Serum albumin concentrations determined by BCP correlated closely with those determined by BCG and quantitative SPE when all samples were included (n = 30; BCP = 1.17 ± (SPE) – 1.0 (r = 0.96). However, for the 14 samples with albumin concentrations <25 g/L there was a lack of correlation between results by the BCP and BCG techniques for low serum albumin concentrations: BCP = 0.81 (BCG) – 3.9 (r = 0.56). Better agreement was found between BCP and quantitative SPE: BCP = 0.83 (SPE) + 0.9 (r = 0.95), indicating that BCP more accurately reflects albumin status at low concentrations.

The degree of overestimation of serum albumin by BCG (BCG albumin minus electrophoretic albumin) correlated closely with the concentration of a2-globulins in the hypoalbuminemia sera (r = 0.86). BCP did not overestimate albumin, and so this correlation was negligible.

In previous studies, serum albumin as determined by BCP vs those determined by electrophoresis have shown a close correlation (9). However, in these investigations this relationship in sera from hypoalbuminemic nephrotic patients with increased a2-globulins was not closely examined. Our results reveal a close correlation between results by the BCP and quantitative SPE techniques in the hypoalbuminemic range, and they further illustrate the significant overestimation of serum albumin by BCG. Results by the Du Pont aca BCP method have been shown to correlate with electrophoretically determined serum albumin in non-nephrotic patients (9). Webster et al. (5) eluted the albumin fraction after electrophoresis and also observed an overestimation of albumin when the BCG procedure was used with hypoalbuminemic specimens. Speicher et al. (7) showed that this overestimate was primarily ascribable to a-globulins. Our results corroborate these observations: we show a correlation of the BCG overestimate of serum albumin concentration with a2-globulins, a relation that is particularly strong for sera with low albumin concentrations and that was not observed with the BCP technique. Patients with the nephrotic syndrome often present with a low serum albumin and increased a2-globulins. In specimens from these patients, an overestimate of serum albumin by the BCG method could become critical, and thus use of the BCP technique would be preferred.

The nephrotic syndrome incurs hypoalbuminuria, hypoalbuminemia, and edema. Increased hepatic production of albumin is accompanied by increased production of other macromolecules, including a-globulins, lipoproteins, and fibrinogen (10). These macromolecules, in the presence of hypoalbuminemia, can interfere with the routine measurement of serum albumin.

We conclude that, for patients with hypoproteinemia, results by the BCP method correlate more closely with actual albumin concentrations, as determined by quantitative SPE, than do those by the BCP method.

It is important for the clinical laboratory and clinical practitioners to realize that the BCP methodology gives lower but more nearly accurate measurements of serum albumin concentrations. Otherwise, the practicing physician, using past medical literature as a base of reference, may consider the nephrotic patient to have a more severe hypoalbuminemia than is actually the case.

References

Detecting Iron Deficiency by Serum Tests

To the Editor:

In a recent Letter, Sheridan and Pearce (1) defended serum ferritin assay as a test for iron deficiency. We would like to draw attention to some aspects of this debate.

Transferrin, the major iron-carrying protein in plasma, increases in response to iron deficiency and estrogen stress. It is also a negative acute-phase protein and decreases during an inflammatory episode. We assess the concentration of transferrin in relation to a protein profile that includes the negative acute-phase proteins, albumin and prealbumin (transferrin), and the estrogen-sensitive proteins, a1-antitrypsin and ceruloplasmin (2, 3).

We find that the most useful indicator of iron deficiency is a disproportionate increase in transferrin, as compared with other negative acute-phase proteins, in the absence of an estrogen response. To support the diagnosis of iron deficiency, this must be accompanied by a decreased saturation of transferrin with iron.

Plasma ferritin is an acute-phase protein; its increase in concentration during inflammation (Figure 1) can mask a diagnostically low result for ferritin. Thus it is important that the ferritin concentration be compared with that of other acute-phase proteins to give some indication of the acute-phase status of the patient. In a review of 350 consecutive requests for serum ferritin assay, we found that 148 (42%) of the samples had ferritin concentrations below the normal reference interval, implying the presence of iron defi-
Evidence for Nonenzymic Glycation of Antithrombin III in Diabetic Patients

To the Editor:

In a recent report, Brownlee et al. (1) described the inhibition of heparin-catalyzed antithrombin III activity induced by in vitro incubation of purified human antithrombin III (AT III) in the presence of glucose at pH 9.5. They propose that nonenzymic glycation of AT III in vivo could account for the underlying hypercoagulable state observed in diabetic patients, the contribution of which has been evoked in the pathogenesis of microvascular disease (2). We present here data showing that the presence of nonenzymically glycated AT III can indeed be demonstrated in the plasma of diabetic patients.

Blood samples were collected in EDTA-containing tubes. Glycated hemoglobin (HbA1c) was measured by automated chromatography on Bio-Rex 70 (3). Sugars bound to plasma proteins through a ketoamine linkage were determined by the colorimetric thiobarbituric assay, after acid hydrolysis (4). Plasmas were submitted to phenylboronate (PB) affinity chromatography (Glycogel B test kit; Pierce Chemical Co., Rockford, IL) by the procedure recommended by the manufacturer. The percentage of the PB-bound protein fraction was deduced from the absorbance of the column eluate at 280 nm. PB-bound fractions were desalted on PD 10 disposable columns (Pharmacia Fine Chemicals, Uppsala, Sweden) and lyophilized. Plasmas and PB-bound fractions were assayed for AT III by radial immunodiffusion and rocket immunoelectrophoresis (Nor-Partigen Antithrombin III plates and Clotimmun Antithrombin III antiserum from Behringwerke, Marburg, F.R.G.).

Figure 1 clearly shows that AT III can be readily detected in the PB-bound fraction in plasmas from diabetic patients (wells 3 and 4), whereas such is not the case for a normal control (well 7). To confirm that binding of AT III to PB in the diabetic samples indeed results from nonenzymic glycation of AT III, we incubated a control plasma at 37 °C for eight days in the presence of 50 and 200 mmol of glucose per liter, respectively, with 100 mg of gentamicin sulfate per liter as preservative. Clearly, the amount of AT III retained on the PB column increases with the extent of in vitro nonenzymic glycation (wells 7 to 9).

Quantitative results are shown in Table 1. The data in this table confirm that the percentage of PB-bound plasmas proteins parallels the extent of nonenzymic glycation, reflected by the amount of protein-bound sugars determined by the colorimetric assay. Actually, on comparing this assay and PB affinity chromatography, we found a correlation coefficient (r) of 0.977 for the samples so determined. We are therefore rather confident that the proportion of PB-bound AT III reflects the extent of nonenzymic glycation of this protein. Because our assay for AT III is based upon its immunological reactivity, our results can be valid only if nonenzymic glycation does not affect the immunological properties of AT III. This is actually the case, because the amount of immunologically reactive AT III does not change after eight days of incubation in the presence of glucose. (In Figure 1, compare wells 10 to 12, all from the same plasma, but with the PB-bound plasma protein fraction varied from 6 to 60%.) In the diabetic samples, however, we observed some discrepancies between the proportions of HbA1c, glycated plasma proteins, and PB-bound AT III, probably a reflection of the different life span of these proteins, which should return to a normal level of glycation at different rates upon equilibration of the hyperglycemic status.

Our data provide evidence for excessive nonenzymic glycation of AT III in diabetic patients. Together with the demonstration of Brownlee et al. of an altered heparin–AT III interaction after in vitro glycation (1), our data support their hypothesis that the heparin-reversible increase in the rate of fibrinogen disappearance induced by hyperglycemia in vivo (6) results from nonenzymic glycation of AT III. Defec-

Table 1. Quantification of Nonenzymically Glycated Proteins

<table>
<thead>
<tr>
<th></th>
<th>HbA1c</th>
<th>Glycated plasma proteins, nmol/mg protein</th>
<th>Phenylboronate-bound fractions, % of total</th>
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<tr>
<td></td>
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<tr>
<td>Normal control</td>
<td>5.1</td>
<td>1.77</td>
<td></td>
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<tr>
<td>Incubated with glucose:</td>
<td></td>
<td></td>
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<tr>
<td>50 mmol/L</td>
<td>9.3</td>
<td>2.30</td>
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<tr>
<td>200 mmol/L</td>
<td>7.8</td>
<td>2.52</td>
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<tr>
<td>Diabetic plasmas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>13.8</td>
<td>2.76</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>17.1</td>
<td>3.25</td>
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</table>

*By colorimetry (4), HMF, 5-hydroxymethylfurfural derivatives of hydrolyzed sugars.

*By radial immunodiffusion.