Experiment 12  
Routine urine examination

[Objectives]
To examine urine for the presence of normal and abnormal constituents by routine urine analysis.

[Principles]
Urine is formed in nephrons by the combined processes of glomerular filtration, tubular reabsorption, and tubular secretion. Then urine is eliminated from the kidney into the ureters, thin-walled tubes that convey urine to the urinary bladder, where it is stored until it can be voided from the body (micturition). The glomerular filtrate is similar to blood plasma in composition, except that large molecules (molecular weights greater than 70,000) are excluded (e.g. plasma proteins, erythrocytes and leukocytes). As the filtrate passes through the renal tubules, some of its constituents are reabsorbed completely (e.g. glucose and amino acids) and some are partly reabsorbed (e.g. sodium). Toxic by-products of metabolism and substances in excess are retained in the filtrate or are secreted into the filtrate and finally excreted in the urine. Thus, the final composition of the urine is quite different from that of glomerular filtrate and reflects the integrity of kidney function and changes in blood composition.

An analysis of urine can yield valuable information about the health of the kidney and the body in general. Various diseases are characterized by abnormal metabolism, which causes abnormal by-products of metabolism to appear in the urine. For example, in diabetes mellitus, glucose appears in the urine (glycosuria). The volume of urine produced and its specific gravity give information about the state of hydration or dehydration of the body. Of course, examination of the urine alone cannot give a diagnosis. However, it is useful when performed in conjunction with other diagnostic methods for assessing various types of abnormal condition.

Normally, the color of urine is light to dark amber (yellow). The color is due to the presence of pigments, such as urochrome, urobilin, and hematoporphyrin, which are normally present in urine. The presence of abnormal constituents may change the color drastically. For example, the presence of hemoglobin will give the urine a brown to red color.
Freshly voided urine is clear and transparent. Cloudiness in freshly voided urine may indicate the presence of pus, blood, or bacteria from urinary tract infections.

The pH of normal urine is 4.8 to 7.5 depending primarily on dietary intake. The acidity of urine increases in acidosis (metabolic and respiratory) and during fever. Alkaline urine may be produced by letting it stand or by storing it in the urinary bladder; this is due to the conversion of urea to ammonia. Other causes of alkaline urine include excessive dietary intake of certain foods (e.g. fruits), the ingestion of alkaline substances (e.g. sodium bicarbonate), and various states of alkalosis (metabolic and respiratory).

The normal range for the specific gravity of urine is from 1.010 to 1.030. Higher values for the specific gravity indicate a more concentrated urine, and lower values indicate a more dilute urine. The specific gravity of urine tends to be low in diabetes insipidus and after excessive quantities of water have been taken. It may be high during fever and thirst, and in patients with diabetes mellitus.

Normally, all of the glucose filtered out of the glomerulus is reabsorbed by the proximal tubule, depending on the Na⁺-mediated co-transport system. The transport system has a maximum capacity which is normally not exceeded. However, when glucose in plasma exceeds 180 mg/dL, the transport capacity is exceeded and glucose begins to appear in the urine.

Ketones (include acetic acid, acetoacetic acid, and β-hydroxybutyric acid) are normally present only in trace amounts in urine. However, the excessive metabolism of fats, due to a high dietary intake of fat or a dependence of the cells on lipid metabolism to produce energy because of fasting, results in the presence of large amounts of ketones in urine.

Usually, only a small amount of protein is filtered from the glomerulus, and most of it is reabsorbed. Excess protein in the urine (proteinuria) reflects an abnormal leakiness or severe damage of the glomerular membrane or both. Various types of nephrosis and nephritis due to infection, vascular degeneration, and other causes may result in proteinuria.

Bilirubin is formed as an end product of heme metabolism. Usually, bilirubin is conjugated with glucuronic acid in liver cells, and is excreted in the bile. Bilirubin appears in urine when there is partial or complete obstruction of the extrahepatic biliary ducts, hepatitis, cirrhosis, or other types of destructive liver diseases.

Normally, the amount of free hemoglobin in the plasma is very small, however, hemoglobinurin occurs when there is an extensive or rapid destruction of erythrocytes.
at a rate that is too fast to allow for the adequate metabolism of free hemoglobin. The causative factors include several types of hemolysis, burns, crush injury, transfusion reactions, and poisons (e.g. snake venoms and mushroom). Some renal diseases (e.g. nephritis), in which the glomeruli are damaged and plasma protein and erythrocytes leak into the kidney tubules, are also positive for occult blood.

Normally, no nitrite is detectable in fresh urine. A positive test indicates the presence of infectious microorganism, such as bacteria, that can transform dietary (urinary) nitrate to nitrite.

Urobilinogen is converted from bilirubin by bacteria in the intestine. Some of the urobilinogen is absorbed from the intestine into the blood, and excreted by the kidney into urine. It is a pigment imparting a yellow to orange color to the urine.

Leukocytes generally are absent from urine, although trace amounts of white cells may not be associated with disease. A small or greater positive test for leukocytes in the urine may indicate some renal diseases, or disease of the ureters, urinary bladder, or urethra.

[Experimental subject]
Student volunteer

[Experimental Equipment]
Clinitek 500 Urine Chemistry Analyzer (Bayer Medical Corporation), Bayer Reagent Strip, glass tube, container, gloves

[Experiment Procedure]
1. Take a container to the restroom and collect 15-20 ml mid-stream urine sample from a student volunteer.
2. Return to the laboratory bench and pour the urine to a glass tube.
3. Examine the urine specimen for color, clarity. Record your observations in the report.
4. Firstly, completely immerse all reagent areas of the Bayer reagent strip in the urine. Then, immediately remove the reagent strip from the urine. While removing the strip, slowly run the edge of the entire length of the reagent strip against the side of the tube to remove excess urine. Finally, place the strip, with reagent area facing up, onto the strip support of the strip loading station, to the right of the small embossed arrow (V) and against the rear wall of the platform. The strip will automatically be advanced along the loading station, under the read
heads for reading color by reflectance colorimetry, then into the waste bin. The results will be printed (including specific gravity, pH, leukocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, and hemoglobin/occult blood).

[Results]
Examine the urine specimen and complete Table 1.

Table 1. Results of urine tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Abbr.</th>
<th>Normal value</th>
<th>Reported results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>COL</td>
<td>Yellow to Orange</td>
<td></td>
</tr>
<tr>
<td>Clarity</td>
<td>CLA</td>
<td>Clear</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>pH</td>
<td>4.8-7.5</td>
<td></td>
</tr>
<tr>
<td>Specific Gravity</td>
<td>SG</td>
<td>1.005-1.030</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>GLU</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Ketone</td>
<td>KET</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>PRO</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Occult Blood</td>
<td>BLO</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Bilirubin</td>
<td>BIL</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Urobilinogen</td>
<td>URO</td>
<td>3.2-16 µM</td>
<td></td>
</tr>
<tr>
<td>Nitrite</td>
<td>NIT</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Leukocytes</td>
<td>LEU</td>
<td>Negative</td>
<td></td>
</tr>
</tbody>
</table>

[Conclusion]
Is the urine of the volunteer normal? If not, what component in urine is abnormal? Why?

[Discussion]
1. Under what conditions would bilirubin be present in the urine?
2. Does the presence of glucose in the urine always indicate diabetes mellitus? Why or why not?
3. Does the specific gravity of urine ever fall below 1.000? Please explain.