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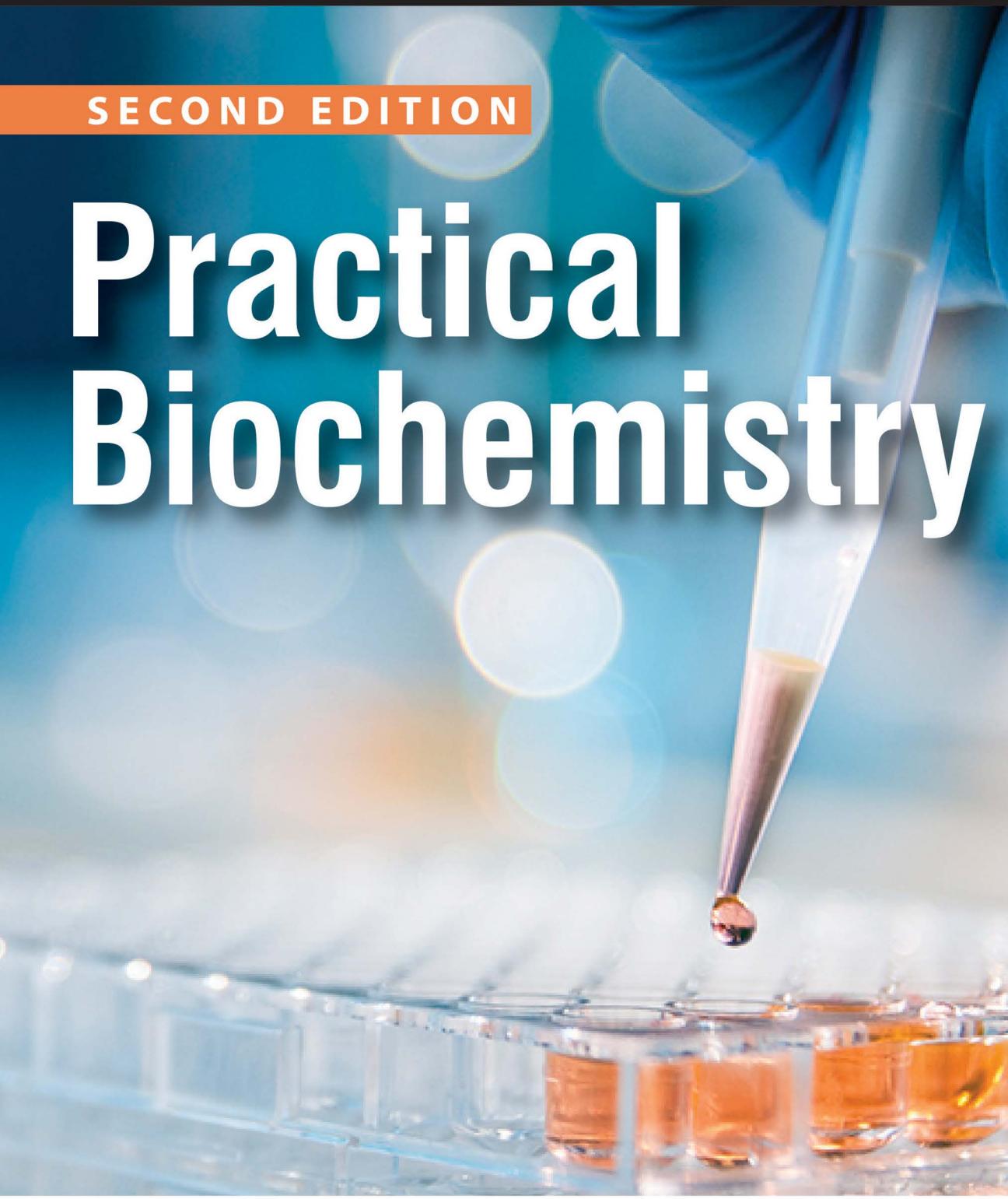

SECOND EDITION

Practical Biochemistry

Geetha Damodaran K

JAYPEE

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CHAPTER

4

Reactions of Urea

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GENERAL REACTIONS OF UREA

Introduction

Urea is the end product of protein catabolism (Fig. 4.1). It is a non-protein nitrogen (NPN). Non-protein nitrogen of the blood and other body fluids include constituents, which are not precipitated as proteins, e.g. Major NPNs of blood urea, uric acid, creatinine, creatine, amino acids and glutathione.

The sources of the constituent atoms of urea (H_2N-CO-NH_2)—carbon from carbon dioxide, one nitrogen from ammonia and the other nitrogen from aspartate. The full set of enzymes required for the formation of urea present only in hepatocytes (liver cells). Hence, urea is synthesized exclusively in the liver. It is mainly excreted in urine, i.e. 80–90% of total nitrogen of human urine is present as urea. *Unlike other NPNs, the concentration of urea in urine decreases* when protein intake is restricted. The amount of urea excreted per day by a normal adult is about 15–30 g per day. Urea content is increased with high protein diet. Blood urea level decreases in severe liver diseases since it is the sole organ concerned with its synthesis.

Physical Properties of Urea Solution

- Appearance — clear
- Color — colorless
- Odor — odorless
- Reaction to litmus — No change

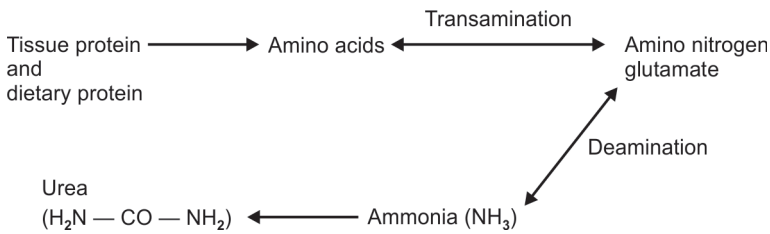


Figure 4.1 Formation of urea

Chemical Reactions

Alkaline Hypobromite Test

Procedure: To 3 mL of urea solution add a few drops of alkaline hypobromite solution (3 mL concentrated NaOH + 2 mL bromine water).

Observation: Brisk effervescence.

Inference: Nitrogen present in urea is liberated as nitrogen gas.

Principle: Sodium hypobromite decomposes urea to CO_2 and N_2. Carbon dioxide is absorbed by the excess sodium hydroxide and the nitrogen is evolved and causing the brisk effervescence. (Fig. 4.2).

Points to Ponder: All ammonium compounds and all compounds containing amino group ($-NH_2$) release N_2 when treated with alkaline hypobromite.

Specific Urease Test (Figs 4.3 and 4.4)

Procedure

- To 2 mL urea solution in a test tube add a drop of phenol red indicator.
- Add 2% Na_2CO_3 solution drop by drop till a pink color develops.
  (pH range of Phenol red: 6.8–8.4 and color range: yellow to red)
- Add 2% acetic acid drop by drop till the pink color just disappears indicating the pH nearer to 6.8.
- Add a 1 mL of urease enzyme extract.
- Keep at 37°C in an incubator for 5 minutes because optimum temperature of urease enzyme is 37°C.
- Set a control tube taking the entire ingredients but using boiled urease enzyme extract.

Observation: Pink color develops in the tube containing urea and no change in control tube.

Inference: Urease decomposes urea to ammonium carbonate. Ammonium carbonate being basic raises the pH. Phenol red used in this test will show pink to red color at basic pH.



Figure 4.3 Specific urease test

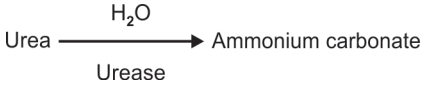
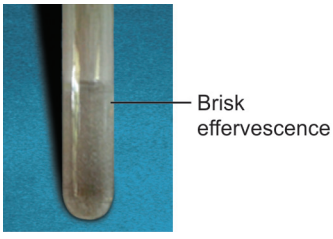


Figure 4.4 Urease action on urea



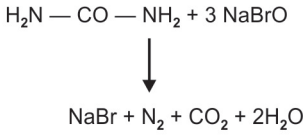


Figure 4.2 Alkaline hypobromite test and reaction

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QUESTIONS

1. Answer to the point:
   a. Number of nitrogen atoms in urea
   b. Sources of nitrogen atoms in urea
   c. Source of carbon in urea
   d. Cells producing urea in the body
   e. Concentration of urea in blood in a normal adult
   f. Two tests to detect urea from a specimen
   g. Main route of excretion of urea from the body
   h. Three non-protein nitrogen compounds in the blood
2. Give the principle of the following tests:
   a. Alkaline hypobromite test
   b. Specific urease test
3. Give brief answers:
   a. Formation of urea by hepatocytes
   b. Urea cycle disorders
   c. Biochemical principle of treatment of urea cycle disorders
   d. Why blood urea is elevated in kidney diseases?

REAGENT PREPARATION

- **Sodium hydroxide concentrated (1N):** Dissolve 40 g reagent grade sodium hydroxide pellets in a few mL of distilled water to make 1 liter. Cool. Allow to stand for 3 days or so. Decant the solution into a bottle fitted with siphon and a calcium chloride tube to prevent entry of carbon dioxide. Standardize by titration with an acid of known strength using methyl red as indicator (secondary standardization) or potassium biphthalate of known strength (primary standardization).
- **Sodium hypobromite (Alkaline hypobromite):** Mix 25 mL of liquid bromine with 250 mL of 40% NaOH.
- **2% acetic acid:** Dissolve 2 mL of glacial acetic acid (99.8%) in 100 mL of water.
- **2% Na_2CO_3**
- **Urease enzyme:** Grind 10 g horse gram or jack bean or soybean with 100 mL 30% alcohol and take the extract.
- **Phenol red indicator:** Dissolve 1 g phenol red in 100 mL distilled water. Add 5.7 mL of 0.05 N NaOH and make the volume up to 250 mL with water.

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Geetha Damodaran K MBBS MD (Biochemistry) is working as an Additional Professor, Department of Biochemistry, Government Tirumala Devaswom (TD) Medical College, Alappuzha, Kerala, India. She has teaching experience in Government Medical College, Kozhikode; Government Medical College, Thrissur; and Government Tirumala Devaswom (TD) Medical College, for 24 years. Her research interest is in the validation of new biomarkers.



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