samples with very unusual or severe electrolyte imbalance where organic anions have replaced chloride ions in the sample being measured. Such samples produce discrepancies (negative biases) because of unequal cation and anion movement at the interface between reference fluid and sample established in the paper bridge that connects the two half cells (11). Improvements have been made by Kodak with development of generation 04 reference fluid, which minimizes the junction-potential effect, and reduces method-to-method discrepancies in patients with extreme electrolyte imbalance (12).

A recent report from the Expert Panel on pH and Blood Gases of the IFCC (13) discussed the activity-vs-concentration issue. That article supported the belief that activity is the more important physiological and biochemical parameter. They did not, however, support the idea of additional reference intervals being introduced.

It was recommended that the measured activity be multiplied by the appropriate constant to obtain the same values as from a flame photometer with normal serum. Kodak has addressed this issue by calibrating with serum-based calibrators, that have values assigned by flame photometry. Reference ranges for all methods would then be similar; however, one would still expect to see inter-method differences in patients with significant volume-displacement or sodium-binding effects.

Eastman Kodak provided the materials necessary for evaluation of generation 04 sodium slides. We thank Mr. Nicholas Van Brunt (Eastman Kodak Co., Clinical Products Division) for technical advice.

References

Biological Variation in Analyte Concentrations in Urine of Apparently Healthy Men and Women

Elizabeth M. S. Gowans and Callum G. Fraser

Analytical, intra-individual, and interindividual components of variation for sodium, potassium, urea, creatinine, calcium, and phosphate were estimated from results for 24-h urine specimens collected from 15 apparently healthy individuals every four weeks for 40 weeks. Expressed as output, mean values differed for men and women, except for calcium. Our data on intra-individual variation were similar to those obtained for 10 men by Shephard et al. (Clin Chem 1981;27:569–72). Calculated analytical goals are easily attained by current methods. Reference values for urine creatinine are useful only when expressed as output and stratified according to gender. The ratios of intra- to interindividual variation generally increase on such stratification; separate reference values for men and women are therefore required for analytes expressed as output. Measurements of sodium and potassium in urine should be reported as concentration, but output terms are favored for the other analytes. Differences for two serial results from an individual must be rather large to differ statistically.

Additional Keyphrases: variation, sources of, electrolytes, urea, creatinine, calcium, phosphate, sex-related differences, output vs concentration as mode of expressing results

Numerical data on analytical, intra-individual, and interindividual biological variation are of considerable interest to the clinical chemist, being useful for (a) assessing desirable standards of performance (1), (b) determining the utility (or lack of it) of conventional population-based reference values (2), (c) evaluating whether serial results obtained from an individual are statistically significant, and (d) deciding which specimen or test provides the most useful clinical information.

There are many data on the biological variation of analytes in serum or plasma (3), but few regarding analytes in
urine. A decade ago, Glenn and Hathaway (4) stated that
goals for urine-chemistry methods could not be set because
neither biological nor analytical variability had yet been
well defined for this milieu. Since then, studies of interlab-
oratory performance in quality-assessment schemes such as
those conducted by the College of American Pathologists (5)
and in Australia (6) have been published, as well as studies on
comparative methodologies for determining proteins (7, 8)
and calcium (9). In addition, analytical goals for some
analytes in urine have been proposed from data on the state
of the art achieved by laboratories (10), and from the
opinions of clinical staff (11). However, despite the widely
held current view that goals should be derived from bio-
logical variation data (1), only one study on this has been
reported (12), with the deficiencies that (a) only men were
studied and (b) only five specimens of urine were collected
from each over five-day and five-month periods.

We therefore assessed analytical, intra-individual, and
inter-individual variation in both men and women, collect-
ing 10 specimens from each subject during 40 weeks.

Materials and Methods

Subjects. We recruited for this study 15 apparently
healthy members of the laboratory staff (seven men and
eight women, ages 20 to 53 years). During the study period,
none took any medication or significant quantities of alco-
hol, and all maintained their usual lifestyles.

Specimen collection and handling. Once every four weeks
for 40 weeks, each subject carefully collected a 24-h urine
specimen into a 2.5-L plastic container that contained 50
mL of 3 mol/L HCl as preservative.

The specimens were accurately weighed, and aliquots
stored at −20 °C until analysis. On the day of assay, we
thawed at ambient temperature all aliquots from a single
subject, thoroughly mixed each one, and centrifuged them to
ensure optical clarity.

Analytical techniques. For each specimen we measured
sodium, potassium, urea, creatinine, calcium, and phos-
phate as follows. For sodium and potassium we used an IL
243 flame photometer with an IL 244 dilutor (Instrumenta-
tion Laboratory, Lexington, MA 02173), and IL reagents.
Calcium, phosphate, and creatinine were determined by o-
cresolphthalein complexone, ammonium molybdate, and
direct (13) alkaline picrate colorimetry, respectively, with a
Technicon RA-1000 "random-access" analyzer (Technicon
Instrument Corp., Tarrytown, NY 10591), and Technicon
reagents. We quantified urea by diacetylmonoxime colori-
metry with a Technicon AutoAnalyzer II continuous-flow
analyzer, and in-house reagents. All the specimens from an
individual were assayed for all analytes on the same day, in
two runs, the order of specimens being randomized between
runs.

Analytical variance was minimized by using single lots of
reagents, standards, and quality-control materials through-
out, and analyses were performed by a single analyist.

Calculation of results. The total variance was divided into
the components attributable to analytical, intra-individual,
and interindividual variance. Because, in assay procedures,
quality-control materials may not behave the same as
specimens from patients (14), we calculated analytical var-
ance from the results of the duplicate analysis of each
specimen. We used only the first of each duplicate result to
calculate the average intra-individual variance and the
interindividual variance. Results calculated from the origi-
nal data were expressed both as concentration units and as
daily output. We used Student's unpaired t-test to assess
whether the means for men and women were different.

Results and Discussion

Analytical Results

Expressed as concentration, the mean values for men and
women showed no significant differences (P > 0.05) for any
of the analytes studied. The data generated were therefore
treated as a single set of data. Expressed as output, the
means for men and women were different for sodium (t =
2.72; 0.02 > P > 0.01), potassium (t = 3.76; 0.01 > P >
0.001), urea (t = 5.76; 0.001 > P), creatinine (t = 10.94; 0.001 >P), and phosphate (t = 5.12; P > 0.001). We therefore
analyzed the output data for the total group, and for men
and women separately. Table 1 lists the mean values, and
the analytical (CVa), intra-individual (CVi), and interindi-
vidual (CVi) variation, as coefficients of variation.

The overall mean values found for the total group of
subjects are lower than those of Shepard et al. (12),
although the mean output values determined for men
are similar. Our analytical CVs are greater than those of
Shephard et al. (12), and, except for creatinine, the overall
interindividual variations are also larger. These findings
were not unexpected because our group comprised both men
and women, not only men, and we assayed larger batches of
specimens. Interestingly, the intra-individual variations we
found for men and women over 40 weeks are very similar to
those found for men over five months (12). Because this is
also true for analytes in serum (15), we believe that this
constancy of intra-individual variation means that (a) exist-
ing data on biological variation, even those obtained over
the short term, can be validly used for a number of purposes,
and (b) setting up complex experiments with large numbers
of subjects is not necessary for deriving valid data on overall
intra-individual variation.

We used the data in Table 1 to:

- derive analytical goals for urine analytes,
- assess the usefulness of conventional population-based
  reference values, and the effects of stratification according to
gender, and
- determine the changes required in serial results, termed
critical differences (16), before significance can be claimed.

Analytical Goals

It is widely stated that analytical error (as SD or CV)
should not exceed one-half of the intra-individual biological
variation (1); that is, CVa ≅ 1/2 CVi. Because analyses of
urine may involve specimens from either sex, with results
expressed as either concentration or output, we decided that
the most stringent goal should be derived from the results
for the group having the least intra-individual variation.
Adoption of this strategy would ensure that a method
meeting this goal would be suitable for all purposes in
clinical chemistry (17). The analytical goals (CVi) are shown
in Table 2. As expected, our goals are similar to those
reported earlier (12), but we have expressed them as CV
rather than as SD because the range of analyte concentra-
tion in urine found in health and in disease is generally
wide, and our strategy allows use of the goal at all concen-
trations.

As previously stated (12), goals for analytes in urine,
derived from data on biological variation, are much wider
than the goals proposed for the same analytes in serum or
plasma (1). Thus, even though laboratories generally cannot
attain goals for serum creatinine or calcium—less than 10%
of laboratories participating in an international quality-assessment program can currently achieve those goals—and less than 20% of laboratories attain the goal for serum phosphate (18), goals for analytes in urine should be more easily achieved in current clinical practice. Indeed, this is demonstrated here, with analytical variance being <0.6% of the total for all analytes. Moreover, the median values for precision obtained recently (1981-1983) in the Australasian urine quality-assessment program (6) are lower than the proposed goals, showing that they can be attained.

Usefulness of Conventional Population-Based Reference Values

The dispersion of conventional reference intervals is due to a composite of analytical, intra-individual, and interindividual variation. When the index of individuality (CVI/CG) is less than 0.6, reference values are of limited utility, but, when CVI/CG is more than 1.4, such population-based reference values are of considerable use (2). Table 3 shows the indices for analytes in urine as concentration and output, and for men and women separately when appropriate.

Creatinine output has a low index (0.46) for the total group, and therefore reference values will be of limited use; in contrast, when stratified according to gender, reference values for creatinine in urine are useful. For all other analytes, the indices of individuality generally lie between 0.6 and 1.4, and reference values will be of some value in assessment of patients’ results, with the caveat that results for some individuals may be unusual for them even if they lie within the population reference intervals.

Compilations of reference data such as those of Eastham (19) and Tietz (20) do not list stratified reference values for men and women, although these are of higher utility, because CVI/CG rises when men and women are considered separately. This is expected because the average intra-individual variations of men and women separately are smaller than that of one composite group.

We believe that it would be useful to re-examine reference values for analytes in urine for both men and women, using modern analytical methods and the strategies proposed by the Expert Panel on Theory of Reference Values of the International Federation of Clinical Chemistry (21). [Ed. note: Chapter 5 in Tietz' Fundamentals of Clinical Chemistry (Saunders 1987), by Solberg, is also a valuable discussion of reference values.]

The highest indices of individuality are found for results expressed as output. Expression of results in these terms is favored when they are being compared with stratified reference values. When individual patients are being monitored, the best test to use is that with the smallest intra-individual variation. Expression as output is generally favored, although for sodium and potassium, concentration or output provides similar information.

Significance of Serial Results from the Same Patient

The critical difference required for two results to be significantly different (P <0.05) is 2.77 √SD2 + SD2. These differences are shown in Table 4 as concentration and output.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>In terms of concn</th>
<th>Total</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>0.72</td>
<td>0.93</td>
<td>0.94</td>
<td>0.81</td>
</tr>
<tr>
<td>Potassium</td>
<td>1.09</td>
<td>0.80</td>
<td>1.14</td>
<td>1.32</td>
</tr>
<tr>
<td>Urea</td>
<td>1.01</td>
<td>0.61</td>
<td>1.24</td>
<td>1.08</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.97</td>
<td>0.46</td>
<td>1.83</td>
<td>1.42</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.85</td>
<td>1.08</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Phosphate</td>
<td>1.21</td>
<td>0.83</td>
<td>1.04</td>
<td>1.41</td>
</tr>
</tbody>
</table>

CLINICAL CHEMISTRY, Vol. 33, No. 6, 1987 849
output, and, where appropriate, separately for men and women.

The differences are mainly due to biological rather than analytical variation, and are large compared with those for the same analytes in serum or plasma (16); we think that this is unlikely to be adequately appreciated.

Because women have lower mean values than men for all analytes except calcium, the critical differences are smaller for the former. For sodium and potassium, expression as concentration leads to smaller critical differences, and reporting of results as concentration has advantage. For all other analytes, reporting as output is further favored.

Overall Conclusions

From our data we conclude that:

- intra-individual biological variation of analytes in urine is similar in the two studies performed; therefore existing data can be validly used, and data on biological variation of analytes not yet the subject of study probably can be adequately generated by using small groups of subjects;

- analytical goals for analytes in urine derived from biological variation data are much less stringent than goals for the same analytes in serum or plasma, and can be easily attained with currently available methodology;

- reference values for creatinine output in urine will be of utility only when stratified according to gender; values for the other analytes we examined will be of rather less value, but stratification according to gender will render them more useful;

- expression of results as concentration is favored for sodium and potassium, and as output for the other analytes; and

- large differences between serial results are required before they are significantly different; these critical differences are larger in men than in women.

### Table 4. Differences in Serial Values Required before Results Are Significantly Different (P < 0.5)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Change in concn, mmol/L</th>
<th>Change in output, mmol/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>70.9</td>
<td>105.1</td>
</tr>
<tr>
<td>Potassium</td>
<td>33.8</td>
<td>41.0</td>
</tr>
<tr>
<td>Urea</td>
<td>168.3</td>
<td>145.4</td>
</tr>
<tr>
<td>Creatinine</td>
<td>5.10</td>
<td>3.88</td>
</tr>
<tr>
<td>Calcium**</td>
<td>2.54</td>
<td>2.57</td>
</tr>
<tr>
<td>Phosphate</td>
<td>15.3</td>
<td>13.4</td>
</tr>
</tbody>
</table>

*No significantly sex-related difference in mean calcium values, as output.

### References