suggested the use of the "Centrifree Micropartition" (Amicon)
ultrafiltration device to separate myoglobin and hemo-
globin. The membranes used in this device have a cutoff at a
relative molecular mass of 30 000; thus they retain hemo-
globin but allow myoglobin to pass. Our experience with
them has highlighted several points of interest to potential
users.

We analyzed urines from normal individuals and hospital
patients, as well as specimens supplemented to give various
concentrations of hemoglobin and myoglobin. The devices
were centrifuged in a fixed-angle (30°) rotor at 1500 × g for
30 min and the filtrate was tested for myoglobin. The
essential findings are:
(a) The simplest, most-sensitive method for detecting
myoglobin in the filtrate is with a "dipstick" based on the
heme peroxidase reaction (we used "Ecor-Test," Boehringer
Mannheim). The detection limit is 0.5 mg/L and the range of
colors developed makes it possible to distinguish three
myoglobin concentrations: <1, 1-3, and 5 mg/L or greater.
We quote a usual value for urine myoglobin of <0.1 mg/L, so
urines that are dipstick negative need not be processed. If
necessary, concentrations >5 mg/L can be quantified by
spectrophotometry, the detection limit being 5 mg/L.
(b) When >200 mg of hemoglobin per liter is present in
urine, enough of it passes through the membrane to produce a
false positive for myoglobin with the dipstick. This
situation is usually identified by the appearance of a red hemo-
globin "pellet" on the membrane. The hemoglobin interfer-
ence is easily eliminated by centrifuging the filtrate in a
new device, as before, and retesting. We recommend this
action routinely for all positive filtrates; it is cost-effective
owing to the infrequency of requests for urinary myoglobin
and the unlimited shelf life of the devices.

The Amicon device offers a rapid, sensitive technique for
semiquantitative measurement of myoglobin in urine, even
in the presence of hemoglobin. A note of caution: use a
positive urine control. We have encountered a few devices
with membranes that, for reasons unknown, retain both
myoglobin and hemoglobin.

Reference
1. Kelner MJ, Alexander NM. Rapid separation and identification
of myoglobin and hemoglobin in urine by centrifugation through a

Urinary N-Acetyl-β-D-glucosaminidase and Albumin: No
Increase in Mildly Hypertensive Subjects, R. W. L.
Siebers, C. W. Lim, and T. J. B. Maling (Dept. of
Medicine, Wellington School of Medicine, and 1 Dept. of
Chem. Pathol., Wellington Hospital, Wellington, New
Zealand)

N-Acetyl-β-D-glucosaminidase (NAG, EC 3.2.1.30), a
proximal tubular lysosomal enzyme, is often increased in
the urine of hypertensive subjects (1), even in the absence of
clinical signs of renovascular disease. An increase in mi-
croalbuminuria has also been demonstrated in subjects with
moderate to severe hypertension (2), probably owing to
increased glomerular filtration of albumin. Anti-hyperten-
sive therapy reduces the increased urinary excretion of
NAG (1) and of albumin (2), suggesting the need for early
and effective treatment of hypertension. However, no data
exist to support increased urinary excretion of NAG or
albumin in mild hypertension, which has prompted us to
investigate whether mildly hypertensive subjects not on any
drug treatment had increased urinary excretion of NAG and
(or) albumin.

We asked 17 mildly hypertensive men with a positive
family history of hypertension and 13 normotensive men
with no family history of hypertension to collect two conse-
cutive 24-h urine specimens. All subjects were of similar
weight and age, and were taking no medication. All the
consecutive 24-h specimens were analyzed for NAG (3) and
albumin (4), and creatinine clearance and mean 24-h values
were calculated.

Our results, tabulated below, indicate that neither 24-h
urinary NAG nor urinary albumin excretion differed signifi-
cantly between the two groups (P >0.05 by nonpaired two-
tailed Student's t-test). These results suggest that urinary
NAG or albumin excretion cannot be used as an index of
antihypertensive therapy in mildly hypertensive subjects
because there is no evidence of underlying renal abnormal-
ity, despite their suggested role as sensitive indicators of
glomerular-tubular dysfunction in more severe hyperten-
sion.

<table>
<thead>
<tr>
<th>Blood pressure, mmHg</th>
<th>Creatinine clearance, mL/s</th>
<th>NAG, mg/L</th>
<th>Albumin, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic</td>
<td>D 1stolic</td>
<td>24 h</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>115.5</td>
<td>80.8</td>
<td>1.92</td>
</tr>
<tr>
<td>SD</td>
<td>9.6</td>
<td>5.4</td>
<td>0.28</td>
</tr>
<tr>
<td>Range</td>
<td>97-136</td>
<td>72-88</td>
<td>1.59-2.37</td>
</tr>
<tr>
<td>Hypertensive men (n = 17)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>134.2</td>
<td>98.9</td>
<td>1.94</td>
</tr>
<tr>
<td>SD</td>
<td>8.4</td>
<td>6.4</td>
<td>0.43</td>
</tr>
<tr>
<td>Range</td>
<td>118-152</td>
<td>90-110</td>
<td>1.28-2.69</td>
</tr>
</tbody>
</table>

*1 mmHg = 133 Pa.

This study was supported by the National Heart Foundation of
New Zealand.

References
1. Alderman MH, Melcher L, Drager DE, Reidenberg MM. In-
creased excretion of urinary N-acetyl-β-glucosaminidase in es-
sential hypertension, and its decline with antihypertensive therapy. N
2. Purvung HH, Morgenstern CE, Jensen HA, Evinr PE. Increased
urinary albumin excretion rate in benign essential hypertension. Lancet
1974;i:1190-2.
3. Lockwood TD, Boemann HB. The use of urinary N-acetyl-β-
glucosaminidase in human renal toxicology. Toxicol Appl Pharma-
4. Fielding BA, Price DA, Houlton CA. Enzyme immunoassay for

Interference by an IgM Paraprotein in the Bromcresol
Green Method for Determination of Serum Albumin,
Roberta G. Reed (Medical Research Institute, The Mary
Imogene Bassett Hospital, One Atwell Rd., Cooperstown,
NY 13326)

A patient with alcoholic liver disease showed serum
albumin below the detectable limit (10 g/L) on the Beckman
"Astra Ideal." When her serum was mixed with an equal
volume of a control serum and re-analyzed, the calculated
value for her serum albumin was 7 g/L. When the serum
was analyzed by electrophoresis, two striking observations
were made. First, albumin was present at 37 g/L and,
second, there was a large paraprotein band at 29 g/L in the beta region, which was determined to be an IgM-kappa by immunofixation electrophoresis.

To determine whether the presence of the paraprotein or an unusual property of this patient's albumin was responsible for the inability to quantify albumin by bromcresol green binding, I isolated the patient's albumin by affinity chromatography on Cibacron Blue-Sepharose and the IgM fraction by chromatography on DEAE-cellulose and G-150-Sephadex. The patient's isolated albumin showed normal ability to react with bromcresol green. When the isolated IgM paraprotein was added to the patient's albumin or to any other serum sample, the albumin concentration was underestimated in proportion to the amount of IgM added.

The kinetics of the reaction of bromcresol green and albumin were monitored in normal serum and in the patient's serum. Albumin normally reacts rapidly with bromcresol green, and color development is complete in <5 s. The presence of the IgM paraprotein in this patient's serum delayed the reaction such that color development required several minutes to reach its final value. Since the Astra Ideal reads absorbance at 10 s and computes albumin concentration as if the reaction were complete, albumin concentration was significantly underestimated.

To determine whether the IgM was an antialbumin, and hence was interfering competitively with the dye, I immobilized albumin and the IgM on agarose (Sepharose), and prepared affinity columns with these (I). The IgM column (12 mg of IgM per 5 mL of Sepharose) demonstrated no affinity for 2 mg of albumin, and the albumin column (120 mg of albumin per 20 mL of Sepharose) demonstrated no affinity for 14 mg of the IgM. Evidently the interaction between albumin and the IgM that interferes with the bromcresol green binding is not a specific antibody–antigen interaction.

To determine whether other IgM paraproteins might also slow the reaction of albumin with bromcresol green, I studied 15 additional serum samples containing IgM paraproteins in concentrations ranging from 0.4 to 10 g/L. None showed any evidence of a slowed reaction between albumin and bromcresol green, all reaching final color development within 5 s.

The suppression of reaction between albumin and bromcresol green that occurred in the presence of this IgM paraprotein does not appear to be a common phenomenon for IgM paraproteins but an isolated property of the specific IgM described.

This work was supported by USPHS Grant AM 32581 and the Stephen C. Clark Research Fund.

Reference


Absence of Statistical Association between Hyperglycemia and Metabolic Acidosis in both Type I and Type II Diabetic Patients on Chronic Dialysis, A. H. Tsamaloukas and P. S. Avasthi (Renal Section, VA Medical Center, Albuquerque, NM 87108)

Renal function contributes to the mechanisms of metabolic acidosis that is frequently seen in hyperglycemia. We studied the effects of absent renal function on metabolic acidosis by comparing concentrations of blood glucose and TCO₂ in 41 (15 type I) diabetics on hemodialysis (HD; 2196 samples) and 14 (eight type I) diabetics on peritoneal dialysis (PD, 258 samples). We used the definitions: euglycemia, glucose 3.3–10 mmol/L; significant hyperglycemia, glucose >30 mmol/L; significantly low TCO₂, below the 99% lower euglycemic confidence limit, or <15 mmol/L.

Frequencies of low TCO₂ were as follows: in HD, euglycemia 9%, hyperglycemia 10% (not significant, NS, by χ²); in PD, euglycemia 14%, hyperglycemia 16% (NS). Frequencies of hyperglycemia were as follows: in HD, normal TCO₂ 11%, low TCO₂ 11% (NS); in PD, normal TCO₂ 14%, low TCO₂ 18% (NS). Low TCO₂ was due to ketoacidosis in three HD and three PD samples with hyperglycemia. No other pathogenetic association between acidosis and hyperglycemia was detected. In 70% of the hyperglycemic samples with low TCO₂, a clinical cause of metabolic acidosis unrelated to hyperglycemia was present. Overall comparison between TCO₂ concentrations at different blood glucose concentrations (variance analysis) showed no statistical significance for either HD (Figure 1) or PD samples. Absence of low TCO₂ means absence of uncomplicated metabolic acidosis.

For both type I and type II diabetics on dialysis, metabolic acidosis is not observed more frequently in hyperglycemia than in euglycemia. When (rarely) metabolic acidosis is caused by hyperglycemia, ketoacidosis is the only recognizable cause. The other causes (lactic acidosis, hypertonicity) of metabolic acidosis in hyperglycemia of patients with intact renal function are absent in hyperglycemic dialysis patients.


This method for assaying urinary oxalate is based on measurement of oxidation products produced by the catalytic activity of the enzyme oxalate oxidase (I–3). Reagents were from Sigma Diagnostics, St. Louis, MO. We used a Multistat III® microcentrifugal analyzer (Instrumentation Laboratory Inc., Lexington, MA).

After extraction of oxalate from control pools and patients' specimens, all calibrators, controls, patients' samples, and