THERMAL STABILITY OF INSULIN MADE FROM ZINC INSULIN CRYSTALS

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A commercial insulin preparation was stored for 2 years, its normal shelf-life, at temperatures from 2° to 36°. Full potency was retained for 2 years when the sample was kept at 2°. However, the activity of the insulin decreased with time as the storage temperature was increased. After one year at room temperature (20° to 25°) the activity of the insulin sample was 20 per cent below that stated on the label. Between 2° and 20° there was a consistent but not significant drop in activity. In the initial period the loss in potency may be essentially a reaction of zero order.

The stability of commercial insulin preparations is important, not only to the manufacturer, but also to the physician and in particular to the diabetic patient. Krogh and Hemmingsen[1] were the first to make a systematic study of the relation between temperature, time, and the destruction of amorphous insulin in a sterile aqueous solution. Their results suggested that the inactivation of insulin at a constant temperature followed a first order reaction, the rate of destruction at any moment being proportional to the concentration. In addition, it was reported that the optimal stability of an aqueous solution of insulin occurred between pH 2 and 4. Sahyun, Goodell and Nixon[2], working with a low-ash insulin preparation, revealed that the addition of Zn^{++} to the aqueous medium improved the stability significantly. However, Lens[3] could not confirm this stabilising effect of Zn^{++}, and reported that the stability of crystalline insulin in aqueous solution was unpredictable. Lens concluded that the inactivation was not due to hydrolysis of the insulin, but could be attributed to denaturation or heat precipitation which was usually followed by an irreversible oxidative process.

These investigations[1–3] were carried out for short periods at elevated temperatures. There is a scarcity of information about the stability of commercial insulin preparations when kept at temperatures encountered under ordinary storage conditions for the expected shelf-life of the product. Consequently an experiment was designed to determine the rate at which insulin made from zinc insulin crystals loses activity when stored for a period of two years at temperatures of 2° to 36°.

Materials and Methods

Insulin Toronto, made from zinc insulin crystals, Lot 942–1, 40 International Units per ml., was used in this study, and was prepared from Master Lot of zinc insulin crystals No 910 which had an activity of 26·2 International Units per mg. on a moisture-free basis. Insulin Toronto, Lot 942–1, contained 5·8 mg. of nitrogen and 0·22 mg. of zinc

for each 1000 International Units of insulin. The diluent consisted of 1·27 per cent (v/v) of glycerol and 0·15 per cent (w/v) of phenol in an aqueous medium which was adjusted to pH 3·0 with hydrochloric acid.

The material was filtered through sterilizing filter pads and, when known to be sterile, was filled into vials made from Type 3 Glass[4]. The vials were stoppered with rubber closures routinely employed in the Connaught Medical Research Laboratories for insulin preparations. This closure was secured by a three-piece aluminium seal.

A sufficient number of vials from Lot 942–1 were obtained and distributed to the collaborating laboratories where they were placed in storage at various temperatures. The stored vials were placed on their side allowing contact of the liquid with the rubber closure. In one of the laboratories the storage temperatures were 2 ± 1°, 11 ± 1°, 20 ± 1°, and 36 ± 1°; while in the other, the temperatures were 2 ± 1°, 14 ± 1°, 24 ± 1°, and 36 ± 1°. One of the 36°-incubators allowed a sudden rise in the temperature one week-end between the 18th and the 21st month of the test.

At intervals of 3 months during the 2-year study, samples were removed from storage and suitable dilutions for the bioassays were made with acid water containing glycerol and phenol. The dilutions from each of the samples were assayed for relative potency against dilutions of the material stored at 2°. To reduce the between assay variation, the three samples stored at temperatures above 2° were assayed at the same time against the reference standard in each individual assay. Several assays, sufficient to give standard errors of approximately ±6 per cent were combined to provide a weighted mean potency ratio for each of the samples. At the beginning of the experiment, as well as from time to time during the study, samples stored at 2°, the reference standard, were removed and assayed against the International Standard.

In both laboratories the insulin activities were determined using mice. The methods have been described elsewhere[5,6]. The individual potency ratios were estimated by means of the angular transformation method[7] in one of the laboratories, and by probits[8] in the other. Satisfactory agreement was found between the two procedures.

Results and Discussion

At the beginning of the experiment, the potency of Insulin Toronto, Lot 942–1, was determined by assaying it against the International Standard. At other times during the experiment similar assays were performed on vials removed from storage at 2°. The results of these assays are indicated in Figure 1. The assay values and their 95 per cent confidence limits are shown as a percentage of the estimate of potency found in each laboratory at the beginning of the experiment. No indication was given of a drop in insulin activity in the material stored at 2°.

The results of the comparative assays of the biological activity of the material in the vials stored at various temperatures against the activity of the sample kept at 2° for each 3-month period up to 2 years are given in Table I. The 95 per cent confidence limits are included in the Table.

TABLE I

PER CENT RELATIVE POTENCY OF INSULIN STORED AT VARIOUS TEMPERATURES

Temp. °	Storage time							
	3 months	6 months	9 months	12 months	15 months	18 months	21 months	24 months
11 ± 1	108 (92–128)	103 (91–117)	114 (101–129)	96 (81–113)	73 (64–84)	86 (76–98)	92 (83–104)	92 (83–103)
14 ± 1	98 (89–111)	100 (85–116)	98 (87–111)	97 (85–111)	93 (83–104)	89 (79–100)	92 (80–106)	91 (85–100)
20 ± 1	94 (80–111)	85 (75–97)	88 (78–99)	81 (69–96)	78 (68–89)	77 (68–88)	92 (81–103)	80 (72–89)
24 ± 1	102 (90–114)	87 (73–104)	92 (83–103)	79 (69–90)	74 (66–83)	74 (66–83)	73 (63–84)	74 (68–81)
36 ± 1 (Lab A)	82 (70–97)	70 (61–79)	67 (59–75)	60 (51–71)	52 (46–60)	51 (45–59)	56 (50–63)	53 (47–58)
36 ± 1 (Lab B)	97 (86–109)	87 (73–103)	78 (70–88)	56 (49–63)	50 (44–56)	47 (42–53)	28* (24–32)	22* (18–26)

* The 36°-incubator allowed a sudden rise in temperature during a week-end.

The results from both of the laboratories for the 36°-storage condition are shown separately.

The effect of the sudden rise in temperature of one of the 36°-incubators is clearly seen by the lower values indicated for the 21st and 24th months.

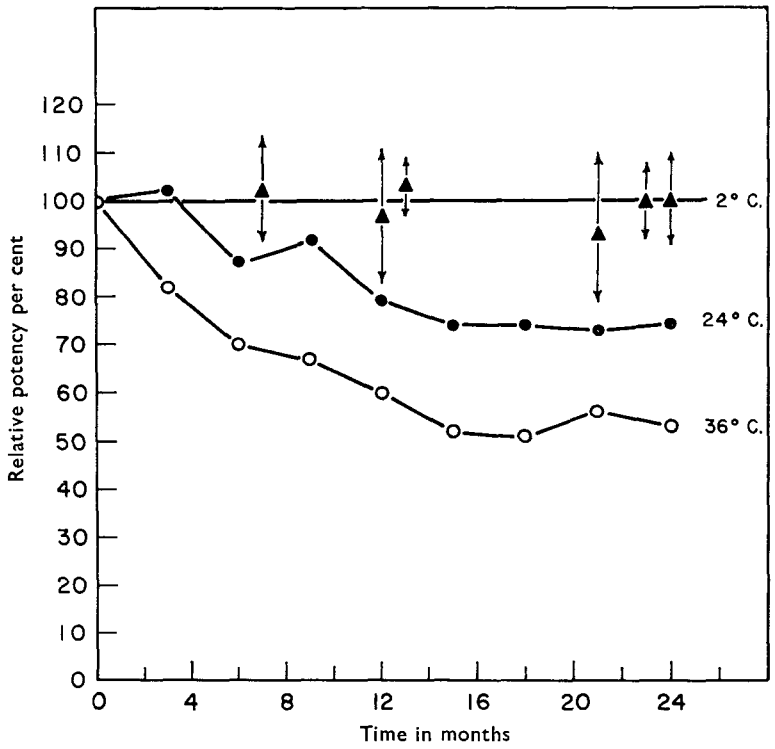

FIG. 1. The relative potency of insulin stored at 2°, 24° and 36° for a period of 24 months.

The relative potencies for two of the temperatures (24° and 36°) have been plotted in Figure 1.

The results of this stability study show that at room temperature (20°) and above, a significant drop in the potency of the commercial insulin preparation has occurred over the 2-year test period. At the other temperatures (11° and 14°) which have been defined as characteristic of a cold place,* the relative potencies do not differ from that of the material stored at 2° at the end of the 2-year period. It is worthy of note, however, that after twelve months of storage, the estimates of the relative potency are consistently below 100 per cent. This suggests that insulin should be stored in a refrigerator, and that the colder the refrigerator without actually freezing, the more stable the insulin.

Attention should be drawn to the relative potency at 11° for the 15th month period; 73 per cent with the 95 per cent confidence limits of 64 to 84 per cent. The significant loss in potency indicated by the assay at this time period was not confirmed at later periods. Apparently this finding can be attributed to between vial difference in stability.

Although the experiment was not primarily designed to investigate the kinetics of insulin inactivation in acid solution, some information is available from the data, concerning the relationship between the loss of biological activity and time, at the various temperatures.

Krogh and Hemmingsen[1] established the reaction to be of the first order. Examination of our data, excluding the experiment at 11° discussed in a previous paragraph, reveals that in certain cases deviation from the straight line between log relative potency and time occurs in excess of the estimated error in the determination of log potency. In other cases, even though such a straight line relationship is not definitely excluded, there is evidence of significant curvature.

An adequate description of the inactivation, according to our results, is given as a straight line between relative potency and time, for the first part of the inactivation, up to approximately 15 months. For this first period the reaction may be of zero order. It is however, followed by a marked levelling off of the rate of inactivation. These observations are in agreement with the work of Lens[3].

If the relationship had been more accurately determined by more precise bioassays and by limitation of the between vial variation, it is possible that they would have served to predict the stability of insulin in aqueous solution for various temperatures and for various periods of storage by the methods outlined by Garrett and Carper[9] and by Garrett[10,11].

Vials stored at 36° or below exhibited neither precipitation nor gel formation over the 2-year period.

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* Pharmacopoeia of the United States XV.

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