Plasma Protein Glycation As Measured by Fructosamine Assay

Andrea Mosca,1 Angelo Carenini,2 Francesco Zoppì,4 Assunta Carpinelli,2 Giuseppe Bantli,3 Ferruccio Carlotti,3 Pierangelo Bonini,3 and Guido Pozza1

The fructosamine test for assessing control of glucose in blood has been extensively evaluated, but some questions remain regarding its validity. From the analytical and clinical evaluation we present here, we conclude that: (a) the test is sensitive to variations in the composition of the sample protein; (b) the fructosamine reaction is almost completely unaffected by labile fractions; (c) the concentrations of fructosamine correlate well with the degree of glycation of total serum proteins, especially with glycated albumins and glycated immunoglobulins, as determined by affinity chromatography; (d) the correlation with glycated hemoglobin (Hb A1c), measured as the stable fraction, is very poor, in diabetics treated with insulin (r = 0.37), or with oral hypoglycemic agents (r = -0.39); and (e) fructosamine and Hb A1c are, in fact, expressions of different periods of metabolic control and therefore have different clinical meanings.

Additional Keyphrases: monitoring diabetes · glycated proteins · glycated hemoglobin

Measurement of the extent of glycation of serum proteins, as determined by the reduction of alkaline Nitro Blue tetrazolium salts (the fructosamine test), has been proposed as a way to estimate glycemic control in diabetic patients (1). Compared with the methods for glycated hemoglobin (2) or glycated albumin in plasma (3), this method is less time-consuming, more practicable, and more suitable for use with automatic analyzers (4–7). Moreover, like the assay of glycated plasma proteins, the fructosamine test can be interpreted as an integrated glycemic index—i.e., reflecting the glycemic control during the two or three weeks preceding the blood analysis—and has been used in some specific clinical cases, such as screening for diabetes mellitus (8), diabetic pregnancy (9), type II (non-insulin-dependent) diabetic patients, during change of therapy (10), and type I (insulin-dependent) diabetic patients in multiple within-day blood analyses (11).

Despite such a large body of information, however, some points remain to be studied in more detail, such as the following:

• Which individual plasma proteins contribute significantly to the fructosamine test?
• Is there a linear correlation between the concentration of fructosamine and total proteins in plasma?
• Is the fructosamine test sensitive to a labile fraction, as is the assay of glycated hemoglobins (Hb A1c) (12)?
• What is the reliability of this new test for monitoring diabetic patients in comparison with the older Hb A1c assay?

To answer these questions, we conducted the experimental and clinical evaluations reported here.

1 Dipartimento di Scienze e Tecnologie Biomediche, Università degli Studi di Milano; 2 C.N.R., Centro Studi di Fisiologia del Lavoro Muscolare; and 3 Istituto Scientifico S. Raffaele, Via Olgettina 60, 20132 Milano, Italy.
4 Laboratorio di Biochimica Clinica ed Ematologia, Ospedale Niguarda Ca’ Granda, 20162 Milano, Italy.
Received December 10, 1986; accepted April 7, 1987.

Materials and Methods

Subjects

In this study we evaluated 87 diabetic patients, 20 of whom (11 female and nine males, ages 13 to 58 years) had type I (insulin-dependent) diabetes mellitus, and 25 of whom (18 women and seven men, ages 39 to 80 years) were originally type II (non-insulin-dependent) diabetic patients but subsequently entered insulin treatment because of failure of therapy with oral hypoglycemic agents or because of long-term diabetic complications. These 45 patients were all hospitalized in our clinical division. The remaining 42 subjects (21 women and 21 men, ages 35 to 80 years) were selected type II diabetic patients being treated only with oral hypoglycemic agents. They were without diabetic sequelae as detected by fundus oculi, electrocardiogram, and 24-h urinary albumin excretion, and had no disorders of protein metabolism detectable by electrophoresis of serum proteins. The mean duration of these patients’ diabetes was six years (range: 0.2–10 years).

Blood specimens were collected from the antecubital vein into Vacutainer Tubes (Becton Dickinson, Meylan, France) for serum separation. The serum was separated within 2 h and stored at 4 °C or stored frozen at −20 °C until assay.

Measurement of Protein Glycation

Fructosamine test. Serum fructosamine was analyzed with a centrifugal analyzer (IL Monarch; Instrumentation Laboratory, Milano, Italy) by the procedure of Johnson et al. (1), based on the ability of ketoamines (fructosamines) to reduce the Nitro Blue tetrazolium salts in alkaline solution. The volume settings were as follows: sample 15 μL, sample diluent 10 μL, reagent diluent 10 μL, reagent 150 μL. The temperature was 37 °C, the wavelength 520 nm; readings were taken at 600 and 900 s. We calibrated results by using the calibrator (glycated bovine serum albumin) supplied by Roche Diagnostica, Milano, Italy. For diluted samples (protein eluates from Sephadex G25 columns) we doubled the sample volume, to increase the sensitivity.

The within-day precision (CV) for duplicate analysis of 60 serum samples, was 0.82%. The between-day precision for quadruple analysis of three different lyophilized control sera, reconstituted on six different days, was 1.11%.

The stability of fructosamine concentration in undiluted serum samples was tested by analyzing, in duplicate, two samples, from normal subjects, stored at different temperatures. The following data were obtained [mmol/L, mean (SD)]: day 0: 2.34 (0.20); day 6 (room temperature): 2.38 (0.18); day 15 (4 °C): 2.30 (0.22); day 15 (−20 °C): 2.39 (0.15). We did not test longer periods, but longer stability at −20 °C (three months) has been reported (4).

The normal reference values (mmol/L) for fructosamine, determined by analyzing samples from apparently healthy subjects (15 men and 23 women, ages 21–73), were: 2.40, SD 0.24, range 1.89–2.91.

Thiobarbituric acid test. The concentration of nonenzymatically glycated proteins in serum samples incubated with
glucose (see below) was assayed, after gel filtration, by a modified thiobarbituric acid method (14).

Affinity chromatography. We assayed the degree of glycation of serum total proteins and of some individual constituents, albumin, and IgG by affinity chromatography, as follows. For the serum samples we used the affinity-chromatographic analysis with "Glyco Gel" columns (Pierce Chemical Co., Rockford, IL), performed according to Rendell et al. (15). Later, we estimated the total protein concentration of the separated fractions (bound and non-bound), by the Coomassie Blue method (16) with a commercial protein assay kit (Bio-Rad Laboratories, Segrate, Milano). Albumin and IgG were measured as described elsewhere (17).

Glycated hemoglobins. The concentration of glycated hemoglobins (as total Hb A1 and "stable" Hb A1c) was assayed by minicolumn chromatography as previously reported (2, 13), at 23 °C.

Glucose Incubation Experiments

We incubated serum and whole blood with D-glucose by injecting a freshly prepared 200 g/L glucose solution (e.g., 40 μL) into the sample. Use of such a concentrated solution minimized dilution of the sample to <2%. We also incubated control samples injected with the same volumes of isotonic saline.

In some experiments, after the incubation, we removed the excess glucose by filtering the sample (1.0 mL) on a 1.3 × 25 cm column of Sephadex G25 (Pharmacia, Uppsala, Sweden) equilibrated with a solution of 40 mmol of NaH₂PO₄ and 128 mmol of NaCl per liter (pH 7.4). We then assayed the protein eluate by the thiobarbituric acid assays after adjusting the total protein concentration to about 10 (±0.5) g/L.

We determined blood glucose by the hexokinase-glucose-6-phosphate dehydrogenase method adapted to an automated analyzer (Hitachi 705; Boehringer Biochemia, Milano, Italy), using reagents supplied by the manufacturer.

Other Reagents

Total immunoglobulins were purified by precipitation with ammonium sulfate as described previously (18). The precipitate was then redissolved with distilled water and analyzed by routine electrophoresis to confirm its purity before the assay.

Human haptoglobin (type 1-1) was purified and lyophilized as previously described (19). Human albumin, transferrin, and α₂-macroglobulin were obtained as crystalline materials from Behring Institute, Milano, Italy.

Statistical Methods

The linear regression analysis was computed as the principal standardized component by the method proposed by Feldmann et al. (20). We used Student's t-test to evaluate differences between groups of data.

Results

Influence of Variations in Total Protein Concentration on Fructosamine Concentration in Serum

We studied this in vitro by analyzing samples with different total protein contents. A serum sample obtained from a normal subject was concentrated by ultrafiltration in a Centricon-10 apparatus (Amicon Co., Danvers, MA) to about 100 g/L. We serially diluted the concentrated serum by mixing it with increasing volumes of the ultrafiltrate, and determined total protein and fructosamine. The results are shown in Figure 1.

It is clear that fructosamine concentration is linearly correlated with total protein concentration. From the slope of the regression equation we calculated that, in the protein concentration range studied, this is a 1.35% increase in fructosamine for every gram per liter increase in total protein concentration. This is in agreement with the data of Van Diejen-Visser et al. (21), who reported a decrease of 23 μmol of fructosamine per liter per gram of serum albumin, or about a 1% decrease per gram of albumin.

Secondly, we studied the possible interference of specific serum proteins on the fructosamine assay by measuring fructosamine concentrations in 10 g/L solutions of purified proteins. The results show that the fructosamine test is sensitive to the following proteins (percentages are percent positive interference): albumin (100%), total Ig (76.2%), transferrin (38.1%), haptoglobin (28.6%), α₂-macroglobulin (14.3%). Thus albumin is not the only plasma protein that reacts positively in the fructosamine test; a greater interference can be expected from the immunoglobulins.

Finally, we report here the results of fructosamine assays of samples from subjects having different forms of alloxulins. We studied five subjects, four being heterozygous carriers of the following albumin variants (22): Catania, Reading, Sondrio, and Venezia; the fifth was a carrier of proalbumin Lille. All the fructosamine concentrations measured in these subjects fell within the normal range.

Sensitivity to the Presence of Labile Fractions

It is well known that the process of non-enzymatic glycation is common to several proteins and that the reactions involved are just a part of a more complex set of reactions, such as those responsible for the browning of proteins during aging (23). The first reaction—i.e., formation of the Schiff base between glucose and protein—is easily reversible and the adduct (aldimine), if not eliminated during the assay, can be responsible for rapid changes in glycated protein concentrations, as for some well-known cases concerning glycated hemoglobins (12).

To verify the sensitivity of the fructosamine test to the presence of labile fractions, we performed some determina-

![Fig. 1. Concentration of total protein and results of fructosamine assay, as measured in aliquots of the same serum specimen treated by ultrafiltration and dilution. Fructosamine concentrations are expressed as a percentage of that in the original specimen (with total protein 85 g/L).](image)
tions by using "in vivo" and "in vitro" experiments.

In a first experiment we wanted to see if the fructosamine test could be influenced by rapid glycemic changes, such as those occurring during an oral glucose tolerance test. The measurements were performed on six patients coming to the hospital for a control visit and showed no apparent changes of fructosamine 120 min after glucose administration [glucose, g/L, mean (SD)]; time 0: 1.08 (0.20); 30 min: 1.66 (0.43); 60 min: 1.60 (0.60); 90 min: 1.46 (0.85); 120 min: 1.38 (0.53); 180 min: 0.96 (0.47); fructosamine, mmol/L: time 0: 2.33 (0.42); 120 min: 2.29 (0.35); average change (±1 SD): ±0.05 (0.09).

Another experiment tested whether fructosamine concentrations could be altered by other glycemic fluctuations, such as those occurring subsequent to a standard meal, in a group of hospitalized type I diabetic patients. In this case also, the test seems to be insensitive to abrupt glucose changes, as for the case of Hb A1c (Table 1). In fact, the differences in glucose, Hb A1c, and fructosamine concentrations before and after a meal are not significant (i.e., P > 0.05).

For the "in vitro" experiments, we incubated a blood sample at 37 °C for up to three days and measured blood glucose, fructosamine, Hb A1c (total), and Hb A1c (stable fraction), obtaining the data reported in Figure 2. In this period of time, the fructosamine and HbA1c are essentially stable, while Hb A1c appears to oscillate like glucose.

In order to have a better evaluation of fructosamine neo-synthesis, we decided to perform a similar incubation only of serum, and to measure, simultaneously, the degree of protein glycation by the fructosamine and thiobarbituric acid tests, the latter being specific for the ketoamine form. Because glucose interferes with the latter test, the specimens were gel filtered before the analysis, and total protein concentration was adjusted to 10 g/L. The results obtained are presented in Figure 3. In this experiment, with respect to that of Figure 2, a significant increase in serum fructosamine was evident after incubation of the sample with glucose (curve A). This is attributable to the higher glucose concentration in the medium during the four days of the latter experiment.

Comparing curves B and C, we can conclude that the increased fructosamine concentrations measured in the gel-filtered samples is mainly due to formation of ketoamine compounds, because the two curves have a nearly identical slope.

Of course, the basal fructosamine concentrations assayed in the original serum sample (curve A) and in that obtained after gel filtration (curve B) are very different, because of the dilution of the sample during gel filtration. However, the relative increase of fructosamine in serum during the first two days of the experiment (from 1.84 to 2.87 mmol/L, i.e., +56.0%) is much greater than that measured on the sample after gel filtration (from 0.49 to 0.57 mmol/L, i.e., +16.3%). This seems to indicate that part of the adduct between glucose and proteins is lost during gel filtration.

At the end of the incubation the increases with respect to the basal levels were (%): +61.4% (curve A) and +40.8 (curve B).

From these data we conclude that a significant amount of the product of the reaction between serum proteins and glucose was formed in this experiment during the first days of incubation, as labile form, which can be rapidly removed by gel filtration. We estimate that, at the end of the experiment, the amount of the labile adduct in the undiluted serum is about a third of the total neo-synthesized products.

Table 1. Concentrations of Blood Glucose, Hb A1c, and Fructosamine in Six Hospitalized Insulin-Dependent Diabetic Patients, before and after a Standard Meal

<table>
<thead>
<tr>
<th>Glucose, g/L</th>
<th>HbA1c, %</th>
<th>Fructosamine, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre Post Pre Post</td>
<td>Pre Post</td>
<td></td>
</tr>
<tr>
<td>1.92 2.27 10.97 11.03</td>
<td>3.99 3.98</td>
<td></td>
</tr>
<tr>
<td>0.72 0.82 10.07 8.99</td>
<td>3.38 3.22</td>
<td></td>
</tr>
<tr>
<td>1.98 2.68 5.68 6.31</td>
<td>1.90 1.90</td>
<td></td>
</tr>
<tr>
<td>1.42 1.37 6.33 6.20</td>
<td>2.46 2.32</td>
<td></td>
</tr>
<tr>
<td>1.52 0.75 8.58 9.30</td>
<td>3.45 3.65</td>
<td></td>
</tr>
<tr>
<td>0.59 0.68 8.43 8.08</td>
<td>2.85 2.95</td>
<td></td>
</tr>
<tr>
<td>Mean SD</td>
<td>1.36 1.43 3.84 3.82</td>
<td>3.01 2.98</td>
</tr>
<tr>
<td>0.59 0.86 2.05 1.87</td>
<td>0.75 0.75</td>
<td></td>
</tr>
</tbody>
</table>

*Values are mean of two determinations.

Fig. 3. Fructosamine (mmol/L) before (A) and after (B) gel filtration of a serum sample incubated with 6 g/L glucose solution at 37 °C: C: fructose equivalents, μmol/L, in the gel-filtered samples as measured by the thiobarbituric acid test; D: glucose, g/L.
Fructosamine Assay of Quality Control Sera

During our experience with this new test, we noticed that it is difficult to find quality-control sera with normal fructosamine values. In Table 2 we report the fructosamine analysis on some of the most popular control materials. As can be seen, only three of 15 can be used as control materials for the normal range. It is likely that some of these sera is present, or has been added, because high fructosamine concentrations are also found when fructosamine is low.

Fructosamine in Diabetic Patients

The mean fructosamine concentration measured in 45 insulin-treated diabetics was 3.21 (SD 0.42) mmol/L. This value differs significantly from that found in the normal group (P < 0.001). Fructosamine values correlated with fasting blood glucose (r = 0.38; P < 0.01) but not with tryglycerides, total cholesterol, or high-density-lipoprotein cholesterol. Moreover, the fructosamine concentrations were not affected by sex, age, or duration of diabetes.

A low correlation of fructosamine with Hb A1c was found in this group of patients (Figure 4, line I), while no correlation of Hb A1c with fasting blood glucose was evident (r = 0.27; P = ns).

In type II diabetics, without complications, the mean fructosamine concentration was 3.67 (SD 0.62) mmol/L, significantly higher than the normal value (P < 0.001). Significant correlation was also found with fasting or postprandial glucose, and their mean (r = 0.56, P = 0.001; r = 0.48, P = 0.01; r = 0.61, P = 0.001, respectively). For comparison, we also evaluated the correlation of Hb A1c with the same parameters (r = 0.59, P = 0.001; r = 0.45, P = 0.01; r = 0.75, P = 0.001).

As reported for the previous group of patients, the correlation between fructosamine and Hb A1c is poor in these subjects also (Figure 4, line H).

A comparison of values for basal glucose, fructosamine, and Hb A1c, in mixed-type diabetics, all having complications and all treated with insulin (group I, n = 45), with type II diabetics, treated only with oral hypoglycemic agents (group II, n = 42), gave the following data (group I vs group II): fructosamine (mmol/L): 3.21 ± 0.42 vs 3.67 ± 0.68 (P < 0.001); Hb A1c (%): 8.10 ± 1.99 vs 7.01 ± 1.55 (P < 0.01).

Although there is no difference between the two groups with respect to their glucose concentrations, fructosamine and Hb A1c seem to be different. In fact, whereas fructosamine is significantly lower in group I, Hb A1c is significantly higher.

Finally, we evaluated the correlation between concentrations of fructosamine and of glycated serum proteins, as measured by affinity chromatography. The study was carried out with 60 mixed-type diabetic patients; the results are reported in Figure 5. A strong correlation was found (Figure 5A: y = 0.927x + 1.077; r = 0.923; Sxy = 0.23), mainly attributable to the glycated albumin fraction (Figure 5B: y = 1.466x + 1.446; r = 0.901; Sxy = 0.26) but also, to a significant extent, to the glycated IgG (Figure 5C: y = 1.142x + 1.143; r = 0.702; Sxy = 0.45).

Discussion

From the results obtained in this evaluation we can confirm that the fructosamine test has certain advantages over other variables used to monitor glycometabolic control, such as Hb A1c. Principally, the test is simple and easy to perform with good precision, both manually and with automated analyzers. Moreover, the procedure can be easily

![Fig. 4. Correlations between concentrations of fructosamine and Hb A1c in serum of diabetic patients treated with oral hypoglycemic agents (O, e) or with insulin (C, f).](image)

$r^2 = 0.439x + 0.594$ mmol/L; n = 41; r = 0.390; $y = 0.248x + 1.206$ mmol/L; n = 42; r = 0.573

**Table 2. Fructosamine and Glucose Concentrations in Some Commercial Quality-Control Sera**

<table>
<thead>
<tr>
<th>Material</th>
<th>Source</th>
<th>Lot no.</th>
<th>Fructosamine, mmol/L</th>
<th>Glucose, g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precinorm U</td>
<td>a</td>
<td>152746</td>
<td>3.91</td>
<td>1.28</td>
</tr>
<tr>
<td>Precipath U</td>
<td>a</td>
<td>152794</td>
<td>3.88</td>
<td>1.09</td>
</tr>
<tr>
<td>Precinorm E</td>
<td>a</td>
<td>2-344</td>
<td>2.16</td>
<td>0.04</td>
</tr>
<tr>
<td>Precipath E</td>
<td>a</td>
<td>2-372</td>
<td>2.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Sclavo</td>
<td>b</td>
<td>3c</td>
<td>5.70</td>
<td>1.52</td>
</tr>
<tr>
<td>Sernorm</td>
<td>c</td>
<td>162</td>
<td>4.68</td>
<td>0.82</td>
</tr>
<tr>
<td>Pathonorm L</td>
<td>c</td>
<td>20</td>
<td>2.08</td>
<td>0.35</td>
</tr>
<tr>
<td>Pathonorm H</td>
<td>c</td>
<td>20</td>
<td>6.00</td>
<td>1.97</td>
</tr>
<tr>
<td>Moni-trol 1</td>
<td>d</td>
<td>LTD-204</td>
<td>6.00</td>
<td>0.88</td>
</tr>
<tr>
<td>Moni-trol 2</td>
<td>d</td>
<td>LTD-104</td>
<td>7.74</td>
<td>2.37</td>
</tr>
<tr>
<td>Validate-A</td>
<td>e</td>
<td>5345-113</td>
<td>7.63</td>
<td>1.92</td>
</tr>
<tr>
<td>Validate-N</td>
<td>e</td>
<td>5334-113</td>
<td>4.42</td>
<td>0.80</td>
</tr>
<tr>
<td>Kontrollogen-L</td>
<td>f</td>
<td>623124E</td>
<td>3.10</td>
<td>1.02</td>
</tr>
<tr>
<td>Kontrollogen-IP</td>
<td>f</td>
<td>623210C</td>
<td>3.42</td>
<td>2.43</td>
</tr>
<tr>
<td>Control-N</td>
<td>g</td>
<td>U0433</td>
<td>4.67</td>
<td>0.84</td>
</tr>
<tr>
<td>Control-P</td>
<td>g</td>
<td>B0637</td>
<td>5.07</td>
<td>1.92</td>
</tr>
<tr>
<td>Control II</td>
<td>h</td>
<td>3465020A</td>
<td>8.70</td>
<td>2.28</td>
</tr>
<tr>
<td>Ortho UN</td>
<td>i</td>
<td>X40802</td>
<td>6.59</td>
<td>2.40</td>
</tr>
<tr>
<td>Ortho NOR</td>
<td>i</td>
<td>020501C</td>
<td>4.42</td>
<td>0.91</td>
</tr>
<tr>
<td>Ortho ABN</td>
<td>i</td>
<td>025501C</td>
<td>4.49</td>
<td>2.99</td>
</tr>
<tr>
<td>Decision 1</td>
<td>j</td>
<td>C401020</td>
<td>1.62</td>
<td>0.45</td>
</tr>
<tr>
<td>Decision 2</td>
<td>j</td>
<td>C401054</td>
<td>2.25</td>
<td>1.24</td>
</tr>
<tr>
<td>Decision 3</td>
<td>j</td>
<td>C401055</td>
<td>2.98</td>
<td>2.66</td>
</tr>
</tbody>
</table>

a: Boehringer Biochemia, Milano, Italy; b: Sclavo Diagnostics, Milano, Italy; c: Nyegaard, Oslo, Sweden; d: Merz & Dade, München, F.R.G.; e: General Diagnostics, Berlin, F.R.G.; f: Behringwerke AG, Marburg, F.R.G.; g: Roche, Milano, Italy; h: Hyland Diagnostics, Malvern, PA; i: Ortho Diagnostics, Milano, Italy; j: Beckman Analytical, Milano, Italy.

1144 CLINICAL CHEMISTRY, Vol. 33, No. 7, 1987
transferred between laboratories (unpublished results). There are, however, some limitations that have to be known in order to give the right interpretation to the analytical data. These are: (a) total protein concentration affects the results; (b) most of the commercial quality-control sera contain some unidentified substance and cannot be used as quality-control materials.

We think that our observations complement, for these points, the actual available literature data (1, 7, 8, 10, 11, 21, 24), which have been focused mainly on clinical applications of the test. Unfortunately, it seems that corrections, in case of abnormalities in the proteic profile, are not easily practicable. The only reasonable correction that can be applied, as reported by Van Dieijen-Visser et al. (21), is for alterations in total protein concentration. Other corrections cannot be performed, because of heterogeneity (in structure and half-lives) among the proteins reactive to Nitro Blue tetrazolium. It's also evident that alterations in serum protein turnover and composition, detectable, at least in part, by the examination of the serum protein electrophoresis pattern, could alter the normal fructosamine concentrations. In these cases, data from the fructosamine test should be taken with caution.

Concerning the sensitivity to the labile fractions, we can conclude that the test is unaffected by it, for the greatest part. This is proved by the incubation experiments of whole blood with glucose (Figure 2) and by the "in vivo" experiments. It is likely, however, that if the labile fractions are present in the sample they may interfere in the test, as shown by the data of Figure 3. This probably may occur, in vivo, only under extreme conditions of unpaired glucose control.

These experiments therefore confirm the conclusions of Johnson and Baker (25), who measured ketoamine formation, during incubation of purified protein fractions with glucose, from the extent of uptake of 14C by proteins. We think, however, that our information is also new, because these latter authors measured the ketoamine synthesis only after an overnight dialysis of the incubated samples against isotonic saline. Potentially, the aldimine form could be lost during such dialysis.

As for the clinical implications of this work, we confirm that diabetic subjects have higher serum fructosamine concentrations than do normal persons. The fructosamine concentrations are related mainly to fasting or basal glucose values and there is no correlation with the glycemia 2 h after glucose load.

This finding, which is in agreement with previously reported results (8, 10, 11), indicates that measurement of serum fructosamine to identify subjects with impaired glucose tolerance is consistent with a conservative diagnosis of diabetes.

We report also a strong correlation of serum fructosamine with glycated proteins and glycated albumin. Clearly, fructosamine assay really reflects the glycation of serum proteins, including albumin.

On the other hand, we did not find the strong correlation between stable glycated hemoglobin (Hb A1c) and serum fructosamine previously reported (5, 8, 11, 26, 27). The different methods used by these authors for assay of glycated hemoglobins, together with some significant differences in studied cases, could explain our findings. In particular, values for fructosamine have been compared with total Hb A1 as measured by ion-exchange chromatography (5, 26) or electrophoresis (27) and with Hb A1c (labile + stable) as

---

Fig. 5. Correlations between fructosamine and total glycated proteins (A), glycated albumin (B), and glycated IgG (C) in 60 mixed-type diabetic patients (regression equations summarized in text)
measured by "high-pressure" liquid chromatography (8) or isoelectric focusing (11). In contrast, in this work we measured stable HB A1c, which is considered to be the only reliable index to glycemic control in diabetic patients during the preceding one or two months (28), and we found no correlation with fructosamine. We also note that the correlation between fructosamine and HB A1c is not improved significantly (r = 0.411, n = 42, for group I of Figure 4) if fructosamine is "normalized" for protein content, as suggested by Sagniez et al. (29). We do not find that a correlation between fructosamine and the stable form of HB A1c has been reported previously.

Moreover, our results indicate that serum fructosamine and glycated hemoglobin reflect different periods of blood glucose control in diabetic subjects, according to the different half-lives of the two proteins. The strong correlation between fructosamine and glycated proteins (albumin and IgG) in serum supports this hypothesis.

It is impossible, from this study, to estimate the interval of blood glucose control that is reflected by the fructosamine test. The literature (1, 10, 11) indicates a period of about two weeks, substantially shorter than that for HB A1c (eight weeks).

The different behavior of fructosamine and HB A1c in serum of the insulin-treated diabetics and in the type II diabetic group suggests other important clinical considerations. It is known that in the first group (insulin-treated diabetics) there are wide circadian glycemyc fluctuations. In contrast, type II diabetics are considered to have stable or controlled diabetes. In clinical practice it is common to find, in insulin-treated diabetics, higher HB A1c concentrations associated with normal blood-glucose concentrations, simultaneously determined, indicating that a previously poor glycemic control has actually improved (30).

In this work, we observed lower values for serum fructosamine in insulin-treated diabetics than in type II diabetics, probably because short-time glucose control is good in these patients, but long-term control is poor. In fact, the value for blood glucose in the fasting patient is similar in the two groups, and in the insulin-treated diabetic group only serum fructosamine, but not HB A1c, correlates with fasting blood glucose.

In conclusion, we believe that the fructosamine test can be considered a new tool for monitoring diabetic status, but use of the test as an alternative to measurement of HB A1c, as proposed by some authors (11, 27), cannot be supported.

References