Liver Histology, Physiology, and Bilirubin

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The Classic Hexagonal Lobule

Hepatic Lobule
A hexagonal shape having the central vein as its center and the portal triads at 3 of its 6 vertices.
Hepatic Lobule

- portal area
- branch or hepatic artery
- branch of hepatic portal vein
- bile duct
- sinusoids
- central vein
- liver cords
  made up of hepatic cells
Portal Areas (Triads):
at site where septa join at angles

Find Here:
1) Hepatic Portal Vein
2) Hepatic Artery
3) Bile Duct
4) Lymphatic
Bile Drainage System

= Portal Lobules (as opposed to Hepatic Lobules)

:Centered on the interlobular bile ducts of portal triads.

: Emphasizes liver's excretory & some of its secretory functions (i.e. bile salts).

:Borders of the portal lobules -- formed by lines joining three surrounding central veins, → a triangular shape.
The Portal Lobule

central vein

portal radicles

THE PORTAL LOBULE

THE CLASSIC HEXAGONAL LOBULE

lines show general course of cords and sinusoids (but both anastomose)
Portal Lobule
Bile Excretory & Secretory functions
Bile flow is towards the portal triad
(central feature in this model)
Hepatic & Portal Lobules Versus Hepatic Acinus
Six Important Liver Functions

1) Glucose Regulation
2) Synthesis of Materials for Export to Either:
   a) The Bloodstream
   b) The Bile
3) Storage of Materials
4) Clearance of Unwanted Materials from the Blood
5) Detoxification of Materials – primarily involving preparing them for elimination by making them water soluble
6) Excretion via the Biliary System
Liver as a Glucostat

A. Interrelationships of liver glucose with glycogen and gluconeogenesis as impacted by the actions of insulin and glucagon in fed vs. fasting states

B. Overall Objective: all the relationships in A. being to maintain blood glucose levels within an acceptable range for the body allowing it to become neither too high nor too low.
Synthetic Roles of Liver

A. Materials exported to the blood

1. Urea
2. Plasma Proteins: many types including albumin and several globulins
3. Lipoproteins critical for transport of fat in the bloodstream
Synthetic Roles of Liver (Contd)

B. Materials Exported to the Bile as Secretions Useful to the Body

1. Bile Acids or their Salts – Needed in the gut to aid solubilization of dietary fat for digestion
   i. Enterohepatic circulation of bile acids – blood levels rise after a meal and drop during fasted state
   ii. Powerful diagnostic tests for liver disease are made by measuring serum bile acid levels during fasting and finding abnormally high levels – a sensitive test for failure of clearance & excretion
   iii. Recently tests of bile acid/creatinine ratio in urine have been shown to be more useful than serum bile acid measurements.
Role of Liver in Storage

A. Glycogen – in relation to glucostat role, it is a very transitory “storage” as its glucose units turn over very quickly

B. Vitamins A & K in fat soluble group

C. Vitamin B₁₂
Role of Liver in Clearance & Detoxification of Materials

A. Bilirubin
B. Ammonia
C. Steroid Hormones
D. Polypeptide Hormones
E. Excess Cholesterol
F. Bile Acids (Salts)
Sources of Plasma Ammonia in the Body

A. Gut – Bacterial action on food proteins
   : 50%

B. Kidney
   : 40%

C. Muscle and Red Blood Cells
   : 5% each
Factors Which Increase Blood Ammonia Levels

A. Increased Dietary Protein
   - Glutamine
   - GI Bleeding
   - Uremia

B. Kidney
   - Alkalosis
   - Muscle – vigorous exercise
   - Fever
   - Infection
Four Organs Capable of Removing Ammonia from Blood

• Primary Organ
  1. LIVER

• These Organs Remove Minor Amounts:
  2. Lungs
  3. Resting Muscle
  4. Brain
Consequences of Failures of Clearances

A. Drugs such as anesthetic agents
   – death: patients never wake up

B. Bilirubin
   - jaundice
   - kernicterus of neonates

C. Steroid Hormone
   - feminization of males w/ cirrhosis
   - Sodium and water retention due to high aldosterone

D. Ammonia – Hepatic Encephalopathy
   1. Association with either Acute or Chronic Liver Disease
      i. Acute: - Widespread liver cell necrosis (death)
      ii. Chronic: Cirrhosis – decreased hepatocyte mass & altered blood flow due to intrahepatic scarring
Role of Liver Cells in Blood Coagulation

A. 8 of the 13 clotting factors of the blood clotting cascade are produced by hepatocytes
   1) 4 are dependent upon Vitamin K for their formation
   2) 4 have their synthesis independent of Vitamin K but of course dependent upon an adequate mass of functional liver cells

B. Clearance & Detoxification of Activated clotting factors requires functional liver
Role of Liver Cells in Blood Coagulation (Contd)

A. Hepatic dysfunction is a cause of coagulopathy (failure of blood to clot and clots to resolve properly)

B. Three General Causes of Coagulation Problems in Liver Disease
   1) Vitamin K Malabsorption
   2) Platelet Abnormalities
   3) Hepatocyte Dysfunction
Most Commonly Altered Functions Associated w/ Severe Liver Injury

A. Altered Glucose Regulation
   1. hypoglycemia

B. Diminished Synthesis of:
   1. Albumin: Diminished plasma osmotic pressure
   2. Coagulation Proteins
   3. Urea

C. Decreased Clearance and Detoxification of:
   1. Drugs
   2. Ammonia
   3. Hormones – Both steroid and polypeptide
   4. Fibrinolysin needed to clear blood clots (i.e. plasmin)
Bilirubin

Why is my baby yellow?

Early detection crucial when treating newborn jaundice
Where it all Starts:
The heme ring as an Iron-Porphyrin

:Heme found in hemoglobin, myoglobin, cytochromes
:breakdown of porphyrin in body is mostly due to turnover of RBC
Heme Ring as Iron-Porphyrin (contd)

Iron from heme is reused, but porphyrin part is discarded

- Converted to Bilirubin (Yellow) by macrophages for eventual excretion via the bile
- Biliverdin (green) is just a more oxidized form of bilirubin
  - found normally in certain species
  - commonly encountered in chronically ill livers of any species (bilirubin stuck in liver oxidizes to biliverdin)

We will continue discussion of ONLY BILIRUBIN
Bilirubin = Major Bile Pigment

- Coloring Agent of Icterus or Jaundice
  - Skin or membrane yellowing
- Porphyrin is first liberated from RBC through destruction by macrophages
  - convert porphyrin to bilirubin and release it to blood
  - it is in water “insoluble” or “free” form
  - “free” only in sense that “not conjugated”
  - Not really free at all, but tightly bound to serum albumin, a blood protein which binds such insoluble materials
Bilirubin (Contd)

• After uptake by hepatocytes:
  – bilirubin becomes “conjugated” (thus no longer “free”)
  – Occurs in smooth ER by linkage w/ glucuronic acid becoming first a monoglucuronide & then a diglucuronide
  – Diglucuronide = completely water soluble
Diglucurononide
("Conjugated" Bilirubin)

- In this form, bilirubin is normally excreted into a biliary tree and into gut
- If this form regains access to the bloodstream (occurs in important hepatobiliary disease) it can pass readily into urine
- “Free” bilirubin cannot do this because it is bound to serum albumin which is far too large a protein molecule to be filtered by the kidney
Tests for Serum Bilirubin

• Van Den Berg Test
  – 3 steps give you both “free” and “conjugated” bilirubin
  1) Mix serum sample w/ appropriate reagents
     a) Color: depth of which is proportional to amount of “conjugated” (aka “direct” or “prompt”) soluble bilirubin present in the serum
     b) At this stage, “free” bilirubin is undetectable b/c bound to serum albumin
Van Den Berg Test (Contd)

2) Mix the serum sample w/ appropriate reagents
   a) Add some alcohol to solubilize the “free” bilirubin, pulling it off the serum albumin so it can react:
   b) See more color which is now the “Total” Bilirubin
Van Den Berg Test (Contd)

3) Step 3 is simple subtraction:

\[
\text{Total Bilirubin} - \text{Conjugated bilirubin} = \text{Free Bilirubin}
\]

- **Free Bilirubin** is also known as “**Indirect**” Bilirubin because value has been obtained indirectly
Trick

• If your lab doesn’t do the complete Van Den Berg Test, but instead gives only a value for “Total” Bilirubin (as many autoanalyzers today do)—If you get a high “total” bilirubin value on blood,—just do a dipstick test on urine for bilirubin. If you get nothing in urine despite the high “total” bilirubin on serum, the bilirubin was of the “free” type rather than the “conjugated” type. Obviously if you get a nice high level on the urine bilirubin test, the serum likely has mostly the “conjugated” type of bilirubin.
Other Related Important Pigments

- Urobilinogen – Formed by bacteria in the intestine that are metabolizing bilirubin
- A synonym is Stercobilinogen
- Oxidation of Stercobilinogen → Stercobilin gives normal color of feces
  --- A very important color of great diagnostic value
Urine Urobilinogen in Diagnosis

• Urobilinogen reabsorbed from the gut is largely just re-excreted into bile by the liver cells but SOME makes its way to the urine where it can be easily detected with dipstick tests.

• Although urobilinogen in urine has diagnostic value its interpretation needs care
Three Steps (Proteins) required in liver cell handling of blood bilirubin

1. A special transport protein required to pluck bilirubin off albumin and get it into hepatocytes cytoplasm.
2. A conjugating enzyme system is needed to place glucuronic acids onto bilirubin making the bilirubin-glucuronides.
3. A special transport protein is required to move the conjugated bilirubin out of the hepatocyte and into the biliary tree at the bile canaliculus.
Summary of Major Types of Jaundice

- 1. Hemolytic - Bilirubin is “Free” and thus never in urine
- 2. Decreased hepatocellular uptake (Gilberts disease) – Bilirubin again is “Free”
- 3. Decreased conjugation of bilirubin (normal in newborns-exaggerated in premature infants with Kernicterus)-Genetic error DZ is Crigler-Najarr syndrome. Bilirubin again is “Free”
- 4. Decreased conjugation also occurs when liver is diseased. Bilirubin again is “Free” but in liver DZ both “Free” and “conjugated” are high.
Summary of Major Types of Jaundice (Contd)

• 5. Impaired movement of conjugated bilirubin into canaliculi—When genetically based (Dubin-Johnson syndrome)—also occurs to some extent whenever liver cells are sick because “sick cells swell” and when the liver cells swell the delicate walls of the bile canaliculi become obliterated---so there can be no exit except back to the blood for the conjugated bilirubin. This is the reason why about half of bilirubin increased during liver DZ is of the soluble “direct reacting” type (shows up nicely when urine tested for bilirubin)
Summary of Major Types of Jaundice (Contd)

• 6. Blockage of Major Bile Ducts—Often by gallstones in man; in animals it is usually due to a tumor, often tumors of the pancreas do this but many other types of tumor can.

   - Bilirubin is entirely of the conjugated type and so shows up nicely elevated in tests for bilirubin in the urine.