College of Medicine, KSU
Medical education Department
Pathology Department
Medical Biochemistry Unit

GIT Block (2nd Year)

Integrated Practical (Biochemistry / Pathology)

Liver Function Tests

Measurement of total bilirubin

By

Medical Biochemistry Unit

Q1. What are the liver function tests (LFTs)?

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Liver chemistry test	Clinical implication of abnormality
Alanine aminotransferase **(ALT)**	Hepatocellular damage
Aspartate aminotransferase **(AST)**	Hepatocellular damage
Bilirubin	Cholestasis, impaired conjugation, or biliary obstruction
Alkaline phosphatase	Cholestasis, infiltrative disease, or biliary obstruction
Prothrombin time	Synthetic function
Albumin	Synthetic function
γ-glutamyltransferase	Cholestasis or biliary obstruction
Bile acids	Cholestasis or biliary obstruction

e.g. Viral hepatitis

Q2. What is bilirubin and how is it produced in the body?

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- Bilirubin is a yellow bile pigment.
- It is produced from the degradation of heme; which is one of the breakdown products of red blood cells.

Q3. Which form of bilirubin is carried to the liver and how?

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- The **unconjugated** form of bilirubin is carried to the liver
- Unconjugated bilirubin forms a **complex with albumin** to be transported

Q4.

- **How & why is bilirubin conjugated?**

- **Mention 2 syndromes due to congenital deficiency of the conjugating enzyme (bilirubin glucuronyl-transferase).**

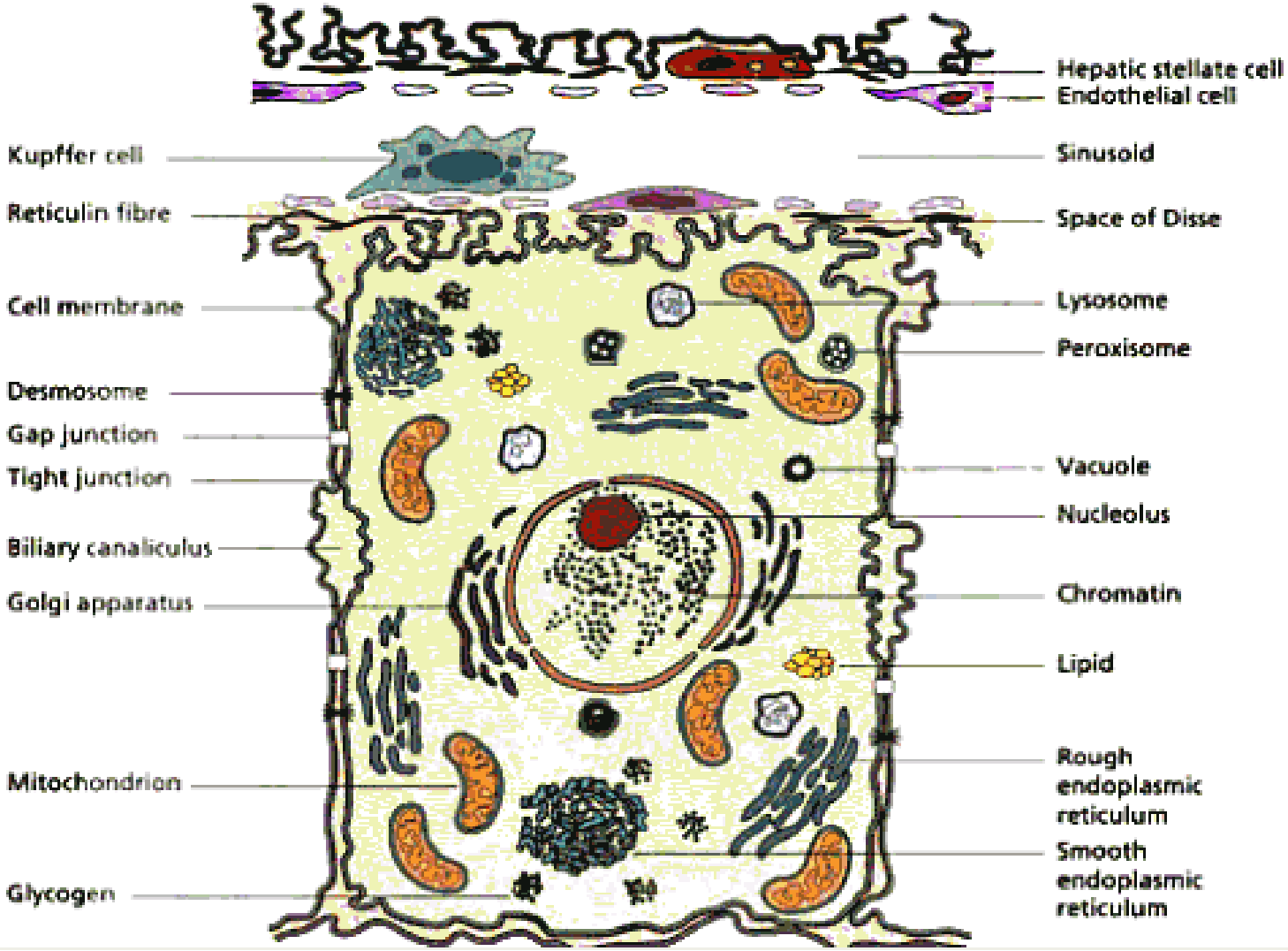
How & why is bilirubin conjugated?

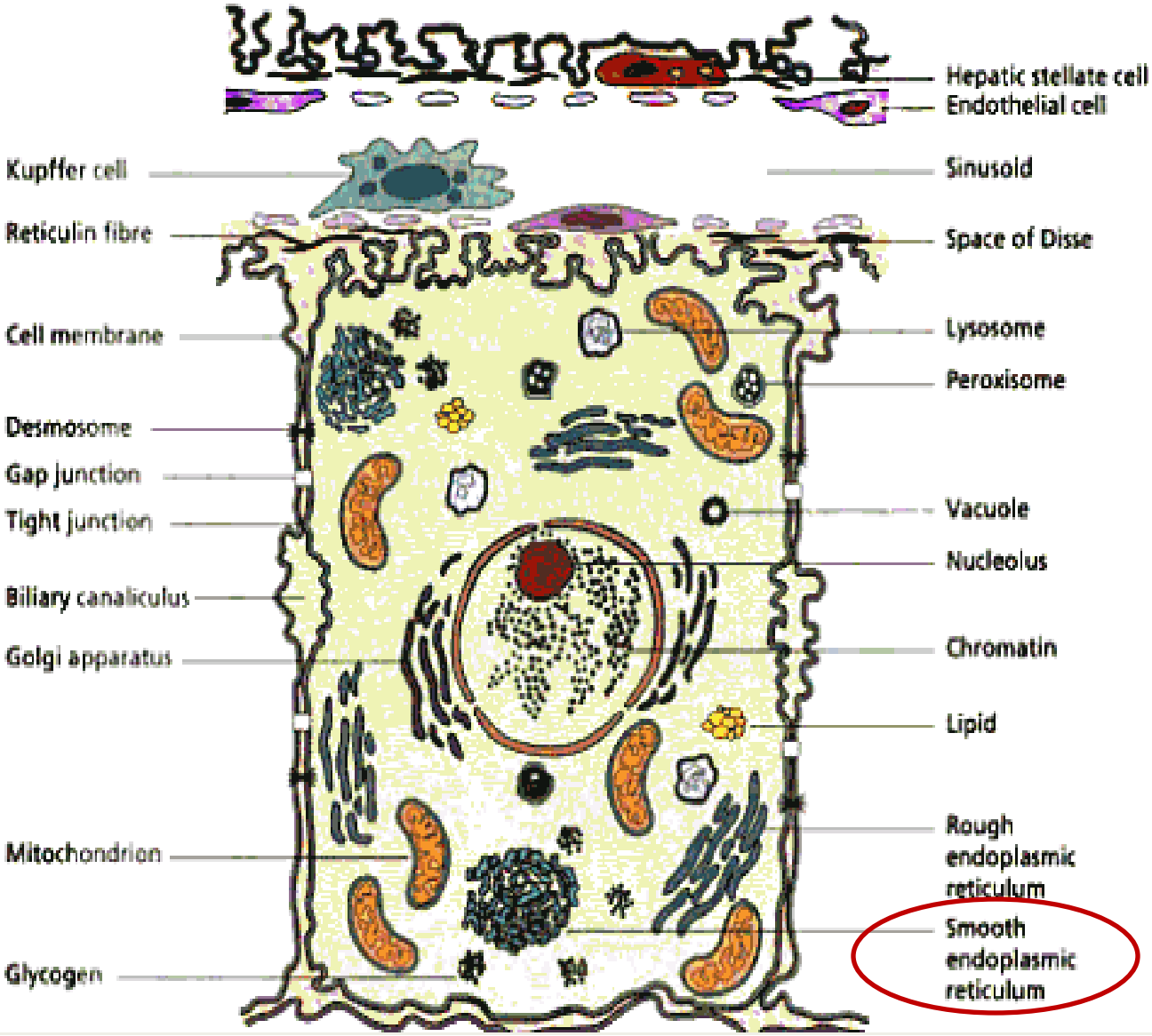
- Bilirubin is conjugated by addition of glucuronic acid in hepatocytes.
- The conjugated-bilirubin is water soluble and can be excreted in the urine and faeces.
- This prevents precipitation and deposition in tissues.

Defective enzymatic conjugation of bilirubin

- Examples of clinical conditions due to congenital deficiency of the conjugating enzyme (bilirubin glucuronyl transferase)
  - Crigler-Najjar syndrome
  - Gilbert syndrome

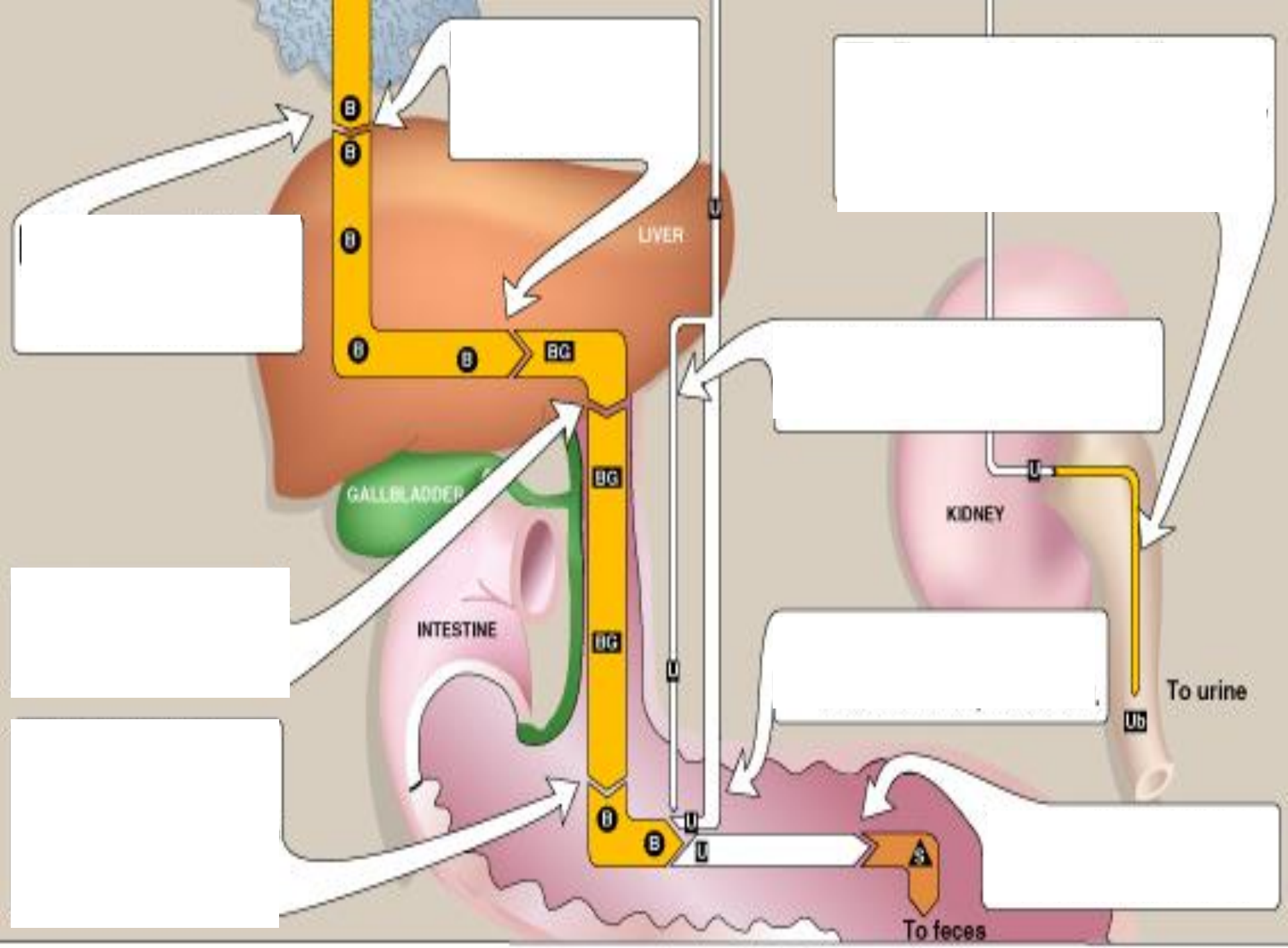
Q5: On the picture, mark the intracellular location for the process of conjugation?

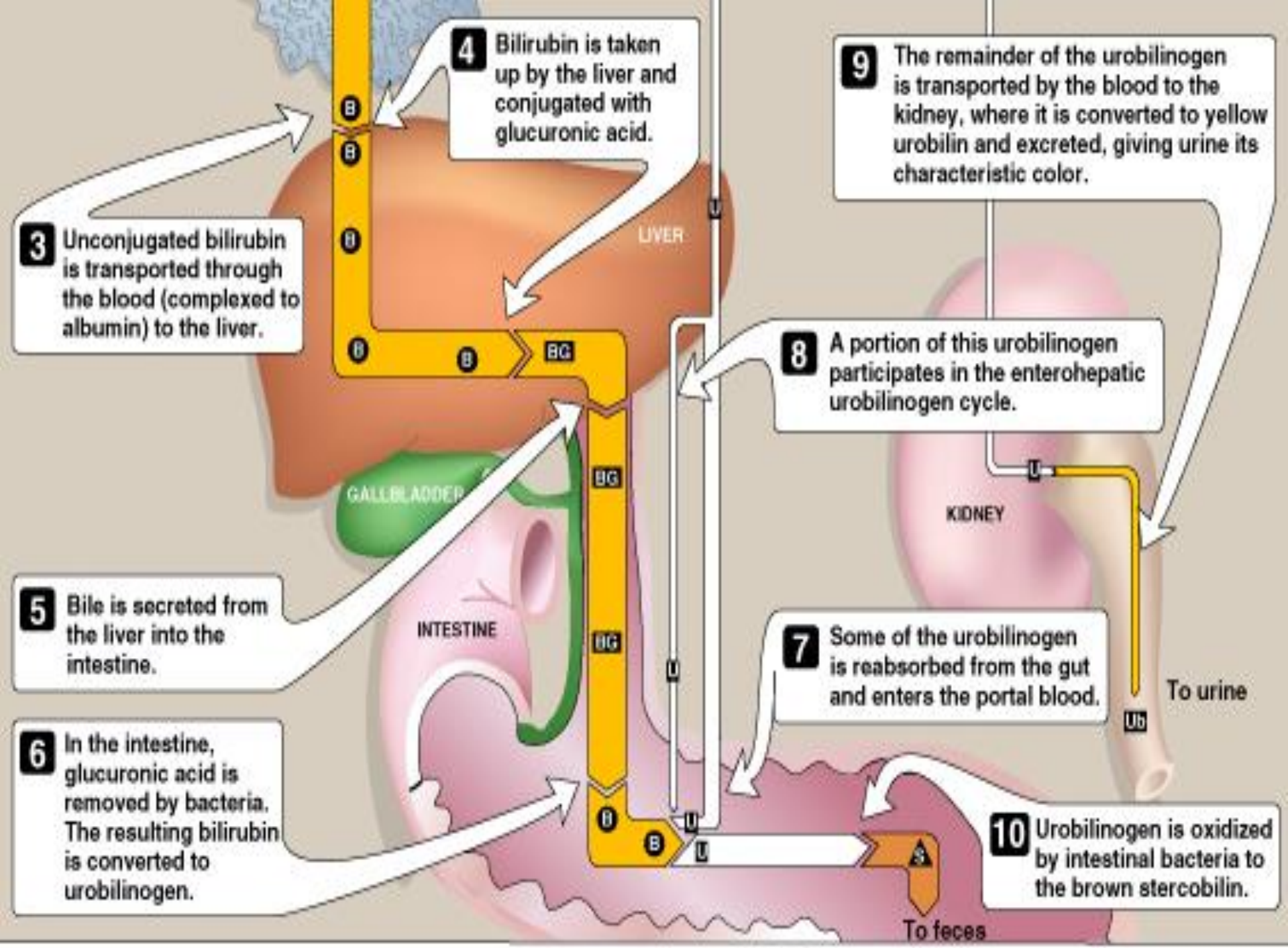



Hepatic stellate cell
Endothelial cell
Kupffer cell
Sinusoid
Reticulin fibre
Space of Disse
Cell membrane
Lysosome
Peroxisome
Desmosome
Gap junction
Tight junction
Vacuole
Nucleolus
Biliary canaliculus
Golgi apparatus
Chromatin
Lipid
Mitochondrion
Rough endoplasmic reticulum
Smooth endoplasmic reticulum
Glycogen

Q5.

A. How is bilirubin eliminated from the body?
B. What are the fates of bilirubin in the intestine?


LIVER
GALLBLADDER
KIDNEY
INTESTINE
To urine
To feces
B
BC
BG
U
Ub
A


4 Bilirubin is taken up by the liver and conjugated with glucuronic acid.
9 The remainder of the urobilinogen is transported by the blood to the kidney, where it is converted to yellow urobilin and excreted, giving urine its characteristic color.
3 Unconjugated bilirubin is transported through the blood (complexed to albumin) to the liver.
LIVER
8 A portion of this urobilinogen participates in the enterohepatic urobilinogen cycle.
GALLBLADDER
KIDNEY
5 Bile is secreted from the liver into the intestine.
INTESTINE
7 Some of the urobilinogen is reabsorbed from the gut and enters the portal blood.
To urine
6 In the intestine, glucuronic acid is removed by bacteria. The resulting bilirubin is converted to urobilinogen.
10 Urobilinogen is oxidized by intestinal bacteria to the brown stercobilin.
To feces

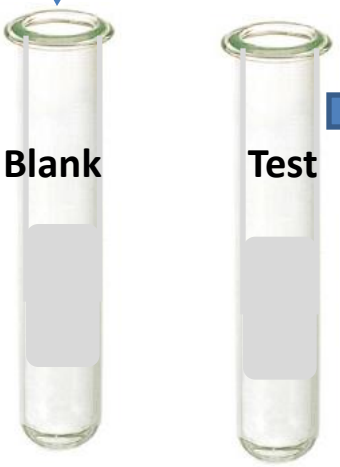
Measurement of Total Bilirubin

1. Add 200µl Reagent 1 To blank and test

2. Add 50µl (1drop) Reagent 2 Only in test

3. Add 1 ml Reagent 3 To blank and test

4. Add 200µl serum



Mix and wait for 5-10 min at RT

5. Add 1 ml Reagent 4 To blank and test

Mix and wait for 5-20 min at RT

6. Transfer(Pour) to cuvette

7. Using blank set the base line at wavelength 578 nm

8. Read test (A) at 578 nm

Calculation of total bilirubin concentration

Conc. of serum total bilirubin:

A × 185 = ... µmol/L

Note- (Normal range: 2 – 17 µmol/L)

Sensitivity

Sensitivity answers the following question:

If a person has a disease, how often will the test be positive (true positive rate)?

i.e.: if the test is highly sensitive and the test result is negative you can be nearly certain that the individuals don't have disease.

A Sensitive test helps **rule out** disease (when the result is negative).

Sensitivity rule out or "Snout"

$$\text{Sensitivity} = \frac{\text{true positives}}{\text{true positive + false negative}} \times 100$$

2 X 2 Contingency Table

Test	Disease	
	+	-
+	**True Positive (TP)**	**False Positive (FP)**
-	**False Negative (FN)**	**True Negative (TN)**

$$\text{Sensitivity} = \frac{TP}{TP + FN} \times 100$$

Specificity

Specificity answers the following question:

If a person does not have the disease how often will the test be negative (true negative rate)?

i.e., if the test result for a highly specific test is positive you can be nearly certain that the individuals actually have the disease.
A very specific test **rules in** disease with a high degree of confidence (when the result is positive).

Specificity rule in or "Spin"

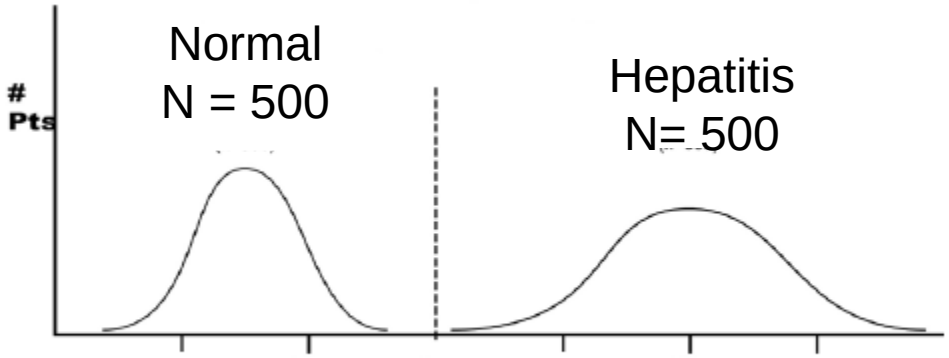
$$\text{Specificity} = \frac{\text{true negatives}}{\text{true negatives + false positives}} \times 100$$

2 X 2 Contingency Table

Test	Disease	
	+	-
+	**True Positive (TP)**	**False Positive (FP)**
-	**False Negative (FN)**	**True Negative (TN)**

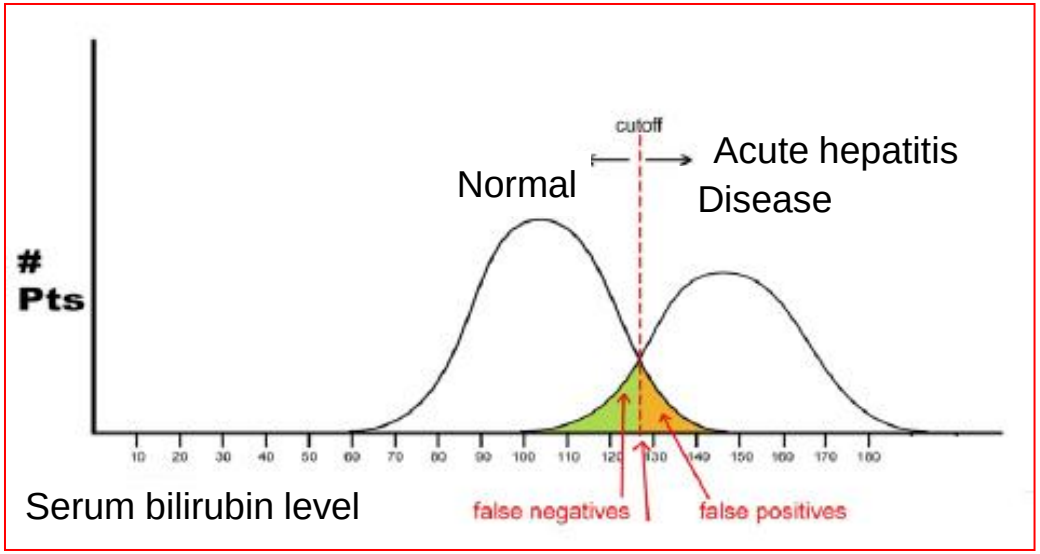
$$\text{Specificity} = \frac{TN}{TN + FP} \times 100$$

An ideal diagnostic lab test results for many subjects (normal and patients)



- A perfect test for acute hepatitis:

The test identifies ALL patients with disease and
All subjects without disease 100% of the time.



- The lab test results in normal and disease conditions overlap.
- To increase the overall accuracy of the test, the centermost point of overlapping is chosen as the cutoff value.
- There are some normal subjects who will have a positive results (False positives)
- There are some patients who will have negative results (False negatives)

Example of calculation

A Lab test to measure serum bilirubin was performed on 1000 individuals. The test gave the following results:

- Number of positive results in patients with acute hepatitis: 440
- Number of positive results in normal subjects: 50
- Number of negative results in normal subjects: 450
- Number of negative results in patients with acute hepatitis: 60
- For this Serum bilirubin test, calculate the following quality measures:

1. The sensitivity
2. The specificity

Answer: draw a 2 X 2 Contingency Table

Test	Disease	
	+	-
+	TP	FP
-	FN	TN

Test	Disease	
	+	-
+	440	50
-	60	450

$$\text{Sensitivity} = \frac{TP}{TP + FN} X\ 100 = \frac{440}{440 + 60} X\ 100 = 0.88\ x100$$

Sensitivity= 88%

$$\text{Specificity} = \frac{TN}{TN + FP} X\ 100 = \frac{450}{450 + 50} X\ 100 = 0.90x100$$

Specificity= 90%


Thank
you