THE DEVELOPMENT OF AN IN VITRO TECHNIQUE FOR MEASURING THE RATE OF SOLUTION OF INSULIN FROM I.Z.S. INJECTIONS

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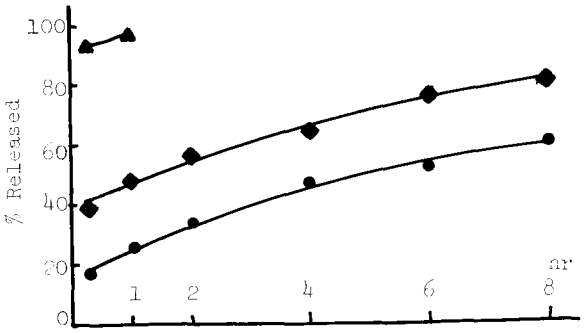
Insulin Zinc Suspensions contain insulin in a form which has a low solubility at neutral pH. The difference in action between the three forms (Ultralente, Lente and Semilente) depends on the surface area and relative amounts of the amorphous and crystalline forms. Izzo and others (1949, 1953) measured the rate of solution of various insulin preparations in aqueous media using a nephelometric assay technique and obtained a reasonable degree of correlation with blood sugar level curves. The object of this work was to develop a simple dissolution method for measuring the rate of solution from I.Z.S. preparations but utilising the native fluorescence of the insulin molecule (Teale, 1960).

A 8,000 units litre^{-1} solution of insulin in pH 6.8 buffer solution was examined spectrofluorimetrically with a double monochromator instrument (Fluoripoint, Baird Atomic Limited) and when excited at 275 nm a fluorescence (emission) peak was obtained at 298 nm. A rectilinear relationship was obtained between concentration and fluorescence in the range 0-400 units litre^{-1}. Experiments were carried out with amorphous Insulin Zinc to determine the saturation solubility; excess material was added to pH 6.8 buffer solution at 37°C, with continuous stirring for 8 hours and samples of filtrate were assayed after dilution against a 400 units litre^{-1} standard solution. The solubility of zinc insulin was determined to be 4,600 units litre^{-1} and, therefore, to obtain sink conditions 400 units litre^{-1} was selected as the maximum concentration for the dissolution test. The B.P./U.S.P. rotating basket apparatus was employed with 1 litre of pH 6.8 buffer solution at 37°C as the test medium and a stirrer speed of 120 r.p.m. Dissolution tests were carried out in duplicate on single batches of Semilente, Lente and Ultralente, 80 units ml^{-1} (Wellcome Foundation Limited). Five ml portions were removed from a freshly shaken vial and added to the dissolution medium via a volumetric pipette. Samples were taken at regular intervals with a syringe and filter holder fitted with a 1.2 µm nylon filter (Millipore NRWP02500). The samples were assayed without dilution as described above, after cooling to 25° ± 2°C, against a standard solution containing 400 units litre^{-1} of insulin. Percentage released values were calculated allowing for the volumes removed during sampling. The results are shown graphically in the figure.



Release of Insulin Zinc in pH 6.8 buffer solution from 80 unit ml^{-1} suspensions; Semilente ▲, Lente ◆, Ultralente ●.

The data obtained suggest that this method is capable of measuring under sink conditions the rate of solution of insulin from I.Z.S. preparations and of differentiating between the three different forms.

Izzo, J.L., Kunz, W. (1949) Proc. Am. Diab. Ass. 9, 225
Izzo, J.L., et al (1953) Diabetes 2, 358
Teale, F.W.J. (1960) Biochem J. 76, 381