



ESTIMATION OF SERUM BILIRUBIN

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Advanced Clinical Biochemistry II (MA 416)

Summer Semester

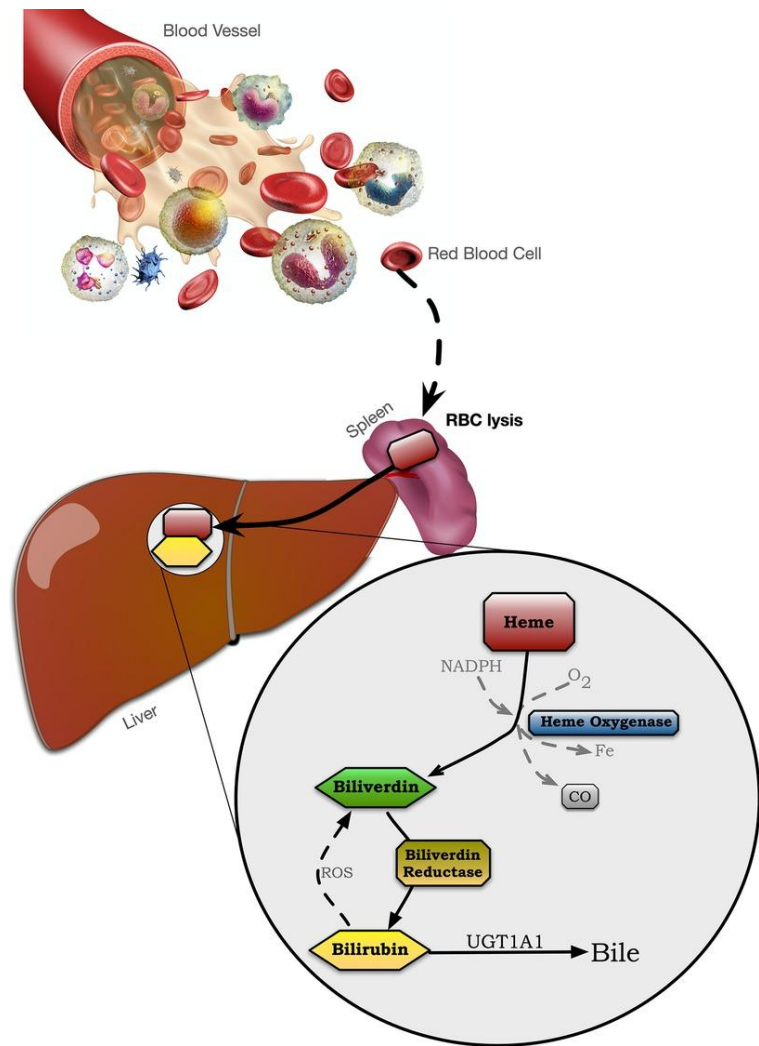
Week 3

24/08/2026

Objectives

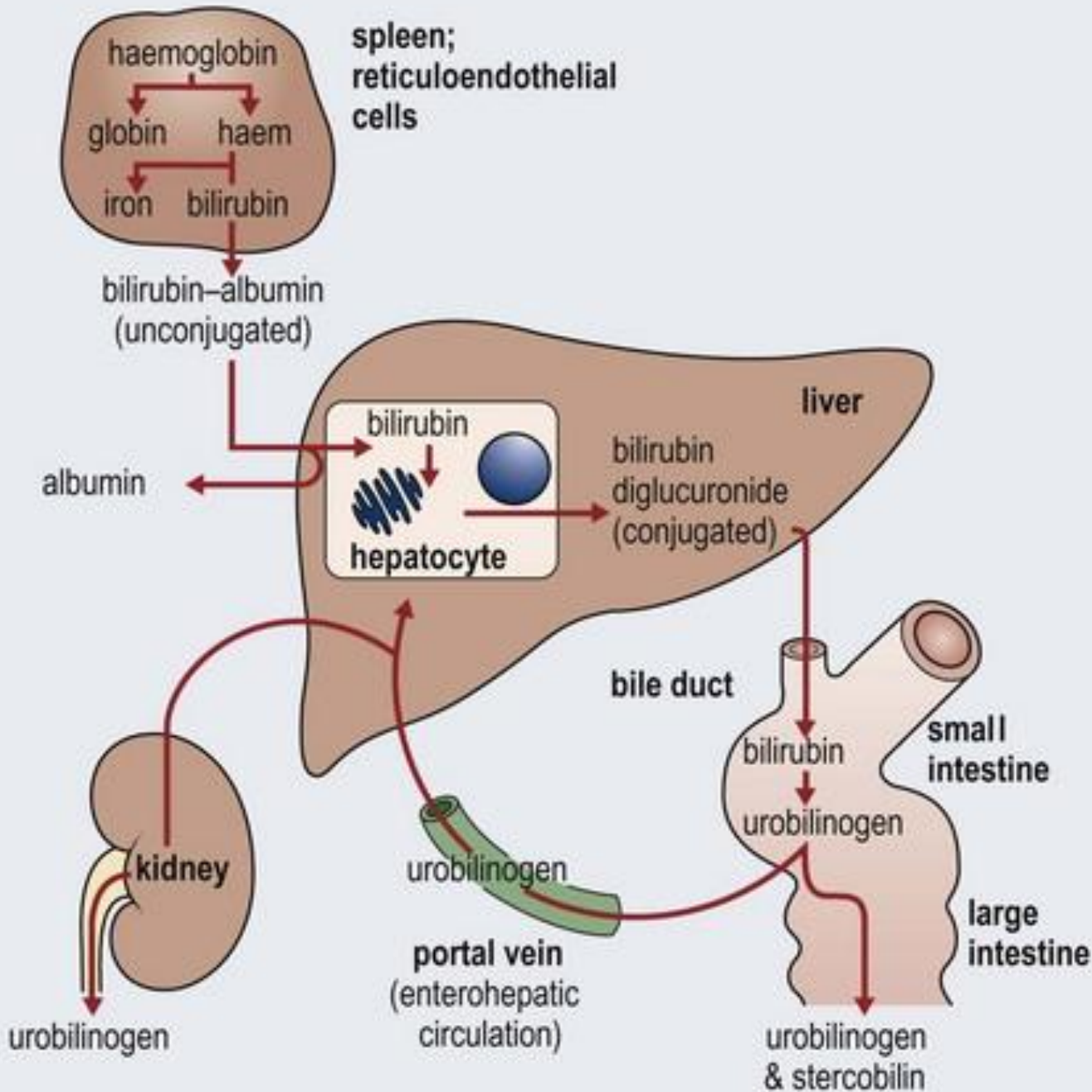


- **At the end of this lesson, the students should be able to:**
- Understand the concept of bilirubin metabolism and associated disorders
- Understand the principle behind the bilirubin test.
- Understand the step-by-step procedure for determination of serum bilirubin.



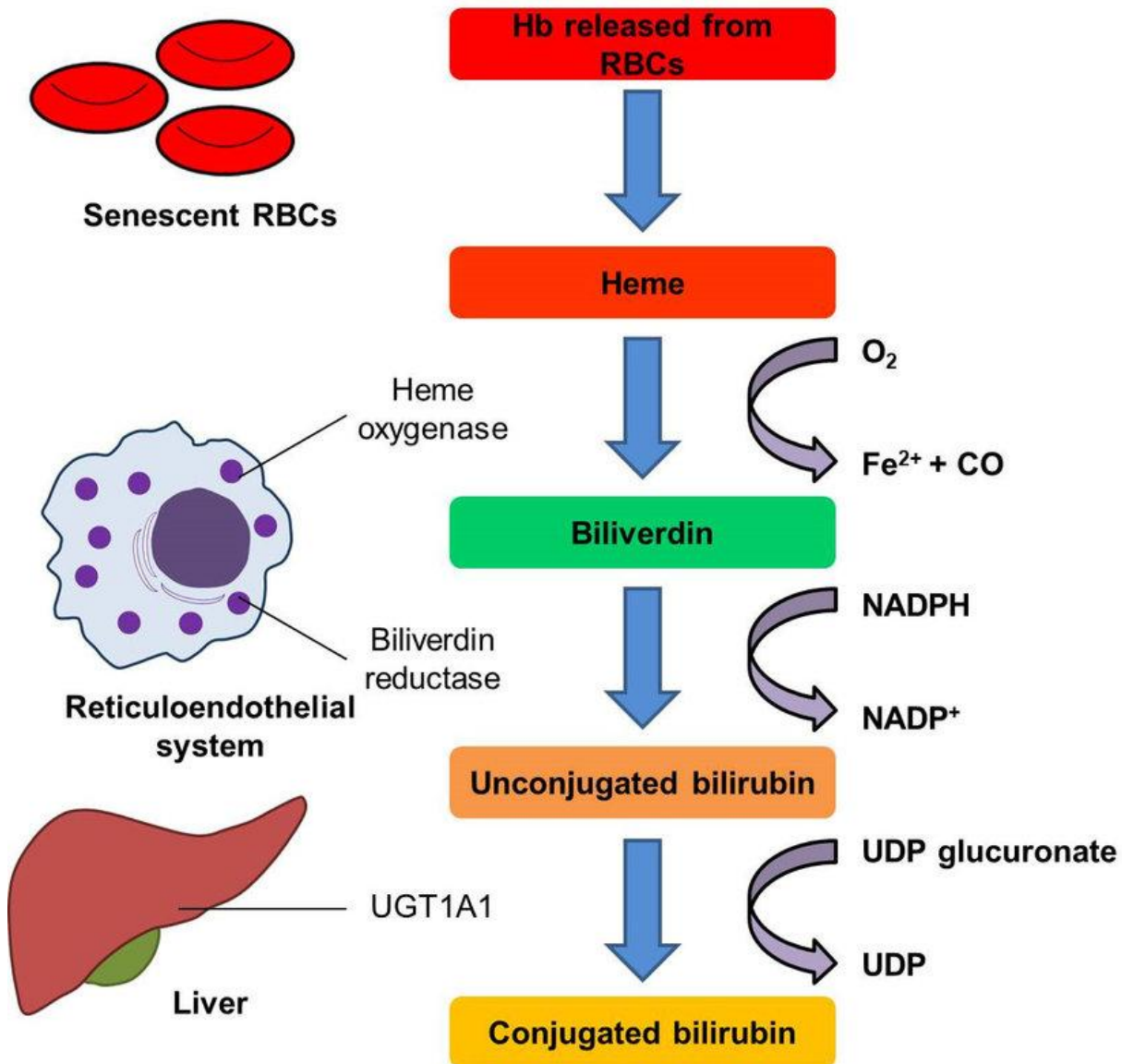
Introduction

- Bilirubin is a yellowish molecule found in **bile**.
- It is made during the normal body process of **RBC breaking down**.
- It is produced when heme molecules of **hemoglobin** break down in the spleen and are mainly secreted into stool and urine.



Cont.

- Bilirubin is responsible for the coloration of stools and urine.
- Elevated levels of serum bilirubin indicate certain diseases.
- A healthy liver removes most of the bilirubin from the body in conjugated form.



Cont.

- It is produced in the form of a water-insoluble molecule (**unconjugated**) and transported to the liver along with albumin.
- The liver converts unconjugated to **conjugated** bilirubin by adding a sugar molecule known as **glucuronate** through a **glucuronidation** reaction.

Types of Bilirubin:

- Total Bilirubin:

direct bilirubin + indirect bilirubin

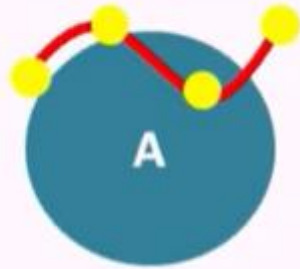
- Indirect bilirubin:

Unconjugated bilirubin (water insoluble, produced in the spleen)

- Direct Bilirubin:

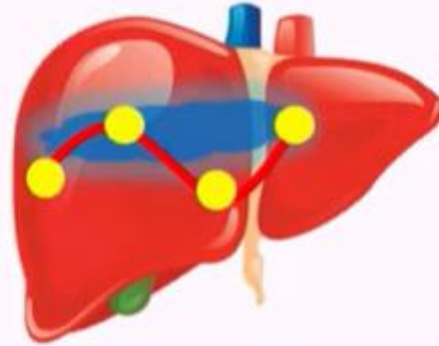
Conjugate (water soluble, converted in the liver).

2 types



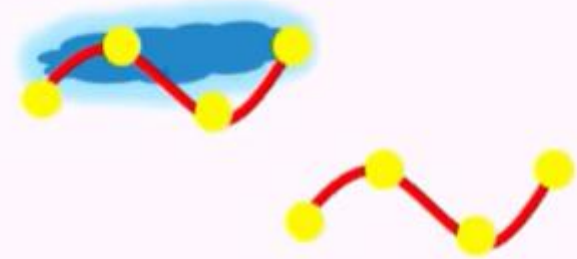
Unconjugated
Bilirubin
(INDIRECT)

Bilirubin



Conjugated
Bilirubin
(DIRECT)

Breakdown of **FAT** 



Total **Bilirubin**

When this test is required ?

JAUNDICE

**Neonatal
JAUNDICE**

GP < 35 wks



**Abdominal
pain**



↓ Appetite



**Dark colored
urine**



**Chills &
fever**

JAUNDICE

JAUNDICE

NORMAL

Yellowish or Greenish
Pigmentation of the
Skin and Whites of
the Eyes

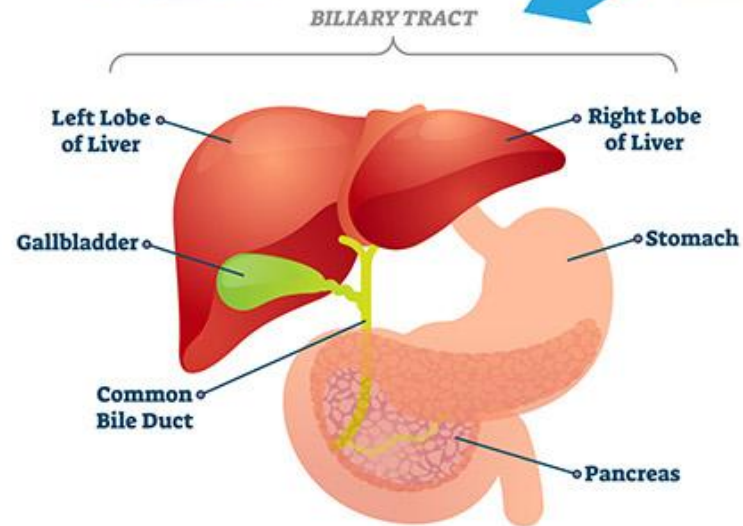
SYMPTOMS

- Itchiness
- Abdominal Pain
- Weight Loss
- Vomiting
- Paler than Usual Stool
- Fatigue
- Dark Urine

**YELLOWING IS FROM
ACCUMULATED BILIRUBIN**
in the Skin, Often Caused by
Liver and Gallbladder
Disorders

CAUSES

- Acute Inflammation of Liver
- Inflammation of the Bile Duct
- Obstruction of the Bile Duct
- Hemolytic Anemia
- Gilberts Syndrome



Principle

- Bilirubin reacts with **diazo reagent** to produce **azobilirubin**.
- **Azobilirubin is purple colored compound.**
- Intensity of color is **proportional to amount of bilirubin in serum.**

❖ Reactions:

▪ Direct Reaction

Involve direct or conjugated bilirubin

The reaction occurs in an aqueous medium

▪ Indirect Reaction

Carried out in methyl alcohol.

Methyl alcohol solubilizes unconjugated bilirubin.

Normal Range

Total bilirubin (5 days – 60 years) =
0.3 – 1.2 or 0.2 – 1.0 (> 60 years) mg/dL

Direct bilirubin (5 days – 90 years) =
<0.2 mg/dL

Normal Range

- **What is bilirubin, and how is it formed?**
- **Where does bilirubin synthesis primarily occur?**
- **What is unconjugated (indirect) bilirubin?**
- **How is bilirubin transported to the liver?**
- **What happens to bilirubin in the liver?**
- **What is conjugated (direct) bilirubin?**
- **What is the clinical significance of elevated unconjugated bilirubin?**
- **What conditions cause elevated conjugated bilirubin?**
- **How is jaundice related to bilirubin metabolism?**

