Decrease in Plasma Glucose Concentration during Storage at \(-20^\circ\text{C}\)

Ottavio Glampietro, Renzo Navalesi, Giuseppe Buzzigoli, Claudio Boni, Luca Benzi

We measured glucose concentrations with the Beckman Glucose Analyzer, before and after storage at \(-20^\circ\text{C}\) for various intervals, in 228 plasma samples from oral glucose tolerance tests on 17 normal subjects and 21 patients with impaired carbohydrate tolerance, and also in 82 control samples collected daily, after overnight fast, from seven insulin-dependent diabetics. We found a significantly \((p<0.001)\) positive relation between storage interval and percentage decrease in glucose concentration. Evidently, the longer plasma is stored, the greater the decrease in glucose.

Additional Keyphrases: plasma glucose storage \cdot Beckman Glucose Analyzer \cdot diabetes mellitus

Blood sugar concentration begins to decrease immediately after the sample is collected, because of glycolytic action of erythrocytes and leukocytes (1, 2). Various methods currently are used to avoid this loss (2), many of which require prompt separation of the cellular component after blood withdrawal. This removal of blood cells allows measurements of glucose in plasma, which more accurately reflects the composition of extracellular fluids and which is independent of hematocrit (2). On the other hand, data are controversial concerning the stability of glucose in plasma stored for long periods (2). Here we report a significant progressive decrease in plasma glucose concentration during storage, even though the routinely recommended procedures for storing plasma samples were observed.

Materials and Methods

To measure glucose in plasma samples, we used a Beckman Glucose Analyzer. This Analyzer determines glucose by means of the oxygen rate method and incorporates an oxygen sensor and an electronic system that measures the rate of change in oxygen concentration when a sample is injected into an enzyme-reagent solution (2). In the reaction

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\beta-D-\text{glucose} + O_2 \rightarrow \text{glucose oxidase} \rightarrow \text{gluonic acid} + H_2O_2,
\]

oxygen is consumed at the same rate that glucose reacts. Thus at all times during the reaction, the rate of oxygen consumption is directly proportional to the concentration of glucose in the sample (4). The technique is free of interference from reducing substances or from agents used to prevent glycolysis or coagulation.

We measured glucose in all blood samples from a standard oral glucose-tolerance test in 17 subjects with a normal carbohydrate tolerance and in 21 with chemical diabetes according to the classification of Fajans and Conn (5). The glucose load consisted of 200 mL of a 500 g/L aqueous solution of glucose (100 g). Blood was sampled from the antecubital vein at 0, 30, 60, 90, 120, and 180 min after glucose administra-

![Fig. 1. Mean (and SD) for plasma glucose concentration after an oral glucose-tolerance test in normal subjects, before and after 4.76 months of sample storage. In Figures 1, 2, and 4, percent mean decreases for each test interval are represented, as are the levels of statistical significance.](image)

1 II Medical Clinic of the University of Pisa.
2 I Medical Pathology of the University of Pisa.
3 C.N.R. Clinical Physiology Laboratory of Pisa.

Address correspondence to the first author at: C.N.R. Clinical Physiology Laboratory, Via P. Savi, 8 56100 Pisa, Italy.

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normal blood concentrations of bilirubin, uric acid, and lipids. The instrument location in the laboratory was unchanged during the entire 21-month period of our assays. For plasma glucose so measured in our laboratory the between-assay CV, estimated on a reference pool, is 3.1%.

Figure 3 shows data on quality control of this plasma glucose assay, accumulated during the entire 21 months of our experimental study. During all 21 months we used the same batch of control sera (Fluinorm N no. 142; Behring Werke, F.R.G). Our quality-control system is based on an internal day-to-day control to check the precision and accuracy (absolute and relative), to assure stability of the assay performance according to Grafnetter and Valek (6).

We used Student's paired t-test to compare, for tolerance-test samples, glucose means at each test time, either with normal or chemical diabetic subjects, before and after sample storage.

We divided the 82 results for samples from insulin-dependent diabetics on the basis of their concentration into five groups: <1 g/L (15 values), 1 to 2 g/L (seven values), 2 to 3 g/L (26 values), 3 to 4 g/L (17 values), and 4 to 5 g/L (17 values). We also compared the glucose means for each group before and after storage, by the same statistical test (Figure 4). Lastly, we correlated for all 310 plasma samples the percentage decrease in glucose with storage duration, to see how glucose loss was related to storage interval (Figure 5).

Results

For the oral glucose-tolerance test in normal subjects, we found a significant difference between mean glucose at the zero (p < 0.05) and 120-min (p < 0.001) intervals only. For the
oral glucose-tolerance test in chemical diabetics and the five
groups of glucose concentrations in insulin-dependent dia-
abetics, we found a highly significant (p < 0.001, almost always)
difference at all the experimental points. Finally, we found
a significantly (p < 0.001) positive relation between storage
duration and decrease in glucose concentration.

Discussion
The changes in plasma glucose values after sample collec-
tion may be considered typical of many compounds of bi-
ological interest (1). Prompt separation of the plasma after
blood sampling (2) avoids glucose degradation owing to gly-
colytic action of erythrocytes and leukocytes. Alternatively,
inhibitors of the glycolytic enzymes (NaF, KF) in combination
with thymol (to prevent bacterial growth) (2) may be used.
These precautions should assure good stability of glucose in
plasma and allow reliable reproducibility of glucose mea-
surements, even after a long time, but our results suggest that
this surmise is incorrect. In fact, we observed a significant and
progressive linear decrease in mean glucose values with storage
interval. Our use of the percentage decrease instead of the
absolute decrease made it possible to correlate duration of
storage with decreases from initial plasma glucose values, even
when these initial values different greatly.

Possible factors leading to our experimental results might
include bacterial contamination of plasma samples. Even
though the patients' samples, after the first glucose assay, were
promptly stored at -20 °C in air-tight containers, we did not
use thymol in them. On the other hand, it is known that thy-
mol may constitute a problem for some glucose assay methods
(2). Lastly, there is some evidence that the higher the glucose
concentration, the more defined the trend to decrease seems
to be. This suggestion derives from the observation that, in
normal subjects, the mean decreases in glucose values (except
the basal and the 120-min points) were insignificant, whereas
in chemical diabetics and in insulin-dependent patients,
groups to which the glucose concentrations were two-or
two-three-fold higher than normal, highly significant mean dif-
ferences (always near to 10%) were found between the two
following glucose measurements. Our data confirm that
long-term stability of glucose in plasma samples, either from
patients with a normal or mild carbohydrate intolerance as
in fasting severely hyperglycemic subjects (as our insulin-
dependent diabetics), even after prompt storage at -20 °C
should not be taken for granted.

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