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Urineysis: Minimizing Microscopy

To the Editor:

The microscopic part of a urinalysis is the most time consuming and thus the most expensive. It has been suggested that a microscopic examination is not necessary if there are no abnormal chemical (macroscopic) results (1). This has not seemed appropriate to us because of the inability to detect the presence of leukocytes by chemical means, but the recent introduction of tests for leukocyte esterase encouraged us to re-evaluate our position.

We performed macroscopic (Chemstrip 9, BioDynamics/bmc) and microscopic analyses on 188 samples submitted for routine urinalysis at our 665-bed acute-care hospital. Results of macroscopic examination were considered normal only if all of the following criteria were met:

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific gravity</td>
<td>1.003–1.030</td>
</tr>
<tr>
<td>Color</td>
<td>yellow or colorless</td>
</tr>
<tr>
<td>Hemoglobin/blood</td>
<td>negative</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>negative</td>
</tr>
<tr>
<td>Urobilinogen</td>
<td>negative</td>
</tr>
<tr>
<td>Leukocyte esterase</td>
<td>negative</td>
</tr>
<tr>
<td>Ketones</td>
<td>≤ trace</td>
</tr>
<tr>
<td>Glucose</td>
<td>≤ trace</td>
</tr>
<tr>
<td>Protein</td>
<td>≤ trace</td>
</tr>
<tr>
<td>pH</td>
<td>4.5–8.0</td>
</tr>
</tbody>
</table>

Results of microscopic examination were considered normal only if all of the following criteria were met:

- <6 erythrocytes or leukocytes/hpf (high-power field)
- <3 hyaline or <1 granular cast/lpf (low-power field)
- Absence of any other casts
- Absence of significant crystals (e.g., cystine)

The results were:

- Reagent-strip abnormal, 91 samples (48%)
- Microscopic abnormal 86
- Microscopic normal 5
- Reagent-strip normal, 97 samples (52%)

Microscopic abnormal 10
Microscopic normal 87

Ten (5%) of the 188 specimens with normal macroscopic analysis had abnormal microscopic findings:

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes/hpf</td>
<td>6–10</td>
</tr>
<tr>
<td></td>
<td>11–20</td>
</tr>
</tbody>
</table>

Improper collection technique appeared to be the source of discrepancy in eight (4%) of the samples. There was discrepancy on two samples (1%) that could not be explained. Both specimens had a negative macroscopic analysis but 6–10 and 11–20 leukocytes/hpf, respectively.

These results are different from those of a similar study (2) done at our hospital in 1979 before the introduction of tests for leukocyte esterase. In that study the disagreement rate was 20%.

Most of the discrepant samples had ≥11 leukocytes/hpf and results of microscopic examination were normal.

Because of the availability of tests for leukocyte esterase, and prompted by this study, we have modified our urinalysis procedure: we now perform microscopic analysis only if there is an abnormal result on macroscopic examination or if a microscopic examination is specifically requested.

References


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Prediction of the Change in Extracellular Sodium Concentration in Hyperglycemia Complicated by Severe Losses of Water and Solute

To the Editor:

The major determinants of extracellular fluid (ECF) sodium concentration are the total body water, the total body sodium, and the total body potassium (1); their relationship to ECF sodium concentration is expressed by the equation (2):

\[ \text{ECF Na} = \frac{(\text{total body K}) + (\text{total body Na})}{\text{total body water}} \]

Katz (3) predicted the changes in ECF sodium concentration that result from hyperglycemia, if the only change created by this hyperglycemia is the dilution of the ECF sodium, resulting from the osmotic water shift from the intracellular fluid (ICF) into the ECF space. The syndromes of diabetic ketoacidosis (4) and nonketotic hyperosmolar hyperglycemia (5) lead to alterations—at times extreme (5, 6)—in the amounts of body water, body sodium, and body potassium. Therefore, direct comparison of Katz’s prediction to the observed changes in ECF sodium concentration is not applicable to these two syndromes.

To study Katz’s principles of osmotic behavior, the net external changes in body water, sodium, and potassium should be incorporated in the analysis of the data from the two hyperglycemic syndromes. External losses of glucose may be omitted from the calculations, because the degree of hyperglycemia observed at any given moment can be considered as the cause of the osmotic events, regardless of any external losses of glucose. One method of accounting for the external changes in water and solute is to correct the observed changes in ECF sodium concentration for the changes in body water and solute, and then compare the corrected changes in ECF sodium concentration to Katz’s prediction. Nanji applied this method to data from patients with hyperglycemia and concluded that Katz’s principles of osmotic behavior were consistent with his findings (7).

I present here an alternative method of incorporating external changes in water and solute in the calculations, applicable to all patients, even the ones with severe potassium deficiency (Nanji’s method is not applicable in this last category of patients). Unlike Nanji’s method, the following method involves the correction of the prediction of the formula \( \Delta \text{Na}/\Delta \text{Gl} \) (glucose), not the patient’s data. Like Nanji’s method it also follows Katz’s principles of osmotic equality between ICF and ECF spaces in the steady state, and of no change in ICF solute and ECF solute during development of hyperglycemia. The correction for the water and solute losses from the hyperglycemia is made by assuming that the hyperglycemia develops in two stages: during the first stage the only event is the loss of water and solute, and during the second stage the only event is the increase in ECF glucose concentration. Further, potassium is assumed to be confined in the ICF space and sodium in the ECF space. For any given degree of hyperglycemia, the changes responsible for the final change in ECF sodium concentration are expressed by the six equations described below. The stage of solute and water losses is described by equations 1–4, and the stage of increase in ECF glucose
concentration by equations 5 and 6.

\[ ICFK = V_{\text{s}} - K_{\text{st}} - V_{\text{ur}} - K_{\text{ur}} \]  
\[ ECFNa = V_{\text{s}} + Na_{\text{st}} - V_{\text{ur}} - Na_{\text{ur}} \]  
\[ Ve_{\text{co}} = (V_{\text{s}} + V_{\text{st}} - V_{\text{ur}}) \cdot \phi_{\text{ECFNa}}/\phi_{\text{ECFK}} + \phi_{\text{ICFK}} \]  
\[ V_{\text{co}} = (V_{\text{s}} + V_{\text{st}} - V_{\text{ur}}) \cdot \phi_{\text{ICFK}}/\phi_{\text{ECFK} + \phi_{\text{ECFK}}} \]  
\[ \Delta Gi \cdot V_{\text{osm}} = [\phi_{\text{ICFK}} + \phi_{\text{ECFK}} - \phi_{\text{ECFK}} - \phi_{\text{ICFK}}] \cdot V_{\text{osm}} + \phi_{\text{ECFK}} - \phi_{\text{ICFK}} - V_{\text{co}} \]  
\[ \Delta Na = (V_{\text{s}} - V_{\text{co}} - V_{\text{osm}}) \cdot Na_{\text{ur}}/(V_{\text{co}} + V_{\text{osm}}) \]  
\[ Na_{\text{st}} - V_{\text{ur}} \]

where: ICFK = total ICF potassium in hyperglycemia and \( \phi_{\text{ICFK}} \) is its osmotic equivalent; ECFNa = total ECF sodium in hyperglycemia and \( \phi_{\text{ECFK}} \) is its osmotic equivalent; \( V_{\text{s}} \) = stable (normoglycemic) ICF volume; \( Ve_{\text{co}} \) = stable (normoglycemic) ECF volume; \( Ve_{\text{co}} \) and \( V_{\text{co}} \) = computed ECF and IC volumes, respectively; ICF = net loss of water during development of hyperglycemia (in the urine mainly); K_{\text{ur}} = average potassium concentration in the fluid lost; Na_{\text{st}} = stable (normoglycemic) ECF sodium concentration; Na_{\text{ur}} = average sodium concentration in the fluid lost; and \( \Delta Na = Na_{\text{adm}} - Na_{\text{st}} \), where Na_{\text{adm}} = admission (hyperglycemic) sodium concentration.

Equation 5 assumes that normal ECF glucose concentration is negligible. I will provide, on request, the derivation of equations 1–6.

Example: A 70-kg man with \( V_{\text{st}} = 28 \text{ L} \), \( V_{\text{s}} = 14 \text{ L} \), K_{\text{st}} = 140 mmol/L, and Na_{\text{st}} = 140 mmol/L develops hyperglycemia (\( \Delta Gi = 110 \text{ mmol/L} \)). Figure 1 shows the effects of various losses in body water, sodium, and potassium on the predicted value for \( \Delta Na/\Delta Gi \).

Negative \( \Delta Na/\Delta Gi \) values indicate that hyperglycemic states create decreases in sodium concentration from the stable baseline value. See text for interpretation.

\[ \Delta Na/\Delta Gi \]

In Fig. 1, all points mark the predicted value for \( \Delta Na/\Delta Gi \) in the body water, sodium, or potassium loss model. The value for sodium loss is an underestimate because sodium loss reduces plasma sodium concentration, and the concentration is used to calculate the predicted value. The value for potassium loss is an overestimate because potassium loss reduces plasma potassium concentration, and the concentration is used to calculate the predicted value. The value for water loss is an underestimate because water loss reduces plasma osmolality, and the concentration is used to calculate the predicted value.

Fig. 1 predicts that, given an appropriate combination of losses in water and solute, patients may present with increased sodium concentration, even in severe hyperglycemia. This finding is in agreement with clinical observations (5). The degree of hyperglycemia shown in Fig. 1 is severe, although not impossible for clinical presentation. To demonstrate the effects of moderate degrees of hyperglycemia, I recalculated the data in Fig. 1 for a \( \Delta Gi \) of 20 mmol/L instead of 110 mmol/L. Now, \( \Delta Na/\Delta Gi \) is equal to 1.82 for zero potassium losses and 4.98 mmol/L per 1 g/L \( \Delta Gi \) for potassium losses of 12.5% of normal.

The table of potassium losses is seen, this model predicts a ratio \( \Delta Na/\Delta Gi \) slightly greater (in absolute terms) in moderate than in severe hyperglycemia, a prediction made by another method also (8). (b) In the presence of potassium loss, the difference between \( \Delta Na/\Delta Gi \) values predicted for moderate and severe hyperglycemia increases greatly. The reason for this latter observation is as follows. \( \Delta Na/\Delta Gi \) in this example reflects two processes: loss of potassium and dilution of ECF sodium by the osmotic fluid shift into the ECF space. Because loss of potassium was assumed to be equal in severe and moderate hyperglycemia, it results in the same decrease in ECF sodium concentration in these states. The decrease in sodium concentration from the hyperglycemic water shift into the ECF space is much larger in severe than in moderate hyperglycemia. Therefore, the relative contribution of potassium to \( \Delta Na/\Delta Gi \) is small in severe hyperglycemia, accounting for only 0.49 mmol/L per g/L \( \Delta Gi \) and large in moderate hyperglycemia, accounting for 3.16 mmol/L per g/L \( \Delta Gi \).

These predictions can be compared directly to patients' data. Because losses in water and solute are usually not known at presentation, the method is applied retrospectively, assuming that the retained (administered minus lost during treatment) water, sodium, and potassium are equal to the losses during development of hyperglycemia (4, 5). The predicted hyperglycemic sodium concentration can then be compared with the actual sodium concentration at admission to test Katz's principles of osmotic behavior.

In the common hyperglycemic states, the observed deviation of plasma sodium concentration from normal represents the sum of the changes created by the osmotic fluid shift from the ICF into the ECF space plus the external losses in water, sodium, and potassium. In anuric hyperglycemia, no losses in water, sodium, and potassium are seen, and the change in sodium concentration is purely the result of the osmotic water shift. Thus, hyperglycemia in anuria is a proper model to test Katz's prediction. This prediction holds true when the body fluid state of anuric hyperglycemic patients at presentation is close to normal (9).

References
Reliability of Urinalysis for Glucose

To the Editor:

In 1961, Kirkland and Morgan (1) reported the very dimal state of routine ward and clinic urine testing for glucose and protein. Simpson and Thompson (2, 3) later observed some improvement, but the results were still far from satisfactory. They sent three contrived urine specimens (of known composition) to wards and clinics, and compared the results with those obtained in their own laboratory. The latter results were considered to be “correct.”

We proceeded to examine the correctness of results of urinalysis for glucose as performed by trained biochemists.

Six hundred random and freshly voided urine samples from outpatients attending our hospital were tested for glucose with “Neostix-3” (Miles India Ltd.) according to the instructions on the package insert. The glucose concentration in each sample was also determined colorimetrically by the o-toluidine method (4). Mannose and galactose, and to a lesser extent lactose, may also react with o-toluidine, but mannose in urine is rare, none of our patients were galactosemic, and none of the female patients were pregnant or lactating.

Neostix-3 is designed to give a positive test for glucose in a concentration of 1 g/L or greater. Twenty samples had a glucose concentration >1 g/L as determined by the o-toluidine method. Neostix-3 gave a positive test for glucose in 17 of these. When all the samples are considered, there was good agreement between the Neostix-3 and o-toluidine methods in 597 (99.5%) samples. However, missing three positive results out of 20 gives a failure rate of 15%, which is undoubtedly unsatisfactory. In these three samples, the glucose concentration was 1.20–1.36 g/L. Of the 17 samples that gave positive results, some had glucose concentrations as low as 1.06 g/L. All the analyses were performed by trained biochemists, so this variation is unlikely to be due to errors in performance of the tests. Variable concentrations of glucose oxidase inhibitors normally present in urine could account for these discrepant results. Pileggi and Szustkiewicz (4) have reviewed such factors.

The minimum concentration of glucose in urine samples prepared by Simpson and Thompson (2, 3) was 1.5 g/L. Even at this concentration, many erroneous results were reported by wards and clinics. Our results show that in urine samples having glucose concentrations between 1.0 and 1.5 g/L discrepant results may be much more frequent.

References

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γ-Glutamyltransferase Activity in Human Milk

To the Editor:

γ-Glutamyltransferase (GT; EC 2.3.2.2) reportedly is richest in the kidneys, with appreciable amounts in liver, pancreas, and prostate, but little in other organs.

While carrying out studies on the various enzymes present in human milk, we detected extremely high concentrations of GT in both colostrum and early breast milk, which we now report. At the time we made these observations we were unaware of similar earlier observations by Landon (1) and Binkley et al. (2).

We collected samples of colostrum within 48 h of delivery and of early breast-milk samples on the fourth postpartum day. Maternal blood samples were collected just before collecting colostrum samples. GT activity was determined according to Szasz (3). Samples of colostrum and early breast milk were diluted 100-fold; maternal serum was processed undiluted.

Measurements were made with a "Quickrate" reaction-rate photometer (Osiris Optical Co., Tokyo, Japan). The mean value ± SD for GT activity in 10 samples of colostrum was 22.99 ± 7.263 (range: 12 200 to 37 200), for 10 samples of early breast milk 4090 ± 2069 (range: 1300 to 8300), and for five maternal sera 8.6 ± 2.97 (range: 4 to 12) U/L. Thus the values for both colostrum and early breast milk were extremely high, as compared with maternal serum (p < 0.001), and activity in colostrum was significantly higher than in early breast milk (p < 0.001).

Our results are slightly lower than those of Landon, who reported GT activity of 28 800 ± 9700 (range: 14 800 to 48 200) U/L for 23 samples of colostrum. This difference is compatible with the observed progressive decrease in values, because Landon's samples were collected within 24 h of labor, while ours were within 48 h postpartum.

Binkley et al. (2), on the other hand, reported GT activity ranging from 490 to 6300 U/L for seven colostrum samples. This difference is probably methodological because the substrate used in their study was γ-glutamyl-β-naphthylamide, whereas γ-glutamyl-p-nitroanilide was the substrate in our study and that of Landon.

The antibacterial activity in human milk has been attributed to the combined action of immunoglobulins and lysozymes (4a). Whether the high concentration of GT also plays a role in this remains to be seen.

In the serum of the newborn the activity of GT is high, decreasing continuously during infancy. Presumably the source of this activity is ingested colostrum and early breast milk, which are rich in this enzyme, colostrum having the greater activity. In the early neonatal period, intact protein can be absorbed by the mucosal cells of the small intestine by pinocytosis. Within a few weeks, owing to the maturation of the intestinal cells, this is no longer the case (5). Many enzyme systems are not fully developed in the newborn. It is not known whether the enzymes in breast milk act as a source for the infant's requirement.

The precise mechanism by which the lactating mammary gland synthesizes enzymes needs to be elucidated. The active mammary gland is very efficient at removing precursors of proteins from blood. Perfusion studies in the goat (4b) have shown that the mammary gland can remove 60 to 70% of several amino acids from the blood in one passage through the gland.

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