the decrease in ACE may have been due to a direct effect of
the steroid on ACE. More recently, decreases in ACE
activity were reported in samples from patients with
asthma or sarcoidosis dosed with prednisolone in vitro (2). We
have made a more comprehensive study of the effects of
prednisolone and methylprednisolone in vitro upon ACE
activity in samples from four distinct patient groups: those
presenting with small-cell bronchogenic carcinoma, pulmo-
nary sarcoidosis, or asthma, and a control group presenting
with no history or evidence of lung disease.

We assessed the following variables: two concentrations of
each steroid, pharmacological (3.3 μmol/L) and supraphar-
macological (1 mmol/L); incubation of steroid with plasma,
from 0 to 96 h; and incubation temperatures of 4 °C or 37 °C.
Incubation of plasma from control and diseased groups of
patients with pharmacological concentrations of either ste-
roid resulted in no significant decrease in ACE activity
under any conditions. At suprapharmacological concentra-
tions, incubation at either 4 °C or 37 °C for up to 48 h
produced no significant inhibition. However, when samples
were incubated for 96 h there was significant inhibition of
ACE activation at both temperatures in samples from all
groups except the lung-cancer group.

Qualitatively, these data support earlier findings (1, 2)
suggesting a direct role for corticosteroids on ACE activity;
they also indicate a similar action for methylprednisolone.
Quantitatively, we conclude that the direct inhibition of
ACE by prednisolone is less likely to interfere with the
clinical usefulness of ACE monitoring of prednisolone ther-
apy of sarcoidosis than was earlier thought. The lack of
inhibitory response seen in the samples from patients with
bronchogenic carcinoma warrants further study with sam-
ple from patients with different lung tumor histopatholo-
gies.

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Magnesium, Surfactants, and Centrifugal Analyzers, J.
Croker and M. J. Peake (Dept. of Biochemistry and
Chemical Pathology, Flinders Medical Centre, Bedford
Park, South Australia, 5042, Australia)

We have recently identified a problem that may cause
sporadic errors in the measurement of magnesium by a
colorimetric method (Clin Chem 1983;29:2120–2121) in the
Cobas Bio centrifugal analyzer (Roche Analytical Instru-
ments, Nutley, NJ 07110).

When using a bioMérieux calmagite reagent system (cat.
no. 61411) as per their recommendations for the Cobas Bio,
we have found that the 30-s time interval after the final mix
of sample with calmagite and base reagents may be insuf-
ficient for stable final absorbance measurements, and that
in some instances inappropriately high absorbances can per-
cist for as long as 2 min after mixing. Duplicate magnesium
estimations have varied within-run by more than 0.2
mmol/L in this and in another Cobas Bio in our department.

We suggest as the most likely cause for this anomaly the
presence of surfactant in the calmagite reagent, which
results in air bubbles and scattering of incident light. A
complete set of results will be invalid if an inappropriately
high absorbance occurs in either the standard cuvets (all
results falsely low), or the reagent blank cuvet (where
values less than the standard concentration will be falsely
low and other values falsely high). Individual results may be
falsely high in other instances. After having the mix cycle
on the centrifugal analyzer verified for correct operation, we
resolved this problem by extending the time for final
absorbance measurements to 4 min, thus improving within-
run precision to approximately 1% by the elimination of
gross errors.

We have noted this problem with other procedures used in
this instrument when large amounts of surfactant are
involved, and we recommend that, during method develop-
ment, unstable absorbance readings be investigated as a
possible reason for unacceptable imprecision. Failure to do
this may result in a potentially reliable method being
unnecessarily rejected.

Effects of Needle Size and Storage Temperature on
Measurements of Serum Potassium, L. Verresen,1 R.L.
Lins,1 H. Neels,2 and M.E. De Broe1, a (1 Dept. of
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Biochemistry, Algemeen Ziekenhuis Stuiwenberg.
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Various sampling, processing, and storage conditions re-
portedly affect the measurement of potassium in blood. The
aim of this study was to evaluate the effect of the puncture
needle size and the storage temperature of the blood.

Using a 23- or 19-gauge needle we collected blood speci-
mens into polystyrene tubes for clotted blood, then mea-
sured the potassium with an Hitachi automatic analyzer.
The results were analyzed by the Wilcoxon matched-pairs
signed ranks test.

The mean (± SEM) concentration of serum potassium in
samples drawn with a 19-gauge needle (n=10) was 4.1 ± 0.5
mmol/L and in those drawn with a 23-gauge needle (n=10),
4.4 ± 0.6 mmol/L. The mean difference between the two
groups was 0.3 ± 0.2 mmol/L (p <0.001). The mean concen-
tration of potassium in blood analyzed immediately (n=30)
was not significantly different from the value observed in
the samples stored for 16 h at 18 °C (n=30): 4.5 ± 0.8 and 4.6
± 0.7 mmol/L, respectively. In contrast, there was a signif-
cant difference (p <0.001) between the untreated samples
and the samples stored at 4 °C for 16 h (n=30) (6.2 ± 0.8
mmol/L).

The accuracy and precision of determinations of serum
potassium decline rapidly when samples are stored. In
particular, when serum is not separated from erythrocytes,
there is a constant interaction between serum and cells. One
of the mechanisms is an active transport system, coupled
with the absorption and phosphorylation of glucose, that
pumps potassium into the erythrocytes (TS Danowski, J
Biol Chem 1941;139:693–705). Reducing the temperature
results in nonspecific enzyme inhibition, blocking this trans-

However, earlier observations provided conflicting data. Our results demonstrate that storage at room temperature does not influence the accuracy of potassium measurements during at least the first 16 h after sampling. This could not be attributed to an increased concentration of serum glucose, which might have continued active potassium transport, because these concentrations of glucose were within normal values (mean 5.23 ± 0.96 mmol/L).

The specimen should never be stored in the refrigerator. If the blood specimen has to be stored overnight without centrifugation, simple storage of a clotted blood specimen at room temperature gives reliable results. Furthermore, needle size can be critical in the collection of blood samples for potassium measurement.

We thank Mr. W. Denissen for analyzing the blood specimens.

### Urinary Alanine Aminopeptidase Determined by Centrifugal Analysis, with Special Reference to Sample Storage, Cl. Philippson, J. L. Sansot, A. Cattaneo, D. Prévot, and Y. Manuel (Biochemistry Dept. and INSERM U 139, Hôpital Henri Mondor, 94010 Créteil, France)

Urinary excretion of alanine aminopeptidase (AAP; EC 3.4.11.2) is a possible index to brush-border membrane activity of the proximal tubule. We have automated determination of the enzyme, using a centrifugal analyzer (Cobas Bio; Roche). Samples were 24-h urines that had been centrifuged at 5000 × g for 10 min. For standard controls we used pooled serum of healthy adults and gel-filtered (Sephadex G25, Pharmacia) urine samples, which we stored at −20 °C. These served as controls to estimate between-day variation of the method.

The assay buffer was Na2HPO4 and KH2PO4 (0.1 mol/L, pH 7.6). The substrates, 1-alanine-4-nitroanilide hydrochloride and 1-leucine-4-nitroanilide, were from Merck, Darmstadt, F.R.G.

From the manual technique of Mondorf et al. (1) and the optimized assay of Jung and Scholz (2) we adapted the kinetic multi-point Cobas assay. After a lag phase of 1 min, the choice of measurement intervals is based on well-defined reaction conditions, during which the enzyme follows zero-order kinetics. The Cobas Bio settings were as follows:

<p>| | | |</p>
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<td>5</td>
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<tr>
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<tr>
<td>11</td>
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</tr>
<tr>
<td>12</td>
<td>Reagent vol, μL</td>
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<tr>
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</tr>
<tr>
<td>15</td>
<td>Time of first reading; s</td>
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</tr>
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</table>

AAP activity was maximum at pH 7.6. The calculated $K_m$ was $3.3 \times 10^{-3}$ mol/L, which corresponds to 83% of $V_{max}$. The standard curve extended to more 200 U/L, well above the values usually found for normal and pathological urine samples.

AAP activity in urine samples was stable for only one week at 4 °C. For a longer storage, remove the low-Mr inhibitors by gel filtration on G25 columns (3, 4), freeze the samples, and store them at −20 °C. The enzymatic activity is fully conserved under these conditions.

Between-day reproducibility (CV), tested on frozen urines samples during several months, was 2.3% for pathological samples (90 ± 2.07 U/L) and 4.9% for normal samples (12 ± 5.88 U/L).

AAP apparently hydrolyzes both alanine-4-nitroanilide and leucine-4-nitroanilide. The high coefficient correlation ($r = 0.915$) indicates low specificity.

This method is rapid, accurate, and useful for investigating a great number of urinary samples each day (90 to 100 determinations per hour).

### References


### Modified Enzymatic Assay for Acetaminophen, Ahmed Alkhayat (Dept. of Chem. Pathol., Withington Hospital, Manchester, U.K.; current address: Dept. of Chem. Pathol., Wythenshawe Hospital, Manchester, U.K.)

Two kits are available for enzymatic measurement of acetaminophen in serum. Each requires 1 mL of enzyme reagent, the primary reason for the high cost of assay, but this can be reduced by the use of automation (1, 2). I have reduced the volume of the phenyl acylamidase (EC 3.5.1.13) enzyme reagent used in the manual assay; use of laboratory-prepared color reagents further decreases the cost, while maintaining acceptable precision at medical decision levels. The modified manual assay costs about 5% as much as the recommended kit method.

Color reagents were prepared as described previously (3). Small aliquots of the reconstituted enzyme reagent were stored at −20 °C. The reference solution was aqueous acetaminophen, 150 mg/L (990 μmol/L). For each determination mix 50 μL of enzyme reagent with 50 μL of blank (water), standard, or test sample and incubate at room temperature for 4 min. Add 1 mL of color reagent A (0-cresol), then 1 mL of color reagent B (ammoniacal cupric sulfate), mix, and incubate for 4 min at room temperature. Measure absorbance at 615 nm. For automation I modified the method described by Hallworth (2), as follows: limit 450 mg/L (2980 mg/L).