EFFECT OF STORAGE PROCEDURES ON INSULIN-ANTIINSULIN COMPLEXES IN PLASMA

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Plasma samples for hormonal assays are stored frozen and on many occasions, the samples have to be taken out more than once, for the sake of extraction and the assay of the biochemical parameters. It is well known that repeated freezing and thawing can produce denaturation or structural changes in the proteins which will affect their biological properties. It is not clear to what extent the insulin antibody titres and the free and total insulin concentrations would be affected by these procedures. In this study, an evaluation of the effect of repeated freezing and thawing on the plasma concentrations of these parameters is made.

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MATERIAL AND METHODS

Plasma samples obtained from eight insulin treated diabetic patients attending to Diabetes Research Centre and M.V. Hospital for Diabetes, Madras, were used for the study. All the patients were receiving conventional insulin therapy for periods ranging from 1 to 5 years. The fasting plasma samples were collected in EDTA, after overnight fast, and atleast 12 hours after the last insulin injection. An aliquot of the plasma sample were extracted prior to the storage. Each unextracted sample was aliquoted into two bottles (I and II). Free insulin was extracted with an equal volume of 30% polyethylene glycol[1] and total insulin was extracted according to the procedure of Nakagawa[2]. The extracted sample and untreated samples (in bottles

and II) were stored at −20 °C. Samples in bottle No. 1 were extracted on two subsequent days with repeated freezing and thawing. All the extracted samples were kept frozen till the assay. Samples in the II bottles were used for antibody estimation by the RIA procedure of Sebriakova and Little[3]. The free and total insulin were measured by RIA procedure of Herbert *et al*[4]. Polyethylene glycol was added in the assay of standards also. The assay of free insulin was repeated with the extract stored again after the first assay.

RESULTS

Table-1 shows the free insulin, total insulin and the antibody binding capacity in the different samples assayed. There was an increase in the free insulin concentration, when the samples were repeatedly frozen and thawed. The corresponding total insulin concentration decreased. The antibody binding capacity increased slightly with repeated freezing and thawing and the antibody index showed a reduction (Table-2). It was noted that the free insulin content of the polyethylene glycol extract was not affected by repeated freezing (16± 13 Vs 17± 13).

TABLE I

Effect of repeated freezing on free insulin and total insulin and binding capacity of the antibody

	Free Insulin uU/ml	Total Insulin uU/ml	Insulin AB BC %
Without Freezing	14.6± 12.8	754± 416	—
Freezing once	11.5± 10.8	91± 66	26± 18
Freezing twice	24± 20	65± 45	33± 19

TABLE II

Effect of repeated freezing on insulin antibody titer

Ab INDEX uU/ml	
Freezing once	Freezing Twice
< 500	< 500
75,150	59,821
2,37,448	2,29,406
11,228	3,658
7,476	2,878
7,818	3,057

DISCUSSION

It was observed from this study, that repeated freezing and thawing caused a dissociation of the antigen antibody complex, whereby the free insulin concentrations were increased. The total insulin concentrations showed a significant reduction, which probably indicated that the immunological binding of the extracted insulin was altered by repeated freezing. If this property had not been affected, we would have expected similar values for total insulin in all aliquots of the same sample. Polyethylene glycol extracts were unaffected by repeated freezing.

The binding capacity of the insulin antibodies also showed an increase with repeated freezing which might indicate that due to the dissociation of the antigen antibody complex, more free sites were available for binding with labelled insulin. This was in agreement with the increased concentration of free insulin obtained in sample I on two consecutive days. The antibody index was also found to be decreased in sample stored after thawing, which could probably be due to the denaturing effect of the procedure. In a recent report, Hanning *et al*[5] from U.K. have also demonstrated similar observations using plasma samples from insulin treated diabetic patients. It is shown by these authors that for accurate estimation of free insulin concentrations in insulin treated diabetic patients, immediate centrifugation of blood and extraction of insulin antibodies are necessary.

Our study also clearly indicates that, free and total insulin should be extracted from freshly collected samples and stored frozen till assayed. Samples repeatedly thawed are not suitable for the study. Even for the evaluation of antibody index, the sample should not be thawed more than once.

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