Heterogeneity of the Fourth International Standard for Insulin by Gel-Chromatography on Sephadex

The Fourth International Standard for Insulin was prepared with a total of 1460 g of insulin donated by 13 laboratories in 7 countries. This starting material contained 52% of bovine and 48% porcine insulin. The material was pooled and subjected to a series of purification processes to obtain finally 1060 g of crystals with the maximum degree of purity according to the methodology employed at that time.

The crystals were finally distributed into ampoules and the biological activity was determined in a collaborative international assay in terms of third international standard. On these bases the fourth international standard for insulin was established with a potency of 24.0 IU/mg[1]. Recently was stated that insulin is synthetized in the β-cells of the islets of Langerhans via a larger molecule of a single polypeptide chain, the proinsulin. This precursor undergoes limited intracellular proteolysis with release of insulin[2-5].

As a consequence of the structural analogy between insulin and part of the proinsulin molecule, in industrial processes of purification of the hormone, the 2 compounds crystallize together. Thus, crystals to be employed for pharmaceutical preparations of insulin contain some proinsulin as well as intermediate forms and insulin dimers[5]. These contaminants may be separated by gel chromatography[6]. These facts caused us to study the homogeneity of the fourth international standard for insulin by gel-chromatography in order to determine the possible presence and percentual incidence of those compounds.

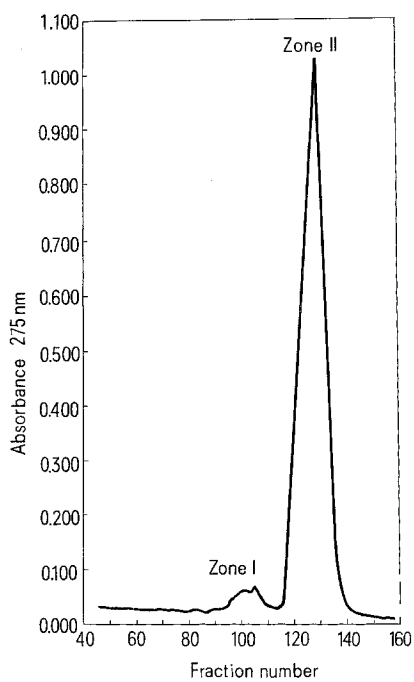
Material and methods. Crystals of the Fourth International Standard for Insulin were obtained from ampoules employed in the Instituto Nacional de Farmacología y Bromatología in biological assays for the control of pharmaceutical preparations of insulin.

The experiments were made by running in each 30 mg of standard dissolved in 10 ml of 1 *M* acetic acid. Fractionation was performed on a 2.4×100 cm column of Sephadex G-50 fine (Pharmacia Fine Chemicals–Uppsala) in 1 M acetic acid, at room temperature. The column was calibrated with blue dextran (Pharmacia-Uppsala-Lot. No. 3408) to determine void volume. 2.3 ml fractions (44 drops) were collected with a Warner Chilcott Lab. model 1205 instrument. Protein content of the fractions was determined at 275 nm in a Beckman DB Spectrophotometer with 1 cm light path.

Results. The elution diagrams obtained with the experiments correspond to the profile showed in the Figure. This demonstrates that, earlier than the appearance of the zone of insulin (II), there is a small zone of higher molecular weight (I). The elution position of this small zone corresponds to proinsulin and proinsulin-like proteins detected by STEINER et al.[5] in crystals of bovine insulin. As shown by the elution diagram, there is a significant difference between the homogeneity of both zones. While zone II (insulin as monomer at this pH) appears homogeneous, zone I shows heterogeneity. From a study of areas relationship zone I represents 4.5% of the total area.

Conclusions. In the present work it was found that the Fourth International Standard for Insulin is heterogenous by gel-chromatography. We think that, being of higher molecular weight, proteins of zone I substances with a low or without insulin-like activity[5], should be considered contaminants.

We suggest that crystals for the Fifth International Standard for Insulin should be subjected to gel-chromatography on Sephadex G-50 in 1 *M* acetic acid and further lyophilization of zone II. This method should give to the next International Standard higher homogeneity and potency.



Elution diagram on gel-chromatography of 30 mg of crystals from the Fourth International Standard for Insulin diluted in 10 ml 1 *M* acetic acid. Eluted from a 2.4×100 cm column of Sephadex G-50 fine in 1 *M* acetic acid at room temperature. $V_o \sim 48$ fractions. 2.3 ml fractions were collected. Zone II corresponds to insulin. Zone I corresponds to proteins of higher molecular weight.

Resumen. Se sometieron cristales del Cuarto Patrón Internacional de Insulina a gel-cromatografía por Sephadex. Se determinó que previa a la hormona, eluye una zona de proteínas de mayor peso molecular, cuya posición de elución corresponde a proinsulina y compuestos intermedios. Su área representa un 4.5% del área total. Se demuestra así la heterogeneidad de dichos cristales, y se sugiere que el Quinto Patrón sea sometido a gel-cromatografía durante su preparación. Este procedimiento daría a dicho Patrón una mayor homogeneidad y potencia.

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