

comparable to those obtainable with a laboratory system, regardless of the level of training of the instrument operator. However, the results were acceptable only when a properly collected blood sample was presented for analysis. The risk in the proliferation of this and similar systems is the assumption that sample collection is trivial, but a proper, professionally collected blood sample is essential. Instrumentation cannot compensate for a contaminated specimen.

We conclude that under proper conditions, the Reflotron is suitable for use in cholesterol screening programs. Its ease of operation, comparability with accepted systems, and, most importantly, its speed of analysis of whole-blood samples facilitate population studies. Its use is justified when all analytical aspects, especially sample collection, are controlled.

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Stability of Serum Fructosamine during Storage

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Stability of serum fructosamine during storage was evaluated in serum specimens obtained from 27 diabetic individuals. The samples were divided into six aliquots, which were stored at -20°C and -70°C for two, eight, and 16 months. The minor systematic differences between the six treatments contrasted with the considerable variation of individual specimens. The mean percentage changes in the six treatments ranged between -4.6% and 7.5% , whereas the changes in individual specimens ranged from -20% to 26.7% . Several factors evidently contribute to this variation, one being progressive *in vitro* glycation, especially at -20°C . Small changes in fructosamine concentrations between consecutively drawn specimens, determined after storage, evidently should be interpreted cautiously. Low temperatures, at least -70°C , are preferable to minimize pre-analytical variation during storage.

Determination of glycated proteins in serum by their ability to reduce nitroblue tetrazolium in alkaline medium (the "fructosamine" assay) has been described as a means of evaluating glycemic control during the preceding one to three weeks in diabetes mellitus (1-4). Such assay is merited by its reasonable costs and technical simplicity, although its feasibility may be compromised by the problems associated with its standardization, the effects of variation in the

reaction matrix, and the lack of specificity of the redox reaction involved (5-6). Furthermore, it is not possible to set the reaction parameters as precisely as required in all automatic analyzers (7). Fructosamine assay might be useful in follow-up studies where simple processing and subsequent analysis of large batches would be desirable. Such an approach assumes adequate stability of fructosamine-containing specimens during storage. The present study was designed to evaluate the effect of storage on results for serum fructosamine.

Materials and Methods

Serum fructosamine concentrations were determined in 27 serum specimens obtained from type I and type II diabetic individuals. Commercially available reagents (Fructosamine Test; Roche, Basle, Switzerland) were used. All serum specimens were then divided into six aliquots, three of which were stored at -20°C and three at -70°C . Samples stored at each of the two temperatures were analyzed two, eight, and 16 months later.

Specimens for the determination of blood glucose with the GA-1220 automatic glucose analyzer (DIC, Kagaku, Japan) were drawn at the same time as those for serum fructosamine. Two pools of sera were used for assessing the between-assay variation of the fructosamine method. Repeated measurements of analysis of variance were used for evaluating the effects of temperature and duration of storage. Blood-glucose concentrations were correlated with the changes of serum fructosamine found after storage at the various combinations of temperature and duration of storage.

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Results

Initial fructosamine concentrations in the specimens examined were 3.5 (SD 0.5, range 2.9 to 4.7) mmol/L. Blood glucose values were 9.1 (SD 3.9, range 1.6 to 16.6) mmol/L. Means of the percentage changes in fructosamine concentrations (i.e., the differences between initial concentrations and those found after storage as a percentage of the initial concentrations) ranged between -4.6% (found after storage for eight months at -70 °C) and 7.5% (eight months at -20 °C). The percentage changes of fructosamine in individual specimens ranged between -20% and +26.7% (Figure 1, Table 1). Analysis of variance revealed a significant interaction of temperature and duration of storage on the changes of fructosamine ($P < 0.001$), indicating significant differences between the subgroups, the changes varying, however, differently with duration of storage at the two temperatures (Figure 1).

Weak but statistically insignificant positive correlations were found between blood glucose concentrations and changes of fructosamine in specimens stored at -20 °C (Table 1). No such correlations were found in specimens stored at -70 °C.

The between-assay CV of the fructosamine method was 2.9% and 1.4% for serum fructosamine concentrations of 2.8 and 4.1 mmol/L, respectively ($n = 11$). Thus, at the diabetic concentration, 4.1 mmol/L, 0.17 mmol/L equaled the limit for an analytical error of three standard deviations attributable to the imprecision of the fructosamine assay. The absolute changes (increases or decreases found after storage in the six specimen batches) exceeded this limit in 44% to 70% of the specimens examined.

Discussion

Considerable variation of fructosamine concentrations was found after storage. Systematic differences between the

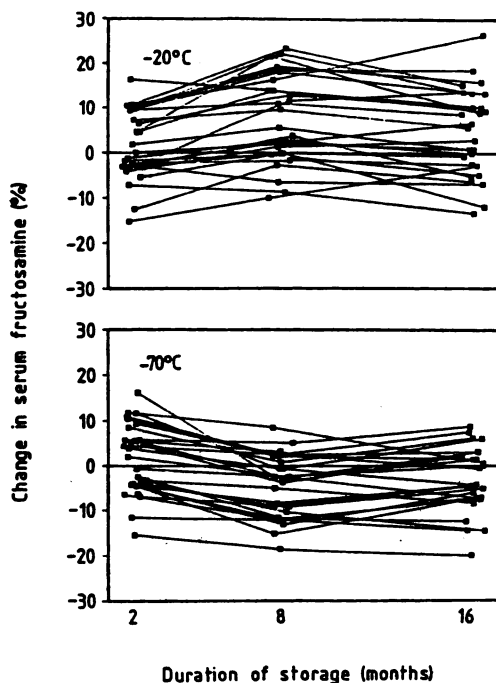


Fig. 1. Percentage differences between serum fructosamine values measured after storage for two, eight, and 16 months at -20 °C and -70 °C

Table 1. Variation in Serum Fructosamine during Storage, and Correlation of the Changes with Initial Blood Glucose ($n = 27$)

Storage temp, °C	Time, months	Change in fructosamine, % ^a	Coeff. of correl. with blood glucose (r)
-20	2	1.7 (-15.3 to 16.5)	0.25
-20	8	7.5 (-10.0 to 23.5)	0.19
-20	16	4.8 (-13.3 to 26.7)	0.30
-70	2	1.4 (-15.5 to 16.2)	0.17
-70	8	-4.6 (-18.5 to 8.5)	-0.03
-70	16	-3.3 (-20.0 to 8.7)	0.05

^a Values are means (ranges in parentheses).

six treatments were minor but statistically significant, whereas the variation for individual specimens was considerable, greater than what would be expected from the imprecision of the fructosamine assay per se. In three treatments the medians of the absolute changes were ≥ 0.2 mmol/L, which limit corresponds to a range of one standard deviation of fructosamine concentrations in a normal population determined in our previous study (3).

The systematic differences between the different time points in this study may in part be due to differences between the batches of reagents for fructosamine analysis. Such differences are difficult to trace by assessing precision with quality-control serum pools, because the pools may be as unstable as individual serum specimens during storage. However, heterogeneity of reagent batches did not contribute to the differences observed between the two temperatures because all analyses at individual time points were done in one session.

Progressive *in vitro* glycation of serum proteins may be one factor increasing fructosamine concentrations. This process can be slowed by storage at low temperatures. However, other factors are evidently involved in the variation, as evidenced by the numerous decreases and the weakness of the correlations between changes in fructosamine and blood glucose concentrations, especially in specimens stored at -70 °C.

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