UNIT: Renal Function
(Creatinine, Urea, Nitrogen, Uric Acid)

Task

Discussion of the clinical significance of creatinine, BUN, and uric acid, methods of determination, and serum determination of uric acid.

Objectives

After completion of this unit, the student will be able to:

1. Determine BUN, creatinine, and uric acid concentrations by colorimetric methods.
2. Explain the clinical significance of abnormal creatinine, BUN, and uric acid values and explain their relative usefulness in assessing renal function.
3. Know the most commonly employed automated and manual methods for determination of BUN, creatinine, and uric acid and be able to explain the principle of the reactions.
4. Calculate creatinine clearance.

Introduction

A. Creatinine — (Crea)

Creatinine is a non-protein nitrogen waste product formed in muscle. It is excreted in the urine; the amount excreted daily is a function of muscle mass and is not affected by diet, age, sex, or exercise.

Creatinine filters easily into the glomerular filtrate and is not reabsorbed by the tubule. Therefore, any condition that reduces the glomerular filtration rate will result in a lessened excretion from the body, with a consequent rise in the concentration of creatinine in the blood. The serum creatinine is a better indicator of renal function than either that of BUN or uric acid because it is not affected by diet, exercise, or hormones.

Expected Results

| Normal range = | 0.5 - 1.5 mg/dl |
| Urine = | 0.8 - 2.0 gm/24 hours |
|           | (800-2000 mg/24 hours) |

Clinical Significance

The serum creatinine is elevated whenever there is a significant reduction in the glomerular filtration rate or when urine elimination is obstructed. The kidney reserve is such, however, that about 50% of kidney function must be lost before a rise in serum creatinine can be detected.

Low serum creatinine values have been associated with muscular dystrophy.
Creatinine Clearance

A very simple, sensitive chemical method of assessing renal function is the creatinine clearance test. This clearance test is an assessment of the glomerular filtration rate, which is an estimate of the amount of plasma that must have flowed through the kidney glomeruli per minute to account for the amount of creatinine appearing in the urine. The test requires complete collection of urine formed during a specified period of time. A serum sample must be obtained from the patient during the urine collection time period. Both the serum and urine creatinine levels are determined in mg/dL. The creatinine clearance is calculated as:

\[
\frac{\text{urine creat. in mg/dL}}{\text{serum creat. in mg/dL}} \times \frac{\text{urine vol. in ml}}{\# \text{ of min. collected}} \times \frac{1.73 \text{ (an average person's surface area)}}{\text{pts surface area in sq. meters}}
\]

Creatinine clearance is usually performed with 24 hour urine collections, although 12 hour and 4 hour clearances are occasionally ordered.

Normal Values

Males = 105 ± 20 mL/min.
Females = 97 ± 20 mL/min.

B. Blood Urea Nitrogen (BUN)

Urea is the characteristic and most abundant nitrogenous end product of protein catabolism in mammals. It is generated by the liver and excreted by the kidney. Urea filters easily through the glomerulus into the ultrafiltrate. It will diffuse passively into the blood as it passes down the renal tubules. Under conditions of normal flow and normal renal function, about 40% of the filtered urea is reabsorbed; when the flow rate is decreased, the amount passively reabsorbed increases. As with creatinine, the serum concentration of urea nitrogen rises with impaired renal function.

The serum concentration of urea nitrogen is influenced by factors not connected with renal function or urine excretion as it is affected strongly by the degree of protein catabolism. A marked change in dietary protein consumption will be reflected in BUN values. The injection or ingestion of steroids produces a rise in BUN as do stressful situations that cause the adrenal gland to secrete additional cortisol. For these reasons, the measurement of serum creatinine is a better indicator of kidney status than is that of BUN although in many cases, they go up and down simultaneously. The various prerenal, renal, and postrenal factors that affect creatinine also influence the BUN.

Azotemia is a term used to refer to any significant increase in the plasma concentration of non-protein nitrogenous compounds, principally urea and creatinine.

Clinical Significance

After considering the effects of high protein diet, administration of cortisol-like steroids and stressful situations, increased BUN is seen in the prerenal, renal, and postrenal factors as described for creatinine. The BUN concentration is low in late pregnancy when the fetus is growing rapidly and utilizing maternal amino acids, in starvation, and in patients whose diet is grossly deficient in protein.
Expected Values

Normal range in serum or plasma 5.0 - 20.0 mg/dL.

C. Uric Acid

Uric acid is a metabolite of nucleic acids, purines, and nucleoproteins and is the end product of protein (purine) metabolism in man. In most mammals, uric acid is oxidized in the liver to allantoin. Higher apes and man appear incapable of destroying uric acid efficiently in this manner. As a result, uric acid accumulates in the plasma (as sodium urate) and is excreted in the urine. Occasionally some persons are afflicted with extensive deposits of uric acid (urates) in their tissues, causing a disorder termed gout. Increased levels of uric acid have been observed in renal failure, chronic lead poisoning, polycythemia, some leukemias, and toxemia of pregnancy.

Methods of Determination

A. Creatinine

Jaffe Reaction — The most popular method for creatinine determinations is based on the reaction originally described by Jaffe.

Creatinine reacts with picric acid under alkaline conditions to form a characteristic yellow-orange complex. Unfortunately, the Jaffe reaction is not specific and a number of substances including proteins in body fluids will interfere.

The color derived from creatinine as well as the non-specific substances, is measured. Upon addition of acid, the color contributed by creatinine is destroyed, while that produced by non-specific substances remains. The difference in color intensity measured at or near 500 nm before and after acidification is proportional to the creatinine concentration.

B. Blood Urea Nitrogen (BUN)

The most commonly employed methods of BUN determination are:

1. Berthelot Reaction — This method is currently one of the most widely used. Urea is split into ammonia and carbon dioxide by the action of urease, an enzyme obtained from Jack Bean meal. The ammonia then reacts with alkaline hypochlorite and phenol in the presence of a catalyst-sodium nitroprusside. The resulting product is indophenol (a blue color) and the concentration of ammonia is directly proportional to the absorbance of indophenol.
2. **Fea ron Reaction** —

\[
\text{CO}_3^2- \xrightarrow{\text{urease}} \text{NH}_4^+ + \text{HCO}_3^-
\]

This method is also very widely used in automated instruments. Urea is heated with diacetyl monoxime in an acid solution to form a yellow diacetyl compound. This color is stabilized by adding an arsenic-sulfuric acid mixture. As a result of the need for critical timing, and the presence of strong acids and oxidizing reagents, this method is not well-suited for manual testing.

3. **Dialdehyde Method** — In this procedure, urea combines with a dialdehyde derivative of benzene. The product of this reaction is then acted upon by a dihydroxynaphthalene compound and results in an intensely colored chromophore which can be measured photometrically.

4. **Urease-Nessler Method of Karr** — To a protein-free filtrate, a buffer solution and urease are added. Upon incubation, urea is split by urease and releases ammonia. Ammonium carbonate is formed. The ammonia in the ammonium carbonate is estimated as dimercuric ammonium iodide (yellow) after adding Nessler's reagent (alkaline potassium iodide and mercuric iodide).

5. **ASTRA** — The BUN Chemistry Module determines blood-urea-nitrogen by means of the enzymatic conductivity rate method employing a Beckman Conductivity Electrode.

6. **ACA** — Urease specifically hydrolyzes urea to form ammonia and carbon dioxide. The ammonia is utilized by the enzyme glutamate dehydrogenase (GLDH) to reductively aminate a-ketoglutarate (a-KG) with simultaneous oxidation of reduced nicotinamide-adenine dinucleotide (NADH).

\[
\text{Urea} + \text{H}_2\text{O} \xrightarrow{\text{urease}} 2\text{NH}_3 + \text{CO}_2
\]

\[
\text{NH}_3 + \text{a-KG} + \text{NADH}(+\text{H}^+) \xrightarrow{\text{GLDH}} \text{L-glutamate} + \text{NAD}^+
\]

The change in absorbance at 340 nm due to the disappearance of NADH over a 17.07-second measurement period is directly proportional to the BUN concentration in the sample.

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**C. Uric Acid**

1. **Phosphotungstic Acid Reduction** — This is the classical chemical method for uric acid determination. In this reaction, urate reduces phosphotungstic acid to a blue phosphotungstate complex, which is measured spectrophotometrically.

2. **Uricase Method** — An added enzyme, uricase, catalyzes the oxidation of urate to allantoin, \( \text{H}_2\text{O}_2 \), and \( \text{CO}_2 \). The serum urate / uric acid may be determined by measuring the absorbance before and after treatment with uricase. (Uricase breaks down uric acid.)
3. **ACA** — Uric acid, which absorbs light at 293 nm, is converted by uricase to allantoin, which is nonabsorbing at 293 nm.

\[
\text{Uric acid} + 2\text{H}_2\text{O} + \text{O}_2 \xrightarrow{\text{Uricase}} \text{Allantoin} + \text{H}_2\text{O}_2 + \text{CO}_2
\]

(Absorbs at 293 nm)                  (Nonabsorbing at 293 nm)

**Procedure 1**

**Creatinine (Sigma #555)**

**Supplies and Equipment**

1. Spectrophotometer(s) capable of measuring transmitted light at 500 nm
2. Appropriate cuvets, test tubes and rack
3. Pipetting devices for the accurate delivery of volumes of 0.1, 0.5, 1.0, and 5.0 mL

**Reagents**

**Warning** — causes irritation. Harmful if swallowed. Do not get in eyes, on skin or clothing.

In case of eye or skin contact or irritation, immediately flush affected area with running water and notify instructor.

1. Sodium Hydroxide Solution — Store at room temperature.
2. Acid Reagent — prepared mixture of sulfuric and acetic acid. Store at room temperature.
3. Creatinine Color Reagent — Picric acid. Store at room temperature. If reagent becomes cold, turbidity may develop. Warm reagent to clear, mix before use.

**Danger:** Dried picric acid may be explosive. If solution has dried out, do not handle. Contact instructor for assistance/get help from fire department.

4. Creatinine Standard(s) — Store in refrigerator.

**Specimens**

1. Serum or plasma, stored at refrigerator temperature or frozen (up to several months) is suitable.
2. Urine specimens are stable at room temperature for 24 hours or for several days when refrigerated.
3. Specimens containing bromsulfophthalein or phenosulfophthlein should not be analyzed.

**Steps**

1. Preparation of alkaline picrate solution. Prepare the appropriate amount of alkaline picrate solution by mixing the designated number of volumes of Creatinine Color Reagent with one volume of Sodium Hydroxide Solution (according to product insert). Mixture is stable for at least one week stored dark at room temperature.
2. If urine samples are being tested, make a X10 dilution (1 part urine + 9 parts DI water). Prepare dilution in separate tubes before preceding to the next step.

3. To appropriately labeled tubes (Blank, Standard(s), Controls, and Patients) ADD:
   - 0.3 mL water to Blank's tube.
   - 0.3 mL creatinine standard(s) to Standard's tube(s).
   - 0.3 mL sample to Control(s) and Patient's tube(s).

4. To all tubes add 3.0 mL Alkaline Picrate Solution (see Step 1).

5. Parafilm, mix and allow to incubate at room temperature for 8-12 minutes.

6. Read and record absorbance of all specimens standard, Controls, and Patients vs. Blank as reference at 500 nm. This is INITIAL A. After reading INITIAL A, return sample to its respective tube.

7. To all tubes, add exactly 0.01 mL Acid Reagent. Parafilm, mix immediately and incubate 5 minutes at room temperature.

8. After 5 minutes, read and record the absorbance (A) of Standard, Controls, and Patients vs. Blank as reference at 500 nm. This is FINAL A.

**NOTE 1:** Urine samples should be diluted 10-15 fold prior to assay. Multiply results by dilution factor.

**NOTE 2:** The colorimetric response obeys Beer's law up to at least 10 mg/dL on narrow bandwidth spectrophotometers and to at least 5 mg/dL for most wide bandwidth instruments, employing 12 mm cuvets.

**NOTE 3:** If TEST value exceeds the limit of linearity for instrument–cuvet system employed, the sample should be diluted, reassayed and result multiplied by the dilution factor.

**NOTE 4:** Bilirubin will increase creatinine results by 0.09 mg/dL for each mg/dL of bilirubin.

**Calculation**

\[
\text{Creatinine (mg/dL)} = \frac{\text{INITIAL } A_{\text{blank}} - \text{FINAL } A_{\text{blank}}}{\text{INITIAL } A_{\text{standard}} - \text{FINAL } A_{\text{standard}}} \times \frac{\text{Standard's Concentration}}{}
\]

**Quality Control**

Two levels of assayed serum controls.

**Expected Values**
**Procedure 2**

**BUN/Urea Nitrogen (Sigma #640)**

*Supplies and Equipment*

1. Appropriate cuvets, test tubes and rack
2. Spectrophotometer(s) capable of measuring transmitted light at 570 nm
3. Pipetting devices for the accurate delivery of volumes of 10 µL, 500 µL, 1 mL, and 5.0 mL

*Reagents*

**Warning:** These reagents are generally corrosive. Avoid contact with skin. Reagents disposed down the sink should be flushed with a large volume of water.

Store all reagents refrigerated.

1. Phenol Nitroprusside Solution
2. Alkaline Hypochlorite Solution
3. Urease Buffer Reagent – (Jack Bean meal) When reconstituted, this dry reagent will be stable for one month.
4. Urea Nitrogen Standard Solution

*Specimens*

1. Serum separated from clot is suitable. Refrigerate or store frozen.
2. Plasma is suitable provided fluoride was not part of the anticoagulant. Also ammonium salts of anticoagulants must be avoided, due to the sensitivity of the reaction.
3. Urine should be assayed within several hours of collection. Refrigeration is recommended to prevent bacterial decomposition of urea. Stability may be prolonged by freezing.

*Steps*

1. Prepare Urease Solution by reconstituting vial of Urease Buffer Reagent with 30 mL of DI ammonia free water.
2. To appropriately labeled tubes (Blank, Standard, Control(s), and Patient) ADD:
   
   - 10 µL water to Blank’s tube
   - 10 µL Urea Nitrogen Standard to Standard’s tube
   - 10 µL sample to control(s) and Patient(s) tubes

3. To each tube, add 0.5 mL Urease Solution. Mix contents by gently swirling.

4. Incubate tubes at room temperature 15-20 minutes (or at 37°C for 5-10 minutes).

5. To each tube add
   
   - 1.0 mL Phenol Nitroprusside Solution
   - 1.0 mL Alkaline Hypochlorite Solution
   - 5.0 mL ammonia free water

   **Mix after each addition.**

6. Allow tubes to develop color at room temperature for 20-30 minutes.

7. Read absorbance (A) of Standard, Controls and Patients vs. Blank as a reference at 570 nm.

**NOTE:** Take care in reading/recording absorbance values. Three decimal places should be recorded.

**Determination of Results**

1. Calculation using a Standard

   \[
   \text{Urea Nitrogen mg/dL} = \frac{A_{\text{test}}}{A_{\text{std}}} \times \text{Standard’s Concentration}
   \]

2. Calibration Curve – Values for Control and Patient tests are read directly from a calibration curve prepared using a series of Standards.

**Quality Control**

Two levels of assayed serum controls.

**Expected Values**

- Serum/plasma BUN 7.0 - 18.0 mg/dL
- Urine urea nitrogen 12 - 20 gm/24 hr

Elevated serum values have been reported in: accelerated protein catabolism
UNIT: Renal Function (Creatinine, Urea, Nitrogen, Uric Acid) (continued)

- water depletion due to decreased intake or excessive loss
- impaired renal function

Decreased serum values have been reported in:
- protein malnutrition
- pregnancy
- rapid rehydration with solutions free of nitrogenous constituents, e.g., glucose infusion

Procedure 3

Uric Acid (Sigma #685 - new in 00) Enzymatic - 520 nm

Reaction

\[
\text{Uric acid} + 2\text{H}_2\text{O} + \text{O}_2 \xrightarrow{\text{Uricase}} \text{Allantoin} + \text{CO}_2 + \text{H}_2\text{O}_2 \\
\text{H}_2\text{O} + 4 \text{AAP} + \text{DHBS} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine Dye} + \text{H}_2\text{O}
\]

(absorbance maximum at 520 nm)

Supplies and Equipment

1. Spectrophotometer(s) capable of measuring transmitted light at 520 nm
2. Appropriate cuvets, test tubes and rack
3. Centrifuge, timer, and the following pipetting devices: 0.05 mL, 5.0 mL.
4. A constant temperature heat block or water bath if performing test at 30°, or 37° C.

Reagents

1. Uricase – enzyme prepared from Candida utilis. Store in foil bag with desiccant.
2. Horseradish Peroxidase
3. 4-Aminoantipyrine
4. 3,5-Dichloro-2hydroxybenzenesulfonate
5. Buffer, also contains non-reactive stabilizers and fillers
6. Uric Acid Standard Solution

*DANGER / PRECAUTIONS:

1. Uric acid reagents are for in-vitro diagnostic use only. Normal precautions should be used as for handling laboratory reagents, and all federal, state, and local laws for handling and disposal followed.
2. Avoid contact and inhalation
3. Consider all blood derivatives as potentially hazardous.

Specimens

1. Serum or plasma (heparinized or EDTA). UA is stable in serum at 2-6°C for 3 days and several months frozen. Slight hemolysis has no effect upon UA level.
2. 24 hour urine specimen in a container to which has been added 10 mL 5% sodium hydroxide to prevent uric acid precipitation. Urine levels are stable for 3 days at room temperature. Refrigeration is recommended only after a 10 fold dilution has been made.
UNIT: Renal Function (Creatinine, Urea, Nitrogen, Uric Acid) (continued)

**Steps**

1. Optimize spectrophotometer at 520 nm, zeroing on deionized water.

2. Appropriately label test tubes (13 x 100 mm) for each Blank, Standard, Control and Patient.

3. Pipet 3.0 mL Uric Acid reagent into each tube, and bring to assay temperature. (RT, 30°C, or 37°C)

4. Add 0.05 mL deionized water, uric acid standard, control or patient specimen to their appropriate tube, and mix by gentle inversion.

5. Incubate for 10 minutes, (if at room temperature) OR 5 minutes if at 30°C, or 37°C. Note: Following completion of the reaction, the absorbance (A) remains constant for 30 minutes.

6. Read and record the absorbance (A) of all tubes, against deionized water.

7. Subtract the absorbance of the blank from absorbance of samples, controls and standard to obtain change in absorbance due to uric acid.

8. Determine the concentration of control and patient samples by either reading from a prepared calibration curve, or by calculation against a standard.

**Calculation (Serum)**

1. First subtract the blank’s absorbance from each of the other specimens (standard, controls, patients).

2. Calculate control and patient results using the following formula.

   \[
   \text{URIC ACID (mg/dL)} = \frac{A_{\text{specimen}}}{A_{\text{standard}}} \times \frac{\text{Concentration of Standard}}{
   \]

**Quality Control**

Two levels of assayed serum controls.

**Expected Values** (It is recommended that each laboratory establish a normal range.)

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<thead>
<tr>
<th></th>
<th>Male</th>
<th>3.6 - 7.7 mg/dL</th>
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<tbody>
<tr>
<td></td>
<td>Female</td>
<td>2.5 - 6.8 mg/dL</td>
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<tr>
<td></td>
<td>Serum</td>
<td>250-750 mg/24 hr</td>
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<td>Urine</td>
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Renal Worksheets

I. Creatinine Sigma # 555

<table>
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<tr>
<th>Identification</th>
<th>Absorbance</th>
<th>Final Absorbance</th>
<th>Δ Absorbance</th>
<th>Concentration (units)</th>
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<tbody>
<tr>
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Calculation formula(s) and examples

<table>
<thead>
<tr>
<th>Creatinine Quality Control</th>
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<tbody>
<tr>
<td>Your Results</td>
</tr>
<tr>
<td>Level 1 ID</td>
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<td>Level 2ID</td>
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Accepting Patient Results? Reason

II. BUN Sigma # 640

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### Renal Function (Creatinine, Urea, Nitrogen, Uric Acid) (continued)

#### BUN Calculation formula(s) and examples

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#### BUN Quality Control

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<th>Your Results</th>
<th>Controls’ range of expected results.</th>
<th>In control?</th>
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<td>Accepting Patient Results?</td>
<td>Reason</td>
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#### III. Uric Acid

- **Wavelength** _____________
- **Linearity** _____________
- **Spectrophotometer Used** ______________________

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### Study Questions

**Instructions:** Legibly write your answers in the space provided. Unless otherwise indicated, each question is worth one point. Using lecture notes, reading assignments and information presented in this lab, answer the following questions.

1. Calculate the creatinine clearance from the following results obtained on a female patient. Show your work and use correct units.
   
   - Serum creatinine = 1.8 mg/dL
   - height = 5'5"
   - Urine creatinine = 59 mg/dL
   - weight = 142 lb
   - 24 hour urine volume 900 mL

   The creatinine clearance on this patient is ________________.

2. Is this result normal?

3. What does an increased serum creatinine?

4. Creatinine clearance is specifically a measure of the ________________________.

5. A yellow-orange color formed when creatinine is treated with picric acid is seen in the ____ ________________ reaction.

6. The majority of creatinine in the glomerular filtrate is reabsorbed in the renal tubules.
   - A. True
   - B. False

7. Urinary creatinine concentration is not affected by diet, sex, or exercise; but seems to be a function of ____________________________.

8. In this creatinine procedure, what is the purpose of treating specimens with acid reagent shortly before taking the final absorbance reading in the creatinine procedure?
9. The Berthelot reaction is used to determine ________________________________.

10. Urea formation takes place in the ________________________________________.

11. The major nitrogenous constituent of normal urine is ________________________.

12. Urea enters the blood stream by active transport through the renal tubules.
   A. True
   B. False

13. List two (2) factors other than renal function or urine excretion that affect BUN concentration. (2 points)

14. What is azotemia?

15. Present two (2) reasons why the diacetyl monoxime method for BUN is less suitable for manual methods than the Berthelot reaction?

16. What enzyme is used in the Berthelot reaction? What products are formed after the enzyme reacts with urea?

17. The uricase method for uric acid assay depends on
   A. measurement of absorption before and after treatment with uricase
   B. collection of evolved gas after uricase treatment
   C. titration before and after uricase treatment
   D. formation of a colored solution
   E. change in viscosity

18. In the uricase method for assay of uric acid, uric acid is
   A. complexed with phosphotungstate
   B. destroyed
   C. precipitated
   D. reduced by phosphotungstate
   E. complexed with uricase

19. Urease is usually prepared from ____________________________________________.

20. Uricase is prepared from ________________________________________________.
21. Which of the following does **NOT** usually cause an elevation of serum uric acid?
   A. gout
   B. impaired renal function
   C. leukemia
   D. impaired liver function
   E. toxemia of pregnancy

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