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References
involved in the increase of serum fructosamine in patients with chronic renal failure.

By a spontaneous reversible reaction, glucose forms Schiff bases with the α- and ε-amino groups of proteins. The Schiff bases can then be converted to stable Amadori products, which in turn can give rise to advanced glycation end products that include covalent cross-links within and between the proteins (1). Damage to proteins by nonenzymatic glycation is thought to be involved in the development of the complications of diabetes mellitus (2) and may also play a role in aging (3). Because nonenzymatic glycation of specific proteins in vivo is proportional to the prevailing glucose concentration during their lifetime, glyated protein measurements in diabetic patients may allow monitoring of the control of glycemia better than would sporadic measurements of blood glucose. A simple test, called fructosamine, was reported in 1982 for measuring nonenzymatically glyated proteins in serum (4). The test, based on reduction of nitroblue tetrazolium (NBT) by Amadori products at alkaline pH, has been evaluated widely in various clinical situations (4–8). Its value in monitoring the control of glycemia in patients with diabetes remains controversial (4–8). A basic anomaly of the conventional test is the fact that it reports about a 10-fold greater concentration of fructosamine in serum than does specific measurement of glycated amino acid residues by HPLC of hydrolyzed serum proteins (4–9). Phillipou et al. (10) have reported recently that the sparse solubility of the diformazan formed during the reaction by reduction of NBT is a source of error in the conventional fructosamine test.

Here we have determined the optimal conditions for the fructosamine assay by monitoring both the specific absorbance and turbidity in the reactions with various detergents. We then automated the optimized reaction. We found that urate is the major source of error in assays of serum fructosamine after solubilization of diformazan and that the error due to it can be corrected mathematically. We present our findings on the performance of the method as well as a study of the factors affecting serum fructosamine in healthy people and in patients with diabetes mellitus, chronic renal failure (CRF), or both.

Materials and Methods

Subjects: We studied 768 healthy individuals and 282 patients with type II diabetes mellitus. The healthy adults were selected from those applying for a general check-up without specified complaints. Blood was sampled after overnight (~10 h) fasting, and the concentrations of glucose, creatinine, electrolytes, albumin, total protein, cholesterol, triglycerides, urate, bilirubin, alkaline phosphatase, lactate dehydrogenase, and alanine and aspartate aminotransferases in serum were determined as before (11). Anyone with glucose >6.1 mmol/L or with test results outside normal reference intervals (except for triglycerides, uric acid, and cholesterol) was excluded from the "normal" group. Apparently healthy children were selected from those attending a pediatric outpatient clinic, on the basis of the same laboratory tests and criteria described for adults. The diabetic patients, diagnosed by conventional criteria (12), were attending different outpatient and inpatient departments. Total protein in sera of the healthy subjects (69 ± 4 g/L) and diabetic patients (69 ± 4 g/L) did not differ significantly, but serum albumin of diabetic patients was lower than those of the age- and sex-matched health subjects (P < 0.001).

We also studied 73 patients with CRF (52 without, 2 with diabetes mellitus). Serum samples from all subject were frozen (~80 °C) on the day of sampling if the fructosamine test was not done on that day.

Chemicals: Bilirubin (lot no. VV327019) was from Merc (Darmstadt, F.R.G.) and urate (lot no. 5061420H) from BDH (Poole, U.K.). The detergents Nonidet-40 (lot no. 9505470E) and Triton X-100 (lot no. K388) were from LKB (Bromma, Sweden). All other chemicals were analytic grade from various suppliers.

Manual fructosamine assay: We used the procedure of Phillipou et al. (10) with the following modifications. Sample (120 μL) was mixed with 1.2 mL of NBT (0.5 mmol/L; 0.1 mol/L carbonate buffer, pH 10.3)3 and with 1.2 mL of 0.1 mol/L carbonate buffer containing various detergents at indicated concentrations (see below). At 0, 10, and 15 min after the start of the reaction, absorbances at 525 and 78 nm (where diformazan shows no specific absorbance) were recorded simultaneously with the dual-wavelength mode of a Beckman DU7 spectrophotometer (Beckman Instruments, Brea, CA). Dihydroxyacetone, the calibration standard, was used at concentrations from 0 to 1.5 mmol/L. Th detergents Triton X-100, Nonidet-40, and Tween-20 were tested at various concentrations to determine the maxima of A525 in reactions of dihydroxyacetone with minimal nonspecific color production (i.e., with H2O as a sample). Tween-20 was relatively ineffective; Triton X-100 at ~20 mL/L and Nonidet-40 at ~30 mL/L (final concentrations) gave the most satisfactory results.

Automated fructosamine assay: We used a centrifuge analyzer (CentrifilChem; Baker Instruments, Allentown, PA) to automate the assay. After a systematic evaluation of various sample/diluent/reagent volumes (see below) we adopted the following conditions and set points: time (T) 600 s, ΔT 5.00 min, filter 520 nm, absorbance limit 0.3 mode terminal, blank autoband, standard value 60 μmol/L, temperature 37 °C, sample volume 20 μL, diluent (H2O) plus sample volume 50 μL, first reagent volume 35 μL, second reagent volume 0 μL (off). Fresh reagent was made before use by mixing equal volumes of 0.544 mmol/L NBT reagent in 0.114 mol/L carbonate buffer, pH 10.3 with 68 mL/L Nonidet-40 reagent in the same buffer. The calibration standard was placed into calibration position in duplicate. In each cycle, aliquots of normal and diabetic serum pools (dialyzed, aliquoted, and stored frozen at ~80 °C) were also analyzed for quality control. Sample carryover, found to be a potential problem with CentrifilChem's pipetter, was eliminated by placing water-filled cups between the sample cups (other instruments with better pipetting ability may also be suitable for automation).

For comparison, we re-assayed, using a fructosamin reagent kit (cat. no. 1101668, lot no. 168818: Boehringer Mannheim GmbH, Mannheim, F.R.G.), 63 normal and 6 diabetic sera that had been assayed for fructosamine by our procedure. The calibrator and quality-control sera were also from Boehringer Mannheim, and we used a Boehringer Mannheim/Hitachi 717 analyzer according to the manufacturer's instructions.

3 The 0.1 mmol/L carbonate buffer concentration that was reported (10) is probably a typographical or typing error.
Other procedures: Sera were dialyzed with the use of 500-Da cutoff dialysis membranes at 4 °C against four or five changes of 5 L of phosphate-buffered saline [pH 7.4, 50 mmol of NaCl, 8 mmol of K2HPO4, and 2 mmol of NaH2PO4, pH 7.4 adjusted (if necessary) with 0.1 mol/L HCl] over 28–32 h. Statistical analyses were carried out with the SPSSx statistical software (SPSS Inc., Chicago, IL).

Results
Adding a detergent to the reagent buffer markedly increased the color generated by reduction of NBT. Table 1 shows that the increase of absorbance at 525 nm between 0 and 15 min after the start of the reaction (the period sed to calculate serum fructosamine (4)] was greater in the presence than in the absence of detergent, both for the hydroxyacetone standard and for serum. However, the relative effectiveness of detergents was much greater for the standard than for serum (Table 1). Therefore, the same normal serum pool analyzed in the absence and presence of detergents gave widely differing serum fructosamine concentrations (~2.0 mmol/L in the absence of detergents and 0.56–0.62 mmol/L in their presence; cf. Table 1). The detergents by themselves caused only marginal color formation (Table 1). Also, when the detergents were added to the reactions that had been completed without detergents, the color intensity quickly increased to the values seen when detergents were present throughout the assay data not shown). Measurements of absorbance at 785 nm where diformazan does not absorb specifically (10) revealed that turbidity increased during the course of reaction of the standard, if no detergent was present (Table 1). Apparently, therefore, the absorbance measurements made the presence of detergents are erroneous because of the poor solubility of diformazan (10).

We adapted to a centrifugal analyzer the fructosamine assay conditions found optimal by the experiments summarized in Table 1. The median value for serum fructosamine measured directly by this automated method was 512 mmol/L in 768 normal subjects (in Table 2, “uncorrected fructosamine”) and correlated strongly with serum urate in these individuals (r = 0.895). On the other hand, the same method yielded fructosamine concentrations of 229–270 mmol/L (range of results for seven separate pools) for pools of normal sera that had first been dialyzed to remove urea as well as other dialyzable serum constituents. We then analyzed urate solutions in water (0–600 μmol/L) as samples and found that each micromole of urate produced color equivalent to 0.824 (SD 0.008) μmol of fructosamine (the intercept of the plotted comparison did not differ from zero). Similarly, when sera that had been analyzed for fructoseamine were supplemented with urate, the measured fructosamine concentrations equaled those calculated on the basis of 0.819 (SD 0.018) μmol of additional fructoseamine for each micromole of added urate (mean of 14 separate runs of a total of 114 normal sera containing as much as 800 μmol of total urate per liter). In a separate set of experiments, serum pools that had been assayed for fructosamine, urate, total protein, and albumin (as well as for the other analytes mentioned in Methods) were dialyzed extensively and re-analyzed for fructosamine, with and without added urate. In these experiments each micromole of added urate gave rise to 0.819 (SE 0.015) μmol of fructosamine (100–600 μmol/L added urate, six separate pools). Finally, when we corrected for the contribution of endogenous urate to the fructoseamine reaction (0.819 μmol of fructoseamine for each micromole of urate), the corrected fructoseamine concentrations showed negligible or no significant deviation from those measured directly in dialyzed aliquots of the same sera when these sera were from people with healthy renal function (Table 3). In sera of the CRF patients, however, −8% (23/301) of the serum fructoseamine appeared to be derived from dialyzable nonurate substances (Table 3).

Serum fructoseamine concentrations in 768 normal and 282 diabetic subjects determined by the automated method and with mathematical correction for the contribution of urate to the reaction are shown in Table 2. On the average, diabetic patients showed significantly greater concentrations of fructoseamine than did healthy subjects (P < 10−8) despite a tendency for the diabetics to have lower concentrations of albumin in their sera (Table 2). Serum fructoseamine normalized for albumin showed relatively smaller overlap between the normal and diabetic populations than did fructoseamine content alone (Figure 1).

Among the healthy subjects, the concentration of serum fructoseamine was slightly higher in men than in women: 253 (SD 36) vs 244 (SD 29) μmol/L (P < 0.001). However, on average, serum albumin was also higher in men than in women—47 (SD 3) vs 45 (SD 3) g/L (P < 0.001)—and the serum fructoseamine/albumin ratio did not differ between

| Table 1. Effect of Detergents on Absorbance Changes during the Fructosamine Reaction |
|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| Sample                          | ΔAabs*                           | ΔAme*                           | ΔAme*                           |
| No                               | Triton X-100, 20 mL/L            | Nonidet-40, 30 mL/L            | No                               | Nonidet-40, 30 mL/L            |
| 0.00                            | 0.004                            | 0.006                           | −0.001                           | 0.000                           |
| 0.001                           | 0.002                            | 0.003                           | (0.001)                          | (0.001)                          |
| 0.012                           | 0.057                            | 0.072                           | 0.003                            | 0.001                           |
| (0.003)                         | (0.008)                          | (0.009)                         | (0.001)                          | (0.000)                          |
| 0.039                           | 0.166                            | 0.210                           | 0.012                            | 0.000                           |
| (0.005)                         | (0.026)                          | (0.016)                         | (0.004)                          | (0.001)                          |
| 0.085                           | 0.117                            | 0.132                           | 0.000                            | −0.003                          |
| (0.008)                         | (0.010)                          | (0.010)                         | (0.001)                          | (0.002)                          |

DHA, dihydroxyacetone. * Change of absorbance between 10 and 15 min of the reaction. ** Mean (and SD) from six to 10 separate assays. *** Sera pooled from healthy adults and kept frozen at −80 °C in single-use aliquots. Effectiveness of Nonidet-40 in increasing the ΔAabs was greatest for DHA (0.072/0.012 = 7-fold and 0.210/0.039 = 5.38-fold), less for normal sera (0.132/0.085 = 1.55-fold), and the least for dialyzed aliquots of normal sera (1.14 = 0.07-fold, data not shown). Thus, the diformazan forming from DHA and from dialyzable serum substances appears to precipitate more readily than the diformazan forming from non-dialyzable (protein) serum constituents.
Diabetic

6.9

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Healthy

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Urate,

Fructosamine,

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population. 

Fructosamine (μmol/L)/albumin (g/L) ratio.

Table 2. Concentrations of Fructosamine, Glucose, Urate, and Albumin in Sera of Healthy and Diabetic Populations

<table>
<thead>
<tr>
<th></th>
<th>Healthy subjects</th>
<th></th>
<th>Diabetic subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>25th</td>
<td>50th</td>
</tr>
<tr>
<td>&quot;Uncorrected fructosamine,&quot; μmol/L</td>
<td>511</td>
<td>389</td>
<td>512</td>
</tr>
<tr>
<td>Fructosamine, μmol/L</td>
<td>249</td>
<td>188</td>
<td>246</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.0</td>
<td>4.1</td>
<td>5.0</td>
</tr>
<tr>
<td>Urate, μmol/L</td>
<td>320</td>
<td>192</td>
<td>312</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>46</td>
<td>39</td>
<td>46</td>
</tr>
<tr>
<td>Fruct/Alb c</td>
<td>5.4</td>
<td>4.2</td>
<td>5.3</td>
</tr>
</tbody>
</table>

*318 men and 450 women, mean age: 34 (SD 13) years. b 156 men and 126 women with type II diabetes; mean age 50 (SD 10) years. Diabetic patients with renal failure were excluded from this population. c Fructosamine (μmol/L)/albumin (g/L) ratio.

Table 3. Fructosamine Concentrations in the Dialyzed and Nondialyzed Sera from Healthy Subjects, Diabetic Patients, and Patients with CRF

Table 3. Fructosamine Concentrations in the Dialyzed and Nondialyzed Sera from Healthy Subjects, Diabetic Patients, and Patients with CRF

<table>
<thead>
<tr>
<th></th>
<th>Nondialyzed sera</th>
<th>Dialyzed sera</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>7</td>
<td>247 (26)</td>
<td>234 (15)</td>
</tr>
<tr>
<td>Diabetic</td>
<td>5</td>
<td>342 (28)</td>
<td>339 (34)</td>
</tr>
<tr>
<td>CRF</td>
<td>7</td>
<td>301 (25)</td>
<td>278 (27)</td>
</tr>
</tbody>
</table>

*Corrected for the urate error (0.819 μmol of fructosamine for each micromole of urate). Concentrations of the directly measured fructosamine (i.e., not corrected for the urate error) in the healthy, diabetic, and CRF sera were 508 (SD 26), 630 (SD 43), and 697 (SD 41), respectively. b Three each of the healthy and diabetic serum pools and one CRF serum pool were also analyzed for fructosamine by the Boehringer Mannheim method (see Methods) and showed, respectively, 12 (SD 8), 8 (SD 7), and 33 μmol/L differences between the nondialyzed and dialyzed samples.

Fig. 2. Age-related alterations of the serum fructose (μmol/L, □), preprandial glucose (G, mmol/L, △), and albumin (A, g/L, ○) in 788 healthy subjects.

Means and SEM for age groups <20, 20–29, 30–39, 40–49, 50–59, and >60 years. The "preprandial" serum glucose of the <20 age group may be artifically high because of the questionable adherence of young children to fasting instructions (excluding results for the children <19 years old from the <20 age group yielded mean serum glucose lower than that of the 20–29 years age group).

a relatively strong correlation between them (r = 0.607, <0.001). In diabetic patients, moreover, serum fructose correlated positively with the duration of disease (r = 0.435, P <0.001). On the average, patients being managed with oral hypoglycemic agents showed lower serum fructose concentrations than did those requiring insulin therapy: 254 (SD 75) vs 344 (SD 111) μmol/L (age- and sex-matched subpopulations P <0.01).

In comparison with the healthy subjects, nondiabetic patients with CRF showed significant increases in serum fructose for each gram per liter increase in serum albumin (Table 4). This increase (not fully attributable to the ~8% contribution of dialyzable nonurate substances fructosamine in CRF sera) was also seen in diabetic CR
Table 4.  Mean (SD) Fructosamine Concentrations Relative to Albumin in Sera of Patients with CRF

<table>
<thead>
<tr>
<th>Peritoneal dialysis*</th>
<th>Hemodialysis*</th>
<th>Peritoneal dialysis and hemodialysis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>uctosamine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>223 (35)*</td>
<td>262 (39)*</td>
<td>238 (68)</td>
</tr>
<tr>
<td>bumin</td>
<td>31 (9)</td>
<td>40 (5)</td>
</tr>
<tr>
<td>ucL/Alb</td>
<td>7.9 (2.5)</td>
<td>6.7 (0.9)</td>
</tr>
</tbody>
</table>

* Five patients with creatinine 1026 (SD 253) μmol/L, urate 511 (SD 107) mol/L, and total protein 56 (SD 6) g/L in serum. * Twenty-two patients with creatinine 1157 (SD 327) μmol/L, urate 506 (SD 116) μmol/L, and total protein (SD 7) g/L. * Twenty-five patients with creatinine 1025 (SD 206) μmol/L, total protein 520 (SD 107) μmol/L, and total protein 58 (SD 9) g/L. Approximately 70% of patients in this group were on peritoneal dialysis at the time of sampling t cross-overs to hemodialysis were frequent for various reasons (e.g., rifamnla). * 279 (SD 37) μmol/L, by the Boehringer Mannheim method. * 318 D (44) μmol/L, by the Boehringer Mannheim method. * Fructosamine (μmol/albumin (g/L) ratio; P < 0.001 for all groups of CRF patients vs healthy subjects (cf. Table 2) and P < 0.05 for the CRF patients on peritoneal dialysis those on hemodialysis.

patients, in comparison with their counterparts without renal failure (data not shown). The CRF patients managed by peritoneal dialysis averaged a greater increase in serum fructosamine for each 1 g/L increase in albumin compared with patients managed by hemodialysis (Table 4). In the group of CRF patients managed by peritoneal dialysis and both peritoneal dialysis and hemodialysis, serum fructosamine and the fructosamine/albumin ratio were correlated inversely with serum creatinine (r = -0.435, P = 0.01; and r = -0.878, P < 0.001, respectively, in the combined population).

We investigated the precision of the method by assaying aliquots of normal and diabetic sera stored frozen at 80 °C. For "uncorrected fructosamine," the within-run V ranged from 1.3% to 2.7% on separate occasions with sera from healthy subjects (n = 12 to 20) and was 1.7% with diabetic pool (n = 22). Between-run CV over a seventh period was 3.7% for a serum pool from healthy subjects. Studies of interference showed no effect of hyperlipidemia on test results for normal- and hyperlipidemic sera mixed at various proportions. Interference from bilirubin, investigated by supplementing serum with bilirubin, gave 0.33 μmol of apparent fructosamine for each micromole of bilirubin. We conclude that, under normal circumstances, interference from bilirubin appears negligible, given the relative concentrations of bilirubin/fructosamine in sera from healthy subjects.

In 130 diabetic and nondiabetic sera, we assayed fructosamine both by our method and by use of a recently marketed reagent kit (Boehringer Mannheim GmbH) involving standards of human albumin and an undisclosed combination of detergents and uricase in the reaction (13). Near-regression analysis of the relationship between the fructosamine concentrations measured by our method (y, range 192–529 μmol/L) and by the commercial method (x) yielded the following equation: y = 0.98x + 7 μmol/L (r = 0.99; 95% confidence interval for slope, 0.90–1.06). Dialysis serum pooled from healthy subjects, to remove all measurable urate, yielded fructosamine results of 236 (SD 7) μmol/L by our method (directly measured) and 211 (SD 4) μmol/L by the commercial method (n = 27 and 17, respectively). When a 300 μmol/L solution of dihydroxyacetone in water was assayed as sample, our method yielded fructosamine results of 296 (SD 10) μmol/L and the commercial one 263 (SD 3) μmol/L (n = 250 and 5, respectively).

Discussion

In the fructosamine assay the Amadori product is usually determined after about 7–10 min from the onset of reaction, in the expectation that other reducing agents in serum will be consumed to insignificant quantities by that time (4–8, 10). However, after solubilization of diformazan by Nonidet–40, we noted that urate (but ordinarily no other measurable dialyzable serum constituent) continues to be a major nonspecific reducing agent after 10 min of the reaction. Considering that serum urate is widely quantified in clinical practice for various purposes with little cost, we evaluated the possibility of mathematical correction for urate interference in the reaction. Concentrations of serum fructosamine determined by mathematical correction for urate in this study (Table 2) may be compared with the 200 μmol/L fructose–lysine residues in normal human serum analyzed by HPLC of hydrolyzed proteins (calculated from ref. 9) and the 247 μmol/L median concentration of fructosamine reported recently for measurements in 562 nondiabetic people by the Boehringer Mannheim fructosamine reagent kit (14). Apparently, therefore, once the errors resulting from poor solubility of diformazan and from interference of urate in the reaction are corrected in one way or another, the fructosamine reaction yields serum concentrations comparable with those shown by the more specific test for the Amadori product (9). Besides this improved accuracy, our automated fructosamine assay has the advantages of simplicity and low cost (a few cents per sample for reagents). Although the test requires assay of serum urate as well, this is unlikely to be a limiting factor in most clinical chemistry laboratories.

Our method and that of Siedel et al. (13) gave similar concentrations of serum fructosamine in healthy subjects and diabetic people without renal failure. On the other hand, the latter method (13) measured greater fructosamine concentrations in the sera of CRF patients than did ours (Table 4). This suggests that either (a) the cross-reacting dialyzable nonurate substances found in CRF sera (cf. Table 3) cause greater color production with the reagents of Siedel et al. (13) or (b) the uricase activity in their system is insufficient to eliminate the high urate concentrations commonly found in sera of CRF patients. The two methods also showed slight differences between fructosamine measurements in dialyzed sera (containing no urate) and in a 300 μmol/L solution of dihydroxyacetone in water (~10% underestimation by the method of Siedel et al. (13)); perhaps the dispersing activities of the detergents used in these two methods differ slightly.

The serum fructosamine concentrations measured in diabetic patients by our automated method were clearly higher in patients with poorer control of glycemia. As such, our test appears to be generally useful in monitoring the effectiveness of treatment regimens and patient compliance. However, when interpreting measurements of serum fructosamine concentrations, one should also consider the possible effects of alterations in protein turnover. Moreover, nonenzymatically glylated proteins may be recognized and catabolized preferentially by macrophages (15). If so, concentration of serum fructosamine may not necessarily be directly proportional to the integrated concentration of serum glucose during the preceding two to three weeks. Interestingly, although serum albumin is a lysine-rich protein (59
lysines per molecule (16), with several of these lysines likely to be accessible in aqueous media (cf. ref. 17), we found less than one glycosylated amino acid residue for each albumin molecule in serum from healthy subjects (calculated from the data of Table 2, assuming Mₐ, 65,000 for albumin).

The finding that serum fructosamine normalized for albumin is increased in patients with CRF, regardless of the cause of disease, deserves attention. Quantitatively, this increase cannot be explained solely by the ~8% contribution of dialyzable nonglycated substances to the fructosamine reaction in CRF sera. Noting that serum fructosamine normalized for albumin was particularly high in the CRF patients managed by peritoneal dialysis (Table 4), we suggest that exposure of patients to hypertonic glucose during dialysis may contribute to the nonenzymatic glycation of proteins in such patients (the patients managed by both peritoneal dialysis and hemodialysis were also predominantly being treated by peritoneal dialysis at the time of sampling). Inverse correlations of serum fructosamine and of the fructosamine/albumin ratio with serum creatinine in patients subjected to peritoneal dialysis appear consistent with such an interpretation. In this regard, Brighton and Furth (18) have reported recently an increase in plasma glucose after peritoneal dialysis and also suggested that this hyperglycemia may promote nonenzymatic glycation. Although these findings implicate an adverse effect of peritoneal dialysis, operation of additional factors as well (e.g., the tendency for glucose intolerance in uremia (19)) is suggested by the greater-than-normal serum fructosamine/albumin ratios in the sera of CRF patients managed by hemodialysis (Table 4).

We thank Drs. N. Abdella and A. Salman for referral of some of the diabetic patients and Dr. N. Nampaory for the dialysis histories of some of the CRF patients. This work was supported by the Kuwait University Grant RA077.

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A Kinetic Assay for Urea in Undiluted Urine Specimens

Pamela Scott and Gerald A. Maguire

We describe a kinetic assay for quantifying urea in undiluted urine samples. The assay linearity extends to urea concentrations of 400 mmol/L, is free from interference from blood (10 mL/L) or bilirubin (1 mmol/L), is sufficiently precise for routine use, and correlates well with an established method for assay of diluted urine samples.

The 24-h urinary excretion of urea reflects the catabolic state of a patient, and provides a guide to the protein requirements of severely ill patients (1). In these patients, measurement of sodium, potassium, and creatinine excretion are also often required. Current methods for measurement of urinary urea, unlike those for these other analytes, require a predilution of the specimen. To obviate the need for this predilution in estimating urea requires that the linear range of current assays be extended.

The most commonly used methods for measuring urea in blood are based on a reaction scheme in which jack bean urease (EC 3.5.1.5) generates ammonia, which in turn is measured by the decrease in NADH absorbance in the presence of glutamate dehydrogenase (GLDH, EC 1.4.1.3). This method may be used in an end-point or kinetic mode. The initial concentration of NADH in this assay can...