitivity defined as two SD was less than 0.01. Removal of C-peptide was checked in each assay by including a sample containing 2 pmol/ml of C-peptide. This sample could not be distinguished from zero.

Recovery and Dilution of Samples

Addition of 0.02, 0.05 and 0.1 pmol/ml to normal sera gave a virtually 100% recovery. Dilution of serum containing 0.03–20 pmol/ml (normal sera 1 h post OGTT and sera from insulinoma patients) has given the expected values within the limits of the assay (e.g., 1:50 and 1:100 – 7.1 and 7.3 pmol/ml; 1:10 and 1:20 – 0.76 and 0.69; undiluted A: 0.09; 1:2 – 0.12; 1:4 – 0.10; 1:8 – 0.10, and undiluted B: 0.18; 1:2 – 0.20; 1:4 – 0.24, 1:8 – 0.18).

The recovery of proinsulin added to serum after acid extraction [12] was determined using porcine and bovine proinsulin. The recovery of both species varied from 80–88% (mean 85%), as determined by the porcine and bovine proinsulin radioimmunoassays, respectively, and it was assumed that the recovery of human proinsulin after extraction would be the same and the results were corrected accordingly.

Reactivity of Proinsulin in the Insulin Radioimmunoassay

In order to calculate the percentage of the IRI due to proinsulin, the reactivity of proinsulin was determined in the insulin radioimmunoassay. The reactivity of bovine proinsulin with anti-insulin serum M 8309 in the 0–0.25 pmol/ml range (0 – approx. 40 μ U/ml) has been shown to be similar (on a molar basis) to that of bovine insulin [16]. At higher concentrations, bovine proinsulin has increasingly lower reactivity. In the same assay, human proinsulin shows approximately 66% reactivity in the range 0–0.50 pmol/ml, that is to say, 0.50 pmol/ml of proinsulin reacts like 50 μ U/ml of insulin (Fig. 4). The percentage was then calculated as follows: the proinsulin concentration in pmol/ml was converted into μ U/ml by multiplying by 100 and this value was then expressed in per cent of the total IRI.

Degradation of Proinsulin in Serum

125 I-labelled bovine proinsulin was added to sera (two normal and two diabetic serum samples) and incubated at 37° C for 24 h. Addition of a specific, non-insulin-binding anti-proinsulin serum [17] revealed that 125 I-proinsulin was not degraded to any detectable degree. Serum samples stored in deep-freezers and rethawed several times have been found unchanged in analyses performed over several months.

Proinsulin, C-Peptide and IRI in Non-Diabetics and in Newly Diagnosed Diabetics

Table 1 shows the glucose, proinsulin, C-peptide and IRI levels in fasting sera and in sera drawn 1 h after 1.75 g of oral glucose per kg of ideal body-weight.

In the non-diabetic group, the IRI increased some 10-fold after glucose (from 0.047 to 0.453 pmol/ml, or from 7.7 to 67.7 μ U/ml) whereas the increase in the C-peptide and proinsulin was only about 5-fold.

In the group of maturity-onset diabetics, the increase in IRI was approx. 7-fold while C-peptide showed only a 3-fold increase and proinsulin doubled. There was a distinct deviation from the non-diabetics in the group of insulin requiring diabetics, whose IRI and C-peptide levels hardly doubled while the mean proinsulin value increased 3-fold.

Table 2 shows the proinsulin calculated as per cent of the IRI. In several of the samples from fasting subjects, the IRI and proinsulin were zero (hence the reduced number of subjects as compared to Table 1). It appears that proinsulin constituted on

Table 1. Blood glucose, serum IRI, C-peptide and proinsulin before and 1 h after oral glucose (1.75 g/kg) in non-diabetics and newly diagnosed diabetics

Group (No. of patients)		glucose mmol/l		IRI pmol/ml		C-peptide pmol/ml		proinsulin pmol/ml	
		basal	1 h	basal	1 h	basal	1 h	basal	1 h
Non-diabetics (24)	mean	4.64	7.84	0.048	0.453	0.38	1.67	0.009	0.053
	range	4.07–5.83	4.40–12.21	0–0.111	0.105–0.930	0.21–0.57	0.70–3.00	0–0.024	0.010–0.110
	SD	0.40	1.92	0.030	0.294	0.11	0.55	0.005	0.023
Maturity-onset-type diabetics (11)	mean	7.46	14.77	0.068	0.476	0.54	1.54	0.022	0.046
	range	5.28–12.65	12.15–21.61	0–0.173	0.111–1.972	0.18–1.00	0.90–3.90	0–0.103	0.028–0.099
	SD	2.84	3.43	0.059	0.575	0.31	0.94	0.0342	0.022
Insulin requiring diabetics (10)	mean	11.12	19.02	0.048	0.087	0.24	0.44	0.010	0.032
	range	7.64–17.16	13.20–26.84	0.012–0.087	0.019–0.099	0.07–0.41	0.08–0.90	0–0.028	0–0.083
	SD	3.52	4.63	0.023	0.029	0.12	0.30	0.009	0.007

average 20%, though with a wide range of variation in the fasting subjects in all three groups. This decreased to about 10% in the non-diabetics and maturity-onset diabetics, all of whom showed a considerable increase in IRI after glucose. In contrast, the insulin requiring diabetics, whose mean IRI increased by 6 μ U/ml, secreted a higher percentage of proinsulin. Only in one patient did the percent proinsulin remain the same after the glucose load.

Proinsulin in Insulin-Treated Juvenile Diabetics

Table 3 shows the IgG binding of insulin, total extractable IRI, C-peptide and proinsulin levels in sera of 10 fasting juvenile diabetics treated with conventional insulin for more than 1 year. All 10 patients had insulin antibodies and, consequently, fasting total IRI levels that were elevated as compared to those in normal subjects (52 normals had a mean fasting IRI level of 7.5 μ U/ml = 0.049 pmol/ml, ranging from 0 to 16, 1 SD = 4.4 μ U/ml). The C-peptide in three patients from this small group was within the normal range (52 normal subjects had a mean fasting C-peptide level of 0.35 pmol/ml, ranging from 0.18 to 0.57, SD = 0.10 pmol/ml). However, their human proinsulin levels were much higher than in normal persons (see Table 1).

Discussion

Methodology, Sources of Error

Since the proinsulin molecule contains both an insulin and a C-peptide moiety, proinsulin reacts in the radioimmunoassays both for insulin and for C-peptide. As serum contains insulin, C-peptide and proinsulin a specific assay for proinsulin includes a thorough separation procedure. So far, the assays were based on two completely different principles: (i) Separation according to molecular size by gel filtration followed by IRI determinations on the indi-

Table 2. Proinsulin, as per cent IRI in sera of non-diabetics and diabetics, fasting and 1 h after oral glucose (1.75 g/kg)

Group	Proinsulin as % IRI: mean (n) and range			
	basal		1 h	
Non-diabetics	19 (16)	6–34	8 (24)	3–20
Maturity-onset-type diabetics	23 (5)	11–43	11 (9)	5–24
Insulin requiring diabetics	16 (8)	7–26	31 (9)	10–100

Table 3. Insulin antibodies, total IRI, C-peptide and proinsulin in insulin-treated juvenile diabetics

Case (initials)	Age (years)	IgG binding mU/ml	Total IRI		C-peptide pmol/ml	Proinsulin pmol/ml
			μ U/ml	pmol/ml		
LP	20	1.044	422	2.78	0.08	0.150
VKA	20	5.655	1168	7.78	0.35	1.80
JLN	19	2.417	512	3.39	0.13	0.325
LJ	19	0.030	48	0.31	0.20	0.105
AUH	19	0.575	304	2.00	0.08	0.163
UH	22	0.175	64	0.42	0.04	0.355
PA	17	1.188	192	1.27	0.11	1.250
SL	12	3.251	640	4.24	0.05	0.072
CL	17	0.097	80	0.53	0.20	0.375
BH	15	0.508	99	0.65	0.12	0.193

vidual fractions [2, 3, 18]. (ii) Enzymatic degradation of insulin followed by determination of the non-degraded proinsulin by insulin radioimmunoassay [6, 19, 20]. The first method is unsuitable for routine analyses of a large number of samples. Furthermore it should be stressed that employing the insulin radioimmunoassay for measurement of proinsulin is only valid when its reactivity has been determined in that particular radioimmunoassay, as pointed out by [21], who found that proinsulin showed less reactivity in their radioimmunoassay

than insulin (about 30%). In the insulin assay used in this study, the reactivity of proinsulin was approx. 66% that of insulin and it gave linear dilutions in the range of 0–0.4 pmol/ml, in contrast to bovine proinsulin, whose reaction in the 0–0.75 pmol/ml range was virtually identical with insulin [16].

The enzymatic degradation method, which is quick and requires only small volumes of serum, seemed an attractive alternative to gel filtration. However, it recorded consistently higher fasting values [5, 7] (e.g., 70% proinsulin in normal women [22]) than those recorded by gel filtration. Lately, it has been demonstrated that the degradation of insulin is only partial and dependent on its concentration, and that proinsulin, too, is degraded to some degree [7]. Furthermore, the activity of the enzyme varied in different plasma samples [5]. In a recent paper published by the group who developed the method, the authors mention that PLM (proinsulin-like material) was determined only in plasma containing more than 30 μ U/ml of TIR (total immunoreactive insulin) due to the incomplete degradation of insulin in fasting plasma [37]. Hence all the earlier values of % proinsulin in fasting plasma obtained with this method may be regarded as erroneous. However, after OGTT, PLM was found to constitute 13% in normals and 16% in maturity-onset diabetics [38, 20], which is in fair agreement with the present results, although it should be mentioned that the standard used for PLM quantitation was porcine insulin, implying underestimation of proinsulin. The same applies to some of the results obtained with gel filtration [4].

The method presented here has several advantages over the other two:

- a) it is quick and simple, its sensitivity is about 0.01 pmol/ml, corresponding to 1 μ U of IRI/ml, the total sample requirement is no more than 1 ml of serum or plasma, and it is suitable for routine processing of a large number of samples;
- b) its specificity is ensured by the fact that proinsulin can bind to S-AIS whilst still binding antibodies to C-peptide;
- c) the separation step is a preliminary to the assays both for C-peptide and for proinsulin. One ml of serum plus S-AIS provides a supernatant in which C-peptide can be determined without interference from proinsulin, and a precipitate free of C-peptide for the determination of proinsulin;
- d) identical treatment of standard solutions of proinsulin and samples.

In agreement with the findings of [21], it was found that unbound proinsulin was less reactive with the antibody M 1181 than C-peptide. But, unexpectedly, proinsulin bound to S-AIS showed practically

the same reactivity as C-peptide (Figs. 2 and 3), which rendered the assay more sensitive. No intermediate forms of human proinsulin were available, but it is fair to assume that they would behave very much like proinsulin, as was the case with bovine and porcine intermediates [14].

No degradation of 125 I bovine proinsulin was observed in serum allowed to stand at 37° C for 24 h, nor did repeated freezing and thawing affect the values. Hence, proinsulin appears to behave as a stable molecule, like insulin, and its resistance to hepatic degradation seems to be better than that of insulin [23]. Long-term experiments have been initiated to establish the storage properties of proinsulin in serum at –18° C, +4° and +25° C.

Since the content of proinsulin in normal sera is 1–5% of C-peptide on a molar basis (see Table 1) it is of utmost importance that all C-peptide is removed from the S-AIS-bound proinsulin. With this in view, the precipitate is washed twice with 2 ml of buffer, and a control tube containing 2 pmol/ml of C-peptide is included in all assays, and it will not be different from the zero standard. The fact that C-peptide does not undergo any non-specific binding to glass or protein makes it no doubt easier to remove all of C-peptide.

Proinsulin Levels

Proinsulin constitutes about 3% of the IRI in the B-cell [14] and a similar ratio was found in portal venous blood during acute stimulation [18]. However, the mean half-disappearance time for proinsulin in 3 insulinoma patients was about four times longer than for insulin [23], due in part to the lower hepatic extraction of proinsulin [24]. This explains the much higher percentage of proinsulin found in peripheral blood. The values reported here: 0–0.024 pmol/ml or 6–34% of the IRI in fasting normal persons, are in good agreement with the findings of [4], [18] and [25]. After stimulation with oral glucose the concentration of proinsulin increased five-fold but, as IRI increased ten-fold, the mean percentage decreased to about 10 (range: 3–20%), as found by [38, 20] using the enzymatic method and by [4] who separated by gel filtration. [21] reported likewise a drop, though from 40 to 20%, in some four persons. The higher figure is partly due to the fact that the calculations were in regard to per cent weight as against per cent molar weight in this report.

Results published about proinsulin in diabetic patients are but few and these publications are especially sparse in regard to the insulin-requiring diabetic. Tables 1 and 2 show that the 9 maturity-onset diabetics (four of whom had ideal body weight

< 110%) had similar values and percentages of proinsulin as the normals. The four obese subjects had no higher mean proinsulin than the rest of the group. These results closely resemble the findings in a group of 7 patients with chemical diabetes reported by [26], as well as the results obtained 1 h after glucose [38]. These groups of diabetics had also higher than normal IRI both in the fasting state and 1 h after oral glucose. Thus, although the secretion of IRI was insufficient to maintain normoglycaemia and glucose tolerance, no striking deviations from the normal peripheral proinsulin levels were found in this type of diabetic. In contrast to the findings reported here and in [26], another study reports no increase in IRP (immunoreactive proinsulin) during OGTT either in 10 normal children or in 11 patients with chemical diabetes, despite massive increments in IRI [27].

There are few reports on serum proinsulin in newly diagnosed insulin-requiring diabetics, who show extremely little, if any, increase in IRI after OGTT at the time of diagnosis. In a group of nine pregnant severe diabetics the percentage of proinsulin determined after gel filtration was higher (19%) than in pregnant mild diabetics (7.6%) or normals (7.4%); the mean IRI increment in the severe diabetics was 27 μ U/ml 1 h after oral glucose in contrast to 103 and 160 μ U/ml in the two other groups [35]. In three diabetics having less than 25 μ U/ml 1 h after oral glucose, the proinsulin component constituted about 37% in the fasting state and was practically unchanged at 2 h, all values being clearly higher than in normal subjects [36]. In the insulin requiring newly diagnosed diabetics investigated in this paper the increase in IRI after oral glucose was extremely small (from a mean value of 0.048 to 0.087 pmol/ml). In the fasting state, the proinsulin levels (ranging 7–26% of the IRI) were similar to those in normals. After oral glucose, the mean percentage of proinsulin increased to 31 (ranging 10–100%) of the IRI. Hence, it appears that the already stimulated B-cell of the fasting insulin requiring diabetic (mean fasting glucose, 11.1 mmol/l) responds to further stimulation with oral glucose with a far higher proportion of the IRI as proinsulin.

Track et al. [28] have shown that degranulated rat islets secrete higher percentages of proinsulin than granulated islets, and state that if proinsulin is not packaged in secretory granules then only a small portion of the proinsulin will be converted to insulin. The findings reported here in regard to juvenile diabetics may thus be a consequence of the complete absence of granules [29] observed with the use of histological techniques.

In a group of 69 insulin treated juvenile diabetics, detectable C-peptide levels were found in 23% [30], the values varying from 0.04 to 0.60 pmol/ml and clearly indicating residual B-cell activity. The concentration of human proinsulin was very high in a small group of such juvenile insulin-treated diabetics, the values exceeding in most cases those of C-peptide (Table 3). Human proinsulin was also estimated by [31] using the C-peptide assay on gel filtered extracts of serum of 6 insulin treated patients and it was found to vary from 4 to 16 ng/ml, a range similar to the one reported here. Due to the presence of insulin antibodies in the sera of all patients except LJ (Table 3), the levels of proinsulin in these patients were most likely higher than in those whose sera contained no antibodies, because of the prolonged half-life of proinsulin-antibody complexes. Assuming that the affinity of human proinsulin to the antibodies equals that of human insulin, the half-life of human insulin will also be prolonged due to the antibodies. If the ratio of secretion of insulin to proinsulin was normal (100:approx. 3 [18]) then the total IRI would have amounted to 20 times the concentration of proinsulin, contrary to what has been established, leaving out of consideration the injected insulin which contributes to increased total IRI. However, when two diabetics with antibodies were injected with 125 I-labelled human proinsulin, the half-life of the tracer was found to be 31 h, in contrast to a half-life of 1.9 h of 125 I-insulin [31]. The latter figure was obtained from one patient only and, considering the great individual variation in antibody characteristics [34], one has to conclude that clarification of this problem has to await the more thorough studies of the kind introduced by [31].

Thus it seems likely that the B-cell continues to hypersecrete proinsulin in insulin requiring diabetics, possibly due to its inability to arrange the proinsulin in secretory granules for adequate proinsulin/insulin conversion [28], as suggested by [36]. Insulin antibodies promote degranulation [32, 33], thereby counteracting the granulation and conversion mechanisms.

Acknowledgements. I wish to thank Dr. J. Schlichtkrull for his constructive criticism and invaluable advice, and Mrs. Marianne Heyden, Mrs. Lisbet Pedersen and Mrs. Bente Hansen for their skilled technical assistance.

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Received: November 1, 1976, and in revised form: April 26, 1977

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