Effect of Storage Temperature on Stability of Commercial Insulin Preparations

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SUMMARY

The effects of storage on stability of six types of commercial insulins have been studied at 5°, 25°, 37°, and 50° C. for periods up to thirty-six months. Samples of insulin from thirty-two batches at concentrations of 40 and 80 U. per ml. were used.

Use of the radioimmunoassay made it practical to perform the large number of potency determinations required to obtain the data in the present report. These data show that no appreciable loss of potency occurred in any of the insulins during thirty months of storage at 5° C. At higher temperatures extent of potency loss was influenced by length of storage and type of insulin. In general, all modified insulins studied were more stable than Regular insulin. Under the same conditions Protamine Zinc and NPH insulins were more stable than the Lente insulins.

The biological reaction (duration of action) of the modified insulins which had been stored for twenty-four months at 5° C. were compared with freshly prepared samples. No significant changes in the response curves characteristic for each insulin type were observed.

Potency determination by the U.S.P. rabbit assay compared satisfactorily with data obtained by radioimmunoassay. DIABETES *17*:499-502, August, 1968.

It is generally believed that insulin deteriorates during storage. This is assumed to occur with Regular insulin and with any of the therapeutically useful modifications which are commercially available. However, except for the work of Stephenson and Romans[1] on Regular insulin, a recent search of the literature did not reveal quantitative data which could be used to define minimal and optimal conditions of storage. Such information is of particular importance to the diabetic, but physicians, pharmacists, and others who deal with this hormone would also find it valuable.

The data in the present paper permit a comparison of rates at which the various insulin preparations of one manufacturer (Eli Lilly and Company) change in potency during storage at several temperatures. Also noted are alterations in certain physical properties of these preparations.

In addition, the data attest to the utility and reliability of insulin potency determinations by radioimmunoassay.

MATERIALS

The study was done on Regular and modified insulin products which are manufactured by Eli Lilly and Company for distribution through regular commercial channels. The pharmaceutical forms represented are identified in table 1. Each of these forms is manufactured at concentrations of 40 and 80 U.S.P. U. per ml. Both concentrations were studied, but only data for the 40 U. per ml. preparations are reported.

In this paper insulin products are referred to by the title in common use (table 1).

The starting materials specified for all modified insulin products are bulk zinc insulin crystals. Manufacturing procedure requires that a quantity of these crystals, called a *master lot*, be assayed for potency by the U.S.P. rabbit procedure and assigned an identifying number. From the master lot modified insulin products are prepared in amounts known as *batches*. For the

TABLE 1

Titles identifying pharmaceutical forms of insulin

Title in common use	Registered title*	U.S.P. XVII title
1. Regular insulin	Regular Iletin	Insulin Injection
2. Lente insulin	Lente Iletin	Insulin Zinc Suspension
3. Semilente insulin	Semilente Iletin	Prompt Insulin Zinc Suspension
4. Ultralente insulin	Ultralente Iletin	Extended Insulin Zinc Suspension
5. NPH insulin	NPH Iletin	Isophane Insulin Suspension
6. Protamine Zinc insulin or PZI	Protamine, Zinc & Iletin	Protamine Zinc Insulin Suspension

*Iletin is the registered trademark of Eli Lilly and Company for insulin.

Presented at the Twenty-eighth Annual Meeting of the American Diabetes Association in San Francisco on June 16, 1968.

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present study samples were taken only from the first batch of each modified product manufactured from a master lot. All samples were taken at random after completion of the final packaging step.

METHODS

The effects of storage at 5°, 25°, 37°, and 50° C. were determined for periods up to thirty-six months. Temperatures were thermostatically controlled with variations not in excess of ±2° C. Throughout the period of storage all samples remained in their original packages until opened for analysis. At this time the required number of bottles was taken out of the cartons and the contents of each examined against a white background under strong light for evidence of change in color and viscosity. In addition, changes in the appearance and behavior of the precipitates of the modified insulins were noted. Following these observations a representative portion was withdrawn from each bottle for potency testing.

Potency was determined by the radioimmunoassay method of Grodsky and Forsham[2] according to the procedure of Probst, Brown, and Henry.[3]

Thirty-two batches are represented in the total samples assayed: seven each by Regular, Lente, NPH, and Protamine Zinc insulins, and two each by the remaining forms, Semilente and Ultralente. Over 2,000 individual radioimmunoassays were performed to obtain the data reported.

All results reported are the actual assay values or a simple average of them. Similar estimates of assay variation were found for Regular, Lente, NPH, and Protamine Zinc insulins stored at 5° C. These estimates were pooled to obtain the average estimate of assay variation. Insufficient data were obtained to warrant similar treatment of the remaining insulin types.

At selected intervals during the test period potency was also determined by the U.S.P. rabbit assay.[4] The number of batches sampled for these assays is given with the data.

All initial potencies are those of the master lot of bulk zinc insulin crystals which served as the starting material for the modified product. In conformity with required manufacturing practice these potencies were determined by the U.S.P. rabbit assay.

Biological reaction (duration of action) of Protamine Zinc insulin was estimated according to the U.S.P. procedure.[4] This method, with appropriate modifications, also was used to estimate duration of action of the remaining modified insulins.[5]

RESULTS

Potency and physical properties

The effect of storage on potency is shown in figure 1. Each graph gives the initial potency, i.e., at time of manufacture, and the potencies after various storage periods at the temperature indicated.

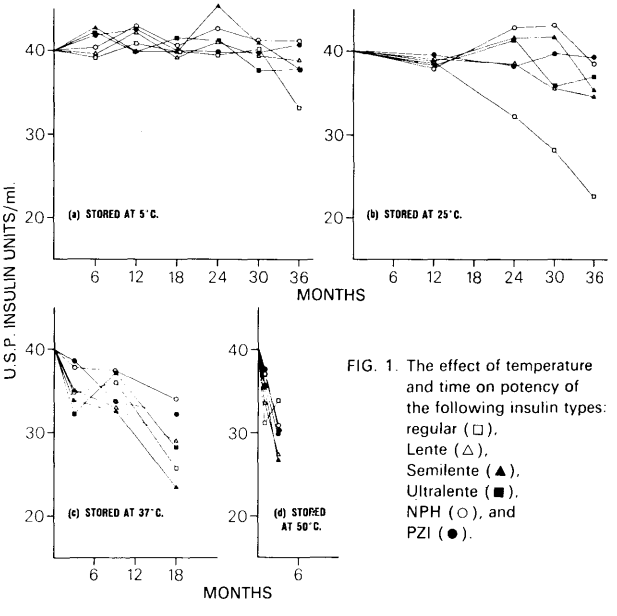

FIG. 1. The effect of temperature and time on potency of the following insulin types: regular (□), Lente (△), Semilente (▲), Ultralente (■), NPH (○), and PZI (●).

Storage at 5° C. In all modified insulin products initial potencies were maintained during thirty-six months of storage (figure 1a). No changes in physical properties were observed. As is normal, a thin layer of fine, easily dispersed precipitate was present. The potency of Regular insulin remained constant for thirty months but had declined by thirty-six months (figure 1a). The decrease was not associated with detectable change in physical properties.

Because the variation inherent in the radioimmunoassay method may not be apparent in the graphs, pooled estimates of variation are given in table 2 for four of the insulins stored at 5° C.

Storage at 25° C. The potency of Protamine Zinc insulin did not diminish during thirty-six months (figure 1b), although slight changes in the color of the supernate and settling of the suspension occurred after thirty months. The thin layer of precipitate was normal in appearance and behavior, i.e., easily dispersed by gentle agitation into the usual fine suspension.

The stability of NPH insulin was similar. After eighteen months the suspension had settled into a thin layer which broke up readily upon shaking. With longer periods of storage, i.e., eighteen to thirty-six

TABLE 2

Variation in radioimmunoassay data

Storage time at 5° C. (months)	Insulin type			
	PZI	Regular	NPH	Lente
6	41.9*±1.64†	39.1±2.47	40.3±2.29	39.6±1.80
12	42.6 ±1.64	40.9±2.38	42.8±2.40	42.1±1.87
18	40.0 ±1.70	39.8±2.47	40.6±2.40	39.2±2.07
24	39.8 ±1.64	39.5±2.47	42.7±2.29	41.1±1.80
30	39.8 ±1.78	40.2±2.38	41.2±2.40	39.4±1.80
36	40.8 ±1.70	33.3±2.47	41.2±2.54	38.8±1.96

*Potency is expressed in U./ml. and is the average of 4-7 batches assayed.

†Pooled estimates of variation were made as described in Methods.

months, the normally fine precipitate formed into aggregates which were increasingly difficult to disperse.

Lente, Semilente, and Ultralente insulins retained potency at 25° C. for twenty-four months. Storage for periods in excess of thirty months was required before losses in potency were significant. Color change in the supernate was noticed after twenty-four months. Also, some easily dispersed aggregates were present in the layer of precipitate which normally forms.

In contrast, Regular insulin followed a more precipitous rate of potency decline (figure 1b). Losses were nearly 10 per cent after only eighteen months, and color change occurred after twenty-four months.

Storage at 37° and 50° C. All insulin preparations lost potency and changed in physical properties at these temperatures. However, the rates at which potency was lost were not identical. In the absence of more definitive data, visual comparison of the graphs in figure 1c and 1d suggests that NPH and Protamine Zinc insulins were somewhat less sensitive to these temperatures than the remaining types.

An increase in viscosity visible to the naked eye occurred in Regular insulin after twenty-four months of storage at 37° C.

Biological reaction

The duration of action of each modified insulin after twenty-four months of storage at 5° C. and of a freshly prepared standard are compared in figure 2. The data show that in no case has storage under these conditions produced significant change in the response curves characteristic for each insulin type.

Similar effects on potency, physical properties, and biological reaction were observed with preparations at concentrations of 80 U. per ml.

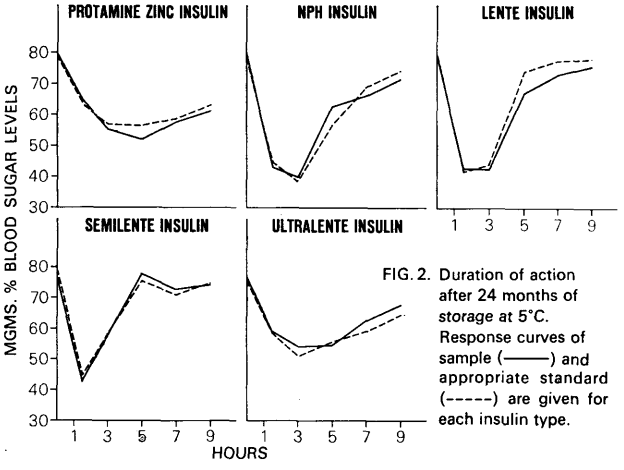

FIG. 2. Duration of action after 24 months of storage at 5°C. Response curves of sample (———) and appropriate standard (-----) are given for each insulin type.

Comparison of assay methods

Potency determinations were also done by the U.S.P. rabbit assay on insulins stored at 5° C. Although data are available for all insulins, to maintain clarity in figure 3 the results of only three are shown, along with comparable data obtained by radioimmunoassay. It is evident that there is satisfactory agreement in the values obtained by these two procedures.

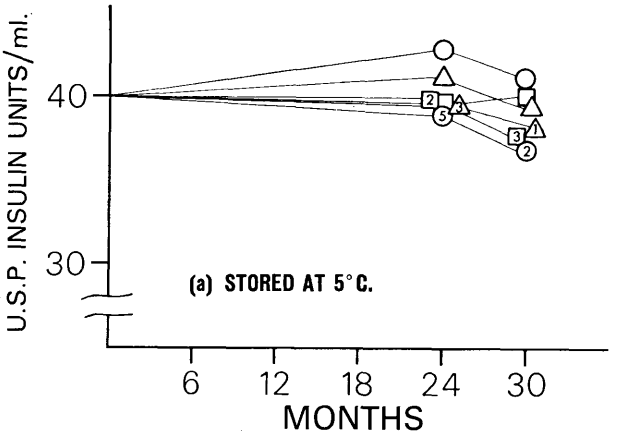

FIG. 3. Comparison of assay results obtained by the radioimmunological method (open symbols) and the U.S.P. rabbit procedure (symbols with numerals). Data are for the following insulin types: Regular (□), Lente (△), and NPH (○). The numerals within the symbols indicate the number of batches assayed.

DISCUSSION

According to the data presented, none of the commercially available insulin preparations of Eli Lilly and Company lost potency during thirty months of storage at 5° C., i.e., at refrigerator temperature. This exceeds the expiration period specified in U.S.P.[4] Furthermore,

the biological reaction of all modified insulins remained unchanged for twenty-four months at 5° C.

During the period of the present study no change in physical properties was seen in samples stored at 5° C. Settling of the suspension of modified insulins into a layer of fine white precipitate is expected. Furthermore, as the time of storage progresses, the volume occupied by the material which has settled diminishes. These events are normal and such precipitates are readily redispersed without loss of potency or change in duration of action in the preparation.

In contrast, precipitates which have formed into aggregates or which have become so tightly packed that they cannot be redispersed indicate that significant deterioration may have occurred.

By thirty-six months at 5° C. potency of Regular insulin diminished, whereas all modified insulins retained their initial activity. Studies for longer periods are in progress.

Both Protamine Zinc insulin and NPH insulin maintained original potency for thirty-six months at 25° C. (room temperature). Lente, Semilente, and Ultralente insulins were less stable than these insulins, but more stable than Regular insulin.

At higher temperatures, i.e., 37° (body temperature) and 50° C., all forms of insulin deteriorated. However, the rate of deterioration for Protamine Zinc insulin and NPH insulin at 37° C. is so low that brief exposure to this temperature may not result in significant potency loss. Exposure at 50° C. should be avoided.

The present data on stability of Regular insulin are essentially in agreement with those of Stephenson and Romans.[1]

Changes in color after storage at high temperature may indicate denaturation of the insulin molecule. It is reasonable, then, that development of color in solutions that were previously colorless is generally accompanied by a decrease in potency.

Viscosity change in solutions of insulin used for therapy occurs rarely unless the period of storage is long and the temperature high. The transformation of solution into gel is invariably accompanied by significant loss in potency.

The preceding discussion and conclusions are applicable to insulins prepared at both 40 and 80 U. per ml., the concentrations in common use.

The two quantitative methods used to obtain the data in the present report depend upon entirely different properties of the insulin molecule. In the rabbit assay the basis of measurement is the hormonal action that occurs in the whole animal. Briefly, the hypoglycemic effect of the U.S.P. reference standard insulin and insulin of unknown potency are compared in rabbits under rigidly defined conditions. The radioimmunoassay, an in vitro method, is based on an immunological reaction, i.e., the binding of insulin to specific antibody. By application of the well-established isotope dilution technic, the amount of insulin in the sample is determined by the per cent change it produces on the amount of radioactive insulin bound to the antibody.

The effects measured by each method, although different, can readily be expressed in units common to both, i.e., units of insulin per ml., a recognized expression of potency. The two methods are analytically equivalent, as data presented here and by others show.[3] Thus, it does not seem advisable to reserve the word potency for insulin measured by the rabbit procedure and to use some other term for insulin determined by radioimmunoassay. Such distinction not only serves no useful purpose but even may be a deterrent to establishing the radioimmunoassay as a U.S.P. method for potency determination.

In addition to its reliability, the radioimmunoassay is rapid. Also, it is especially useful when a large number of potency determinations is required, as in the present study in which the rabbit assay is impractical.

ACKNOWLEDGMENT

The authors are grateful to K. W. Wantland for his keen interest, advice, and encouragement, and to C. B. McClintock for valuable assistance.

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[5] Procedures developed by the Insulin Testing Department, Eli Lilly and Company.