rapid, accurate, precise, and adaptable to routine clinical laboratory analysis. Because our method is automated it is substantially more convenient than former methods (3–5). Use of serum or plasma, rather than an ultrafiltrate or protein-free supernate, and preincubation of specimen with NAD⁺ to eliminate interfering side reaction of endogenous dehydrogenases and their substrates simplifies the procedure. The working reagents are conveniently prepared from stock reagents with long-term stability. This assay should be useful in evaluating patients at risk of developing D(-) lactic acidosis.

References

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Salivary Urea Nitrogen as an Index to Renal Function: A Test-Strip Method
Toshihiro Akai,1 Keiichi Naka,1 Chikao Yoshikawa,1 Kiyoshi Okuda,1 Teruo Okamoto,2 Seiji Yamagami,3 Takashi Inoue,4 Yasuo Yamao,5 and Shigeki Yamada5

To investigate the feasibility of using salivary urea nitrogen as an index of renal glomerular filtration rate, we developed and applied a new analytical system consisting of a urease-containing test strip and an automatic reflectance spectrometer. The concentrations of urea nitrogen so determined correlate well (r = 0.93) with concentrations in serum. These preliminary data suggest that our method can be used routinely as a simple and reliable means of detecting abnormalities of renal function.

Additional Keyphrases: urea nitrogen  •  glomerular filtration rate  •  kidney disease

Measurement of urea nitrogen in blood is valuable for diagnosing renal diseases, particularly those associated with a reduction in glomerular filtration rate (GFR). However, specimens are obtained by venipuncture, an invasive technique. We have developed an alternative, noninvasive technique, a dry-reactant test strip. Use of the strip is based on the observation (1) that the concentration of urea nitrogen in saliva reflects its concentration in serum so that one may validly substitute saliva for serum as the sample.

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Here we report our development of a simple, reliable system for measuring salivary urea nitrogen, and its application as an indicator of renal function.

Materials and Methods

Reagents. The reagent layer of the strip consists of chromatographic paper (no. 52; Toyo Chromatographic Paper Co., Ltd., Osaka, Japan) that was immersed in 1 mol/L Tris buffer (pH 8.5) containing 2 g of urease (EC 3.5.1.5, from jack beans; Toyobo Co., Ltd., Osaka, Japan) per liter. The indicator layer of the strip consists of cellulose acetate film that was soaked in acetone containing 1.6 g of bromresol green (Wako Pure Chemical Industries Co., Ltd., Osaka, Japan) per liter.

In the urease–indophenol comparison method (2), we determined urea nitrogen in serum and saliva by using the "Urea NC Autotest" (Wako Pure Chemical Industries). Instrument. An instrument that measures reflectance automatically was used. In this system, the intensity of a color developed on a test strip is measured as the reflectivity of a single wavelength of light (583 nm) by use of a spherical integrator.

Procedure. Moisten the reagent layer by touching it to the tongue or by applying one drop (about 50 μL) of serum. Then remove the strip, peel off the protective layer from an adhesive patch, and place the adhesive patch onto the exposed surface of the reagent layer containing the buffered urease (Figure 1).
In this process, salivary or serum urea is hydrolyzed by the urease-containing reagent layer to produce ammonium hydroxide and carbon dioxide (see Figure 2). The ammonium hydroxide produces ammonia gas under alkaline conditions (pH 8.5), which changes the color of the brom cresol green in the indicator layer from yellow to blue. After a 15-min interval at room temperature during which the blue color develops, peel the indicator layer (bottom layer in Figure 1) from the strip, insert the layer into the reflectance spectrometer, and measure the intensity of the reflected light at 583 nm.

To calibrate this system, we used a strip having the same reflective intensity as that produced by 200 mg of urea nitrogen per liter.

**Patients.** We studied 44 patients, ages 16 to 68 years, with kidney diseases in various stages. Ten of the men and five of the women were regularly being treated by hemodialysis; the other 29, 19 men and 10 women, were not.

In addition, 12 patients with normal concentrations of urea in their serum and no history of nephropathy were the control subjects: seven men and five women, ages 18 to 62 years.

**Results**

*Precision.* Table 1 summarizes the within-run and day-to-day precision of the present method. In the study of day-to-day precision, saliva or serum specimens stored at -20 °C were allowed to thaw at room temperature before assay.

*Effect of ambient temperature.* As Figure 3 shows, the urea nitrogen values for saliva as determined by the present method appear to increase with increases in ambient temperature. We observed this same tendency when serum was used as the specimen.

*Effect of reaction time.* Duration of reaction time directly affected the measured concentrations of urea in saliva (Figure 4). Again, we saw the same tendency with serum samples.

*Clinical findings.* The mean concentration of urea nitrogen in serum of patients with kidney diseases who were receiving hemodialysis was 841 (SD 132) mg/L. In those not receiving hemodialysis it was 526 (SD 237) mg/L, and in control subjects, 147 (SD 43) mg/L. The respective concentration of urea nitrogen in saliva were 954 (SD 166), 556 (SD 231), and 159 (SD 45) mg/L.

*Comparison with the urease–indophenol method.* Figure 5 shows the relation between results by the present method and those by the urease–indophenol method for serum and saliva specimens.

Table 1. Statistical Summary of Results* on Six Specimens of Saliva, Six of Serum

<table>
<thead>
<tr>
<th></th>
<th>µg/L</th>
<th>SD</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within-run</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saliva</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>163</td>
<td>9.7</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>263</td>
<td>21.0</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>468</td>
<td>36.4</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>Day to day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saliva</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>87.3</td>
<td>6.2</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td>505</td>
<td>45.5</td>
<td>9.0</td>
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</tr>
<tr>
<td>997</td>
<td>26.3</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>149</td>
<td>6.4</td>
<td>4.3</td>
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</tr>
<tr>
<td>285</td>
<td>17.4</td>
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</tr>
<tr>
<td>503</td>
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<td>Day to day</td>
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<tr>
<td>934</td>
<td>42.0</td>
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</table>

*n = 10 for each set.
Discussion

Previous workers have suggested that concentrations of salivary urea nitrogen can be used for diagnosis of kidney diseases (3–6), but experimentally this has been difficult to confirm. One difficulty is that bacteria contaminate the saliva specimen, and the bacterial urease interferes. Albrecaen and Thaysen (7) demonstrated that use of parotid saliva obviated these problems, but such specimens are difficult to collect. In addition, they and other investigators who used the same sampling method (7–9) reported variations in the saliva/serum urea ratios (0.70–0.75).

Using our method, we assayed urea nitrogen in whole saliva and found (Figure 6) a saliva/serum urea ratio of approximately 1.03. We ascribe the higher ratio to the fact that salivary urea is measured so quickly, virtually eliminating the problems associated with bacterial contamination. Because our preliminary study showed a gradual increase in urea nitrogen with reaction time, we stop the reaction time at 15 min.

Urea nitrogen concentration also increased with temperature, but the method serves to provide clinically useful results when run at room temperature (about 25°C).

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References