Estimation of Serum Urea and Urine Urea

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Lecture Overview

Urea

Source and fate
  - Production
  - Reabsorption
  - Excretion

Blood Urea

Urine Urea

Urea Clearance

BUN/Cr ratio

Experiments
  - 1-Estimation of Blood Urea
  - 2-Estimation of Urine Urea
Source and fate of urea

Production:

Urea is the final degradation product of protein and amino acid metabolism. In protein catabolism the proteins are broken down to amino acids and deaminated. The ammonia formed in this process is synthesized to urea in the liver. This is the most important catabolic pathway for eliminating excess nitrogen in the human body.

the **Rate of urea cycle** dependent on nitrogen and intake and internal breakdown.
Source and fate of urea

Reabsorption:
Approximately 40%-50% of the filtered urea undergoes passive reabsorption in the proximal tubule.
Source and fate of urea

Excretion:
Most excreted through the kidney, small amount through the bowel and skin.
1-Estimation of Blood Urea

The determination of serum blood urea nitrogen is widely used for screening and evaluation of kidney function. The test is frequently requested along with the serum creatinine since simultaneous determination of these 2 compounds appears to aid in the differential diagnosis of prerenal, renal and post renal.

Increased blood urea nitrogen (BUN) may be due to:
- Impaired renal function (renal failure)
- Volume depletion (increase of urea reabsorption)
- High protein diet
- Catabolic States:

Decreased blood urea nitrogen (BUN) may be due to:
- Liver Disease
- Malabsorption
- Reduced protein intake (Starvation, anorexia)
2-Estimation of Urine Urea

- The **urine urea nitrogen test** determines how much urea is in the urine to assess the amount of protein breakdown. The test can help determine how well the kidneys are functioning, and if the intake of protein is too high or low.

- The urine urea nitrogen test is performed by collecting a 24-hour urine sample.

**Low levels of urea in the urine may suggest:**
- malnutrition
- too little protein in the diet
- kidney issues

**High levels of urea in the urine may suggest:**
- too much protein in the diet
- too much protein breakdown in the body
**Urea Clearance**

Inulin, creatinine, and Urea Clearance

- Inulin is freely filtered at the glomerulus and is neither secreted nor reabsorbed; thus, inulin clearance is the gold standard for the glomerular filtration rate.
- Creatinine is also freely filtered and not reabsorbed, but creatinine is secreted in the distal nephron, so creatinine clearance exceeds inulin clearance.
- Urea is freely filtered and not secreted but undergoes reabsorption in the distal nephron. Reabsorption of urea is flow dependent, so that more urea is reabsorbed at lower urine flow rates.

\[
Cu = \frac{Uu \times V}{Pu}
\]

where, 
- \(Cu\) = urea clearance in ml/minute
- \(Uu\) = urine urea in mg/ml
- \(V\) = volume of urine in ml
- \(Pu\) = urea in mg per ml of plasma

*Urea Clearance*
BUN/Cr ratio

BUN/Cr. Ratio = (BUN in mg/dL)/(serum creatinine in mg/dL.)

• For a normal individual on a normal diet, the reference interval for the ratio ranges between 12 and 20, with most individuals being between 12 and 16.
• The ratio is indicative of pre-renal injury when the BUN/Cr. ratio is greater than 20 lower ratios:
  low protein intake, starvation
  severe liver disease.
High ratios with normal creatinine levels may be noted with:
  high protein intake
  catabolic states of tissue breakdown prerenal azotemia.
High ratios associated with high creatinine concentrations:
  postrenal obstruction.
Objectives:

1-Estimation of Blood Urea
2-Estimation of Urine Urea
Principle:

Urea is hydrolyzed in the presence of urease enzyme and water to yield ammonia and carbon dioxide. The ammonia reacts with α-ketoglutaric acid and reduced nicotinamide adenine dinucleotide (NADH) in the presence of glutamate dehydrogenase (GLDH) to yield glutamic acid and nicotinamide adenine dinucleotide (NAD).

The rate of oxidation of NADH to NAD is measured at 340 nm over a limited urea concentration range and limited time period, and is proportional to the concentration of urea.

\[
\begin{align*}
2\text{NH}_3 + \text{CO}_2 & \quad \text{UREASE} \quad \text{NH}_2 - \text{CO} - \text{NH}_2 + \text{H}_2\text{O} \\
\text{AMMONIA} & \quad \text{UREA} \\
\text{NH}_3 + \text{HOOC-(CH}_2\text{2-CO-COOH} + \text{NADH} + \text{H}^+ & \quad \text{GLDH} \quad \text{HOOC-(CH}_2\text{2-CH(NH}_2\text{)-COOH} + \text{NAD}^+ + \text{H}_2\text{O} \\
\alpha-\text{KETOGLUTARIC ACID} & \quad \text{GLUTAMIC ACID} \\
\text{GLDH: Glutamate Dehydrogenase}
\end{align*}
\]
Material:

- **BUN-ZYME Reagent**: UREASE, GLDH, NADH, α-KETOGLUTARIC ACID, buffers and stabilizers.
- **BUN-ZYME Standard solution** 25 mg/dl (nitrogen = 53.57 mg/dl)
- **BUN-ZYME Serum sample**
  - Water bathe 37 °C
  - Micro pipette
  - Quartz cuvett
  - Stopwatches
Method:

<table>
<thead>
<tr>
<th></th>
<th>Standard</th>
<th>Serum</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reconstituted Reagent</td>
<td>3ml</td>
<td>3ml</td>
<td>3ml</td>
</tr>
</tbody>
</table>

Pre-warm at 37°C for 2 min. and add:

<table>
<thead>
<tr>
<th></th>
<th>Standard</th>
<th>Serum</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>0.025/25µl</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>serum</td>
<td>-</td>
<td>0.025/25µl</td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>-</td>
<td>-</td>
<td>0.025/25µl</td>
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• After exactly 30 seconds, read and record absorbance A1 against distilled water at 340 nm.
• At exactly 60 seconds, read and record the absorbance A2 and determine ∆A.
# Reference Values

<table>
<thead>
<tr>
<th>SPECIMEN</th>
<th>UREA NITROGEN</th>
<th>UREA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum/Plasma</td>
<td>5-23 mg/dL</td>
<td>10-50 mg/dL</td>
</tr>
<tr>
<td>Urine 24 h</td>
<td>9-16g/24h</td>
<td>20-35 g/24 h</td>
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</tbody>
</table>
## Calculations of the Results:

<table>
<thead>
<tr>
<th></th>
<th>UREA NITROGEN</th>
<th>UREA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>SERUM OR PLASMA</strong></td>
<td></td>
</tr>
<tr>
<td>Urea Nitrogen (mg/dL)</td>
<td>[\Delta A (\text{Sample}) \times 25 \times \Delta A (\text{Standard})]</td>
<td>Urea (mg/dL) = [\Delta A (\text{Sample}) \times 53.57 \times 50]</td>
</tr>
<tr>
<td>Urea Nitrogen g/24 h</td>
<td>[\text{mg/dL (Urea Nitrogen)} \times \text{ml. Urine /24 h} \times 100 \times 1000]</td>
<td>Urea(g/24 h) = [\text{mg/dL (Urea)} \times \text{ml. Urine /24 h} \times 100 \times 1000]</td>
</tr>
</tbody>
</table>
Discussion:

Comment on the level of Urea in serum and urine.
References:

• Sarre, H., Nierenkrankheiten, Georg Thieme Verlag Stuttgart (1959).
• http://link.springer.com/chapter/10.1007%2F978-88-470-0552-5_8#