The Physiology and Pathophysiology of Gastrointestinal and Hepatopancreaticobiliary Disorders

Preparing for Professional Competence

A. B. R. Thomson

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THE WESTERN WAY

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Thank you.

A. B. R. Thomson

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Prologue

Like any good story, there is no real beginning or ending, just an in-between glimpse of the passing of time, a peek into a reality of people's minds, thoughts, feelings, and beliefs. The truth as I know it has a personal perspective which drifts into the soul of creation. When does life begin, when does an idea become conceived, when do we see love or touch reality? A caring, supportive, safe, and stimulating environment creates the holding blanket, waiting for the energy and passion of those who dream, invent, create – disrupt the accepted, challenge the conventional, ask the questions with forbidden answers. Be a child of the 60's. Just as each of us is a speck of dust in the greater humanity, the metamorphosis of the idea is but a single sparkle in the limitlessness of the Divine Intelligence. We are the ideas, and they are us. No one of us is truly the only parent of the idea, for in each of us is bestowed the intertwined circle of the external beginning and the end….

The child will grow, the images will expand, the learning of all aspects of our craft will develop and flourish amongst persons of good will. Examinations will become second nature, as each clinical encounter, each person, each patient, becomes our test, the determination of clinical competence, of caring, of compassion. May these three C's become part of each of our live's narrative. And from this start comes Capstone Academic Publishing, an innovation for the highest quality and value in educational material, made available at cost, speaking in tongues, in the languages of many cultures, with the dialect of the true North strong and free, so that knowledge will be available to all.

Outstanding medical practice and true dedication to those from whom we receive both a privilege and pleasure of care, comes within, comes from those rare teachers who inspire us. True, we need all of these to jump over a very high bar. But to be a truly outstanding physician, you need to care for and care about people, and you must respect the dignity and rights of all others. You must strike a balance between love and justice, and you place your family and friends at the top of your wish-list of lifetime achievements.

For the skeptics who ask "What do you want from me?" I just simply say "You are the future; I trust that in time you too will help young people to be the best they can be."

May good luck, good health, modesty, peace, and understanding be with you always. Through medicine, all persons of the world may come to share caring, respect, dignity, and justice.

Sincerely,

Emeritus Distinguished University Professor, University of Alberta
Adjunct Professor, Western University, London, ON, Canada

"They may forget what you said, but they will never forget how you made them feel."
Carl W. Buechner, on teaching.

"With competence, care for the patient. With compassion, care about the person."
Alan B. R. Thomson, on being a physician.

Dedication

To

Rebecca, Maxwell, Megan, Henry, Felix, Toby and Grady,

You fill the world with joy.

You promise the hope of a future of Love, Justice and Peace.

“With glowing hearts

we see thee rise.”

Acknowledgments

Patience and patients go hand in hand. So also does the interlocking of young and old, love and justice, equality and fairness. No author can have thoughts transformed into words, no teacher can make ideas become behavior and wisdom and art, without those special people who turn our minds to the practical - of getting the job done!

Thank you, Naiyana, for translating those terrible scribbles, called my handwriting, into the still magical legibility of the electronic age. Thank you, Sarah, for your creativity and hard work. Becky, thank you for your marvelous artistry. My most sincere and heartfelt thanks go to the excellent persons at JP Consulting, and CapStone Academic Publishers. Jessica, you are brilliant, dedicated enthusiastic and innovative and caring. Thank you.

When Rebecca, Maxwell, Megan Grace, Henry, Felix, Toby and Grady ask about their Grandad, I will depend on James and Anne, Matthew and Allison, Jessica and Matt, Benjamin and Jeannette to be understanding and kind. What I was trying to say and to do was to make my professional life focused on the three C's - competence, caring, and compassion - and to make my very private personal life dedicated to family - to you all.

Clinical Physiological Challenges and Sample Questions

CONTROL OF FOOD INTAKE

CLINICAL PHYSIOLOGICAL CHALLENGE

- Give the mechanisms involved in the control of food intake.

→ In your answer, give the role of each of the following:

- Mouth and GI tract
- Adipocyte, Leptin, Insulin, Ghrelin, Incretins
- POMC – secreting neurons (pro – opoimelanocortin)
- ARC (arcuate nucleus)
- PVN (paraventricular nucleus), LHN (lateral hypothalamic area hunger centre)
- NTS (nuclear tractus solitaries)
- CCK and GLP-1
- PYY
- Cerebral orexigenic and anorexigenic pathyways

- Source
  - Adipocytes, lesser amounts in gastric chief cells and placenta, as well as in breast milk
- Composition
  - 167- amino acid protein
- Receptor
  - 5 different forms
    - Blood brain barrier – may be defective in obesity
    - Hypothalamus – activated JAK stat (Janis kinase signal transduction and translation system)
- Action
  - Short term ↓ food intake
    - ↓ NPY neuropeptide Y, a transmitter in central and peripheral nervous system (PNS)
    - ↑ α-MSH (α-melanocyte – stimulating hormone)

- Give the mechanism by which CCK has an orexigenic effect.
- Give 5 satiety signals arising from the GI tract.
- Give 3 GI signals which increase appetite.
- Give an explanation of the mechanism of the effect of Apo– A IV.
- Give the compensatory mechanisms which are involved to reduce the impact

  of developing deficiencies in energy and/ or protein when a patient is starved.
- Give two compensatory metabolic mechanisms blunted in obesity, and thereby represents an adaptation which slows the effect of fasting on weight reduction.
- Give 5 GI conditions in which there has been demonstrated to be superior clinical outcome with the use of parenteral nutrition orders

MOUTH AND SALIVARY GLANDS

CLINICAL PHYSIOLOGICAL CHALLENGE – salivary secretion

Background:

- Salivary acinar cells secrete $KHCO_3$ and absorb NaCl.
- The apical (luminal) membrane contains a Na^+/H^+, Cl^-/HCO_3^- and a H^+/K^+ exchanger as well as epithelial Na^+ channels (ENaC) and CFTR (cystic fibrosis transmembrane regulator). The basolateral membrane contains a Na^+/H^+ exchanger, $NaHCO_3$ cotransporter, Na^+/K^+ ATPase, as well as K^+ channels and non-CFTR Cl^- channels.
- Draw a diagram which depicts the steps in the salivary secretion of fluid and electrolytes.
- Give the steps in the salivary secretion of fluids and electrolytes.

CLINICAL PHYSIOLOGICAL CHALLENGE – Salivary secretion

Background: The salivary gland is innervated by the parasympathetic and sympathetic nervous systems.

- Give the explanation for the development of xerostomia in some persons with Sjogren's syndrome, or treated with anticholinergic drugs.

ESOPHAGUS

CLINICAL PHYSIOLOGICAL CHALLENGE – GERD: “Reflux” disease

Case: A 45 year old man with an increased BMI of 29 began to develop severe retrosternal burning discomfort and acid regurgitation when his new –onset hypertension was treated with a calcium channel blocker. His symptoms of gastroesophageal reflux disease (GERD) were worsened by bending forward after meals, and by eating chocolate.

- Give the pathophysiology of GERD.

➢ In giving your answer, please include the manometric pressure valves of the upper (UES) and lower esophageal sphincter (LES), as well as the characteristics of the peristaltic waves of the esophageal body

  - Function of body of esophagus
  - Tone and relaxation of LES
  - Peripheral and central pathways mediating transient lower esophagial sphincter relaxation (LESR)
  - Conditions associated with GERD
  - Maintainance of integrity of esophageal mucosal membrane

CLINICAL CORRELATION

  - The tongue, like the face and palate, has a bilateral upper motor neuron innervation in most people, so a unilateral upper motor neuron lesion often causes no deviation.
  - A clinically obvious upper motor neuron lesion of CN-XII is usually bilateral and results in a small immobile tongue.
  - The combination of bilateral upper motor neuron lesions of the ninth, tenth and twelfth nerves is called pseudobulbar palsy.
  - A lower motor neuron lesion of the twelfth nerve causes fasciculation, wasting and weakness. If the lesion is bilateral, it causes dysarthria.
  - Movement disorders may affect the tongue.

- Give 10 factors which physiologically modulate LES pressure.
- Give the physiology of transient LES relaxation (TLESR).
- Give 6 differences in the physiology of tLESRs and swallow-associated declines (relaxation) in LES pressure (sLESRs).

- Give mechanisms of action of gamma β receptor agonists (e.g. baclofen) on the physiology of the esophagus and stomach which make this class of drugs potentially useful in persons with refractory GERD.
- Give a pathophysiological classification of GERD and GERD symptoms, and use this to classify the drugs used to treat persons with GERD.
- Classify the drugs used to treat GERD.
- Give the manometric features of the esophageal motility disorders (manometry) tracing abnormalities which characterize three major clinical disorders of esophageal motility.

STOMACH

- Give the cells of the stomach and their function.
- Give the physiological effect of trefoil factors.
- Give 4 factors responsible for increasing and/or decreasing the release of somatostatin from the D cells.
- Give 5 peptides other than somatostatin which inhibit acid secretion by way of ↓ ECL histamine release.
- Give the mechanism of this surgically induced increased acid secretion.
- Give the mechanism of action of the H3 receptors to alter gastric acid secretion.
- Give the name of the Gastrointestinal Peptide Hormones, their source and their action on the stomach.Give the cellular acid secretion by the parietal cell.
- Give the cellular acid secretion by the parietal cell.
- Give the scientific basis for the calcium-infusion and the secretin tests for Zollinger_Ellison Syndrome, and the rational of measuring BAO and MAO to calculate ration BAO/ MAO.
- Give the pathophysiological mechanism(s) for the phenomenon of acid rebound which occurs when anti-secretory treatment is suddenly stopped.
- Give the pharmacokinetics and pharmacodynamics of PPIs, and explain why PPIs must be taken half an hour before the intake of food.
- Give the explanation for the increase in the plasma concentration of gastric following the injection of secretin in the patient with a gastrinoma.
- Give the reason why the ratio BAO / MAO is much higher with a gastrinoma than health.
- Give the explanation why secretion of pepsinogen is essential for normal secretion of gastric acid, and why secretion of gastric acid is essential for normal function of pepsinogens.

- Give the reason why the basal and maximal outputs of pepsinogen increased with gastrinoma.
- Give the physiological function of the antrum and the pylorus.
- Give the key physiological factors involved in gastric motor activity.
- Give the key physiological factors involved in the modulation of rate of gastric emptying.
- Give 20 factors which alternate the rate of gastric emptying.
- Give the hormone related factors which modify gastric emptying.
- Give 8 clinical tests of gastric emptying.
- Give 10 pathophysiological processes involved in the gastroparesis associated with type I diabetes.
- Give the pathophysiological defects leading to symptoms and pharmaceutical approaches in diabetic gastroparesis, and describe the pharmaceutical approaches used to control these symptoms.
- Give the **treatment** of delayed gastric emptying.
- Give the mechanism (s) of action of 6 prokinetic drugs used for the treatment of symptoms of gastroparesis.
- Give the pathophysiology of gastroparesis.
- Give 6 tests of gastric function which might be used in this patient to document the suspected presence of gastroparesis.
- Give the basis of the dietary recommendations for gastroparesis.
- Give the receptors on gastric smooth muscle which may be targeted for her pharmaceutical treatment.
- Give 10 non-pharmaceutical maneuvers for the treatment of nausea and vomiting during pregnancy.
- Give the explanation why gastric acid secretion falls with acute or chronic H. pylori pangastritis, yet why gastric acid secretion rises with antral gastritis.
- Give 10 bacterial factors and 10 host factors which are important in the H. pylori-associated development of peptic ulcer, lymphoma and gastric cancer.
- Give the postulated mechanisms by which H. pylori causes DU (duodenal ulcer disease).
- Give the reason why the rate of symptom relief with Hp-triple therapy lower in functional dyspepsia where there has been an EGD,, than in uninvestigated dyspepsia (UD).
- Give 5 molecular factors which increase the risk of carcinogenesis from H. pylori.

- Draw the main types of bariatric surgery used for weight loss.
- Give the pathophysiological basis of 3 late complications of gastric surgery, and their suggested endoscopic treatment.
- Name 3 bariatric procedures, and list 3 complications for each, and give 5 complications common to all bariatric surgical procedures.
- Give 10 **late complications** common to all bariatric surgical procedures.
- Give the mechanisms for the digestion of iron- and vitamin B12- containing foods.
- Give how these normal processes are deranged after gastric surgery.
- Give the pathophysiology of the diarrhea and Steatorrhea which may arise as a result of gastric surgery.
- Give 8 examples of the common genetic abnormalities in gastric cancer (GCa).
- Give 7 premalignant conditions for GCa.

PANCREAS

- Give the gastrointestinal peptide hormones acting on the pancreas and gallbladder.
- Give 10 proteins secreted from the acinar cells of the pancreas.
- Give the organelles involved in the synthesis of enzymes and proenzymes, and trafficking of vesicles to the apical membrane of the pancreatic acinar cell.
- Give the physiology of the secretion of digestive enzymes by the pancreatic acinar cells.
- Give the cellular steps involved in the secretion of digestive proteins from pancreatic acinar cells.
- Give the mechanism by which CCK stimulates pancreatic acinar cell secretion of enzymes.
- Give 5 mechanisms that protect the acinar cells from autodigestion by its own proteases.
- Give the cellular physiology of the pancreatic acinar cell secretion of water and NaCl.
- Give an outline of the process of normal pancreatic fluid secretion, and thereby explain the principle of the secretin test for pancreatic HCO_3^- secretion.
- Describe the process of normal pancreatic secretion of enzymes and proenzymes, and thereby explain the principle of the Lundh test meal for pancreatic enzyme secretion.

- Give the mechanisms by which the pancreas prevents autodigestion, including the normal post-prandial process of inhibition of the cephalic, gastric and intestinal phases of pancreatic secretion.
- Give 6 major factors involved in the pathogenesis of acute pancreatitis.
- Give examples of four major susceptibility genes which cause or predispose to pancreatic disease.
- Give the pathophysiology of acute alcohol-associated damage to the pancreas.
- Give 6 systemic diseases associated with pancreatitis.
- Give the pathophysiology of the failure of 3 organs other than the pancreas other than the pancreas in necrotizing pancreatitis.
- Give the pathophysiology of chronic pancreatitis due to alcohol abuse.
- Give evidence for 3 alternate theories for the pain of chronic pancreatitis.
- Give the factors responsible for the development of diabetes mellitus in chronic pancreatitis.

HEPATOBILIARY SYSTEM

CLINICAL PHYSIOLOGICAL CHALLENGE- Portal Hypertension

Case: A 45 year old man develops esophageal varices. He consumes over 20 ounces of alcohol a day, and is suspected to having portal hypertension (PHT) from cirrhosis.

Question: Describe the portal circulation, define PHT, and explain the mechanism of development of PHT in prehepatic, intrahepatic and posthepatic disorders.

- Give the pathogenesis of the systemic and splanchnic (extrahepatic) vasodilation which leads to the hyperdynamic circulation in portal hypertension.
- Give the mechanism(s) of action of 5 drugs used to treat portal hypertension (PHT).
- Give the 4 zones of venous drainage in EV.
- Give the pathogenesis of esophageal varices (EV).
- Give an explanation for why liver transplantation (LT) is effective therapy for both PHG (portal hypertensive gastropathy, as well as for GVE / GAVE).
- Give the effect of reduced ileal uptake of bile acids on the mechanisms controlling the hepatic synthesis of bile acids. Explain the pathophysiology for a short ileal resection (<100 cm) causing choleriac diarrhea, whereas a longer ileal resection (> 100 cm) results in steatorrhea).

- Give the pathophysiology of why a resection of < 100 cm of ileum results in choleretic diarrhea, whereas a > 100 cm resection results in steatorrhea.
- Give the change in pathophysiology which occurs with a loss of > 100 cm of ileum, leading to steatorrhea.
- Draw a diagram which depicts the steps in the cholangiocyte secretion of $NaHCO_3$ and water.
- Give the physiology of bile acid-dependent and independent canaliculated secretion of water (bile flow / secretion).
- Give 8 clinicopathological presentations of DILI, and for each give an example of a causative drug.
- Give 10 possible risk factors involved in the pathogenesis of DILI.
- Give the drug, environment and host factors involved in the pathogenesis of DILI (drug-induced liver injury).
- Give the role of the metabolic and host immune systems in the pathogenesis of acetaminophen hepatotoxicity.
- Give the hepatic components protecting against drug-induced liver injury (DILI).
- Give the reason why DILI resulting from drugs metabolized by phase I reactions (e.g. acetaminophen) is localized in zone 3 (around the terminal hepatic venules [THV]).
- Give 5 major toxic mechanisms of drug-induced liver injury, leading to hepatocyte apoptosis and necrosis.
- Give the pathogenesis of NAFLD (non-alcoholic fatty liver disease) and NASH (non-alcoholic steatohepatitis).
- Give 6 factors which activate HSC (hepatic stellate).
- Give the regulated changes in gene expression which occur during activation of HSCs.
- Give the pathophysiological steps leading to non-alcoholic fatty liver disease (NAFLD).
- Outline the initiation and perpetuation steps leading to steatohepatitis and fibrosis.
- “Go for Purple.” List the regulated changes in gene expression which occur during activation of HSC (hepatic stellate cells).
- Give the pathophysiology of autoimmune hepatitis (AIH).
- Give the pathophysiology of primary biliary cirrhosis (PBC).
- Give the molecular biology of hepatitis B virus (HBV).
- Give the role of hepcidin in the control of iron balance.

- Give the speculated mechanism of HFE in sensing of body iron stores (BIS).
- Give the non-HFE forms of HH.
- Give the changings in iron binding, sensing; and transport proteins in HFE and non-HFE HH.
- Give the changes in iron binding, sensing, and transport proteins in inflammation, which lead to iron overload.
- Give the mechanism of iron overload in ALD and HCV.
- Give the speculated role of ↑ Fe in hepatocytes and the development of fibrosis.
- Give the pathophysiology of Wilson disease.
- Give 20 clinical features that suggest the diagnosis of WD.
- Give the histopathological changes in the liver in WD.
- Give a classification of the other causes of reduced ceruloplasmin levels.
- Give the reasons why measurement of liver copper may be normal or non-diagnostic in early WD.
- Give the mechanism for the iron deficiency which may occur the WD.
- Give 7 laboratory features that suggest the diagnosis of WD.
- Outline why is it important to appreciate that it takes ~ 6 months for D-penicillamine and trientine to improve neurological symptoms and liver enzymes in WD.
- Give adverse effects of therapy of WD (Wilson disease).
- Give the pathogenesis of α1-AT deficiency.
- Give the histopathology of α1-AT deficiency.
- List 7 liver diseases/conditions/presentations in HIV-infected persons even in the post-HAART era.
- Give 4 physiological mechanisms which contribute to the altered metabolism of drugs during pregnancy.
- Give the maternal and fetal factors implicated in the pathogenesis of HELLP.
- Outline the contribution of the small and large intestine, liver, skeletal muscle, kidney and brain to the pathogenesis of hepatic encephalopathy (HE) in patients with liver failure and HE (hepatic encephalopathy).
- On the basis of your knowledge of the pathogenesis of hepatic encephalopathy (HE), give the treatment of episodic and persistent HE, and provide the rationale for each treatment.
- Give the coagulation disorders in cirrhosis.

- Give the immunopathological steps involved in liver rejection after orthoptic liver transplantation (LT), and the pharmaceutical agents used to block each step required for T-cell maturation.

GALLBLADDER

CLINICAL PHYSIOLOGICAL CORRELATION – Gallbladder Absorption

Background:

- The basolateral membrane (BLM) epithelium contains Na^+/K^+ATPase, as well as K^+, Cl^- and H_2O channels.
- The apical / luminal membrane (LM) of the gallbladder contains both Na^+/H^+ exchanger (NHE) and HCO_3^-/Cl^- exchanger, as well as H_2O channels.

- Draw a diagram which depicts the absorption of H_2O by the gallbladder epithelium.

- Give the steps in pathogenesis of cholesterol gallstones.
- Give 5 genetic abnormalities associated with the formation of cholesterol gallstones. Consider your understanding of hepatocyte uptake, synthesis, catabolism and secretion of cholesterol.
- Give 5 mechanisms leading to "hypomotility" by which the gallbladder contributes to the formation of gallstones.
- Give the explanation for the formation of bile saturation with cholesterol which may occur normally with fasting.
- Give the role of liver and the small intestine in the development of lithogenic bile and cholesterol gallstones.
- Give 8 protein and non-protein factors that accelerate the nucleation and crystallization of cholesterol in bile, leading to the supersaturation of cholesterol and the formation of biliary sludge or cholesterol gallstones.
- Give the pathophysiology of the formation of cholesterol gallstones.
- Give the role of the hepatocyte in the metabolism of CH (cholesterol).
- Give the role of small intestine to the development of gallstones.
- Give 5 changes in bile acid metabolism which occur during pregnancy, and which increase the risk of cholelithiasis.
- Give the genetic basis for cholestatisis of pregnancy seen in about 15% of cases.
- Give the potential role of SAM (S-adenosyl – L- methionine) in the treatment of cholestasis of pregnancy.

- Give 4 benefits of UDCA in the management of cholestasis of pregnancy.
- Give the mechanisms for each of these four classes of drugs which lead to their increasing the risk of the development of cholelithiasis.

CLINICAL PHYSIOLOGICAL CHALLENGE

Case: A 45 year old woman with primary biliary cirrhosis (PBC) has hypercholesterolemia and xanthelasma.

- Outline the role of the liver in maintaining normal concentrations of cholesterol in the blood.

CLINICAL PHYSIOLOGICAL CHALLENGE - Gallstones

Case: A 28 year old mother of four healthy children develops RUQ pain and tenderness. Acute cholecystitis is diagnosed.

- Give the steps in the metabolism of bilirubin
- Give the pathophysiology of the development of bilirubin gallstones.
- Explain the colour of the urine and stools in persons with obstruction of the common bile duct (CBD)

CLINICAL PHYSIOLOGICAL CORRELATION – Bilirubin Metabolism

Case: A 32 year old women develops malaise, abdominal discomfort and jaundice.

- Draw a picture of bilirubin metabolism to distinguish between prehepatic, hepatic and posthepatic causes of jaundice.
- Give the pathophysiology of the development of hyperbilirubinemia and jaundice.

SMALL INTESTINE

- Give the pattern of smal and colonic motility which occur with feeding and fasting.
- Give the physioligical explanation for the “law of the intestine.”
- Give 8 pathophysiological causes of postoperative ileus.
- On the basis of small intestinal manometric changes, indicate which of the following clinical conditions is caused predominantly by myopathic or neuropathic changes.
- Give the most likely cause of intestinal obstruction.
- Give the usual bacterial presence (10^x/ml) of different sites along the gastrointestinal tract.
- Give the immunological characteristics which make immunoglobulin ideal for its function in the GI tract.
- Give 8 components of the GI mucosal barrier, and for each give their function to protecct against enteric infection.
- Give the physiology of the intestinal aabsorption of water.
- Give the standing-gradient hypothesis in the cintext of understanding how a glass of water is absorbed.
- Give the amount of Na^+ and H_2O which moves through this water channel.

CLINICAL PHYSIOLOGICAL CHALLENGE - Diarrhea

Case: A 25 year old woman returns from a holiday in a Central American Country, complaining of watery (non-fatty and non-bloody) diarrhea. She is unable to tolerate drinking milk because of bloating, excess flatus and worsening diarrhea; but taking a mixture of sugar and salt improves her dehydration.

- Give the normal process of absorption of salt and water.
- Explain how the intestine normally protects itself from luminal bacteria.
- Give the scientific basis for the tests used to diagnose SIBO (small intestinal bacterial overgrowth).
- Explain the basis for her lactose intolerance.
- Give the scientific basis for the use of oral rehydration solutions.

- Give the effects of aldosterone, glucocorticosteroids and catecholamines on Na^+ / H_2O absorption by the small bowel and colon.
- Give the effect of systemic pH on Na^+ / H_2O transport.
- Give the steps which are involved in the transduction of an external signal into a charge in cellular function, such as occurs with secretagogues.
- Give three classes of Cl^- channels.
- Give the mechanism by which K^+ channels modulate the hyperpolarization of the cell interior resulting from Na^+, K^+ -ATPase on the basal membrane (BM) of the enterocyte or H^+ , K^+ -ATPase on the apical membrane (BBM) of the parietal cell, and promote Cl^- secretion.
- Give the role of PK2 involved in secretory diarrhea.
- Give the role of the intestinal immune cells in the physiology of Cl^- secretion.

CLINICAL PHYSIOLOGICAL CORRELATION – More Diarrhea

Background: Diarrhea may result from an imbalance between intestinal secretagogues and absorptagogues. The treatment of diarrhea may include measures to normalize this imbalance.

- Give the role of the intestinal immune cells, endocrine cells and enteric nervous system in the control of fluid and electrolyte balance by the intestinal tract.

CLINICAL PHYSIOLOGICAL CORRELATION – Chloride Flux

Background:

- One process by which diarrhea occurs in through the decreased absorption of chloride (Cl^-) in the upper portion of the villus, and the increased secretion of Cl^- is the lower portion of the crypt-villus unit.
- The movement of Cl^- may be electroneutral or electrogenic and involves transporters and channels in the brush border membrane (AM) and the basolateral membrane (BM).
- Secretion of Cl^- is fundamental to the process of secretory diarrhea, such as occurs in cholera.

- Draw a picture of Cl^- absorption and secretion from along the length of the small intestinal villus and in the colon.

- Give the names of 4 major pancreatic proteolytic enzymes their cleavage sites and their products,
- Give the name of the proteins which are resistant to proteolysis in the lumen of the small intestine.
- Pancreatic proteases break down exogenous and endogenous protein. Give 3 other of their functions.
- Give the process of digestion and absorption of dietary carbohydrates.
- The intestinal brush border membrane (BBM) LPH (lactase-phylorizin hydrolase) gene expression is regulated single-nucleotide polymorphism (SNO). Give the method to distinguish in Caucasians between primary and acquired lactase deficiency, on the basis of LPH SNPs.
- Give the processes of emulsification, micellar formation, digestion and absorption of dietary trglycerides.

- Give the names of the three pancreatic enzymes involved in the lipolysis of trglyceride (TG).

- Give the differences between pancreatic PLRP2 (pancreatic lipase-related protein) and TG lipase.

- Give the effects of pancreatic enzymes on phospholipids and cholesterol.

- Give the mechanism by which cholesterol is transferred to the liver from non-hepatic tissues.

- Give the correct mechanism of the lipoprotein – poor lipoproteins in abetalipoproteinemia, as well as the often quoted incorrect/ incomplete mechanism.

- Giev the reason for the unexpected lack of lipemic serum in cholestatic liver disease when the serum TG levels are increased.

- Give the physiology of the absorption of dietary folic acid.

- Give the physiology of the absorption of cobalamin (vitamin B12).

- Give the Ca^{2+} transporters which are vitamin D (1, 25 – $\{OH\}_2$) responsive, and name which one is rate-limiting to the absorption of Ca^{2+}

- Give the names of 10 proteins innvolved in the absorption of iron, and in the balance of body iron stores (BIS).

- Give the role of hepatic hepcidin and IL-6 in the control of body iron absorption and balance.

- Give the role of HFE, B2-microglobulin and TR (transferrin receptor) in the duodenal crypt cells in the control of body stores, and the impact of defective HFE, as in HH (hereditary hemochromatosis).

CLINICAL PHYSIOLOGICAL CORRELATION – Iron Metabolism

Background: In *HFE*-related hereditary hemochromatosis, loss of functional HFE protein (hepcidin) leads to aberrant hepatocellular sensing of plasma iron, with inappropriately low levels of hepcidin, diminished macrophage iron stores, and inappropriately greater duodenal iron absorption.

- Draw a picture of the absorption of dietary iron by the duodenal enterocyte, and the role of both the intestine and the liver in
  - Causing hereditary hemochromatosis
  - Responding to either decreased or increased body iron stores
  - Responding to a chronic inflammatory process

- Give 10 components of GALT (gut-associated lymphoid tissue).
- In the context of immune – mediated non-toxic reactions to food, give the physiologic and immunologic barriers of the GI tract.
- Give the defective innate immunity, abnormal autophagia, and excessive response in adaptive immunity which occur in Crohn disease, and contribute to the pathogenesis of this chronic inflammatory condition.
- Give 10 genetic, microbiome and mucosal epithelial defense mechanisms which may have a pathophysiological implication in the development of CD.
- Give 5 immunosuppressive agents commonly used in gastroenterology or hepatology (for example for Crohn disease, ulcerative colitis, autoimmune hepatitis, liver), and for each give their mode of action, common toxic effects, and recommended monitoring.
- Give the immunopathogenesis of celiac disease.
- Give the suggested immunopathogenesis of refractory celiac disease (RCD).
- Give 5 clinical or laboratory features associated with IgE-mediated food-induced symptoms.
- Give the steps in IgE – and non-IgE-mediated food allergy.
- Give the anastomotic circulation between the celiac axis (CA) and the superior mesenteric artery (SMA), and between the SMA and the inferior mesenteric artery (IMA).
- Give the mechanism of tissue injury which occurs with reperfusion following a period of ischemia.
- Give 3 factors which are associated with ↑ risk of AMI (acute myenteric ischemia) in young persons.
- Give the limitation of ultrasound studies, including Duplex and Doppler flowmetry, in diagnosing AMI.

COLON

- Give the physiology of colonic motility.
- Give the location of the nerve cell bodies which occur in plexuses in the various histological layers of the colon.
- Give the normal physiology of colonic transit. Classify the medications used to treat constipation. Give the safety of these medications during pregnancy and lactation.
- Give 4 proposed pathophysiological causes of IBS.
- Give 8 observations which suggest that inflammation may play a role ("trigger") in the pathophysiology of post-infectious IBS.
- Give a classification of laxatives, their mechanism(s) of action, and their potential limitations.
- Name 6 major intestinal gases.
- Give why it is suggested to persons with "intestinal gas" to eat slowly and to drink at the end of meals.
- Give the mechanism to explain why breath Hydrogen levels may be falsely low and thereby misrepresent the presence of SIBO (small intestinal bacterial overgrowth).
- After eating garlic – containing foods, why does flatus not smell of garlic?
- Give the normal 24-hour volume of passage of flatus which is passed per rectum, and the number of individual passages.
- Give the relative rate of passage of substances along the GI tract.
- Distinguish between the terms "bloating" and "distention".
- Give the muscles involved in defecation.
- Give the nerves involved in defecation.
- Give the anatomical components of fecal continence.
- Give the physiology of defecation.
- Give the approximate normal values of anorectal sphincter pressure and volumes of balloon distention to achieve rectal sensation.
- Give the tests used to investigate fecal incontinence, including sensory, motor, and biomechanical function, as well as rectal capacity and compositive sensory motor function.
- Give the medical, endoscopic and surgical treatments of fecal incontinence.
- Give the process of normal defecation.
- Give the approximate % contribution of various mechanisms to the development of fecal incontinence.
- In the context of fecal incontinence, what is "rectal agnosia"?

- What is the “anal wink”, and what is the significance of its loss?
- What is the use of neurophysiologic testing to measure PNTML (pudendal nerve terminal motor latency time)?
- What is “biofeedback therapy”, and in what type of incontinence is biofeedback training most effective?
- Give 4 techniques used to investigate fecal incontinence.
- Give the cellular processes through and by which a balance of cell growth is achieved, describe the programs which trigger apoptosis and control proliferation, and use colorectal cancer as an example of the disruption of normal cell proliferation leading to oncogenesis.
- Give the pathophysiology of apoptosis.
- Give the histopathological signs of apoptosis.
- Give the genes associated with CRC.
- Give the abnormal molecule pathways involved in the pathogenesis of sporadic colorectal cancer (CRC).
- Give the name of the gene in the APC gene family which increases the risk of colon cancer (CRC) in Ashkenazi Jewish persons, and the gene which may be altered and to thereby increase the risk of CRC from the metabolism of nutrients and environmental agents.
- Give 10 examples of molecular genetic changes associated with sporatic or familial / hereditary colorectal cancer (CRC).
- Give the role of APC gene function, nuclear β-catenin, the Wat signal pathway, the LEF (lymphoid enhancer factor) / TCF (T-cell factor) family in the development of CRC.
- Give 4 biochemical changes that occur during the development of CRC.
- Give the proposed mechanisms of beneficial action of probiotics in the GI tract

OVERVIEW OF THE GI TRACT

Luminal Anatomy of the GI Tract

- Definition of the GI Tract: "Just a long tube, with a couple of accessory secretory glands, with immune storage, digestive and absorptive functions"

- Function of the GI tract: to produce a sequential and coordinated secretory and absorptive response to a meal, and to help modulate food intake.

Functions

- Appetite and food intake

- Mouth and Saliva Glands
  - Chops up big bites of food (mastication)
  - Lubricates the smaller pieces of food with saliva
  - Begins the digestion of carbohydrates (salivary amylases)
  - Pushes the food and fluid into the upper esophagus (transfer phase of swallowing)

- Esophagus
  - Pushes food and fluid from the upper to the lower esophageal sphincter
  - The lower esophageal sphincter (LES) reduces the regurgitation of food and fluid from the stomach into the esophagus

- Stomach
  - Secretes HCl and pepsinogen, as well as gastrin and somatostatin, to aid in the digestion of carbohydrate, fat and protein
  - Partially destroys the bacteria in food
  - Churns the food bits into even smaller pieces, and emulsifies lipids
  - Stores the partially digested and ground up "mush" until it is ready to be pushed by smooth muscle peristalsis into the duodenum
  - Secretes ghrelin, which has some longterm control over appetite and food intake
  - Turns off salivary secretion
  - Relaxes ileocecal valve

- Small Intestine
  - More digestion as well as absorption
  - Pushes remaining mixture of what is left of the meal and drink into the large bowel
  - Releases hormones to stimulate secretions from pancreas, liver and gallbladder, as well as secreting hormones to reduce appetite and food intake
  - Turns off gastric secretion

- Pancreas
  - Secretes enzymes to further digest carbohydrate, fat and proteins
  - Secretes HCO_3 to neutralize gastric HCl in the duodenum
  - Secretes hormones which influence the metabolism of absorbed nutrients

- Liver
  - Synthesizes albumin, clotting factors, bile acids and cholesterol
  - Metabolizes bilirubin, drugs
  - Secretes bile, phospholipids, cholesterol, fluid

- Gallbladder
  - Stores bile acids and bilirubin until released into duodenum
  - Absorbs water to concentrate the bile acids

- Colon
  - Ferments the left-overs into a short chain fatty acids (acetic, butyric, proprionic acids) and flatus
  - Absorbs water until feces are formed
  - Stores feces until the colon is ready to be evacuated
  - Helps prevent fecal incontinence

Digestion and Absorption of Macronutrients

Macronutrients		Digestion	Absorbed units
➢ Carbohydrates	○ Starch ○ Soluble/insoluble fiber ○ Disaccharides ○ Monosaccharides	○ Salivary/ pancreatic amylase ○ BBM disaccharides	○ Monosaccharides
➢ Lipids	○ Triglyceride ○ Cholesterol ○ Fat-soluble vitamins (FSV) ○ Bile salts	○ Lingual, gastric and pancreatic lipase	○ Fatty acids ○ Free cholesterol ○ FSV ○ Bile salts

Macronutrients		Digestion	Absorbed units
➢ Protein	○ Peptides	○ Gastric pepsinogen (pepsin) and pancreatic trypsinogen (trypsin) ○ BBM dipeptides	○ Peptides (in minute amounts) ○ Small peptides (including di- and tripeptides) ○ Amino acids

Abbreviations: BBM, intestinal brush border membrane; FSV, fat soluble vitamins

Daily Intake: Calories, average baseline needs, 30 kcal/day; protein, 0.5 mg/kg; fluids, 1.5-2.5 L/day

Approximate daily volume output: Saliva 0.5 L/day; Stomach 1.0-1.5 L/day; in small intestine –8 L/day, ↑ bile; 0.5 L/day; colon 1-1.5 L/day; in feces

Daily Output of "poo" (stool, feces) about 250 g/day, depending on daily intake of fibre : stool consists of non-digested/non-absorbed food and fluids; intestinal bacteria.

Systems

- Integration
  - Digestion/absorption (assimilation = uptake across the brush border membrane [BBM], and transfer across the basolateral mambrane [BM]), secretion, and motor activity (leading smooth muscle to peristalsis)
  - Neural, hormonal and paracrine responses, with sensor and transmission processes
  - Afferent and efferent components (turn "on" and turn "off") the functions
  - When a function occurs at one site of the intestinal tract, the proximal portion becomes turned off, and the distal site is turned on (e.g., when the stomach is active, the salivary glands and esophagus "relax" their stimulated function, and the hepatopancreaticobiliary functions become ready to be stimulated.

- Neural
  - CNS (central nervous system; vagus nerve) and ENS (enteric nervous system; GI "mini-brain") activate secretion and peristalsis through neuro transmitters.
  - Submucosal (Meissner's) plexus): ENS of small and large intestine
  - Myenteric (Auerbach's) plexus: ENS from esophagus to rectum in between the inner circular and outer longitudinal muscle layers

- Afferent sensory neurons: mechanoreceptors for smooth muscle tension, chemoreceptors (chemical, enteric toxins, pH, nutrients and osmoreceptors)
- Interneurons efferent secretomotor neurons (interneurons)
- Brain-gut axis modifies input from the parasympathetic nerves (vagus for the GI tract, except for the distal third of the colon), and from the autonomic ganglia (through the spinal cord, medulla and brain)

- Neurotransmitters
  - Excitatory motor neuron neurotransmitters (such as Ach [acetylcholine], encephalins, VIP [vasoactive intestinal peptide], and NO [nitric oxide]
  - Inhibitory motor neuron nerotransmitters (such as serotonin [5-HT; 5-hydroxytryptamine], somatostatin and substance P)

- Hormonal
  - Mucosal receptors (mechanical, osmotic, chemical) act on neural pathways.

- Paracrine
  - Sensor cells release transmitters into adjacent effector cells.

- Parasympathetic
  - Pre- and post- ganglionic fibers (using Ach for pre ganglionic fibers, and for post-ganglionic fibers, as well as other neurotransmitters).
  - Efferent fibers pass to the medulla.

- Sympathetic
  - Pre-ganglionic fibers and post-ganglionic neurons pass to, into post-ganglionic sympathetic fibers which synapse on either ENS (enteric nervous system)or on effector cells.

Old age makes you redundant

but

It's OK to be redundant – but only if you're a gene!

Grandad

CONTROL OF FOOD INTAKE

Control of Food Intake

- To reach the stage of following a hamburger, fries and shake through the gastrointestinal (GI) tract, we must first have the desire to eat and the willingness to ingest this high fat, high carbohydrate and high calorie meal. This control of food intake is a complex and integrated process, and only an overview will be given here.

Useful Background: Terms used to describe the balance of energy expenditure

- RMR – Resting metabolic rate (~2,100 kcal/day in a 70-kg adult)
- AREE – Activity-related energy expenditure
- NEAT – Non-exercise-associated thermogenesis (e.g. typir looking around: varies 3-to 10- fold between persons about 500 kcal/day
- DIT – Diet-induced thermogenesis – about 10% of daily ener expenditure

Weight = energy in – energy out

Weight gain occurs from positive energy balance (in > out)

Useful Background: Calculations of Total Energy Expenditure
TEE = REE + PAEE + TEF

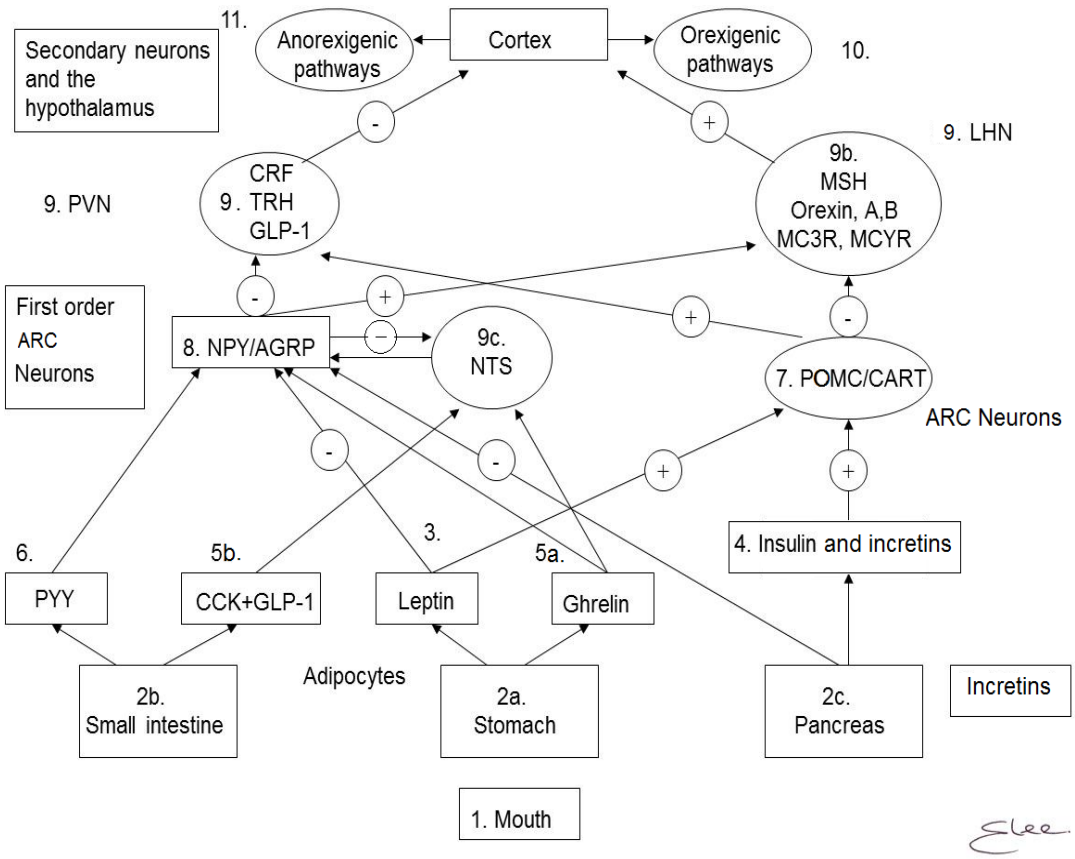
Abbreviation: PAEE, physical activity energy expenditure (20% of TEE); REE, resting energy expenditure (70% of TEE); TEE, total energy expenditure; TEF, thermal effect of feeding (10% of TEE)

CLINICAL PHYSIOLOGICAL CHALLENGE

- Give the mechanisms involved in the control of food intake.

→ In your answer, give the role of each of the following:

- Mouth and GI tract
- Adipocyte, Leptin, Insulin, Ghrelin, Incretins
- POMC – secreting neurons (pro – opoimelanocortin)
- ARC (arcuate nucleus)
- PVN (paraventricular nucleus), LHN (lateral hypothalamic area hunger centre)
- NTS (nuclear tractus solitaries)
- CCK and GLP-1
- PYY
- Cerebral orexigenic and anorexigenic pathyways



Abbreviations: AGRP, agouti-related protein; ARC, arcuate nucleus of the hypothalamus;CART, cocaine and amphetamine regulated transcript; CCK, cholecystokinin; CNS, central nervous system; CRF, corticotrophin releasing factor; GLP-1, glucagon-like peptide-1; LHA, lateral hypothalamic area; MSH, melanocyte-stimulating hormone; NPY, neuropeptide Y; NS, nervous system; NTS, nucleus tractus solitaries; POMC, pro-opiomelanocortin; PNV, paraventricular nucleus of the hypothalamus; PYY, peptide YY3-36; TRH, thyrotropin-releasing hormone; VMN, venteromedial nucleus satiety centre

Adapted from: Foxx-Orenstein AE. *ACG Annual Postgraduate Course Book*: 2008, page 148. Quoted in GI Practice Review, 2010; *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Figure 48.9, page 1041.

Networks Involved in the Control of Food Intake

1. Mouth- oropharyngeal reflexes are stimulated by chewing and swallowing, and may reduce food intake after a certain threshold.

2. GI tract
   a) Stomach - releases leptin and ghrelin
   b) Pancreas - releases insulin
   c) Small intestine - releases PYY, CCK, GLP-1

➢ Stomach

3. Leptin and Adipocytes

   - Leptin↑ leptin in blood with food intake lowers (leptin lowers, ghrelin gains) further food intake by acting through the arcuate nucleus.
   - Leptin tells the brain how much fat is stored.
   - Plasma leptin concentration increases as the mass of the adipocyte tissue increases (↑ adipocytes → ↓ leptin → ↓ intake of food).
   - Leptin is a 17-kDa protein, made mostly in adipocytes
   - Leptin binds to its receptor Ob-r.
   - Ob-r is a tyrosine kinase- associated leptin receptor that signals through JAK-2 and STAT.
   - Leptin from the stomach and insulin from the pancreasare satiety-producing (anorexigenic) signals

➢ Pancreas

4. Insulin

   - ↑ POMC (pro-opiomelanocortin) / CAR (cocaine and amphetamine regulated transcript neurons) in ARC neurons
   - This causes ↑ PVN (paraventricular nucleus of hypothalamus)
     - CRF (corticotropin releasing factor)
     - TRH (thyrotropin releasing factor)
     - ↑ GLP-1 (glucagon-like peptide – 1)

- Insulin concentration rapidly increases in response to food intake, and is more of a short-term regulator of ARC, while leptin is more long-term.
- A deficiency of Ob-r leads to leptin resistance.
- T½ of leptin is about 75 min; fasting or short term changes in food intake have little effect on leptin concentrations.
- Leptin from the stomach and insulin from the pancreas
  - ↓ neuropeptide Y (NPY) and the agouti-related protein (AGRP) in the first-orderneurons in arcuated nucleus (ARC) .
- Leptin
  - ↑ POMC / CART
  - ↓ NPY / AGRP

  } in first order are neurons

- Give the physiological effects of leptin.

Source	Adipocytes, lesser amounts in gastric chief cells and placenta, as well as in breast milk
Composition	167- amino acid protein
Receptor	5 different forms ▪ Blood brain barrier – may be defective in obesity ▪ Hypothalamus – activated JAK stat (Janis kinase signal transduction and translation system)
Action	Short term ↓ food intake ▪ ↓ NPY neuropeptide Y, a transmitter in central and peripheral nervous system (PNS) ▪ ↑ α-MSH (α-melanocyte – stimulating hormone)

5. Ghrelin

- Source
  - Gastric fundic P/D_1 cells
  - Minute amounts in
    - Small intestine
    - Pancreas
    - Pituitary
    - Kidney
    - Placenta
- Composition
  - 28 – amino acid peptide
- Receptor
  - GHR (growth hormone [GH] secretagogue receptor)
- Action
  - Plasma levels increase with the development hunger i.e., fasting
  - Acts through vagal afferents to stimulate (ghrelin GAIN)
  - Ghrelin → ↑ food intake and ↑ gastric motility
  - Ghrelin level then falls
  - Pre-meal and post-meal decrease in GH concentration in blood
  - ↑ GH secretion
  - ↑ GHR levels before meals (fasting)
  - When BMI is low, ↑ GHR
  - Signal to begin eating
  - Activates NPY and agouti – related protein producir neurons in arcuate nucleus of hypothalamus
  - Acts on vagus nerve to ↑ gastric contraction
- Clinical
  - Bariatric gastric bypass surgery removes the norm premeal increase in ghrelin, thereby contributing to t desired weight loss
  - Prader – Willi syndrome (congenital obesity grow hormone deficiency, hypogonadism) ↑ GHR circulation that fails to fall after eating, resulting hyperphagia and ↑ BMI

➢ Definition: the enteroinsular axis and incretins are the GI peptid which stimulate pancreatic islet cell insulin release, and thereby play role in glucose homeostasis.

- GLP-1 and GIP are the most important incretins.
- Amylin and secretin

- ↓ release of glucagon (amylin), → ↓ gastric emptying (amylin, secretin) → ↓ rate / amount of carbohydrate reaching the upper small intestine. They are not considered to be incretins because they do not stimulate the release of insulin.

➢ Incretins

- Absorped glucose stimulates the secretion of GLP-1 and GIP (glucose-dependent insulinotropic peptide) from the small inestine

- The functions of GLP-1 are:
  - GLP-1 acts on GLP-1 receptors (in the periventricular nucleus [PVN], dorsal medial hypothalamus [DMH] and the arcuate nucleus [ARC] of the hypothalamus).
  - Stimulates insulin secretion from the pancreatic beta cells (enhance glucose-simulated insulin release).
  - Delays gastric emptying
  - ↑ satiety (↓ appetite), ↓ food intake

- GLP-1 and GIP (glucose-dependent insulinotrophic peptide) are incretins, GI peptides which stimulate the entero-insular axis to release insulin.

- Others incretins include
  - CCK
  - Gastrin
  - GRP (gastrin releasing peptide)
  - Motilin
  - PACAP (pituitary adenylate cyclase- activating peptide)
  - VIP (vasoactive intestinal peptide)

Amylin (islet amyloid peptide) is a 37-amino acid peptide secreted from the pancreatic islet cells with insulin. The action of amylin is to ↓ glucagon release, and along with CCK and secretin, to slow gastric emptying.

Proglucagon ⟶ Glucagon + $\underline{GLP_1 + GLP_2}$

↑ ↑

Pancreas Small intestine

This helps to regulate postprandial blood glucose concentrations [GC], GI peptides also regulate postprandial [GCI] by stimulating insulin release.

SO YOU WANT TO BE A GASTROENTEROLOGIST!

Incretins are responsible for about half of the postprandial release of insulin. The major incretins are GLP-1 and GIP. What role do these two incretins play in type II diabetes mellitus?

- GIP – normal secretion but ↓ response
- GLP – ↓ secretion

- CB-1/CB-2 binds endocannabinoids, endogenously produced arachidonic acid derivatives.
- Food releases ghrelin
- These cause short-term ↓ intake.
- Gastric ghrelin is the only GI hormone which stimulates food intake.
- Ghrelin acts on both the brainstem solitary nucleus (NTS) as well as on the hypothalamus.
- Ghrelin released from the gastric mucosa from the presence of food binds to GHSR in arcuate nucleus (ARC) and vagal afferents to ↓ food intake.
- Gastric leptin, small intestinal PYY, CCK and GLP-1 as well as pancreatic insulin lower food intake.
- Small intestinal PYY acts on ARC, whereas small intestinal CCK and GLP-1 act on NTS., CNS networks.

➢ Small intestine

- Food releases from the small intestine
  - Glucagon, GLP-1 (glucagon-like peptide-1), gastrin-releasing peptide (GRP), SS, peptide YY (PYY), and CCK* (cholecystokinin).

6. CCK

- Give the mechanism by which CCK has an orexigenic effect.

  - While CCK-RF stimulates the ECL cell to release CCF, CCK-RF reduces pancreatic secretion by negative feedback.
  - CCK and secretin are released from ECL cells by intraluminal releasing factors (e.g., CCK releasing factor [CCK-RF]).
  - With food intake, CCK-RF stimulates CCK release into the blood. As the food stimulus lessens, trypsin in the intestinal lumen digests CCK-RF, and the release of CCK is diminished.

7. PYY

  - Peptide tyrosine (PYY) is
    - Present in the brain as well as in the L cells of the ileum and colon.
    - PYY1-36 and PYY3-36 bind to their receptors and reduced food intake.
    - PYY has come into focus as an anorexigenic peptide with the finding that the weight loss after Roux-en-Y gastric bypass in part due to the enhanced release of PYY after bariatric surgery.
    - PYY increases the intestinal synthesis of apo-A IV, which itself acts centrally to reduce food intake.

  - GLP-1
    - ↓ antral motility
    - ↑ gastric volume
    - ↑ fullness
    - ↓ food intake
  - Apolipoprotein A-IV
    - ↑ with absorption of triglycerides
    - ↓ gastric motility
    - ↓ food intake

➢ Hypothalamus

- POMC

  - POMC / CART and NPY / AGRP comprise the ARC (first order neurons in hypothalamus)
  - POMC / CART are or exogenic, whereas NPY / AGRP are anorexigenic.
  - POMC-secreting neurons

- The POMC-secreting neurons have receptors for leptin and for insulin, and are stimulated by these two peptides.

- When stimulated by leptin or insulin, these neurons produce either the neuropeptide POMC (pro-opiomelanocortin) from one class of neurons, or neuropeptide Y (NPY) and agouti-related protein (AGRP) from another class of neurons.

- CART

  - The first order POMC neurons also synthesize and release CART (cocaine-amphetamine-related transcript), which is also anorexigenic.

- α-MSH

  - From the synapses of POMC neurons, a cleavage product of POMC is released, α-MSH (melanocortin α - melanocyte – stimulating hormone).

  - α-MSH binds to second – order neurons which contain MC3R and MC4R melanocortin receptors.

  - Stimulation of MC3R/MC4R by α-MSH from POMC is anorexigenic (↓ food intake and ↑ satiety), as well as increases energy expenditure by way of activating descending sympathetic pathways.

8. NPY / AGRP are also first order neurons

   - ARC has both inhibitory and stimulating influences on PVN and LHN.

   - The NPY/AGRP neurons activate the NPY receptors Y1R/Y5R (GPCRs, cannabinoid receptors) on secondary neurons, leading to ↑ food intake.

   - NPY inhibits PVN.

   - Second order neurons in PVN inhibit the anorexigenic pathways to ↑ satiety ↓ food intake.

   - AGRP binds to MC4R melanocortin receptors on the secondary neurons of the POMC pathway, reducing the anorexigenic effect of α-MSH, thereby producing an orexigenic effect.

   - AP in the cortex is stimulated by the corticotrophin releasing factor (CRF), thyrotropin-releasing hormone (TRH), and glucagon-like peptide-1 (GLP)-1.

➢ Secondary neurons of the hypothalamus (PVN, LHN, NTS)

- Paraventricular nucleus (PVN)
  - The PVN positively influences the cortical anorexigenic pathways (AP), and the LHN positively influences the cortical orexigenic pathways (OP).
  - Reacts to
    - Psychosocial factors
    - Social norm
    - Cortical control
    - Environmental factors
- Lateral hypothalamic nucleus (LHN)
  - NPY/AGRP neurons stimulate and POMC neurons inhibit the secondary neurons in the LHN, producing the orexigenic peptides melanin-concentrating hormone (MCH) and orexins A and B
  - Cortical OP is stimulated by melanocyte-stimulating hormone (MSH) as well as by orexin A and B in LHN.
  - CB-1/CB-2
    - The GPCRs are canabinoid receptors in the basal hypothalamus, other parts of the brain, vagal afferents, as well as in peripheral tissue.
  - PVN
    - Paraventricular nucleus
    - Neurons project to the brainstem and to the cerebral corte
  - NTS – nucleus tractus solitaries a second order neuron in the hypothalamus
  - VMN – Venteromedial nucleus satiety centre
  - DMN – Dorsomedial hypothalamic nucleus
  - Nucleus tractus solitaries (NTS) receives input from PVN, and integrates sensory information from the viscera by way of vagal afferents
  - Distention triggers vagal afferents, which act through the NTS

Control of Food Intake (anorectic peptides which ↓ meal size)

- Give 5 satiety signals arising from the GI tract.
  - Stomach, adipocytes
    - Leptin
    - GRP (gastrin-releasing peptide)
  - Pancreas
    - NPY (neuropeptide Y)
    - Pancreatic polypeptide)
  - Small intestine
    - CCK (cholecystokinin)
    - PYY_{3-36} (peptide YY)
    - GLP-1 (glucagon-like peptide 1)
    - Oxyntomodulin
    - Apolipoprotein A-IV
    - Somatostatin
- Give 3 GI signals which increase appetite.

Trick question! There is only one GI peptide which stimulates appetite, ghrelin (leplin, lowers appetite; ghrelin, gain in appetite).

SO YOU WANT TO BE A GASTROENTEROLOGIST!

- Give an explanation of the mechanism of the effect of Apo– A IV.
  - Produced in
    - Enterocytes, in response to dietary fat release PYY, and NPY (neuropeptide Y) PP → ↑ enterocyte production of Apo – A IV
    - Arcuate nucleus of hypothalamus
  - Anorectic effect of Apo – A IV acts centrally

Starvation

Postoperatively (post-op)a patient is kept NPO and is hydrated with intravenous normal saline.

- Give the compensatory mechanisms which are involved to reduce the impact of developing deficiencies in energy and/ or protein when a patient is starved.

➢ First 24 hr post-op
  - Circulating nutrients – glucose, fatty acids (FAs) , triglycerides) 1200 kcal
  - Stores – liver & muscle glycogen → ↑ glycogenolysis → ↑ glucose
  - Liver metabolism - ↓ glucose production & oxidation
  - Whole body - ↑ lipolysis → ↑ FAs, ↑ ketone bodies

➢ Next several days
  - Oxidation of protein (proteolysis):
  - Glucogenic amino acids (alanine, glutamine) → ↑ deamination - ↑ renal production of glucose [especially for brain & blood cells; (gluconeogenesis)]

➢ Up to 2 wk of starvation
  - Adipose tissue
    - ↑ lipolysis → FA, from ↑ shift of AA from somatic (muscle) to visceral organ compartment eventual loss of visceral protein
    - ↓ insulin
    - ↑ epinephrine
    - ↑ adipocyte sensitivity to catecholamines
  - Liver
    - ketogenesis
    - ↑ FA from ↑ lipolysis (see above)
    - ↑ glucagon/ insulin
    - ↑ ketone bodies use by brain, in place of glucose
    - reduces brain need for glucose, and need for oxidation of protein
  - Heart, kidney, skeletal muscle - ↑ use of FAs & ketone bodies
  - Bone marrow, renal medulla, peripheral nerves

- ↑ anaerobic glycolysis →↑ pyruvate (P) & lactate (L) →↑ glucose production of from P&L via the Cori cycle

- ↑ Sympathetic nervous system (SNS) activity
- Thyroid - ↑ inactive hormone (net effect: TG → FA → glucose)
- Physical activity ↓ from
  - ↓ active thyroid hormone
  - ↓ SNS activity

➢ After 2 wk of starvation

- Organs shrink
  - Limit of TG stones reached (lethal depletion: 70% to 95%)
  - ↑ muscle protein breakdown (lethal depletion: 30% to 50%)
  - ↓ BMI (lethal reduction: < 13 kg/m^2 (males), < 11 kg/m^2 (females)
- Plateau reached in adaptive processes
- Kidney
  - ↓ urea nitrogen (N) production & excretion (1 g of urinary N arises from the breakdown of 30 g of protein)
  - ↓ urine output
- Death

- Give two compensatory metabolic mechanisms blunted in obesity, and thereby represents an adaptation which slows the effect of fasting on weight reduction.
  - ↑ lipolysis
  - ↓ glucose production

Protein – Energy Malnutrition (PEM)

- Importance of assessment of nutritional status
  - PEM is associated with poor clinical outcomes
  - Two useful tools
    - SGA (subjective global assessment)
    - MNA (mini-nutritional assessment)
- Primary PEM
  - ↓ intake of protein ± calories, or
  - ↓ intake of good quality protein (inadequate essential amino acids)
- Secondary PEM
  - ↑ systemic inflammatory response (eg IBD) → ↑ IL-1, TNF-α, interferon & IL-6
  - ↑ protein catabolism
  - ↑ REE
- PEM in the presence of active inflammation
  - Even with nutritional support, lean body mass (including muscle mass) will not correct until the systemic inflammatory response from associated inflammation is corrected.
  - If a gain in weight occurs in the person with secondary PEM, before associated inflammation is treated, the weight gain is mostly fluid (edema) and fat
- Special consideration: cancer wasting syndrome
  - PEM is accelerated by the production of proteolysis – and lipid – metabolizing factors
  - PEM must be corrected slowly, to avoid (the refeeding syndrome)
    - If glucose is fed rapidly, insulin is released, phosphate is rapidly taken up into cells, the serum PO_4^- falls, and complications develop:
      - Neurological (seizures, paresthesias, hyperosmolar coma) and cardiovascular (CCF, death)

- GI – diarrhea, death
- RBC fail to release O_2 normally – VT
- Acute thiamine deficiency (wet beriberi) initiation of parenteral nutrition, including peripheral as well as total parenteral nutrition.

– The cells in PEM are depleted of K^+ & Mg^{2+}, and refeeding with glucose/ AAs shifts these back into cells, and may lead to serious cardiovascular adverse effects (prolonged QT interval, ventricular trachycardia (VT)

– Remember that the refeeding syndrome may occur with

o Predigested monomeric or oligomeric elemental or semielemental diets are not superior to polymeric diets or whole food

o In order for any hypocalcemia to be fully corrected, it may be necessary to correct any associated body depletion of Mg^{2+} (best assessed from urinary levels after IV infusion rather than from serum concentrations)

SO YOU WANT TO BE A GASTROENTEROLOGIST!

- Give 5 GI conditions in which there has been demonstrated to be superior clinical outcome with the use of parenteral nutrition orders
  - Pre- but not post-operative nutritional support given to surgical patients (including gastric and colorectal cancer ↓ morbility by 35%, as well as ↓ mortality (by a lesser amount)
  - Persons with decompensated alcoholic liver disease or alcoholic hepatitis have ↓ mortality & mortality, as well as faster recovery, when nutritional support is provided with enteral or parental nutrition.
  - Patients with acute pancreatitis do better with nutritional support
  - Cancer patients receiving radiotherapy have a superior quality of life with nutrition support
  - Tumour obstruction of bowel – PN is beneficial
  - Bone marrow transplantation associated mucositis (may have associated diarrhea) – PN is beneficial
  - Curiously, persons with IBD treated with TPN have a worse outcome

MOUTH AND SALIVARY GLANDS

THE SECRETORY ENDPIECES AND DUCT SYSTEM
OF THE HUMAN SUBMANDIBULAR GLAND

CLINICAL PHYSIOLOGICAL CHALLENGE – salivary secretion

Background:

- Salivary acinar cells secrete $KHCO_3$ and absorb NaCl.
- The apical (luminal) membrane contains a Na^+/H^+, Cl^-/HCO_3^- and a H^+/K^+ exchanger as well as epithelial Na^+ channels (ENaC) and CFTR (cystic fibrosis transmembrane regulator). The basolateral membrane contains a Na^+/H^+ exchanger, $NaHCO_3$ cotransporter, Na^+/K^+ ATPase, as well as K^+ channels and non-CFTR Cl^- channels.
- Draw a diagram which depicts the steps in the salivary secretion of fluid and electrolytes.

Structure

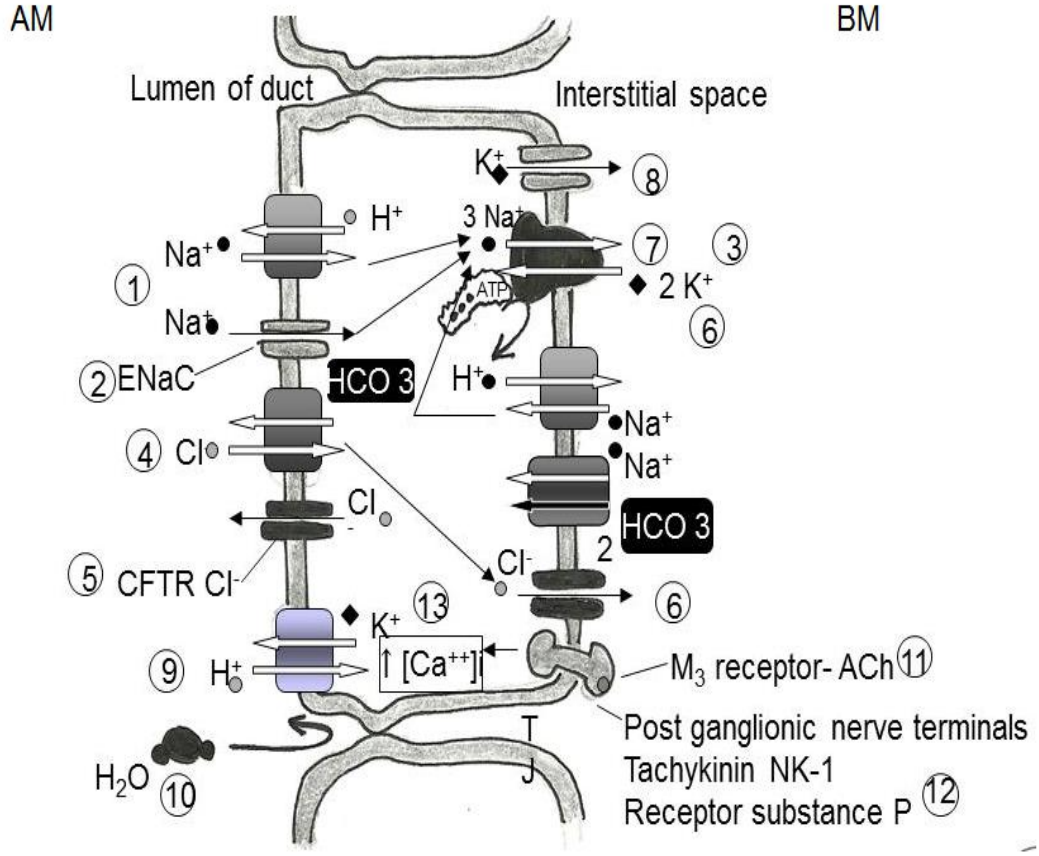
- The duct cells alter the composition of the secretion from the acinar cells
- The duct cells have many mitochondria, and their basal portion is striated
- The tightness of the tight junction (TJs) of the salivary glands lead to their low permeability to water, and to hypotonic secretion at low flow rates
- Acinar cells of the parotid gland secrete a serous watery fluid which contains amylase activity
- Sublingual glands secrete a highly glycosylated mucin glycoprotein
- The submandibular glands secrete both serous and mucinous fluid from separate serous- and mucus- acinar units. Highly glycosylated proline-rich proteins are also secreted in both the serous- and the mucous- acinar units.

Function

- Grinding of food (mastication) and lubrication with saliva
- Some digestion of carbohydrate in the mouth through lingual amylase.
- Secretion of saliva

Gland	Secretion	
	Mucin	Serous
Parotid	-	+
Sublingual	+	-
Submandibular	+	+

STEPS IN THE SALIVARY SECRETION OF FLUID AND ELECTROLYTES



Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Figure 43-10, page 928,

- Give the steps in the salivary secretion of fluid and electrolytes.
  - AM
    - NHE (Na^+ / H^+ exchanger)
    - ENaCs (epithelial Na^+ channels)
    - CFTR (cystic fibrosis transmembrane receptor)
    - HKE (H^+ - K^+ exchanger)
  - BM
    - CC (Cl^- channel)
    - $NaHCO_3E$ (Na^+/ HCO_3^- exchanger)
    - Na^+ - K^+ ATPAse
    - KC (K^+ channel)

➢ Na^+

- In
  - 1) Na^+ enters the salivary cell from the lumen of the duct across the brush border membrane (AM) ENaCs (epithelial Na^+ channels) and the Na^+ / H^+ exchanger (NHE)
- Out
  - 2) The intracellular Na^+ concentration acts through the ubiquitin-protein ligase (U-PL) to provide feedback inhibition on ENaC, reducing further Na^+ entry into the cell across AM.
  - 3) Na+ leaves the salivary cell from the Na^+ - K^+ pump

➢ Cl^-

- In
  - 4) Cl^- enters the salivary cell by the AM Cl^- / HCO_3^- exchanger.
- Out
  - 5) CFTR secretes Cl^- out of the enterocyte across the AM.
  - 6) The non-CFTR channels in the basolateral membrane (BM) provide another pathway for the exit of Cl^- from the enterocyte.

Abbreviations: AM, brush border membrane; BM, basolateral membrane

➢ K^+

- In
  - 7) K^+ enters the salivary cell by the BM Na^+ - K^+ exchanger.
  - 8) K^+ possibly exits by a BM K^+ -channel.
- Out
  - 9) In the AM, K^+ is exchanged for H^+, with K^+ leaving the cell, and H^+ entering
  - The K^+ secreted by the H^+/ K^+ exchanger binds to the HCO_3^- secreted by the AM Cl^- / HCO_3^- exchanger.
- Water
  - 10) The tightness of the tight junctions (TJs) leads to low permeability of the AM to water, and thereby to the hypotonic secretion of saliva at low flow rates

- Give the steps in the regulation of salivary secretion of fluid and electrolytes.

➢ Parasympathetic: Stimulation

- Stimulation
  - Preganglionic parasympathetic fibers (CN V, VII, IX) → postganglionic nerve terminals → Ach → duct BM M3 receptors → ↑$[Ca^{2+}]$I

    ↑ $KHCO_3$ secretion ↑ NaCl absorption

  - The sublingual and submandibular salivary glands are supplied with preganglionic parasympathetic fibers in cranial nerve (CN) VII and less so in CN V.

  - The parotid gland receives preganglionic parasympathetic fibers mainly in CN IX, and less so through CN V.

    V — sublingual
    VII — * submandibular
    Parotid — IX *

  - 11) Parasympathetic stimulation releases Ach from postganglionic nerve terminals, which acts on salivary duct cell BM M3 receptors to increase intracellular Ca^{2+}

  - 12) Substance P acts on the BM tachykinin NK-1 receptor to increase fluid secretion through the Ca^{2+} signaling pathway

  - 13) ↑$[Ca^{2+}]$i enhances fluid secretion by activating the Ca^{2+}- signaling pathways to increase KHCO3 secretion and NaCl absorption.

CLINICAL PHYSIOLOGICAL CHALLENGE – Salivary secretion

Background: The salivary gland is innervated by the parasympathetic and sympathetic nervous systems.

Problem**:** Give the explanation for the development of xerostomia in some persons with Sjogren's syndrome, or treated with anticholinergic drugs.

- Sympathetic system: Inhibition

  - Superior cervical ganglia postganglionic sympathetic fibers → norepinephrine (Nor') → ↑ blood flow
    - ↑ salivary adrenergic receptors and Ca^{2+} signalling pathways
    - ↑ CFTR - ↑↑ NaCl absorption
    - ↑ amylase, ribonucleases
    - ↑ stimulation of myoepithelial cells -↓ resistance to flow - ↑ salivary flow

  - Postganglionic sympathetic fibers from the superior cervical ganglia reach the parotid, sublingual and submandibular salivary glands in the blood vessels that supply these glands.

  - Sympathetic stimulation increases blood flow to these glands, and this enhanced salivary gland blood flow increases salivary gland secretion.

  - Sympathetic stimulation releases norepiniephrine from sympathetic nerve terminals, norepinephrine released from the sympathetic nerve terminals stimulates CFTR, and thereby increases NaCl absorption.

  - Because the cholinergic stimulation is more limited than the adrenergic, NaCl reabsorption may exceed KHCO3 secretion, and salivary secretion falls, causing a dry mouth (xerostomia).

  - Norepinephrine (Nor') released through the sympathetic nerve terminals also acts through adrenergic receptors and the Ca^{2+} signalling pathway.

  - When the stellate-shaped myoepithelial cells in the salivary gland acinar and intercalated duct cells are stimulated by the sympathetic system, the resistance to salivary flow is reduced, and salivary gland flow increases.

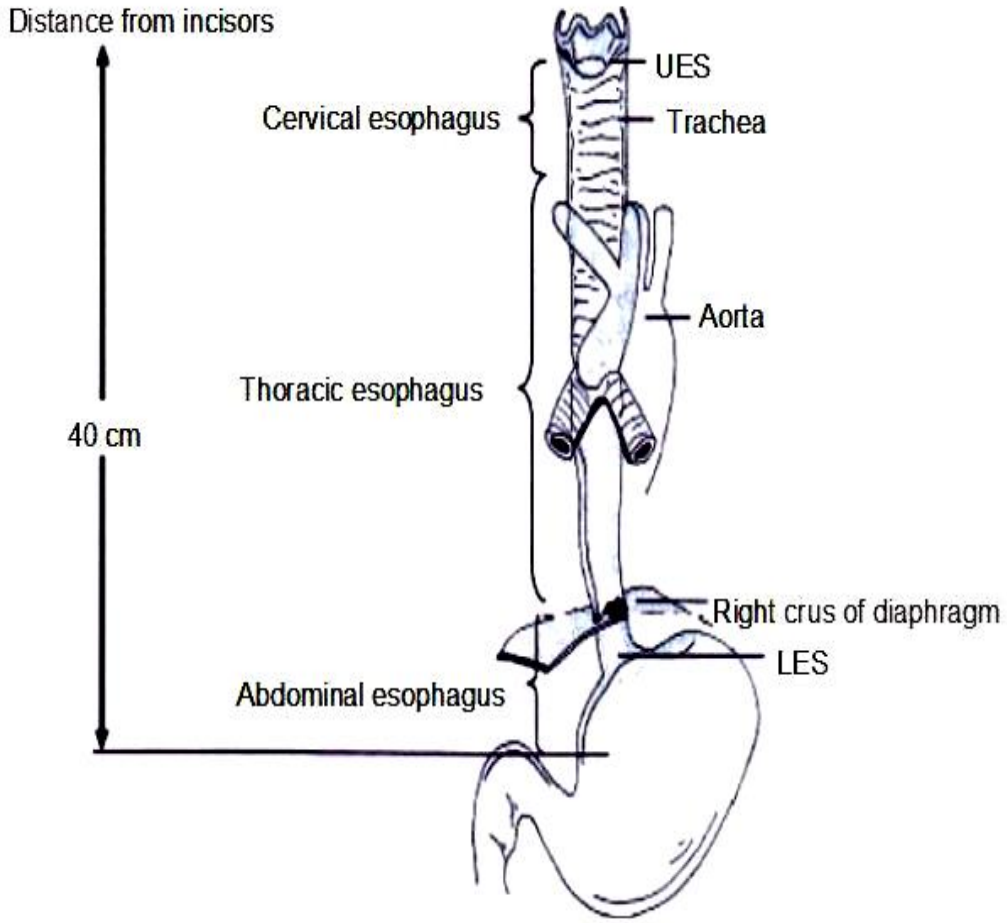
  - This effect of norepinephrine on adrenergic receptors and the Ca^{2+} signalling pathway cause the salivary duct cells to secrete amylase and ribonucleases into the mouth, using the cAMP pathway sympathetic control.

  - IgA secretion into the mouth by endocytosis is facilitated by the secretory component.

  - Secretion of IgA also occurs through BM polymeric IgA receptors

ESOPHAGUS

Gross Anatomy of Esophagus



e.

Main Diseases

- Motility disorders(scleroderma, achalasia, diffuse esophageal spasm, hypertensive [“nutcracker”] esophagus)

- Gastroesophageal Reflux Disease (GERD)

Functions

- To facilitate the passage of food and fluids from mouth to stomach (transit)

- To prevent the regurgitation of gastric contents back up the esophagus (gastroesophageal reflux)

Structure

- Hollow muscular tube, functionally closed at the upper (pharyngeal) end by the upper esophageal sphincter (UES), and at the lower (gastric) end by the lower esophageal sphincter (LES).

- The UES is a high pressure area formed from the tonic contraction of the striated cricopharyngeal and the caudal fibers of the inferior pharyngeal constrictor muscles.

- The muscle of the proximal (cervical) 30% of the esophagus is also striated, and the distal 70% is smooth muscle.

- The distal portion of this smooth muscle thickens at or just below the diaphragmatic hiatus to form the LES.

- The LES has circular “clasp” fibers on the gastric lesser curvature side of this junction between the esophagus and stomach; on the greater curvature side of the LES the “sling-like” muscle fibers form the long oblique gastric muscle fibers.

- The venous blood drainage of the lower esophagus involves the azygous and the portal systems.

- If pressure is increased in the portal circulation, this pressure is transmitted through the left gastric vein, and the submucosal venous plexus enlarges. If the portal pressure continues to increase (portal hypertension, such as hepatic cirrhosis), esophageal varices result.

- If the portal pressure exceeds 12 mm Hg, these esophageal varices may burst, leading to life-threatening bleeding (variceal upper GI bleeding).

- The histology of the esophagus varies slightly from the usual pattern of the GI tract:
  - Stratified squamous epithelium down to the LES, where there is columnar epithelium at the point where the esophagus meets the stomach.
  - The outer layer of the wall of the esophagus is only a thin layer of connective tissue, rather than the distinct serosal layer, so that the esophaguslacks this protective layer and is more prone to perforation.

CLINICAL PHYSIOLOGICAL CHALLENGE – GERD: “Reflux” disease

Case: A 45 year old man with an increased BMI of 29 began to develop severe retrosternal burning discomfort and acid regurgitation when his new –onset hypertension was treated with a calcium channel blocker. His symptoms of gastroesophageal reflux disease (GERD) were worsened by bending forward after meals, and by eating chocolate.

- Give the pathophysiology of GERD.

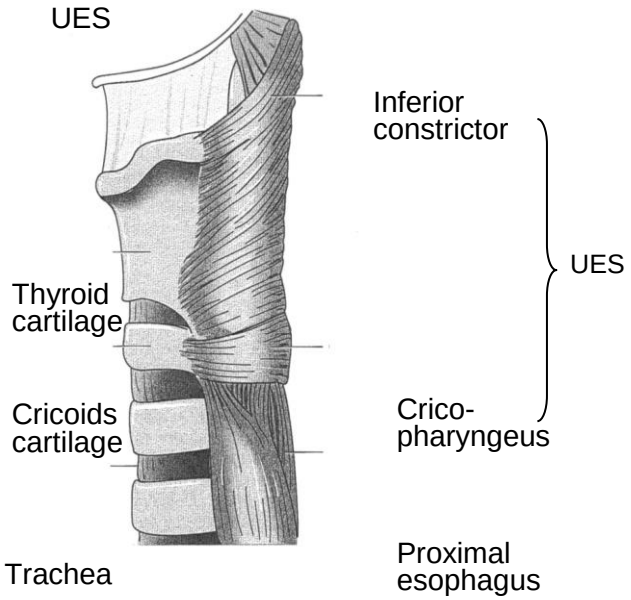
➢ In giving your answer, please include the manometric pressure valves of the upper (UES) and lower esophageal sphincter (LES), as well as the characteristics of the peristaltic waves of the esophageal body
  - Function of body of esophagus
  - Tone and relaxation of LES
  - Peripheral and central pathways mediating transient lower esophagial sphincter relaxation (LESR)
  - Conditions associated with GERD
  - Maintainance of integrity of esophageal mucosal membrane

Preventing Gastroesophageal Reflux of Gastroduodenal Contents

- UES

Normal Esophageal Manometric Values

- Resting Pressure 30-120 mm Hg
- Residual Pressure after swallowing < 8 mm Hg
- Coordination with swallow Yes

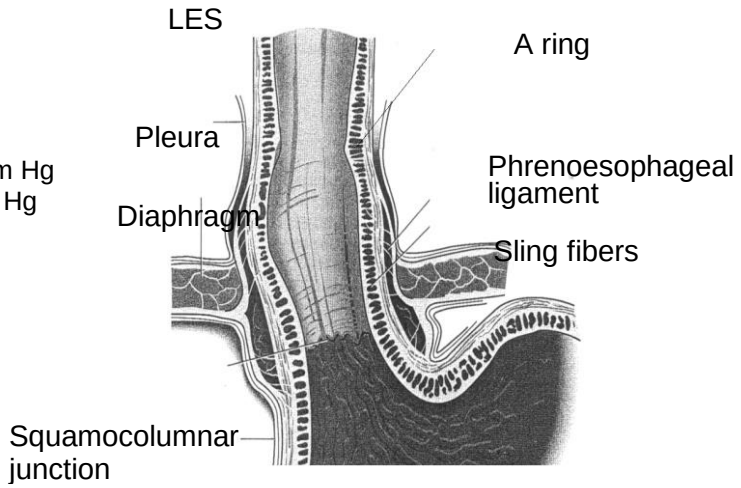


- Esophageal Body

Contraction: Peristaltic	> 80%
Simultaneous	< 20%
Non-conducted waves	0%
Mean contraction amplitude	30-180 mm Hg
Duration	< 5.8 secs
Low amplitude contractions	< 30%
High amplitude contractions	0%

- LES

- Resting Pressure 13-43 mm Hg
- Residual Pressure after swallow < 13 mm Hg
- Hiatus hernia No



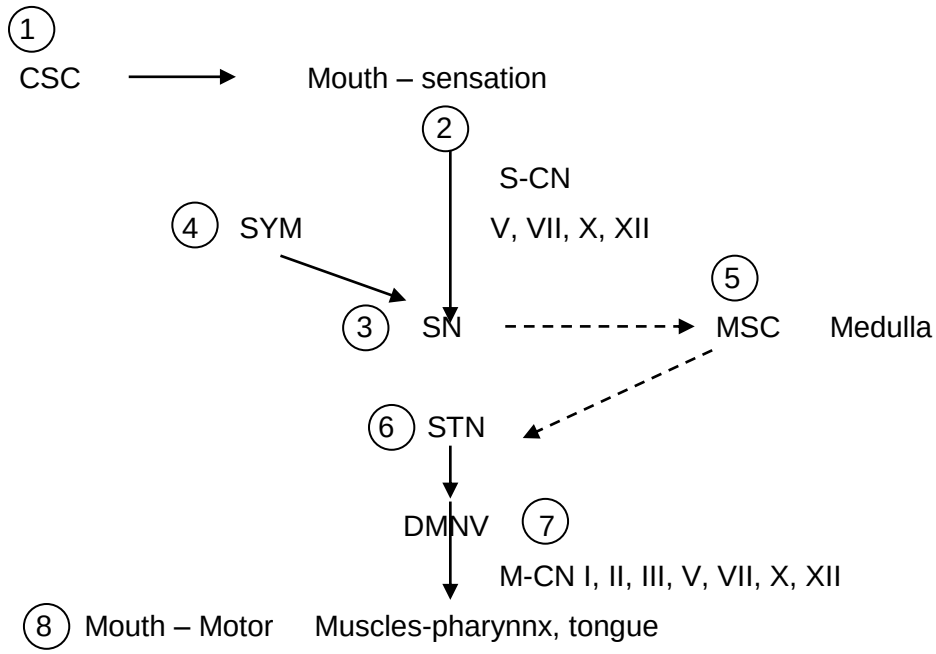
Abbreviations: LES, lower esophageal sphincter; UES, upper esophageal sphincter

Adapted from: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Figure 31-1, page 552, Ninth Edition, 2010

Esophageal Motility and Swallowing

- Give the neurological control of swallowing.
  - 1) Voluntary initiation of swallowing begins in the cortical swallowing centre(CSC) in the frontal cortex with food being placed in the mouth
  - 2) Sensory receptors in the oropharynx, larynx and esophagus act through afferent pathways to the sensory nuclei of cranial nerves (sen-CN) V, VII, IX and XII.
    - Esophageal muscle mechanoreceptors are in Auerbach myenteric plexus
    - Mucosal / submucosal chemoreceptors are in Meissner submucosal plexus
  - 3) The solitary nucleus (SN) in the pons receives afferent input from
    - S-CN (2)
    - Thoracic sympathetic afferents pathways (4)
    - Vagal parasympathetic afferent pathways from Auerbach and Meissner plexuses
    - Sensory components MSC (medullary swallowing centre) (5)
  - 6) Solitary tract nucleus (STN) receives afferent input from motor component of MSC.

NEUROLOGICAL CONTROL OF SWALLOWING



Abbreviations: CSC, cortical swallowing centre;; S-CN, sensory nuclei of cranial nerves; SN, sensory nucleus; MSC, medullary swallowing centre; STN, solitary

tract nucleus;SYM, thoracic sympathetic afferent pathways; DMNV, dorsal nucleus of vagus; M-CN, motor nuclei of cranial nerves

- Mouth / tongue and oropharynx
  - After ingestion and mastication of food, saliva is secreted, and a food bolus is formed and swallowed.
  - Muscles of soft palate, tongue and pharynx shorten the lumen of the pharynx.

CLINICAL CORRELATION

- The tongue, like the face and palate, has a bilateral upper motor neuron innervation in most people, so a unilateral upper motor neuron lesion often causes no deviation.
- A clinically obvious upper motor neuron lesion of CN-XII is usually bilateral and results in a small immobile tongue.
- The combination of bilateral upper motor neuron lesions of the ninth, tenth and twelfth nerves is called pseudobulbar palsy.
- A lower motor neuron lesion of the twelfth nerve causes fasciculation, wasting and weakness. If the lesion is bilateral, it causes dysarthria.
- Movement disorders may affect the tongue.

  - Coordinated transfer of bolus from mouth to upper esophagus
  - Closure of inlet to larynx
  - Pharyngeal components of swallowing
    - Coordination of patterned activation of motor neurons and muscles to transform the oropharynx from a respiratory pathway to an esophageal transfer pathway
    - Nasopharynx-soft palate elevation and retraction
      - UES
        - Opens
        - Pressure rises
        - Pharynx constrictors clear the pharynx, moving food bolus into area of upper esophageal pressure (UES)
- UES (upper esophageal sphincter)

- Cricopharyngeus forms the 1 cm area of ↑pressure of the UES (upper esophageal sphincter)
- UES pressure maintenance and coordination of relaxation of swallowing is achieved by vagal trunks from the nucleus ambiguous
- ↑ UES pressure
  - Distention of esophagus
  - Stress
  - Inspiration swallowing
- ↓ UES pressure
  - Belching (causes relaxation of UES and closure of the glottis)
  - Anesthesia
  - Sleep

➢ Esophageal Components of swallowing

- Give the physiology of the esophageal transit of food.

➢ Esophagus

Muscle	Site	Efferent nerves (motor)
○ Striated	Proximal 5% Middle 35%	- Lower motor neurons cell bodies in nucleus ambiguous - Excitatory vagal innervations - Activation of motor units in a craniocaudal sequence through the release of Ach and stimulation of nicotinic cholinergic receptors on motor endplates of striated muscle cells - Peristalsis of striated muscle of esophagus is controlled by the medullary swallowing centre
○ Smooth	Middle 35% Lower 60%	- Dorsal motor nucleus of the vagus - Excitatory ganglionic neurons predominate proximally, inhibitory ganglionic neurons dominate distally

Muscle	Site	Efferent nerves (motor)
	○ High-resolution esophageal pressure topography shows a transition zone between the skeletal and smooth muscle, characterized by - ↓ amplitude contractions - ↓ progression of peristalsis - ↑ likelihood of failed transmission	- Superior laryngeal nerve, cell bodies in nodose ganglion - Recurrent laryngeal nerve, esophageal branches of vagus
	○ ANS autonomic nerve system	- Myenteric plexus between longitudinal and circular muscle layers - Meissner plexus between muscularis mucosa and circular muscle

- Upper straited muscle
  - Peristalsis
    - Primary (1^o) wave – initiated by swallow $1^o > 2^o$ wave pressure
    - Secondary (2^o) wave – initiated by distention of esophagus (food, fluid, air; balloon)
    - A “milking” wave clears the esophagus from the top to the bottom
    - When the pressure of the 1^o/ 2^o peristaltic milking wave is low (<30 mmHg), the esophagus is less likely to be completely cleared
    - If there are two swallows made in rapid succession, in such a way that the first peristaltic wave is still in the esophagus, the inhibitory ganglionic neurons in the myenteric plexus rapidly cause hyperpolarization of the circular smooth muscle in the lower third of the esophagus
    - This hyperpolarization of the circular smooth muscle inhibits the peristaltic contraction of the first swallow (deglutitive inhibition)
    - There is no deglutitive inhibition in the longitudinal muscle layer
    - With contraction of the outer longitudinal and inner circular smooth muscle, the esophagus may shorten (about 1 inch, 2.5 cm)
    - There may be a central origin to deglutitive inhibition

- Thus, the esophageal clearance is less efficient with low pressure esophageal peristaltic waves, on with rapid swallowing causing deglutitive inhibition

- Skeletal muscle
    - From the swallowing centre in the medulla (MSC), vagal efferent fibers are excited, and discharge in spike bursts during 1^{o}/ 2^{o} peristalsis
    - The Ach stimulates nicotinic cholinergic receptors on the motor end plates of the striated muscle cells
    - The MSC vagal efferent fibers discharge first the vagal fibers in the upper and then the middle of the esophagus
    - This sequential firing of skeletal muscle first in the proximal and then in the middle esophagus leads to peristalsis
    - These discharges leading to peristaltic muscle contraction are enhanced when the afferent sensory fibers are activated by the bolus passing along the esophagus
    - These vagal motor efferents are inhibited during the pharyngeal component of swallowing, or when the proximal esophagus is distended (deglutitive inhibition)

    - The action of cholinergic (Ach) activity on M3 receptors stimulates the esophageal body circular and longitudinal smooth muscle, whereas NO (nitric oxide) has an inhibitory effect on this smooth muscle.

    - Swallowing evokes sequential esophageal contractions that pass from the straited-to the smooth muscle segments of the upper one-third and lower two-thirds of the esophageal body, respectively.

    - The striated muscle in the upper third of the esophagus is innervated by the vagal efferent fibers in the recurrent laryngeal nerve.

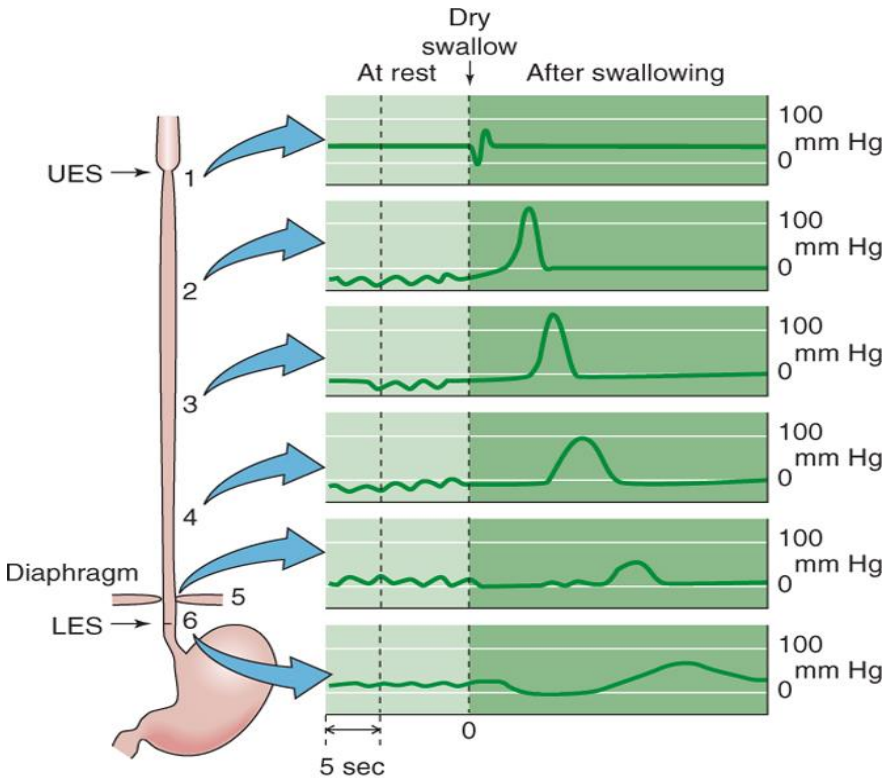
    - In the striated muscle esophagus, vagal stimulation causes contractions that occur only during the period of stimulation.

    - The lower portion of the tracing shows the paristaltic contractions of the esophageal body resulting in the movement of a food bolus from the upper to the lower end of the esophagus in about 10 seconds.

    - The peristaltic waves (“esophageal transit of food”) shows sequential “peristaltic” contractions in the esophageal body, with full LES relaxation occurring with a swallow.
    - After a dry swallow, the pressure wave of primary peristalsis moves sequentially down the esophagus.

- Thus, the striated muscle esophagus is dependent on central neuronal sequencing for its peristaltic contraction.
- Stimulation of the vagus nerve, evokes peristaltic contractions only in the smooth muscle segment of the esophagus.

ESOPHAGEAL TRANSIT OF FOOD



- Smooth muscle
  - Vagus nerve also controls 1º peristalsis in lower esophagus (smooth muscle)
  - Stimulation of the vagal efferent (motor) may ↑ or ↓ the smooth muscle activity:
  - Control of peristalsis by smooth muscle : medullary swallowing centre

- Stimulation by swallowing (vagus):
  - Longitudinal muscle
  - Depolarization with superimposed spike bursts
- Circular muscle: hyperpolarization, then depolarization and spike bursts
  - Myenteric plexus – necessary for peristaltic propagation
- Smooth muscle contraction caused by ganglionic cholinergic neurons in response to swallowing
- This vagal nerve stimulation simultaneously reaches the ganglionic neurons in the middle and distal third of the esophagus
- There is a short (2 sec) latency in the proximal smooth muscle in terms of the time of the arrival of the nerve stimulation and muscle contraction
- There is a longer (5 to 7 sec latent period in the distal esophagus)
- This latent period may result "....from a neural gradient along the esophagus, where in excitatory ganglionic neurons dominate proximally and NO&VIP-releasing inhibitory ganglionic neurons dominate distally" (Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, page 682)
- Intrinsic neuronal mechanisms are capable of producing a persistaltic sequence in the smooth muscle segment.
- Hyperpolarization and no inhibition of the smooth muscle proceeds the cholinaergically mediated smooth muscle contraction.
- The distally increasing duration of the latency gradient caused by NO inhibition/hyperpolarization of the smooth muscle, followed by cholinergic contraction and a distally moving contraction pressure wave, leads to progressive movement of the bolus along the length of the esophagus by way of this primary peristalsis.
- The LES pressure falls in anticipation of a bolus of food/fluids passing along the length of the esophagus, through the area of now relaxed LES, and into the stomach.
- Sensory receptors in the body of the esophagus may be stimulated by distention, and result in secondary peristalsis; this secondary peristalsis helps to return to the stomach the small amounts of food/fluid which may be normally regurgitation into the esophagus from the stomach.

Competence of the lower esophageal sphinceter (LES)

- The prevention of excessive reflux from the stomach into the esophagus is achieved through the effects of the resting LES (lower esophageal sphincter), and the transient lower esophageal spincter relaxation (TLESR).

➢ **Resting Pressure of LES**

- The inherent property of the smooth muscle at the junction between the esophagus and the stomach forms the tonic contraction (myogenic) which results in the high-pressure LES zone.
- Between swallows, the pressure in the LES is between 10 to 35 mm Hg.
- On swallowing, the vagal efferent fibers synapse on inhibitory neurons in the myenteric plexus, which release NO and cause relaxation of the LES.
- Within <2 seconds of a swallow, the LES relaxes and remains relaxed for 5 to 7 seconds, until the esophageal pressure wave from the migrating contraction pressure wave of the primary peristaltic wave reaches the distal esophagus.
- Tertiary peristalsis results from an intramural mechanism which is unrelated to the swallowing centre.
- Tertiary peristalsis is a pathological process, which may include diffuse esophageal spasm resulting in dysphagia for liquids, as well as NCCP (non-cardiac chest pain).
- In a clinical setting, the acidity and the motility of the esophagus can be measured, and demonstrate the pattern of the esophageal body and the pressure of the UES and LES.
- There are variety of modulators of LES pressure

Modulators	↑ LES Pressure	↓ LES Pressure
➢ GI hormones/ peptides	○ Gastrin ○ Motilin ○ Substance P	– Secretin – Cholecystokinin – Somatostatin – VIP
➢ Neural agents	○ α-Adrenergic agonists ○ β-Adrenergic antagonist ○ Cholinergic agonists	– α-Adrenergic antagonist – β-Adrenergic agonists – Cholinergic antagonists

Modulators	↑ LES Pressure	↓ LES Pressure
➢ Foods	○ Protein	– Fat – Chocolate – Peppermint
➢ Drugs		
○ Histamine		
○ Antacids		
○ Metoclopramide		
○ Domperidone		
○ Cisapride		
○ Prostaglandin $F_{2\alpha}$		
○ Baclofen		

Abbreviations: LES, lower esophageal sphincter; VIP, vasoactive intestinal peptide

Printed with permission:*Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Table 43-1, page 708, Ninth Edition, 2010

- LES (lower esophageal sphincter)
  - The high pressure at the esophagogastric junction (EGJ) is caused by
    - LES (lower esophageal sphincter) pressure
      - 3-4 cm in length
      - Tonically contracted
    - Crural diaphragm
      - 2 cm length
      - ↑ in crural diaphragm contraction, with ↑ EGJ pressure
      - Inspiration (abolished by mechanical ventilation)
    - ↓ crural contraction, ↓ EJJ pressure
      - Distention of esophagus
      - Belching air, vomiting
    - Sling and clasp fibers of the middle layer of the musculature of the gastric cardia (including the angle of His)
  - The high pressure in EGJ goes form 1-1.5 cm proximal and 2 cm distal to the SCJ (squamocolumnar junction)

- The LES is tonically contracted
- This myotonic LES tone"... varies directly with membrane potential and superimposed electrical spike activity that leads to an influx of Ca^{2+}." (Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, page 683)
- The spike activity with influences Ca^{2+} flux is itself regulated by K^{+} - and Ca^{2+} - activated Cl^{-} channels
- Vagal (cholinergic) input partially determines LES basal tone
- Adrenergic (sympathetic) input acts through the myenteric neurons)
  - Stellate and proximal thoracic ganglia – with
    - splachnic nerve
    - celiac ganglion
    - α – adrenergic receptors on myenteric neurons (excitatory, as well as inhibitory)
- Sympathetic (adrenergic, α – adrenergic receptors) activity
  - ↑ excitatory neurons activity
  - ↓ inhibitory neuron activity
  - The net effect of α – adrenergic stimulation is
    - ↑ LES pressure
    - ↓ body muscle (relaxation)
- Hormonal factors
- Mechanical factors

➢ Relaxation of LES

- An intramural process
- Mediated by post ganglionic nerves, ".... mediated by vagus
- Caused by swallowing or distention of esophagus
- Post-ganglionic transmission is by way of nicotinic and muscarinic receptors
- Transmitters of post ganglionic neurons causing LES relaxation and ↓ LES pressure is mediated by NO (nitric oxide, produced from the amino acid L-arginine, by way of the enzyme NO synthase)
- Postganglionic deglutitive relaxation of the LES may also occur with other peptides such as VIP (prejunctional neurotransmitter acting on nerves containing NO synthase), as well as possibly PHI (peptide histidine isoleucine), CGRP (calcitonin gene-related peptide), galamin, and PACAP pituitary adenylate cyclase activation peptide)

- Give 10 factors which physiologically modulate LES pressure.
    - Membrane potential
    - K^+ and Ca^{2+} - activated Cl^- channels
    - Spike activity
    - Ca^{2+} flux
    - Pressure
        - Gastric distention
        - Intra-abdominal pressure (e.g. obesity, ascites)
    - Food
    - Peptides
    - Hormones
    - Medications
    - MMC (migrating motor complex (MMC phase III ↑ LES to mmHg; normal resting LES pressure/ tone is 10 – 30 mmHg)
    - Neurogenic factors
        - Vagal (cholinergic input partially determines LES basal tone
        - Adrenergic (sympathetic, acting through the myente neurons)

➢ **Transient LES relaxation (TLESR)**

- Give the physiology of transient LES relaxation (TLESR).
    - Physiological reflux of gastric contents may occur during these times of transient lower esophageal pressure (TLES) relaxation.
    - TLES may be more important than LES pressure in the pathogenesis of gastroesophageal reflux disease.
    - The TLESR leads to some small “physiological” amounts of exposure of the lower portion of the esophageal mucosa to gastric hydrochloric acid (HCl).
    - Glutamate or GABA (γ-aminobutyric acid) are neurotransmitters.
    - TLESR occurs when distention of the gastric fundus inhibits the GABA pathway.
    - Some spontaneous and transient relaxation of the LES may occur independently of a swallow, relaxation of the LES, some EG reflux, and thus possibly related to distension of the gastric fundus, and again mediated through release of the neuroinhibitor, NO.
    - Peripheral and central pathways mediate transient lower esophageal sphincter relaxations (TLESRs) and associated inhibition of the crural diaphragm (CD)

- To reduce TLESRs, block the vagal stimulation (DMN – NTS) or stimulate GABA system
- Glutamate/GABA inhibit neurons in the NTS (nucleus tractus solitarius)
- Neurons in NTS synapse with neurons in DMN (dorsal motor nucleus) of vagus nerve
- Neurons in vagus from DMN project to LES (causing lower esophageal sphincter relaxations)
- Giving baclofen, a GABA type B agonist, inhibits DMN, NTS, vagal inhibition of LES, and reduces TLESRs
- AEs (adverse effects) of baclofen include dizziness, weakness, nausea, confusion (about 10% incidence of each)

Adapted from: Vakil et al., *Am. J. Gastro*. 2011; 106: 1427-1438

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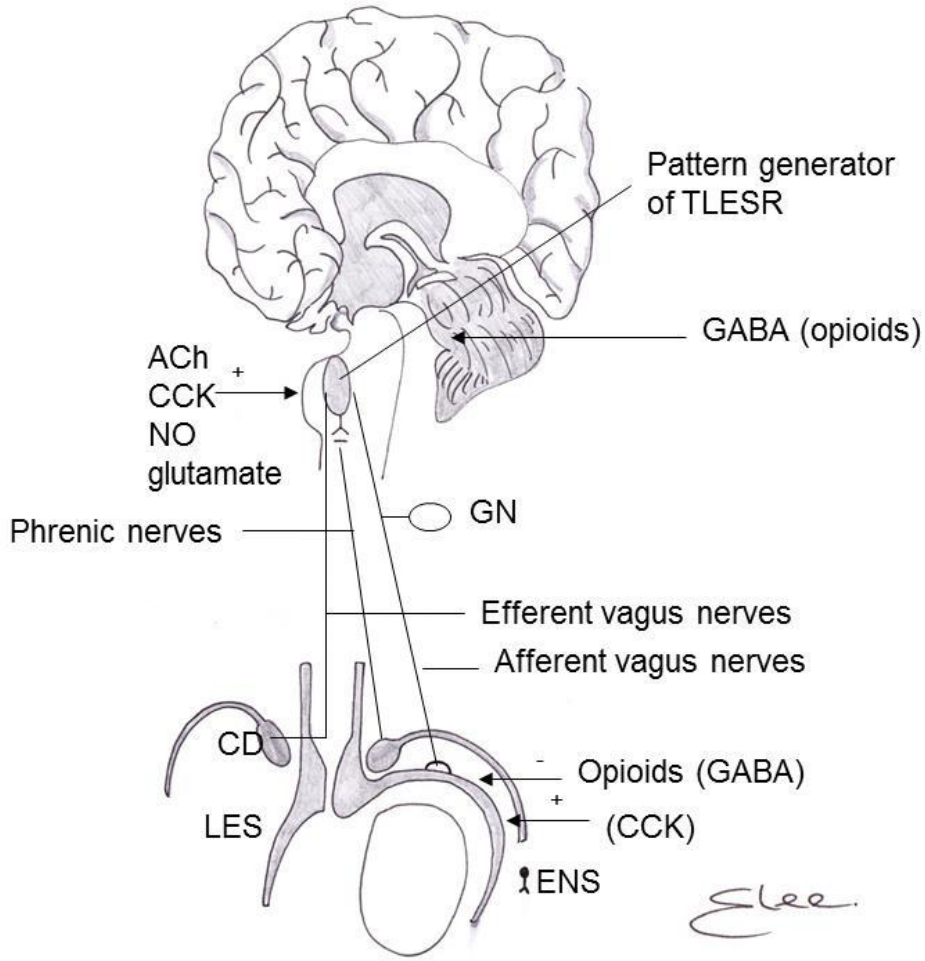
- Give mechanisms of action of gamma β receptor agonists (e.g. baclofen) on the physiology of the esophagus and stomach which make this class of drugs potentially useful in persons with refractory GERD.
    - Gamma β receptor agonists have the following physiological effects
        - ↓ TLESR (~50%)
        - ↑ LESP (basal)
        - ↑ rate of gastric emptying
        - ↓ reflux episodes (~40%)

Transient Lower Esophageal Sphincter Relaxation (TLESR) and Inhibition of the Crural Diaphragm (Cd) Relax The LES



Abbreviations: Ach, acetylcholine; CCK, cholecystokinin; ENS, enteric nervous system; GABA, gamma aminobutyric acid; GN, nodose ganglion; LES, lower esophageal sphincter; NO, nitric oxide

Adapted from: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Figure 41-1, page 668, Ninth Edition, 2010

➢ Transient lower esophageal sphincter relaxations (**tLESRs**)

- Give 6 differences in the physiology of tLESRs and swallow-associated declines (relaxation) in LES pressure (sLESRs).

	tLESRs	sLESRs
○ Stimulus	○ Distention of gast cardia/ fundus, ev without swallowing peristalsis	○ Swallowing peristalsis
○ Association w pharyngeal swallowing	No	Yes
○ Contraction of dis esophageal longitudir muscle	Yes	No
○ Shortening of esophagus	Yes	No
○ Associated w synchronized peristalsis	No	Yes
○ Inhibition of cru diaphragm	Yes	No
○ Frequently in post-prand state	Yes	No
○ Afferent neural pathway	○ Gastric afferents the subdiaphragma vagus	○ Pharyngeal a superior ○ Laryngeal nerve
○ Central neural circuit	○ Caudal dorsal mo nucleus	○ Subnuclei of t medulla
○ Efferent limb in t preganglionic vag inhibitory pathway to LES	Yes	Yes

Abbreviation: tLESRs, Transient lower esophageal sphincter relaxations; sLESRs, swallow-associated lower esophageal sphincter relaxations

Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Figure 41-4, page 891.

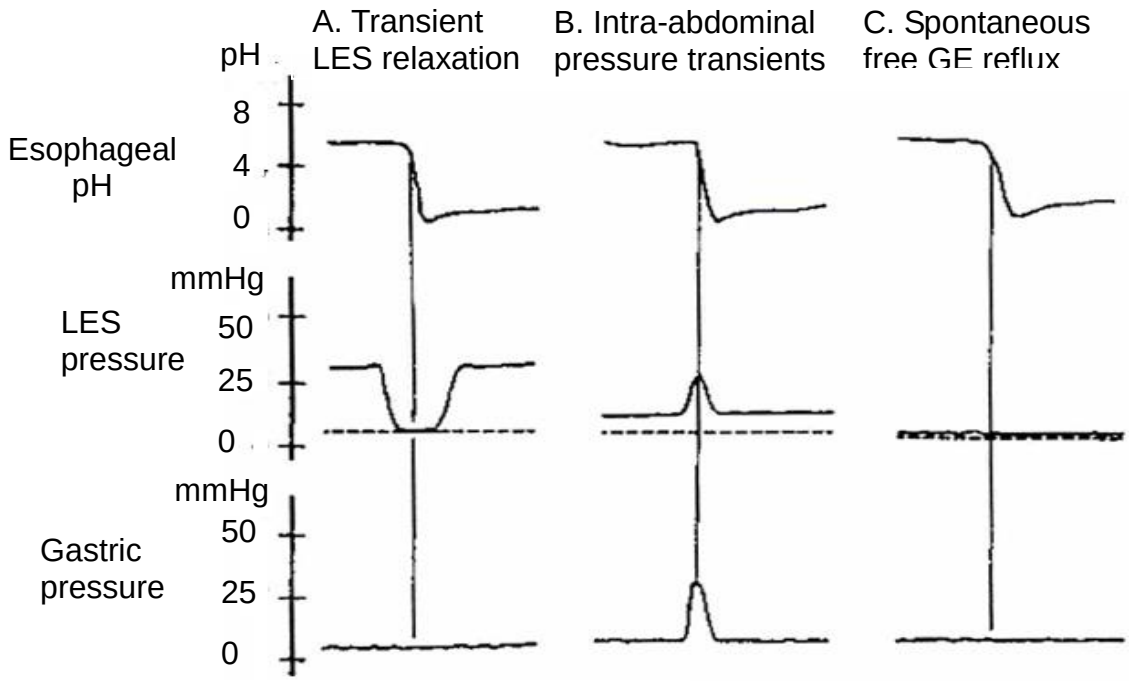
Esophageal Motility Disorders

- Given the complexity of the swallowing process, and the integration of factors necessary to prevent gastroesophageal reflex, it is not surprising that there are a number of esophageal motility disorders.

- The commonest esophageal motility disorder is GERD (gastroesophageal reflux disease), and the commonest disorder of the esophagus is GERD.

➢ Gastroesophageal Reflux Disease (GERD)

MECHANISMS OF GERD



Abbreviations: LES, lower esophageal sphincter; GE, gastroesophageal

In some GERD patients, when life-style changes (such as weight loss in the setting of increased BMI [obesity]) and medical therapeutics fail, the surgical enhancement of LES pressure and surgical correction of an associated hiatus hernia may be considered.

- The commonest disorder of the esophagus is GERD (gastroesophageal reflux disease)

- GERD is due to abnormalities of the physiology of the esophagus which prevents excessive reflux of gastroduodenal contents into the esophagus (please see previous section).

- GERD is caused by regurgitation of gastric acid into the esophagus for intervals which are longer than normal ('physiological")

- The mechanism of GERD include excessive TLESR (transient lower esophageal sphincter relaxation, intraabdominal transients, and spontaneous GE reflux. In many GERD patients, the baseline LES pressure is reduced.

- Give a pathophysiological classification of GERD and GERD symptoms, and use this to classify the drugs used to treat persons with GERD.

➢ Motility disorders

- ↑ transient lower esophageal relaxations (tLESRs)

- ↓ lower esophageal sphincter pressure (LESP)
  - Cholinergics (bethanecol)
  - GABA receptor agonists [baclofen])
  - Hiatus hernia
  - Stomach (gastroparesis), obstructive sleep apnea
  - Prokinetics

- ↓ esophageal peristalsis
  - Scleroderma and CREST syndrome

- Delayed gastric emptying

➢ Damaging factors

- Normal HCl secretion, but ↑ reflux of acid
  - Alginate, antacids, H_2RAs, PPIs
- Increased gastric acid production
- Bile and pancreatic juice
  - Sucralfate

➢ Resistance factors

- Reduced saliva, HCO and EGF production
  - Chewing gum
- Diminished mucosal blood flow
- Growth factors, protective mucus

- Perception
  - TCAs, SSRIs

Note: The gastric secretion of acid is usually normal in GERD. The problem in GERD arises as a result of ↓tLESR, so acid refluxes: there is too much acid in the esophagus because of the abnormal function of the barrier function of the LES.

Abbreviations: GERD, gastroesophageal reflux disease; H2RA, histamine 2 receptor antagonist; LES, lower esophageal sphincter

Printed with permission: Murray JA. *Mayo Clinic Gastroenterology and Hepatology Board Review* 2008: pg. 3.

- Classify the drugs used to treat GERD.
  - tLESP – (GABA receptor agonist (baclofen)
  - ↑ LESP – cholinergic (bethanecol)
  - ↓ sensation – TCA
  - ↓ coating – alginate
  - ↓acid – PPI, H_2RA, antacids
  - ↑ gastric emptying – prokinetics (e.g. domperidone)
  - ↑saliva – chewing gum
  - ↓ bile acids cholestyramine sucralfate

As you climb the ladder of success, be sure
it's leaning against the right building.

H. Jackson Brown, Jr.

- Other Esophageal Motility Disorders

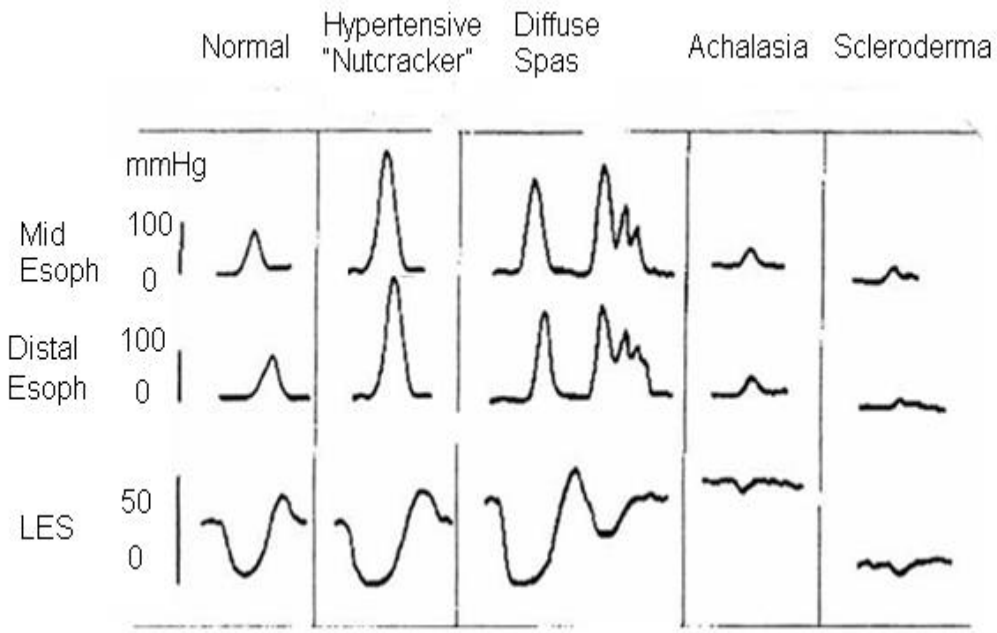
Disorder	Chicago Classification Criteria
Normal EGJ Relaxation (Mean Intergrated Relaxation Pressure < 15 mm Hg)	
○ Aperistalsis	– 100 % of swallows with absent peristalsis
○ Hypotensive peristalsis	– More than 30% of swallows with peristal defects ≥ 3 cm in 30 mm Hg pressu isocontour
○ Intermittent	– 70% or more of swallows with peristal defects ≥ 3 cm in 30 mm Hg pressu isocontour
○ Frequent	– Normal CFV, mean DCI > 5000 and < 80 mm Hg.s.cm or LES after-contraction > 1 mm Hg
○ Hypertensive peristalsis	– Normal CFV, mean > 8000 mm Hg.s.cm
○ Spastic nutcracker	
○ Diffuse esophageal spas (DES)	– Normal EGJ relaxation and spasm (CFV > cm/s) with ≥ 20% of swallows
Impaired EGJ relaxation*	
○ Classic Achalasia	– Impaired EGJ relaxation and aperistalsis
○ Achalasia w esophageal compression	– Impaired EGJ relaxation, aperistalsis, a panesophageal pressurization with ≥ 20% swallows
○ Spastic Achalasia	– Impaired EGJ relaxation, a peristalsis and spas (CFV > 8 cm/s) with ≥ 20% of swallows (see F 42-13C and D)
○ Functional EGJ obstructi	– IBP > 30 mm Hg compartmentalization betwe the peristaltic wavefront (normal or nutcrack and EGJ

*mean integrated relaxation pressure ≥ 15 mm Hg

Abbreviations: CFV, contractile front velocity; DCI, distal contractile integral; EGJ, esophagogastric junction; IBP, intrabolus pressure; LES, lower esophageal sphincter.

Printed with permission:*Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Table 42-2, page 699, Ninth Edition, 2010

- Give the manometric features of the esophageal motility disorders (manometry) tracing abnormalities which characterize three major clinical disorders of esophageal motility.



  - In **hypertensive** ("nutcracker") esophagus, the pressure waves are of high amplitude, but are still peristaltic.

  - In **diffuse esophageal spasm**, normal peristaltic waves are interspersed with high-pressure, nonpropulsive (simultaneous) contraction waves that are often repetitive.

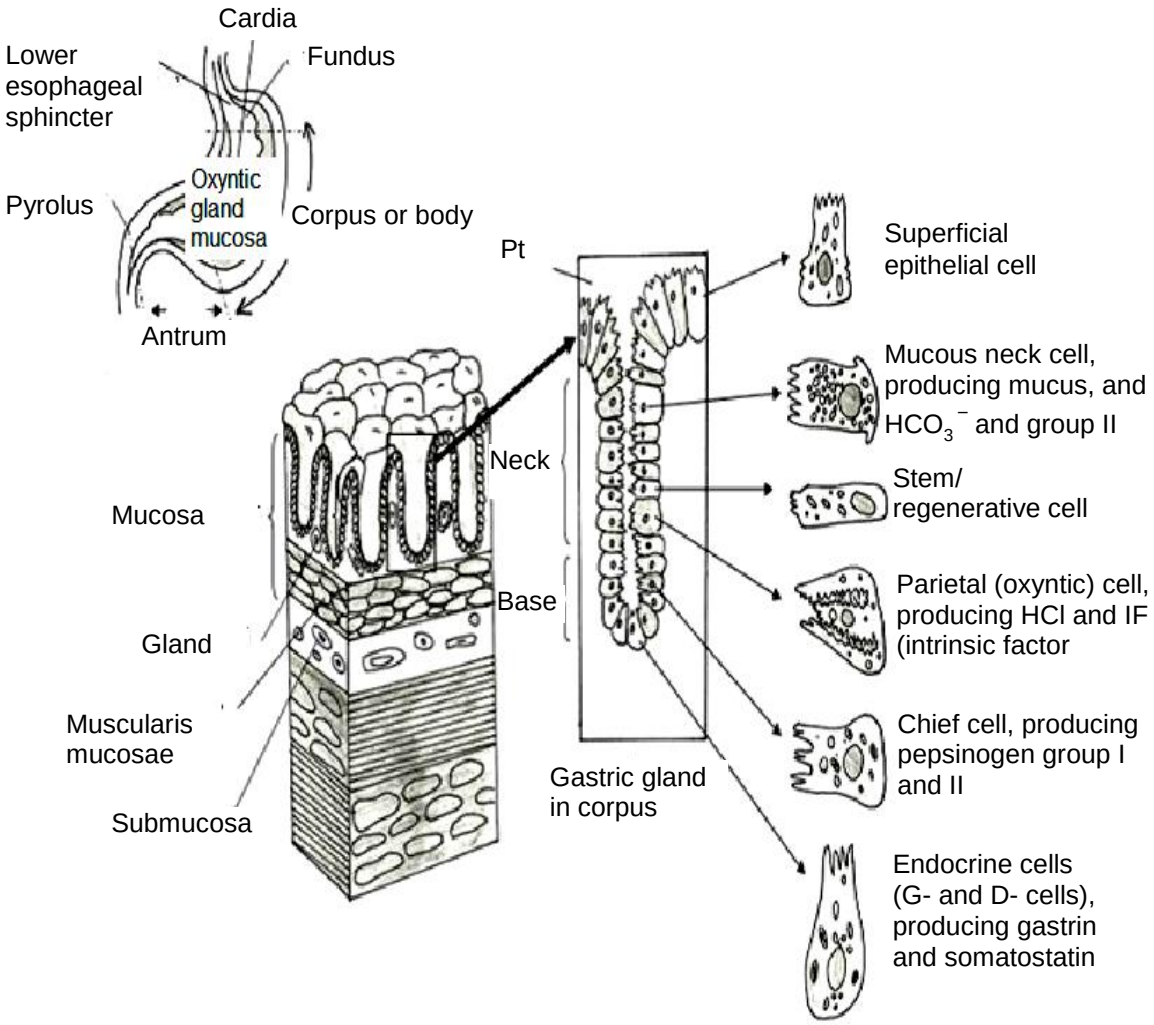
  - In **achalasia**, the resting LES pressure may be abnormally high , contractions of the esophageal body are simultaneous and propulsive, resting intraesophageal pressure is elevated, and swallow-induced LES relaxation is either absent or incomplete.

  - **Scleroderma** is characterized by the presence of weak, nonperistaltic esophageal contractions, and a marked hypotensive LES that relaxes normally with swallowing.

  - Medical management of esophageal motility disorders, can be understood from their derangement of the normal ordered process of swallowing and esophageal dysmotility.

STOMACH

Gastric Anatomy



- Give the cells of the stomach and their function.

Cells	Function
➢ Parietal (oxyntic) Cell	
○ Hydrochloric acid	– Provides optional pH for pepsin and gastric lipase (please see below) – Assists duodenal inorganic iron absorption (reduction of food Fe^{3+} to Fe^{2+}) – Negative feedback of gastrin release (when intragastric acidity falls [↑ pH], serum gastrin concentration rises)

	– Stimulation of pancreatic HCO_3^- secretion – Suppression of growth of microorganisms ingested in food
○ Intrinsic factor	– Binding of vitamin B_{12} for subsequent ileal absorption
➢ Chief (Peptic) Cell	
○ Pepsins	– Initial hydrolysis of dietary proteins – Liberation of vitamin B_{12} and Fe^{3+} from dietary protein
➢ Superficial epithelial cell	– Maintains barrier function of gastric membrane
➢ Endocrine Cell	
○ Gastrin secreting somatostatin G- cells	– Stimulate acid secretion
○ Somatostatin secreting D-cells	– Inhibit acid secretion
➢ Mucous Neck Cell	
○ Mucin/HCO_3^-	– Protection against noxious agents including hydrochloric acid and pepsins

*In addition to assisting digestion and absorption of dietary protein, hydrolysis certain dietary proteins may render them harmless in individuals who may be allerg to these proteins (i.e., pepsin may prevent the risk of developing certain fo allergies).

"All life is an experiment. The more experiments you make, the better"

Ralph Waldo Emerson

SO YOU WANT TO BE A GASTROENTEROLOGIST!

The surface of the stomach and intestine possess many defensive mechanisms, including trefoil factors.

- Give the physiological effect of trefoil factors.
  - Source
    - pS2 gastric mucus neck cells
    - spasmolysin gastric antrum and pancreas
    - intestinal trefoil factor goblet cells in small intestine and colon
  - Composition
    - Cloverleaf shape
    - G cysteine residues and 3 disulfide bonds
  - Action
    - Secreted mucus – mucosal surface
    - Growth – promoting properties

Main Diseases:

- Peptic ulcer disease
- Helicobacter pylori infection
- Acid hypersecretion disorders
- Gastroparesis

Gastric function:

- Reservoir for food
- Synthesizing and secreting HCl and pepsinogen as well as mucus and intrinsic factor
- Mixing of food with HCl and pepsinogen
- Control the release of partially digested food into the duodenum
- Assist in the control of appetite and food intake.
- Gastric alcohol dehydrogenase may metabolize small amounts of ingested alcohol.

In order to understand acid sensitive disease in the stomach, such as peptic ulcer disease causing gastric and duodenal ulcers, it is necessary to understand the initiation and termination of hydrochloric acid secretion at the organ and the cellular level.

Gastric Acid Secretion

An understanding of gastric parietal cell secretion of HCl and G cell secretion of stimulatory gastrin as well as D cell secretion of inhibitory somatostatin, is important to understand the pathophysiology of dyspepsia, peptic ulcer disease, as well as the effects of hypergastrinemia, including the ZES (Zollinger-Ellison Syndrome) of the sporatic or MEN-1 / types.

- Parietal cells
  - Secretory vesicles are lined with membrane containing the H^+ / K^+-ATPase acid-secreting pump.
  - With food stimulation the secretory vesicles traffic to and are incorporated into the luminal membrane of parietal cells, forming the secretory canaliculi.
  - The pathway for K+ to reach the H^+ - K^+ ATPase is in a short-circuited state during fasting.
  - Upon stimulation this pathway allows access of the K^+ to the H^+ - K^+ ATPase, exchange of H^+ for K^+ and thus HCl secretion.
  - The parietal cell is stimulated by the cephalic, gastric and intestinal components, with activation occurring through
    - ↑ Ca_i^{2+} (intracellular Ca^{2+})
    - ↑ cAMP → cAMP-dependent PK (protein kinase) cascade
  - When activation of the parietal cell ceases, or inhibition increases, the H^+-K^+ ATPase in the parietal cell membrane moves back (reinternationalization) into the cytoplasmic side of the cell.
  - Reinternalization of the H^+ - K^+ ATPase occurs by way of the cytoplasmic tail of the beta subunit of the enzyme.

- **Fasting – Basal Gastric Acid Secretion**

- Gastric membrane permeability
  - The stomach produces large amounts of acid (HCl), to begin the digestion of food.
  - The gastric membrane is designed to reduce back diffusion of acid into the wall of the stomach.
  - Mucus is produced and released from
    - Glands in the gastric antrum (a neutral glycoprotein)
    - Surface and neck cells contains both neutral as well as acidic glycoproteins.
  - Strong permeability or diffusion barrier for H^+ and Na^+ is created by

- Tight junctions (TJs) between the gastric epithelial cells,
- Hydrophobicity of the lipid membrane of the cells
- Secreted mucous and HCO_3^- from glands in the gastric antrum
- Prostaglandins
- Normal blood flow and capillary function
- Normal cell proliferation

- This alkaline microclimate adjacent to the gastric cellular membrane forms part of the unstirred water layer which provides a pre-epithelial barrier to the back diffusion of HCl

- Mucus is a viscous gel, which contains
  - Water
  - Electrolytes
  - Phospholipids
  - Glycoproteins

- Mucus is secreted as the result of Ach release from the vagus nerve.

- The vagus nerve becomes stimulated by irritation by
  - The direct contact of food on the gastric mucosa, or
  - The indirect effect of chemicals such as pickles and red peppers.

➢ Basal (interdigestive) gastric acid secretion

- Basal acid output (BAO), ~5mEq/L

- BAO – low in morning, higher later in the day

- BAO increases with the number of parietal cells, which increases in proportion to a person's body weight, and in response to chronic hypergastrinemia

- The maximal stimulated acid secretion is called the maximal acid output (MAO).

- When gastric secretion is constantly stimulated, such as in patients with hypergastrinemia from the Zollinger-Ellison Syndrome (ZES), the basal acid secretion is increased more than the stimulated secretion (BAO / MAO ~ 60%).

- **Turning "on" Acid Secretion - Stimulated Gastric Acid Secretion**

➢ Stimulated gastric HCl secretion

- 3 pathways
  - Neurocrine (direct) pathway
  - Hormonal (indirect) pathway
  - Paracrine pathway

➢ Calcium

- Phospholipase C (PLC) cleaves PIP2 (phosphatidyl inositol 4, 5-b1 phosphate) in the BM of the parietal cell to IP3 (inositol 1,4,5-triphosphate) and DAG (diacylglycerol).

- IP3 releases Ca^{2+} stored in the ER (endoplasmic reticulum) of the cytosol of the parietal cell.

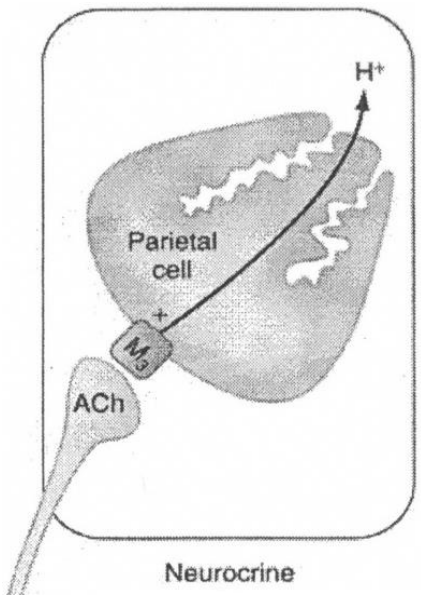
- M3 activates a Ca^{2+}- channel in the BM of the parietal cell, leading to a further increase in intracellular Ca^{+2} $[Ca^{+2}]i$

- Ca_i^{+2} is also increased from the Ca^{2+}-channels activating calmodulin-dependant protein kinase.

- **Neurocrine ("direct") pathway**
  - There are the overlapping cephalic (CNS), gastric and intestinal phases, which enhance acid secretion.
  - Acetylcholine (Ach; neuronal stimulation), gastrin (hormonal) and histamine (paracrine) are the gastric stimulants.
  - Each of these have receptors (M3, CCK_B and histamine – 2 receptors, respectively) on both the parietal cell (direct pathway) and the ECL (enterochromafin cell, indirect pathway).

"A journey of a thousand miles begins with a single step"

Lao Tzu



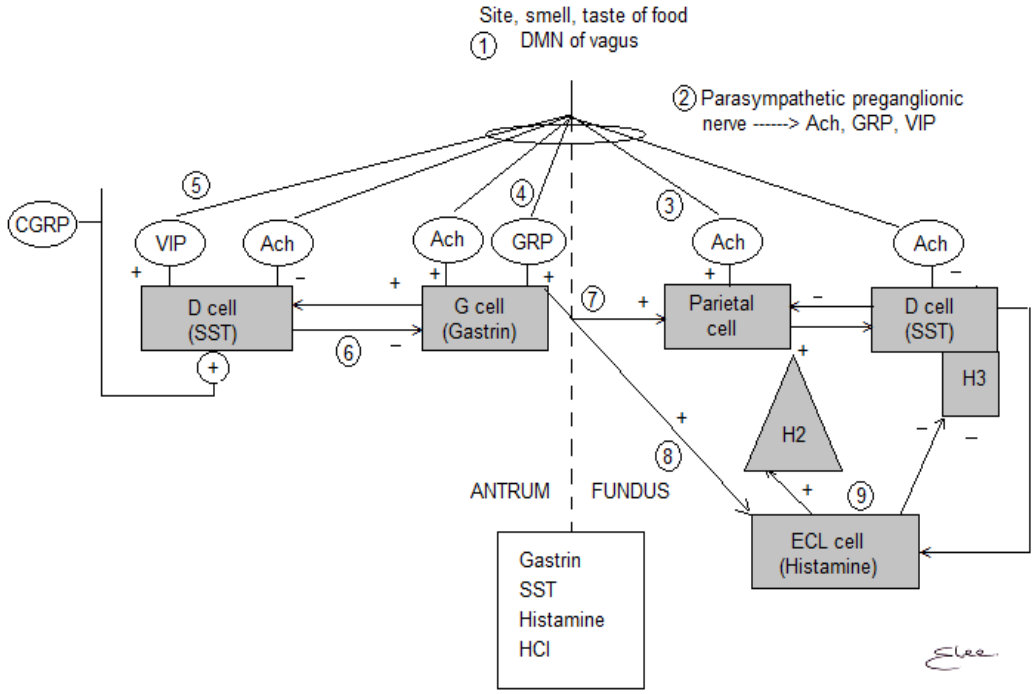
Parasympathetic preganglionic nerves

(aka direct pathway)

Abbreviation: Ach, acetylcholine; M3, muscarinic receptor

- Acetylcholine
  - The cephalic phase is responsible for about 30% of total stimulated acid secretion.
  - The gastric phase is responsible for about 60-65% of total stimulated acid secretion.
  - 1) The site, smell, and taste of food activate the dorsal motor nucleus (DMN) of the vagus nerve in the medulla.
  - 2) The parasympathetic preganglionic nerves release acetylecholine (Ach), gastrin-releasing peptide (GRP), and vasoactive intestinal peptide (VIP) neurons.
  - 3) Ach binds to M3 receptors directly stimulates (+) parietal cells in the fundus (oxyntic mucosa), to secrete HCl.
  - The hormonal (“indirect”) pathway (release of gastrin from the G-cell, and its action on the CCK-2 receptor of the parietal cell, as well as the release of the gastrin – releasing peptide (GRP) from the vagus nerve, and the action of GRP on the G-cell.

- Gastrin and their receptors (CCK1 and CCK2)



Abbreviations: Ach, acetylcholine; CGRP, calcitonin gene related peptide; ECL, enterochromafin-like cell; DMN, dorsal motor nucleus; GP, gastrin releasing peptide; SST, somatostatin

- GRP from PPP/ENS neurons as well as Ach stimulate the antral G cells to release gastrin.
- GRP neurons, activated by luminal protein, also stimulate gastrin secretion. VIP neurons, activated by low grade gastrc distention, stimulate SST and thus inhibit gastrin secretion.
- 4) Ach and GRP stimulate (+) G-cells to release gastrin (G).
- VIP stimulates (+) D cells to release and Ach inhibits (-) the D cells to release somatostatin (SST).
- 6) Ach neurons stimulate gastrin secretion directly as well as indirectly by inhibiting SST secretion (thus eliminating its restraint on gastrin containing G-cells).
- 7) Gastrin binds to CCK-2 receptors on BM of parietal cells to stimulateHCl secretion.
- 8) Gastrin stimulates ECL cells to produce histamine.

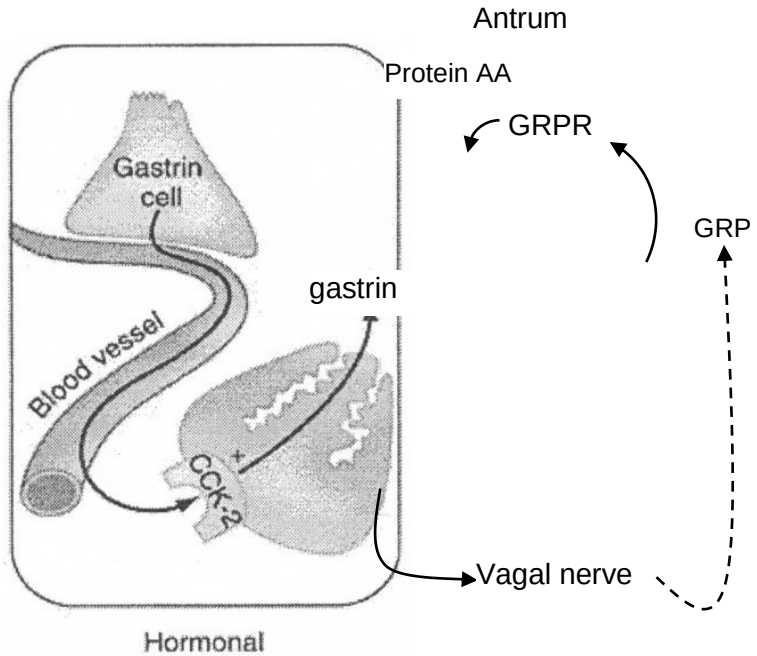
- 9) Histamine stimulates (+) parietal cells (↑ HCl) and inhibits (-) H3 receptors on D cells (↓ SST)

- Gastrin is released from G cells in the gastric antrum as a result of
  - Amino acids (AA) protein and peptides) in the stomach and from
  - Distention of the stomach.
- Gastrin binds to the
  - CCK2 (aka CCK-B, or gastrin) receptors on
    - Parietal cells
    - ECL cells in body of stomach, adjacent to parietal cells
  - CCK1 (aka CCK-A) receptors on D cells, pancreas, gallbladder and brain

➢ Gastrin (G)
- Acutely
  - Binds to CCK2 on
    - Parietal cells → ↑ secretion
    - Gastric ECL cells
- ↑ synthesis of histamine in ECL cells
- ↑ release from ECL cells of
  - Histamine
  - PACAP (pituitary adenylate cyclase-activating polypeptide)
  - VIP (vasoactive intestinal peptides)
- ↑ histamine binds to CCK1 receptors on
  - D cells → ↑ somatostatin (SST)
  - Gallbladder
  - Brain
  - ↑ somatostatin acts on G cells → ↓ gastrin parietal cells → ↓ HCl
- Note paradoxical effect of gastrin
  - ↑ histamine release from ECL cells
    - ↑ HCl secretion
  - ↑ somatostatic release from D cells
    - ↓ G release
    - ↓ HCl secretion

- Major inhibitory mechanism of somatostatin
  - Reduction of gastrin-stimulated release of histamine from ECL cells
- Antigens stimulate gastric mucosal mast cells – release of histamine
- Chronically
  - Causes hypertrophy of parietal cells and ECL cells

- Hormonal ("indirect") Pathway



Abbreviations: CCK, cholecystokinin; GRP, gastrin releasing peptide; GRP-P, gastrin releasing peptide receptor

- Products of protein digestion (i.e., peptides and amino acids)
  - Stimulate the G cells to release gastrin
  - Stimulate GRP (gastrin-releasing peptide) release from the vagal nerve endings

- Gastrin, released from antral G cells, travels in the systemic blood circulation to reach the parietal cell.

- The gastrin binds to the CCK_2 receptor directly activates cholecystokinin-2 (CCK-2) receptors on the parietal cells and leads to the release of intracellular calcium.

- This stimulates the post-translational modification of G-101 (the 101 amino acid containing gastrin) in the trans-Golgi apparatus of the endoplasmic reticulum (ER) of the G cells.

- GRP releases gastrin from antral G cells, and reduces the release of somatostatin (SST) from gastric body and antral D-cells.
- The release of gastrin from the G-cells is mediated from the luminal and anti-luminal sides of the G-cell.

- This post-translational modification of G-101 results in G-17 and G-34.

- G-17 and G-34 are present as gastrin I (non-sulfated) and gastrin II (sulfated).

- In the plasma, G-17 is inherently more active, but is degraded faster than the less active but more slowly degraded G-34.

- Because of the differences in their inherent activity and rates of degradation in the plasma, G-17 and G-34 have similar potency, mole per mole.

- The parietal cell H^+ secretion is stimulated by way of CCK_B, Cq, PLC, IP3, and DAG.

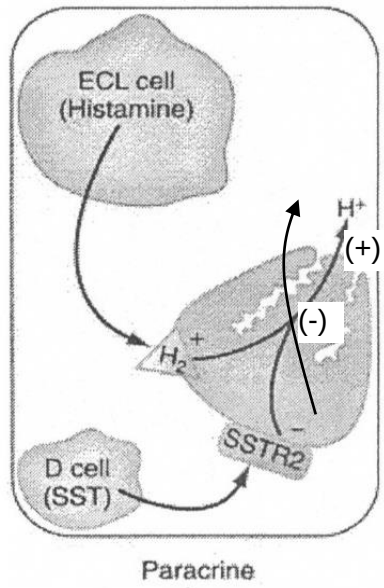
- The M3 and CCK_B receptors couple to a GTP-binding protein. The Gqi receptor activates PLC (phospholipase C).

➢ Histamine

- Ach and gastrin binding to the M3 and CCK_B receptors on the outer membrane of the ECL release histamine from the ECL cells
- Histamine released from oxyntic ECL cells diffuse to the parietal cells (paracrine effect), and stimulates the release of GRP from
  - peptidergic postganglionic parasympathetic (PPP) neurons
  - as well as ENS neurons.
- Histamine released from ECL and from mast cells from
  - binds to the histamine H_2 receptor on the basolateral membrane (BM) of the parietal cell.
- Histamine coupled to GIs
  - couples to G1s (another GTP-binding protein)
  - activates AC (adenyl cyclase) to form cAMP
- cAMP activates PKA (protein kinase A).

- Somatostatin
  - Paracrine Pathway
    - Somatostatin acts by a paracrine pathway, and acts to ↓ gastrin release, which in turn ↓ acid secretion

Fundus



(aka ECL [enterochromafin pathway])

Adapted from: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Figure 49-1, page 818, Ninth Edition, 2010

- Release of acid into the lumen of stomach restores SST secretion in both the fundus and the antrum is mediated via the release of calcitonin gene related peptide (CGRP) from extrinsic sensory neurons.
- Acid in the lumen of the gastric antrum releases SST
- SST inhibits antral G-cell gastrin release, and thereby inhibits HCL secretion (protein → ↑G, ↑Ach - ↑ HCl - ↑ SST - ↓ G → ↓ HCl)
- Distention of the gastric wall by food activates indirectly stimulates acid secretion by activating the vagal afferent and efferent pathway (vagovagal) and ENS reflexes.
- Alcohol, coffee, and peptones (partially digested protein) in the gastric lumen also stimulate the release of antral G cell gastrin.
- SST from D cell represents a redundant regulatory pathway, which inhibits acid secretion.
- SST binds $SSTR_2$ on basolateral membrane of parietal cell, reducing acid recretion.

- SST acts directly on ECL cells to reduce histamine release, and to thereby further decrease acid secretion.
- SST reduces
  - antral gastrin release from antral G cells
  - inhibits histamine release from ECL cells
  - SST-28 is more abundant that SST-14. SST secretion by the D cells.
- Histamine released from ECL cells act via H3 receptors on D cells to inhibit SST secretion.
- Acute infection with Helicobacter pylori (Hp) also activates CGRP neurons to stimulate SST and thus to inhibit gastrin secretion.
- In duodenal ulcer patients who are chronically infected with Hp, the organism or cytokines released from the inflammatory infiltrate inhibit SST, and thus stimulate gastrin (and thereby acid) secretion.

Adapted from: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* Figure 49-10, page 822, Ninth Edition 2010.

- Give 4 factors responsible for increasing and/or decreasing the release of somatostatin from the D cells.

  - ↑ relea (SST)
    - Gastric acidity (↑ H^+ → somatostatin → ↓ H^+ [fee back loop])
    - Minor distention of stomach, acting through ν neurons
    - ↑ VIP activation
    - ↑ gastrin → ↑ somatostatin
      - ↓ ECL secretion of histamine
      - ↓ gastrin release
  - ↓ release
    - Major distention of stomach
    - Ach

  - SST arises from D cells in the gastric antrum and fundus, in the duodenum, the islet cells of the pancreas, and by neurons of the hypothalamus
    - SST is increased by luminal acid in the duodenum, but luminal acid has no effect on the G cells in the antrum.
    - SST secretion is decreased by vagal Ach acting on the D cell.
  - SST binds to G1 coupled SSTR2 receptors on parietal cells, as well as on ECL cells and G cells tonically restrains acid secretion.

- This restraint is exerted directly on the parietal cell as well as indirectly by inhibiting histamine secretion from ECL cells and gastrin secretion from G-cells.
- SST directly inhibits acid secretion by the parietal cells.
  - The SST receptor and the prostaglandin receptors for prostaglandin E2 (PGE2) couple to a GTP-binding protein on the BM of the parietal cell, (Gq, coupled by M3 receptor and CCK_B receptor, Gs coupled by histamine – two receptors, all in the cytosol of the parietal cell).
  - G1 inhibits the activity of Gs, and thereby reduces cAMP, PKA, and H^+-pumping by the canalicular membrane proton pump H^+, K^+-ATPase.
  - PGE2 binds to EP3 (prostaglandin receptor) on BM of parietal cell, PGE2-EP3 couple to G1.
  - Release of histamine from the ECL cell.
  - Binding of histamine to the H_2 receptor of the parietal cell.
  - Stimulation of acid secretion.
  - Release of somatostatin [SST] from the D cell.
  - SST binds to the $SSTR_2$ on the parietal cell, reduing acid secretion.
  - SST redues histamine release from ECL cell.
  - Neuroendocrine, hormonal, and paracrine pathways directly regulate parietal cell acid (H+) secretion.

- Give 5 peptides other than somatostatin which inhibit acid secretion by way of ↓ ECL histamine release.
  - CGRP (calcitonin gene-related peptide)
  - PYY (peptide YY)
  - Prostaglandins
  - Galanin
  - CCK2 and CCK2 receptors are G-protein-coupled receptors
  - Signaling by way of pertussis toxin-insensitive G-proteins
  - Agonist stimulation of receptors

➢ Prostaglandins
  - Produced and stored in gastric macrophages and capillary endothelial cells.
  - ↓ acid secretion by way of inhibiting
    - Gastrin-stimulated histamine release
    - Histamine-stimulated parietal cell acid secretion

- Distention
  - Initially, little distention
    - VIP neurons are activated to release somatostatin
    - Somatostatin inhibits antral G cells
  - Then, more distention
    - Stimulation of release of acetylcholine (Ach; cholinergic)
      - Ach directly stimulates M3 receptors on parietal cell
      - ↑ gastrin
      - ↓ somatostatin

- AA in stomach
  - Direct effect of AA → G cells → ↑ gastrin
  - Indirect effect of AA
    - Activate Ach neurons
    - Activate GRP (gastrin related peptide) neurons → G cells → ↑ gastrin

SO YOU WANT TO BE A GASTROENTEROLOGIST!

PYY (peptide YY) is contained in ECL cells in the terminal ileum and colon. Resection of the terminal colon (R. hemicolectomy) may be associated with increased gastric acid secretion, which sometimes results in diarrhea which responds to the use of a PPI.

- Give the mechanism of this surgically induced increased acid secretion.
  - PYY inhibits gastrin-stimulated histamine release
  - This inhibition is lost with ileal resection and R. hemicolectomy

SO YOU WANT TO BE A GASTROENTEROLOGIST!

Histamine released from ECL cells act on the H2-receptors on the basal membrane of gastric body parietal cells, increasing HCl secretion.

- Give the mechanism of action of the H3 receptors to alter gastric acid secretion.
  - Histamine stimulates H3 receptors to ↓ secretion of somatostatin from D-cells; the ↓ somatostatin leads to ↓ inhibition of parietal cell acid inhibition, and thus ↑ HCl secretion (inhibition results in stimulation).

Phases of Gastric Acid Secretion

- **Cephalic and Gastric Phases**
  - There are numerous peptide-hormones produced by the GI tract, and many of these are involved in the regulation of gastric hydrochloric acid secretion.

- Give the name of the Gastrointestinal Peptide Hormones, their source and their action on the stomach.

Hormone	Source	Action
Gastrin (G)	G cells, antrum of stomach	↑ H^+ secretion
Gastrin-releasing peptide (GRP)	Vagal nerve endings	↑ Gastrin release
Acetylcholine (Ach	Vagal nerve endings	↑ HCL secretion
Histamine	ECL cells; Releases GRP fr PPP and ENS neurons	↑ G from G cells, ↓ SST from D cells
Secretin (S)	S cells in small intestine	↑ HCO3- and fluid secret by pancreatic ducts
Somatostatin (SS	D cells of stomach a duodenum, islet cells pancreatic islets	↓ Gastrin release
Motilin	Endocrine cells in upper tract	↑ Smooth muscle contracti
Peptide YY (PYY)	Endocrine cells in ileum a colon	↓ Vagally mediated a secretion

Printed with permission: *Medical Physiology*, Second Edition, Boron Walter F. a Boulpaepemile L. 2009; Table 41-1, page 889.

- **The Intestinal Phase**

➢ Stimulation of Gastric and Acid Secretion

  - Protein digestion products (partially digested peptides and amino acids) stimulate duodenal G cells to secrete gastrin, which stimulates the parietal cell.

- Protein digestion products act on an unknown intestinal endocrine cell to release an unknown hormone (named entero-oxyntin), which stimulates the gastric parietal cell to produce hydrochloric (HCl) acid.

➢ Inhibition of Gastric Acid Secretion

- In addition to the intestinal phase which stimulates gastric acid secretion, there is an intestinal phase which inhibits acid secretion.

- Loss of Gastric Stimulation
  - Loss of the stimulatory Ach, gastrin and histamine, as well as the emptying of the stomach and loss of gastric stimulatory signals, causes “inhibition” of parietal cell acid secretion.

- Gastric Inhibitory Peptide (GIP)
  - GIP is released from K cells in the duodenum and jejunum as the result of fat and glucose in the lumen.
  - GIP reduces antral gastrin, and directly reduces acid secretion by the parietal cell.

- Secretin
  - Fat as well as acid in the duodenum release secretion from S cells in the small intestine.
  - Gastric acid entering the duodenum initially causes an acidic environment in the lumen.
  - As pH falls below 4.5, secretin is released from the duodenal S cells.
  - Secretin acts as an enterogastrone (inhibitor) in the intestinal phase of gastric acid secretion, inhibiting gastric parietal cell acid secretion.
  - Secretion also stimulates pepsinogen secretion from the gastric chief cells.

- Prostaglandin E2 (PGE2)
  - PGE2 reduces histamine release from ECL cells, and inhibits gastrin release from antral G cells.

- Enterogastrones
  - In addition to SST GIP, secretin and PGE2 other enteric hormones inhibit gastric acid secretion (enterogastrones): CCK, VIP, gastric inhibiting peptide (GIP; also better known as “glucose-dependent insulinotropic polypeptide”), neurotensin (NT), and peptide YY (PYY).

CLINICAL PHYSIOLOGICAL CHALLENGE – Turning Acid Secretion On and Off

Background:

- The stomach has a series of integrated control systems to mediate the secretion of HCl and pepsin to initiate the process of digestion of food.
- However, an excess of HCl and pepsin can damage the mucosa of the stomach and duodenum, causing ulceration with its associated problems of hemorrhage, obstruction and perforation.

Problem: Describe how the stomach turns on and then turns off hydrochloric (HCl) acid secretion

DMNV	Parasympathetic Pregandlionic nerves	"Direct" effects
	Ach	↑ parietal cell H^+ ↑ G cell gastrin ↓ D cell somatostatin (SST)
	GRP	↑ D cell gastrin
	VIP	↑ D cell SST ↓ G cell gastrin

"Indirect"

G cell	Gastrin (G)	↑ parietal cell H^+
D cell	somatostatin (SST)	↓ D cell
ECL cell	Histamine	

Abbreviations: DMNV, dorsal motor nucleus of the vagus; ECL, enterochromaffin-like cell; CGRP, calcitonin gene related peptide; GRP, gastrin releasing peptide; SST, somatostatin

- Parietal cell H^+ ↑ SST
- G cell gastrin ↑ SST
  ↑ H^+
- ECL cell histamine ↓ SST
  ↑ H^+

Gastric Hydrochloric Acid Secretion at the Parietal Cell (Cellular) Level

➢ The vocabulary of the components

- Parietal cell

AM	Cytosol	BM
H^+ / K^+ ATPase	Carbonic anhydrase (CA)	Na^+ / H^+ exchanger (NHE)
K^+ channel		Na^+ / K^+ ATPAse
Cl^- channel		K^+ channel
		Cl^-/ HCO_3^- exchanger

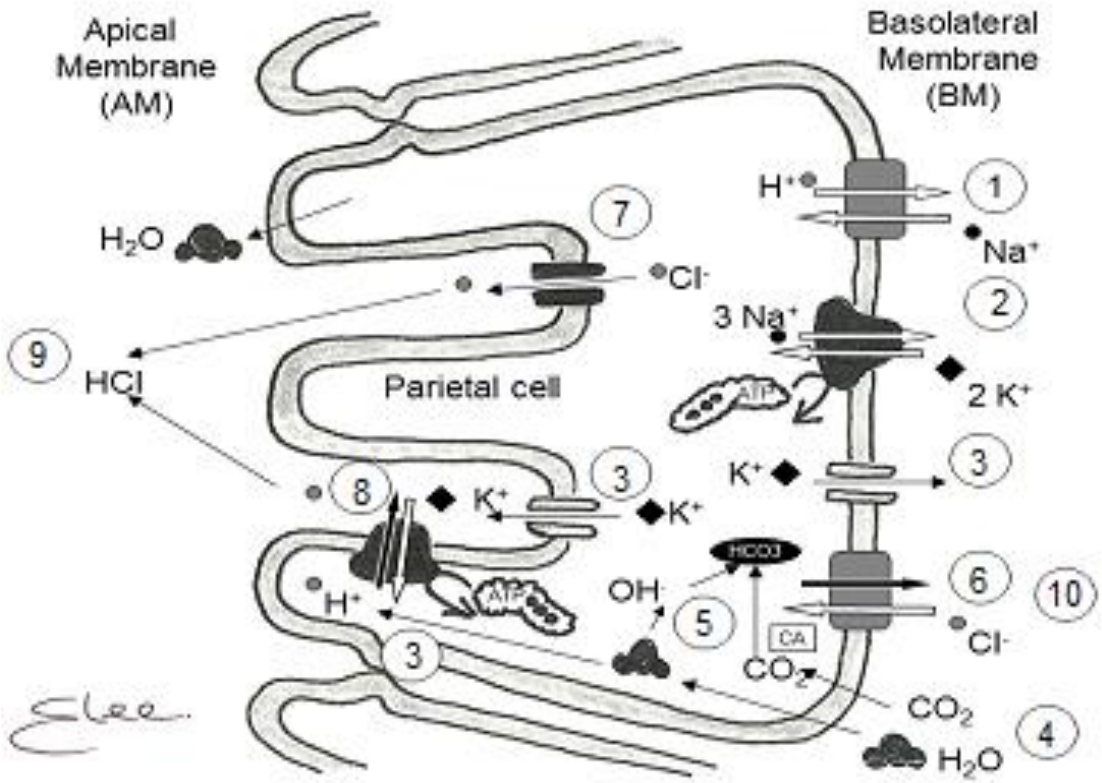
- Requirements of the system
  - H^+ formed in the parietal cell is secreted across the apical membrane (AM)
  - Cl^- is needed to form HCl with the H^+.
  - Cl^- enters the parietal cell from the action of the basolateral membrane (BM) Cl^- / HCO_3^- exchanger.
  - Cl^- leaves the cell through the AM Cl^- channel.
  - The process is "energized" by the BM Na^+ / K^+ ATPase.

See Figure "Parietal cell and H^+ secretion"

- Give the cellular acid secretion by the parietal cell.
  - Na^+enters the parietal cell across the BM by way of
    - 1) Na^+ / H^+ exchanger
    - 2) Na^+ / K^+ ATPase
  - K^+ enters the parietal cell across the BM by Na^+ / K^+ ATPase.
  - 3) To avoid accumulation of K^+ in the parietal cell, K^+ may exit the parietal cell through the AM or BM K^+ channels.
  - 4) CO_2 and water diffuse across the BM and into the cytosol of the parietal cell.

- 5) Carbonic anhydrase (CA) in the cytosol of the parietal cell forms H^+ plus HCO_3^- (from OH^- plus CO_2).
- 6) Cl^- enters the parietal cell across the BM by the Cl^- / HCO_3^- exchanger.
- 7) The Cl^- needed to form HCl in the gastric lumen moves from the cytosol of the parietal cell across a Cl^- channel in the AM.

PARIETAL CELL AND H^+ SECRETION



Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Figure 42-4, page. 898.

- 8) Parietal cells in the gastric body have numerous tubulovesicles (TVs) which contain inactive, H^+, K^+ -ATPase (aka "the acid [or proton] pump"
- The beta subunit of the H^+, K^+ - ATPase is the actual H^+ pump or catalytic unit.
- With stimulation of the parietal cell (neural, hormonal and paracrine factors), the activated PKA, PCA, Ca^{2+} activate H^+, K^+, - ATPase.

- The tubulovesicles (TVs) which now contain activated H^+, K^+ - ATPase are inserted into the AM.
- The H^+ is actively secreted into the gastric lumen, in exchange for K^+ in the lumen.
- The H^+ in the cytosol of the parietal cell is removed by the proton pump on the AM, pumping H^+ into the external side of the AM.
- 9) H^+ and Cl^- form HCl, which streams through the overlying mucus and into the gastric lumen.
- The mucus layer reforms, and the mucus layer prevents the back diffusion of HCl and pepsin towards the AM.

- The surface epithelial cells in the gastric body and antrum secrete HCO_3^-.
- The HCO_3^- is trapped in the mucous / unstirred water layer adjacent to the luminal side of the gastric membrane.
- This trapping of HCO_3^- in the mucus / unstirred water layer form the alkaline microenvironment close to the AM.
- This alkaline microclimate is protective to the luminal surface of the gastric cells, and thereby forms part of the diffusion or permeability barrier.
- The high concentration of H^+ in the gastric lumen does not readily diffuse through the mucus gel layer, and the alkaline microenvironment.
- If any pepsin or acid manages to diffuse back into the mucous layer, the pepsin will be rendered inactive because of the alkaline microclimate.
- The back-diffusion of acid from the gastric lumen into cytosol of the parietal cell will also be partially neutralized.
- 10 / 6) The cytosolic HCO_3^- formed from the CA effect on $CO_2 + H_2O$ exits across the BM by the Cl^- / HCO_3^- exchanger.

Abbreviations: CCK, cholecystokinin; VIP, vasoactive intestinal peptide; PYY, peptide YY; ENS, enteric nervous system

Teaching is the art of acting
I don't teach Gastro', I teach about myself

Grandad

CLINICAL PHYSIOLOGICAL CHALLENGE – Hypergastrinemia and Acid Hypersecretion

Case: A 35 year old man with dyspepsia and diarrhea was found on upper GI endoscopy to have three ulcers in the second portion of the duodenum. He did not take ASA or NSAIDs, and his antral biopsies taken before acid lowering therapy were negative for H. pylori. A fasting serum gastrin concentration was 750 pg/ml. You suspect that he may have a gastric hypersecretory state.

- Give the scientific basis for the calcium – infusion and the secretin tests for Zollinger-Ellison Syndrome, and the rational of measuring BAO and MAO to calculate ratio BAO / MAO.

Abbreviations: BAO, basal acid output; MAO, maximal acid output

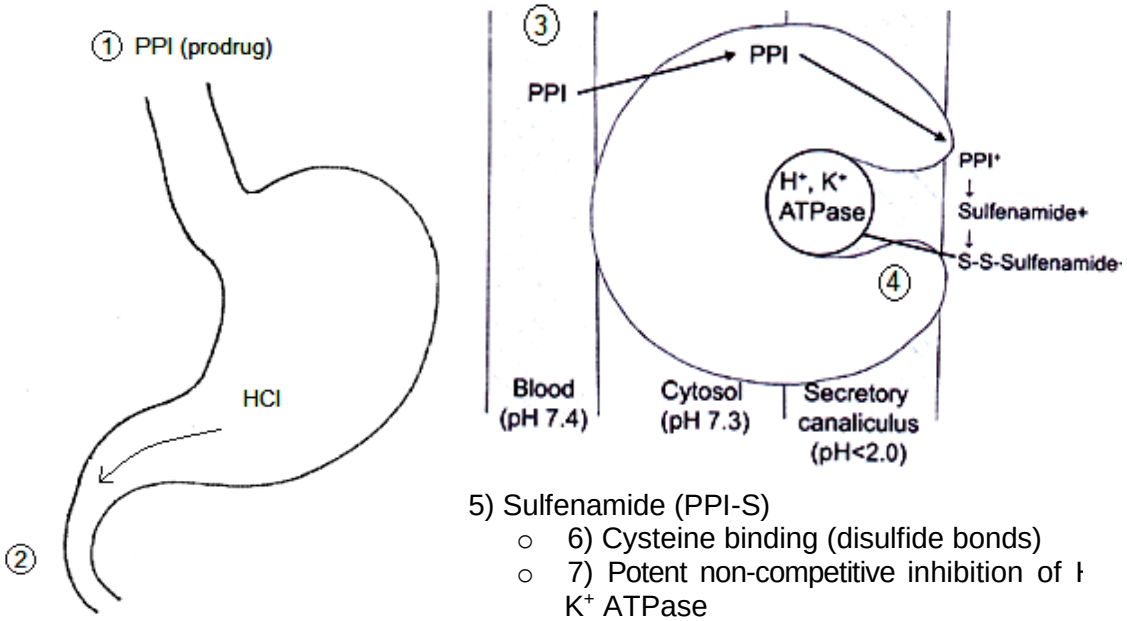
Acid Rebound

- Definition: An increase in acid secretion to above pretreatment values when an anti-secretory drug (e.g. PPI, H2RA) is suddenly discontinued.
  - The magnitude of the acid rebound, which is usually ~15%, may be sufficient to cause a post-treatment recurrence of symptoms.

- Give the pathophysiological mechanism(s) for the phenomenon of acid rebound which occurs when anti-secretory treatment is suddenly stopped.
  - Acid secretion inhibition → ↑ gastrin
    - → Up-regulation (hypertrophy / hyperplasia) of
      - Parietal and G cells (H^+ and gastrin)
      - Ach pathways
    - → Down-regulation of D-cells (somatostatin [SST])
  - Suddenly stopping PPIs
    - A given stimulus causes more release of stimulatory gastrin a Ach and less release of inhibitory somatostatin from t increased number of parietal cells

Pharmaceutical Control of Acid Secretion

- The Mechanism of Action of Proton Pump Inhibitors (PPIs)



5) Sulfenamide (PPI-S)
- 6) Cysteine binding (disulfide bonds)
- 7) Potent non-competitive inhibition of H K^+ ATPase

Adapted from: *Sleisenger and Fordtrans's Gastrointestinal and Liver Disease*. Figure 49-15, page 825, Ninth edition, 2010.

CLINICAL PHYSIOLOGICAL CHALLENGE – PK/ PD of PPIs

Case: When an 18 year old man's dyspepsia fails to respond to once a day proton pump inhibitor (PPI), he is reminded to take the medication half an hour before breakfast, and he now enjoys symptom relief.

- Give the pharmacokinetics and pharmacodynamics of PPIs, and explain why PPIs must be taken half an hour before the intake of food

- 1) PPIs are pro-drugs, coated to protect against damage by gastric HCl.
- 2) PPI are rapidly absorbed in upper small intestinal enterocytes and into the portal circulation, with C_{max} of the PPIs achieved in about one hour
- 3) PPIs reach parietal cell from the portal and systemic bloodstreams, diffuse through the cytoplasm of the unstimulated, fasting, basal state parietal cell and accumulate in the acid environment of the secretory canaliculus.

- Proton pump (H^+, K^+ ATPase) in the membrane of the canaliculi in the cytosol of the parietal cell are inactive during fasting, and do not bind PPI or its metabolites.
- 4) With food intake, the parietal cells are stimulated.
- In the stimulated state, the canaliculi with inactive H^+, K^+ -ATPase ("inactive canaliculi") traffic to the apical membrane of the parietal cell.
- 5) In the stimulated, active canaliculus, the PPI becomes protonated, is trapped as a sulfenic acid, and then is degraded to sulfenamide (PPI-S).
- 6) The canaliculi, in the AM undergo a change in configuration with activation, exposing their cysteine sites
- The sulfenamide in PPI-S binds covalently to the disulfide bonds in one or more cysteines of the H^+, K^+ - ATPase.
- All PPIs bind to cysteine 813; omeprazole also binds to cysteine 892, lansoprazole to cysteine 321, and pantoprazole to cysteine 822.
- 7) PPI-S bound to canaliculi blocks H^+, K^+ -ATPase (proton [H^+] pump) in the canaliculi of the AM and cause potent non-competitive secretion of HCl, regardless of method of stimulation (Ach, gastrin, histamine)
- Therefore, taking PPI half hour before meals provides the time necessary for
  - The PPI to be absorbed across jejunal enterocytes, into portal circulation, across the BM of the parietal cell, and to be metabolized to the PPI-sulfenamide (PPI-SS); and for
  - The canaliculi to be stimulated to traffic to and to be inserted into the AM, where the PPI-S can now bind to the exposed cysteine molecules in the H^+, K^+ ATPase and to markedly and non-competitively reduce HCl secretion arising from all forms of stimulation
- While the t1/2 of PPIs in the blood is about 1 hr, the irreversible binding of PPI to the H^+, K^+-ATPase provides a biological half-life (inhibition of H+ secretion of about 14-18 hr.
- After one dose of PPI, only the H^+, K^+-ATPase in the activated secretory canaliculi are inhibited, but some inactive canaliculi remain and more are formed.
- With each dosing of PPI, more of the canaliculi become inactivated.
- This explains why it takes several days of dosing with PPIs for a steady state of maximum acid inhibition to be achieved.
- With each dosing of PPI, more of the canaliculi become inactivated.

Hypergastrinemia: Gastrin and Disorders of Acid Secretion

In health, the serum gastrin level increases with food. Gastrin also goes up when the antrum is distended (again, by food or disease which shows the emptying of the stomach. Gastrin is metabolized in the kidney will have an ↑ gastrin concentration. There is negative feedback between the gastrin level and acid secretion, so when the parietal cell produces less acid, the G (gastrin-entering cell) lose the feedback inhibition resulting from HCl, more gastrin will be released, and the gastrin concentration will be very high, and because the G-cell secretion from the tumor is not under the usual feedback control, the serum gastric will continue to increase, and acid levels in the stomach will also go up and up. Thus, in order to understand the disease due to hypersecretion of gastrin and acid, we need to understand the role of gastrin and acid, we need to understand the role of gastrin in the physiology acid secretion.

- Overview
  - Where there is
    - ↑ gastric H+, there is ↓ serum gastrin concentration
    - ↓ gastric H+, there is ↑ serum gastrin concentration
  - Any condition which reduces parietal cell number (gastric atrophy, atrophic gastritis) or function (PPI, H2RA, vagotomy) will lead to ↑ serum gastrin.
  - In chronic renal disease, there is ↓ breakdown / metabolism of gastrin, leading to ↑ serum gastrin.
  - In short bowel syndrome, loss of ileal inhibitors of the release of gastrin result in ↑ serum gastrin.
  - When a person eats, the food (amino acids) and the distention result in ↑ gastrin.
  - When there is a sporatic or MEN-1-associated gastrin secreting tumor (gastrinoma), there is also ↑ serum gastrin.
- Approach to hypergastrinemia
  - Is the patient fasting?
  - Have acid inhibitory medications been stopped?
  - Is renal function normal?
  - Is gastric acid being secreted? (for example, in atrophic gastritis, there is no H^+ produced because of the atrophy of the parietal cells, and this leads to ↑ serum gastrin concentration; but no acid peptic disease occurs because the ↑ gastrin cannot produce ↑ H^+ from the reduced number of parietal cells).
  - The presence of a gastrinoma can be investigated by diagnostic imaging to find the tumor (CT scan, EUS [endoscopic ultrasound]
    - By laboratory testing to determine if there is
      - ↑ acid secretion
      - ↑ serum gastrin concentration after an IV infusion of Ca^{2+}
      - ↓ serum gastrin concentration after an injection of secretin

CLINICAL CHALLENGE – Secretin Test for Gastrinoma

Case: A patient with aggressive peptic ulcer disease complicated by hypercalcemia and diarrhea has fasting hypergastrinemia. A secretin infusion test is undertaken to determine if the patient has biochemical evidence of gastrinoma.

- Give the explanation for the increase in the plasma concentration of gastrin following the injection of secretin in the patient with a gastrinoma.

Calcium Infusion

- Ca^{2+} infusion causes ↑ serum gastrin concentration in health, but a higher increase with gastrinoma.

Secretion Test for Gastrinoma

- In health, secretin causes ↑ gastrin and ↑ SST (somatostatin) release.
- The ↑ SST acts to inhibit gastrin release, so the balance is for secretin to have little effect on serum gastrin concentration.
- In gastrinoma, the functional coupling of the release of gastrin and SST is lost, so that the secretion of gastrin is unopposed by the injection of secretin, and the serum concentration of gastrin rises.
- The second approach to investigating whether hypergastrinemia is due to gastrinoma is to give the patient an injection of secretin
  - If the administration of secretin
    - ↑serum gastrin - suggests gastrinoma, or
    - ↓ serum gastrin - suggests there is no gastrinoma

Measurement of basal and maximal acid output

- In response to injection of pentagastrin, in health there is ↑ BAO and ↑↑ MAO
- In gastrinoma, there is ↑↑↑BAO and ↑ MAO

- Give the reason why the ratio BAO / MAO is much higher with a gastrinoma than health.
  - In gastrinoma there is ↑
  - In gastrinoma, there is ↑ secretion of gastrin in both the fed and the fasting state, rather than just the ↑ gastrin secretion in response to food.
  - This means the acid secretion is stimulated all the time, even when the gastrinoma patient.

Gastric Pepsinogen and Pepsin

- Pepsinogen and Pepsin

  - Ach, secretin, VIP, β_2-adrenergics, PGE2 and nitrogenous protein breakdown products (PBP) are agonists of pepsinogen secretion from chief cells in the gastric body

  - The nitrogenous protein breakdown products (PBP) of pepsin also stimulate the secretion of gastrin from antral G cells, and CCK from I cells in the duodenum.

  - The affinity of the CCK_A receptor is higher for CCK than for gastrin, whereas the CCK_B receptor has a greater affinity for gastrin than for CCK.

  - AA, PBP → antal G cells → ↑ gastrin → CCK_B receptor
    AA, PBP → duodenal I cell → CCK → CCK_A receptor
    S cell secretin → CCK_A
    Vagus Ach → M3
    Ach secretin →
    CCK_B receptor / CCK_A receptor → chief cells ↑ Ca^{2+} → Pepsinogen
    Ach secretin → Partial cells ↑ HCl

Abbreviations: AA, amino acids; PBP, protein breakdown products

  - Ach binds to M3 receptors on chief cells → ↑ $Ca^{2+}{}_I$ → ↑ pepsinogen

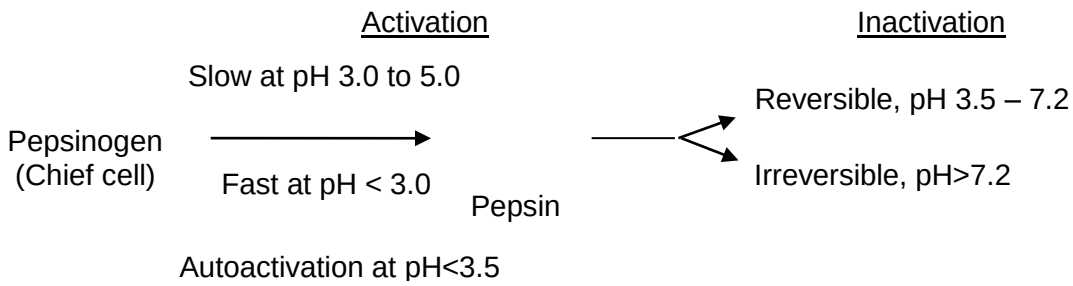
  - There are receptors on the chief cells for secretin.
    - M3 muscarinic receptor for Ach and the CCK_A receptor for CCK.
    - M3/CKA stimulate chief cell pepsinogen secretion through an increased intracellular concentration of Ca^{+2}.

  - Pepsinogens are a group of proteolytic proenzymes, (aka “zymogens”). There are three isoforms of gastric pepsinogen.
    - Group I pepsinogens, the main pepsinogen which is secreted from the gastric chief cells located in the body of the stomach.
    - Group II pepsinogens are secreted from chief cells as well as from mucous neck cells in the gastric cardia, body and antrum.
    - Group III pepsinogen is cathepsin E.
  - The pepsinogen secretory granules fuse with other secretory granules in the chief cells, and then fuse with the outer plasma membrane of the chief cells.

- Both preformed and newly synthesized pepsinogen are secreted into the gastric lumen by the progress of compound exocytosis.
- The pepsinogen secreted into the gastric lumen is an inactive enzyme precursor, which must be activated before it has proteolytic activity and can begin to digest protein.

➢ Autoactivation of pepsinogen by pepsin

- While most protein digestion occurs in the duodenum as the result of pancreatic trypsin, gastric pepsin still plays an important role as an endopeptidase,
- The endopeptidase pepsin breaks down dietary protein into relatively large peptides, as well as some amino acids (AA).
- At gastric pHs of between 3.0 to 5.0, there is slow cleavage of a small terminal fragment of pepsinogen to form pepsin.
- At pH less than 3.0, the activation of pepsinogen to pepsin is very fast.
- If the pH rises above 3.5, the pepsin becomes reversibly inactivated.
- Pepsin is the active proteolytic component of pepsinogen.
- If the gastric pH exceeds 7.2, the pepsin enzyme activity is destroyed (irreversible inactivation).



- Give the explanation why secretion of pepsinogen is essential for normal secretion of gastric acid, and why secretion of gastric acid is essential for normal function of pepsinogens.

    - Pepsinogens are converted to pepsins, which begin the process digestion of dietary proteins to amino acids (AA).
    - AA directly stimulate G cells to ↑ gastrin, and indirectly activate A neurons as well as GRP neurons.
    - This ↑ gastrin leads to ↑ histamine released from ECL cells, which turn stimulates the H2 receptors on the parietal cell to stimula secretion of H^+.
    - Pepsins become inactive at pH > 4, so the low intragastric pH (↑H maintains pepsins in an active form, which then maintains the A stimulation of release of gastrin from G cells, as well as release Ach and GRP from neurons.

CLINICAL PHYSIOLOGICAL CHALLENGE – BAO/MAO vs. pepsin output

Background: In hypergastrinemia arising from a gastrin-producing tumor, the increase in BAO is greater than the increase in MAO, such that the ratio BAO/MAO becomes > 60%.

- However, the ratio of gastric BAO/MAO (basal acid output divided by maximal acid output) is much higher than the ratio of basal to maximal pepsinogen secretion.

- Give the reason why the basal and maximal outputs of pepsinogen increased with gastrinoma.
    - The Ach-responsive HCl secretion stimulates a local cholinergic reflex to release even more Ach, and thereby more pepsinogen from the chief cells.
    - When HCL empties into the duodenum, secretin is released from duodenal S cells, and secretin also stimulates the chief cells to secrete pepsinogen.
    - In gastrinoma, there is ↑ H^+ entering the duodenum, ↑ release of secretin from duodenal S cells, and ↑ secretin-associated secretion of pepsinogen from the chief cells.

Motor Function and Gastric Emptying

- In order to appreciate the rational approach to the investigation and management of disorders of gastric emptying, it is first necessary to understand the complex, integrated process of gastric filling, accommodation, grinding / mixing and emptying.

- Gastric emptying is achieved through several motility related factors:

- The time for gastric emptying or the half-time (t ½) for emptying, depends upon a number of factors, including amount and composition of the ingested food.

➢ The intake of food begins the processes of

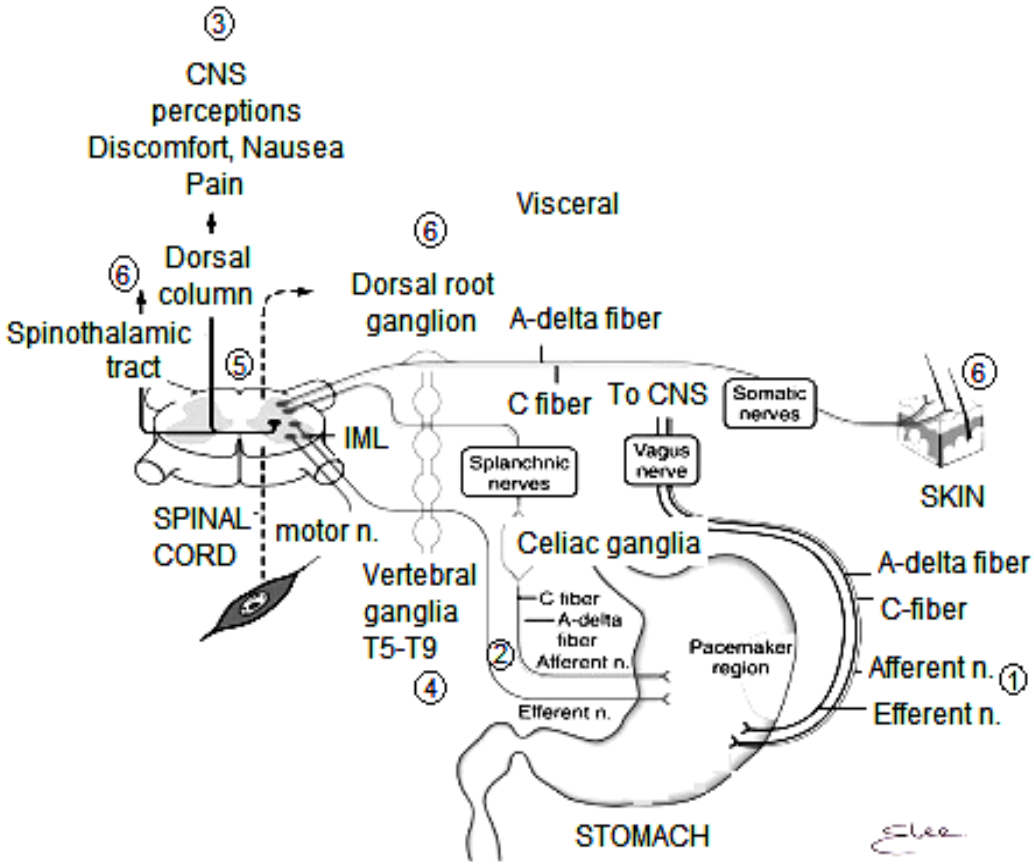
- Release of peptides which act on
  - ENS
  - Vagal function
    - Afferent
    - Efferent
  - ICCs
  - Smooth muscle
- Fundic receptive relaxation
- Body grinding and mixing ("trituration")
- Antral peristalsis
- Antropyloroduodenal coordination
- Emptying of 2 to 4 ml of chime, containing particles < 4 mm, by varying resistance of pylorus and antrum

Neural component

The components which are important for gastric motor function include

- Autonomic nervous system (ANS)
- Enteric nervous system (ENS)
- Interstitial cells of Cajal (ICCs)
- Smooth muscle cells
- Peristaltic reflex

- The afferent vagal neurons and the vagovagal reflexes involve
  - Nucleus of tractus solitarius
  - Vagal dorsal motor nucleus
  - Vagal efferents



Afferent and efferent neural connections between the stomach and central nervous system (CNS)

- 1) Low threshold mechano- and chemoreceptors stimulate visceral sensations such as stomach emptiness or fullness, and symptoms such as nausea and discomfort.
- 2) The vagus nerve contains afferent nerves with A-delta and C- pain fibers with cell bodies in the nodose ganglia with connections to the nucleus tractus solitarius (not shown).
- Changes in gastric electrical rhythm, excess amplitude contractions, or stretch on the gastric wall are peripheral mechanisms that elicit changes in

afferent neural activity (via vagal and/or splanchnic nerves) that may reach consciousness to be perceived as visceral perceptions (symptoms) emanating from the stomach.

- 3) These stimuli are mediated through vagal pathways and become conscious perceptions of visceral sensations if sensory inputs reach the cortex.
- 4) The splanchnic nerves also contain afferent nerves with A-delta and C fibers that synapse in the celiac ganglia with some cells bodies in the vertebral ganglia (T5-T9).
- 5) Interneurons in the white rami in the dorsal horn of the spinal cord cross to the dorsal columns and spinothalamic tracts and ascend to sensory areas of the medulla oblongata.
- These splanchnic afferent fibers are thought to mediate high-threshold stimuli for visceral pain.
- 6) In contrast to visceral sensations, somatic nerves such as from the skin carry sensory information via A-delta and C fibers through the dorsal root ganglia and into the dorsal horn and then through dorsal columns and spinothalamic tracts to cortical areas of somatic representation for somatic pain.

Abbreviations: IML, intermediolateral nucleus; n., nerve.

Printed with permission: Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadephia, 2010 Figure 48-17, page 801.

- **Autonomic nervous system (ANS)**
  - **Parasympathetic nervous system (PNS)** pathways from
    - DMN (dorsal motor nucleus)
    - NA (nucleus ambiguous)
    - TS (tractus solitarius)
  - **Vagus efferents pass to MP (myenteric plexus)** in wall of stomach
  - PNS
    - Stimulatory vagal nerves
    - ↑ gastric contraction
    - ↑ gastric secretion

- **Sympathetic nervous system (SNS) pathways**
  - From IML (intermediolateral columns) in T5 to T10 levels of spinal cord from
    - Celiac ganglia to splanchnic efferents
      - Myenteric ganglia — norepinephrine
    - Splanchnic efferents to
      - Submucous ganglia } norepinephrine and somatostatin
      - Circular muscle
      - Blood vessels — norepinephrine and NPY
      - Pyloric sphincter
      - Non-sphincteric muscle
  - Inhibitory

- **Enteric nervous system (ENS)**
  - Main networks
    - SM submucosal
    - Myenteric
    - Deep muscular
      - Interstitial cells of Cajal (ICC)
      - Plexi involved in pacemaking
      - Muscle propulsion (MMC)
        - Sensation
        - Secretion
  - "Stimulation of excitatory enteric neurons leads to depolarization of IM-ICCs".
  - "The depolarization [of IM-ICCs]……causes positive chronotropic effects on the frequency of gastric slow waves", and also……..increases the contractile response of smooth muscle to slow wave depolarizations……..." (Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, page 793).
  - Thus, the ICC networks provide the control of frequency and propagation velocity for the circular muscle contractions that comprise gastric peristalsis waves" (Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, page 792).

Abbreviations: MMC (aka IMMC), interdigestive migrating motor complex; NPY, neuropeptide Y

- **ICC(interstitial cells of Cajal), aka pacemaker cells),** slow waves, plateau or action potentials
  - ICCs arise from c-kit-positive mesenchymal cell precursors
  - This increased activity of the plateau or action potentials causes the gastric peristaltic contractions.
  - The fundus does not have MY-ICCs or a slow wave, so the fundus does not participate in the linking of slow wave to the plateau or action potentials, and therefore has no role in producing peristaltic contractions
  - The IM-ICCs in the fundus
    - Sensory cells for mechanoreception
    - Innervated by inhibitory vagal neurons which regular tone and receptive relaxation in the fundus
  - Electrode placed on the anterior wall may be used to detect the myoelectrical activity of the slow waves arising from the pacemaker region.
  - This recording of the gastric myoelectrical activity is employed clinically as the EGG (electrogastrogram).
- MMC (migrating myoelectrical [motor] complex)
  - ICCs at the junction of the gastric fundus and body at the proximal part of the greater curve of the stomach have innate activity and spontaneously produce a slow wave (aka pacesetter potential, gastric myoelectrical activity)
  - MMCs originate in the stomach / duodenum and travel to the ileum.
  - MMCs begin and continue through non-vagal mechanisms.
  - MMCs occur in other parts of GI tract besides stomach and duodenum: LES, sphincter of Oddi, gallbladder.
  - MMC I to III recur every 90 to 120 min
  - ICCs present in multiple layers of stomach wall
    - Myenteric (MY-ICCs)
    - Intramuscular (IM-ICCs)
    - Submuscular
    - Subserosal
  - Slow waves from occur at 3 cpm (cycles per minute) move circumferentially and distally at 14 mm/sec towards the antrum
  - MY-ICCs

- When the uptake of the slow wave depolarizes, there is a reduction in the threshold for the circular smooth muscle to contract.
- When the membrane potential reaches the threshold potential, the force of contraction quickly increases (steep slope of the voltage-contraction curve)
- The slow wave becomes linked with the plateau or action potentials.
- "Action potential is superimposed on the plateau potentials in the terminal antrum and pylorus (Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, page 792)
- The action potentials are associated with increased amplitude of the contraction of the smooth muscle.
- Cyclical contractile activity
- Phase 1:
  - Quite; little contractile activity
- Phase 2
  - Random, irregular contractions
- Phase 3:
  - Bursts of "regular, high – amplitude phasic contractions ["activity front"] that last from 5 to 10 min….and migrate from the antrum to the ileum (Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, page 794) in 90 to 120 min.
  - Motilin is of importance to Phase 3 contractions
  - Cyclical contractile activity beginning in phase 3 of MMC seen in stomach and small bowel, as well as,
    - LES (lower esophageal sphincter)
    - SoD (sphincter of Oddi)
    - Gallbladder

- Smooth muscle cells
  - Receptors in the excitable membrane of the smooth muscle cells
    - Muscurinic
    - Central and peripheral dopamine (D2)
    - 5 HT3 / 5 HT4
    - Motilin
    - α-adrenergic
    - Somatostatin

- Phosphodiesterase

- These receptors bind to amines and peptides which reach the smooth muscle cells from neurocrine, paracrine and endocrine pathways
- The excitable membrane of the smooth muscle cells fires spontaneously, causing action potentials that cause the muscle cell to contract
- The muscle cells are joined as a syncytium, which provides electrical coupling of neighboring muscle cells
- The contraction resulting from the spontaneous depolarization, action potential and syncytial coupling cause contraction in the circumferential and longitudinal axes.

- **Electrophysiology of smooth muscle**

Site	RMP, mV	PP/AP
Fundus	-50	-
Effect of inhibitory vag input	Receptive relaxation fundic tone)	
Corpus / antrum		
Effect of Ach or stretch	-60 / -70	↑ amplitude and duration of P occurrence of PP, occurrence AP → low or high-amplitu contractions
Pylorus		

- Electrical barrier between slow wave of distal antrum (3 cpm) a duodenum (12 cpm), i.e., the duodenal slow wave is 12 cpm [contractio per minute].

Abbreviations: AP, action potential; PP, plateau potential; RMP, Resting membrane potential

- **Peristaltic reflex**
  - Responsible for the “law of the intestine”
  - Ascending contraction: stimulus in the lumen, usually from distention by food
    - Transmitters of excitation of smooth muscle
      - Acetylcholine
      - Serotonin acting on 5HT4 receptors on cholinergic interneurons
      - Tachykinins
        - SP (substance P)

- SK (substance K)

- Descending contraction: inhibition of smooth muscle below area of stimulation causes descending inhibition
  - Resistance to the movements of the food bolus which is being pulsed distally into the relaxed area by the ascending contraction and the proximal smooth muscle excitation.
  - Transmitters of inhibition of smooth muscle
    - NO (nitric oxide)
    - VIP (vasoactive intestinal peptide)
    - Somatostatin
    - GABA (gamma-aminobutyric acid)
    - Endogenous opiates
  - Pylorus (antroduodenal junction)
    - 0.6 cm to 1.6 cm long, and maintains an area of high resting pressure
    - 3 times per minute phasic contractions sweep across this antroduodenal junction, emptying particles which are 1 mm to 2 mm in size
    - These phasic pyloric contractions are mediated by NO, Ach and opiates.

- Muscle activity: circular, oblique and longitudinal muscle layers of stomach, which leads to
  - Relaxation of fundus (accommodation)
  - Contraction of antrum (chemical digestion, shearing and pulverization, propulsion, rates pulsion, sieving, emptying of 1 mm to 2 mm particles)

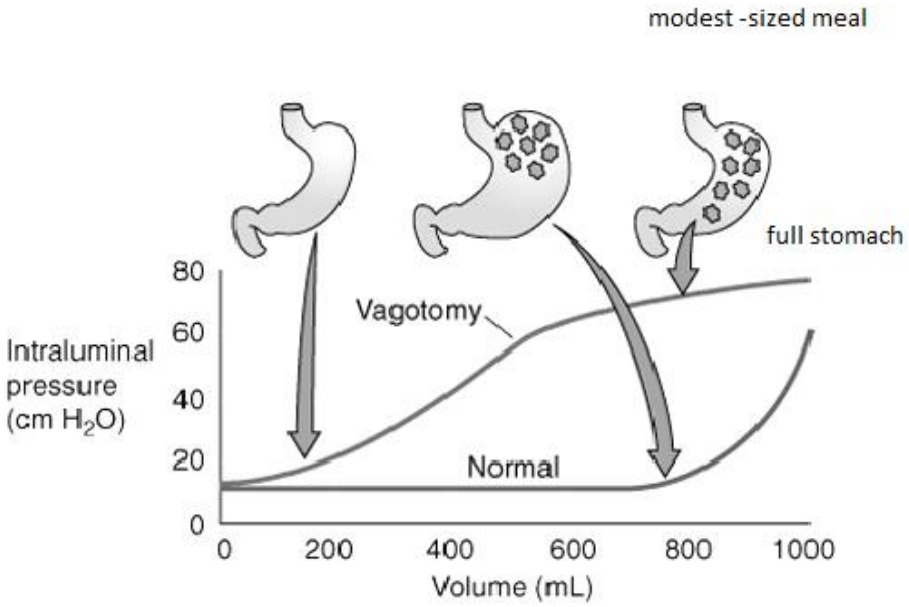
Gastric Accommodation and Receptive Relaxation

- Receptive relaxation
  - Vagus and NO (nitric oxide)
    - Ingested food and fluid stretch the fundus and stimulate mechanoreceptors and chemoreceptors.
    - Secretin is released from enterochromaffin cells.
    - IPANs (intrinsic primary afferent neurons) in the submucosa or myenteric plexus of the stomach.
    - Activated mechano- / chemo-receptors stimulate IM-ICCs.
    - The IM-ICCs activate vagal efferent and vago-vagal reflexes.

- The nucleus of the tractus solitaries, periventricular nucleus and dorsal motor nucleus of the vagus are stimulated.
- There is inhibition of the vagal excitatory neurons.
- NO and VIP (vasoactive intestinal peptide) are released.
- NO and VIP are also inhibitory to the normal tone of smooth muscle of the fundus.
- The end result is for filling of the fundus to result in receptive relaxation.
- This process begins before mixing and grinding in the gastric body.

➢ Other factors

- Distension
  - Antrum
    - Capsaicin-sensitive afferent vagal nerves mediated by
      - $5\text{-}HT_3$ (5-hydroxytryptamine)
      - GRP (gastrin-releasing peptide)
      - Duodenal CCK_A receptors
  - Duodenum
  - Colon
- Perfusion of duodenum
  - Acid
  - Lipid
  - Protein
- Receptive relaxation for liquids is in the fundus, as occurs with solids, as well as in the gastric body and antrum.
- With the intake of food/fluid, the stomach initially stretches, without any increase in its basal intraluminal pressure of about 10 cm H_2O.
- This "accomodation" of intragastric volume is mediated by the afferent and efferent pathways of the vagus nerve.
- Relaxation is effected through stimulation of an inhibitory, nitrergic neuron.
- The inhibitory motor neuron may also be stimulated by Ach and serotonin.



- The mechanoreceptors are also stimulated by
  - IM-ICCs in wall of the fundus
  - Vagovagal reflexes
  - Activated vagal afferent neurons
  - Distention of antrum, duodenum, colon
  - Chemicals in lumen: lipid, protein or H^+ in duodenum
- Gastric enterochromaffin cells release serotonin (5-HT).
- 5-hydroxytryptamine-3 (5-HT3), GRP (gastrin-releasing peptide), and CCK_A receptors act on the capsaicin-sensitive gastric afferent vagal nerves.
- 5-HT activates gastric IPANs (intrinsic primary afferent neurons) in gastric submucosal and myenteric plexus.
- IPANs signal afferent vagal neurons (vasovagal reflex) and also signal reflexes and SNS (sympathetic nervous system).
- Afferent vagal neurons connect to nodose ganglia (cell bodies) and NTC (nucleus tractus solitarius), as well as to ICCs connecting to circular muscle.
- NTC connect to hypothalamus and cortex.

- Pain stimulates splanchnic / spinal primary afferent neurons.
- These primary afferent neurons connect to dorsal horn of spinal cord, and from there to spinothalamic and spinoreticular tracts in dorsal column.
- NO and VIP (vasoactive intestinal peptide) are released and stimulate the vagal inhibitory neurons.
- The vagal excitatory neurons are inhibited
- CCK
  - Released from enteroendocrine cells in duoden mucosa in response to fatty acids (from digest triglycerides) in the duodenum.
  - Activation of CCK receptors → ↑sensation fullness → ↓ food intake
- CCK activates CCK_A receptors on the vagal afferent neurons.
- Vagal afferent neurons ascend to the nucleus tractus solitarius (NTS).
- Nerves from NTS ascend to the periventricular nucleus (PN).
- PN participation leads to satiety.
- Vagal dorsal motor nucleus (DMN-V) connect to descending vagal efferent neurons.
- Descending vagal efferent neurons reduce gastric emptying.
- The net effect of CCK is to inhibit gastric emptying.
- This represents the duodenal “brake”
- PYY (polypeptide-YY)
  - Released from enteroendocrine cells ileocolonic mucosa
  - ↓ gastric emptying and small bowel rate of tran (“ileal brake”)
  - ↓ appetite, ↓ food intake
- CRF (corticotrop releasing factor)
  - Responds to emotional stress
  - Acts through central pathways in periventricu nucleus
    - Central dopamine 1 and 2

- Vasopressin (AVP) pathways

➢ CRF and AVP

- As part of the stress response gastric emptying slows from
  - CRF (corticotropin-releasing factor)
  - AVP (vasopressin) acts on pathways in PN
  - Acts through central dopamine 1 and 2.

- SCF (stem c
  - ↑ BS (increased blood sugar), i.e. hyperglycem → ↓ SCF
  - ↓ SCF → ↓ ICCs and ↓ contraction of smoc muscle

factor)

Antral Filling and Pyloric Pump

- Give the physiological function of the antrum and the pylorus.

  - The food contents of the fundus are mixed with saliva, gastric acid and pepsin.

  - The body and antral peristaltic waves grind the food into 1- to 2- mm solid particles (titurition).

  - The period of grinding of solid food into small particles before emptying begins the lag period.

  - Antral peristalsis occurs at the rate of 3 per minute.

  - With co-ordinated relaxation of pyrolus, 2-4 ml of chyme containing the 1- to 2- mm solid particles are emptied through the pylorus, usually in little pulsatile, “squirts”.

  - The amount of material emptied across the pyrolus (“stroke volume”) is set not by volume but by calories.

  - The rate of caloric emptying is 3 to 4 kcal/min.

  - This caloric emptying rate, and the rate of emptying of the volume which contains the calories, is determined by

- The amplitude (normally 10 to 40 mm Hg) and length of the peristaltic contraction
  - Intragastric perssure
  - The pressure gradient across the pyloric sphincter
- Some gastric peristaltic contractions may push chyme into the antrum, and then stop.
- Other peristaltic contractions continue to the pyrolus.
- If the pylorus is closed, the chyme pushed as far as the pyrolus will return to the gastric body for more grinding (mixing, titurition).

Food-related factors

- Response to Ingestion of Liquids
  - Receptive relaxation for liquids is in the fundus, as occurs with solids, as well as in the gastric body and antrum.
  - Liquids are emptied faster than solids
    - Liquids do not require grinding before passing through the pylorus.
  - Water empties faster than calorie-dense liquids
- Response to ingestion of solid food
  - With the intake of food/fluid, the stomach initially stretches, without any increase in its basal intraluminal pressure of about 10 cm H_2O.
  - This “accomodation” of intragastric volume is mediated by the afferent and efferent pathways of the vagus nerve.
  - Relaxation is effected through stimulation of an inhibitory, nitrergic neuron.
  - The inhibitory motor neuron may also be stimulated by Ach and serotonin.
  - When the volume of the stomach distends beyond the ability of the stomach to accommodate (usually about 800 ml), the intragastric pressure rises, and the person may begin to feel “full”.

➢ Body contractions

- Give the key physiological factors involved in gastric motor activity.

- Neurons of the ENS (enteric nervous system) are close to MY-ICCs and IM-ICCs.
- Excitatory neurotransmitters include Ach (acetylcholine) and substance P (SP).
- Inhibitory neurotransmitters include NO (nitric oxide) and VIP (vasoactive intestinal polypeptide).

- The spread of the slow wave and the smooth muscle contraction is integrated by IM-ICCs.
- IM-ICCs are electrically coupled by way of gap junctions to the smooth muscle cells.
- The MY-ICCs produce the slow electrical waves which depolarize the smooth muscle membrane depolarization.
- IM-ICCs slow wave activates the voltage-dependent L-type calcium channels in the smooth muscle cell membrane
- The depolarization which occurs during the upstroke of the slow wave redepolarizes to the value of the RMP, and the contraction ends.
- MY-ICCs initiate, and IM-ICCs mediate neurotransmission: they
  - Integrate slow wave activity and smooth muscle activity, and
  - Coordinate the spread of slow waves.
- Relaxation of the fundus and proximal portion of the gastric body is replaced by contractions of the fundus and proximal corpus.
- This pushes food into the rest of the body and antrum, where mixing and grinding occur.
- This initial postprandial interval of receptive relaxation, mixing and grinding, is called the “lag phase”.
- The lag phase duration depends on the composition of the meal (usually about 45 to 60 min).

➢ Emptying

- Trituration of solid food into particles 1 to 2 mm in size begins the linear phase of gastric emptying.
- The gastric peristaltic waves arise from the electrical activity of ICC, the plateau and, action potentials.
- These peristaltic waves sweep waves through the body of the stomach at 3 cpm (cycles per min).
- The clearance of the pyloric sphincter and the contraction of the duodenum present the emptying of the particles > 2 mm.
- The antral contraction waves pump, pulsatile “squirts” of food and fluid into duodenum.
- Depending on the strength of contraction (10 to 40 mm Hg) and duration of antral peristaltic wave, and the resistance provided by pyloric sphincter

and duodenal contraction, usually 3 to 4 kcal/min is emptied into the duodenum.

- About 50% of a meal will be emptied in 90 min ($T_{1/2}$ of gastric empting), and 95% has been emptied by 4 hours.

- Non-caloric and then caloric liquids are emptied first without a lag phase and in a "mono-exponential emptying", pattern, then digestible material during the linear phase as a result of lower amplitude pressure waves.
- The rate of gastric emptying may be modified by the amount and composition of the ingested food and fluids.
  - Volume
  - Viscosity
  - Osmolarity
  - Nutrient density
  - For further details, please refer to Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, Table 48.1, page 800.
  - The ICCs in the deep muscular plexus junction in conjugation with the ENS (enteric nervous system)
  - The ICCs in the myenteric plexus do not depend on the ENS.
  - ICCs in the pylorus are associated with inhibitory neural activity in this region, and thereby are important in the context of the rate of gastric emptying.

- Give the key physiological factors involved in the modulation of rate of gastric emptying.

  - Vagal and splanchnic nerve activities modulate the neuromuscular activities of the stomach.

  - Vagal stimulates and splanchnic inhibits

  - The balance between excitatory and inhibitory nerves to the stomach leads to slower or faster gastric emptying.

  - There is slow wave gastric myoelectrical activity during fasting.

  - GI peptides are involved in gastric emptying.

  - In the postprandial period there is summation of the slow wave activity linked to plateau and action potential activity.

  - Only a large meal, hypoglycemia and reduced fundic accommodation (relaxation) speed the emptying of the stomach (reduced $T_{1/2}$ of emptying).

- All other gastroduodenal, ileal and colonic neuromuscular factors, as well as meal-related factors, delay gastric emptying (longer $T_{1/2}$).
- Gastric emptying may be slowed by either bradygastria (1 cpm [cycle per minute]) of low- or high- amplitude or by tachygastria (6 cpm), where the gastric dysrhythmia is not coordinated with opening of the pylorus.

- Give 20 factors which alternate the rate of gastric emptying.

➢ Gastric emptying is influenced by

- Food
  - Calorie density
  - Viscosity
  - Acidity
  - Contents of fat > protein > carbohydrate
  - Fatty acids in duodenum or ileum
  - Osmolality
  - Fiber
  - Tryptophan
  - Volume
- Motility-related factors
- Hormones

➢ The Motility-related Factors

- Fundic accomodation
- Antral peristaltic contraction characteristics
- The pressure gradient between stomach and duodenum
- The degree of anthropyloroduodenal coordination
- Pylorospasm
- Presence of dysmotility (e.g., tachygastria)
- The amount of duodenogastric reflex

- Give the hormone related factors which modify gastric emptying.
    - Peptides CCK, CCK_A receptors, GLP-1, PYY, CRF, SCF, dopamine, and GLP-1.

➢ GLP-1 (glucagon-like polypeptide -1)

- GLP-1 is released in proportional response to hyperglycemia.
- Hyperglycemia releases insulin (incretin effects of GLP-1), which causes
    - ↓ antral contractions
    - ↑ gastric dysrhythmias
    - ↓ sensation of fullness from fundic distention
    - ↓ gastric emptying
- Hypoglycemia and GLP-1 or hyperglycemia (> 220 mg/dL)
    - ↑ dysrhythmias
    - ↑ contractility
    - ↑ emptying
    - ↑ dysrhythmias

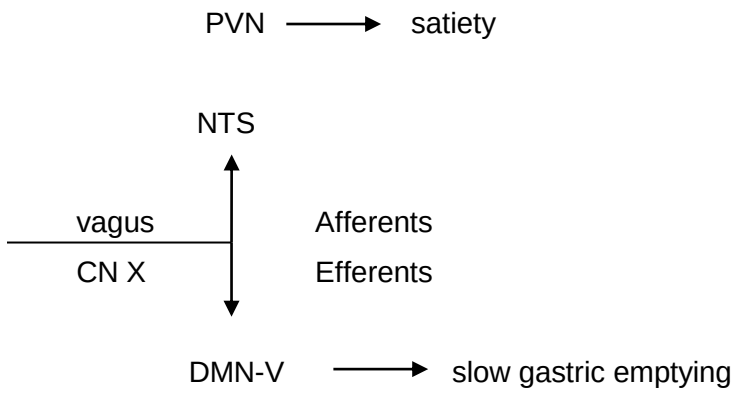
➢ CCK and the “Ileal Break”

- Fatty acids in the ileum stimulate an enterogastric reflex (the “ileal break”) to slow gastric emptying.
- The ileal break involves PYY, CCK and GLP-1.
- Duodenal mucosal receptors for FA (fatty acids), AA (amino acids) and carbohydrates limit the rate of gastric emptying to ~ 150 kcal/hr.
- Fat in the ileum slows small bowel emptying through the release of GLP-1 and -2, as well as PYY (peptide YY).

➢ BMI (body mass index)

- As BMI increases, gastric emptying becomes faster
- Medium- and long- chain fatty acids (FA) release CCK.

- CCK slows gastric emptying
  - ↓ fundic tone
  - ↓ antral contraction
  - ↑ pyloric tone



Abbreviations: DMN-V, dorsal motor nucleus of the vagus; NTS, nucleus tractus solitaries; PVN, paraventricular nucleus

- Usual causes related to impaired motility (gastroparesis) and mechanical obstruction
- Sometimes the term “gastroparesis” will be used interchangeably with delayed gastric emptying, and then implies a process rather than mechanism.

Clinical Tests of Gastric Emptying

- Usual causes relaed to impaired motility (gastroparesis) and mechanical obstruction
- Sometimes the term “gastroparesis” will be used interchangeably with delayed gastric emptying.

- Give 8 clinical tests of gastric emptying.

➢ Emptying
  - Scintigraphy
  - Capsule technology
  - Breath tests

- Volume recovery tests
- Ultrasonography
- CT/MRI technology

➢ Antral contractions
- Antroduodenal manometry
- Capsule technology

➢ Myoelectrical activity
- EGG (electrogastrography)
  - Tachygastrias (> 3.7 cpm)
  - Bradygastrias (< 2.5 cpm)

➢ Gastric relaxation
- Barostat
  - Measurement of intragastric tone and volume (not pressure)
- SPECT – single photon emission CT
  - Scintigraphy, ultrasonography, MRI

- Give 10 pathophysiological processes involved in the gastroparesis associated with type I diabetes.

➢ Abnormal function of the fundus
- In response to distention of fundus phasic contraction, ↓ accommodation due to failure of recovery of ↓ tone of fundus during the fasting (postprandial) period,
- Possible defect in the NO pathway, ↓ (50%) of IMMC during fasting associated with antral contraction and the clearance of undigested solids > 1 mm to 2 mm.

➢ Abnormal function of the antrum
- ↑ lag time for emptying of stomach
- ↓ frequency of distal antral contractions (< 1 per minute), causing hypomotility
- ↑ IPPN (isolated pyloric pressure waves, “pylorospasm”)
- ↓ proximal gastric contraction, with failure of food still in stomach to be redistributed, again entering the antrum for trituration and emptying.

- Abnormal electrical slow wave rhythms, possible from ↑ prostaglandins
  - Brachygastria
  - Tachygastria
  - Mixture of brachygastria and tachygastria
- Vagal dysfunction, e.g. autonomic neuropathy, hyperglycemia in type 1 diabetes
- ↓ ICC in deep muscle plexus, e.g. in diabetes possible due to ↓ insulin and IGF-1 → smooth muscle cell produces ↓ stem cell factor
- ↑ oxidative stress (as in diabetes)
- ↓ inhibitory nitric oxide containing neurosis
- ↑ phosphodiesterase -5 activity
- Associated neuropathic or myopathic abnormalities of small bowel motility

CLINICAL PHYSIOLOGICAL CHALLENGE – Gastroparesis

Case: A 29 year-old diabetic lady develops bloating and fullness after meal. She is diagnosed with possible gastroparesis, and is referred to you for management. You recommend alterations in her diet, and explain her choices of prokinetic drugs.

- Give the pathophysiology of gastroparesis (not just in this patient).
- Give 6 tests of gastric function which might be used in this patient to document the suspected presence of gastroparesis.
- Give the basis for the dietary recommendations for gastroparesis.
- Give the receptors on gastric smooth muscle which may be targeted for her pharmaceutical treatment.

- Give the pathophysiological defects leading to symptoms and pharmaceutical approaches in diabetic gastroparesis, and describe the pharmaceutical approaches used to control these symptoms.

Symptoms	Pathophysiology	Pharmaceutical approaches
➢ Early satiety a postprandial fullness	○ Defective accommodati from ↓ in NOS neurons	– NO donors
➢ Nausea a vomiting	○ Gastroparesis secondary enteric nervous syste neuropathy ○ Abnormal pacemaker activi	– Prokinetic agents – Gastric – ↓ pacing
➢ Epigastric pain	○ Sensory neuropathy in gast wall	– Tricyclic antidepressants – Neurostimulation
➢ Persistent nausea	○ Tachygastria / bradygast from abnormal pacemak activity	– Control of blo glucose – Domperidone

Abbreviations: NO, nitric oxide; NOS, nitric oxide synthethase

Adapted from: Owyang C. *Gastroenterology* 2011;141:136

- Give the **treatment** of delayed gastric emptying.
  - ➢ Nutrition (diet)
    - ○ Low fat diet
    - ○ Low non-digestible fiber
    - ○ Frequent, small meals
    - ○ Liquid meals (including enteral nutritic po / PEJ (percutaneous endoscopica placed jejunostomy tube)
  - ➢ Complications to be corrected
    - ○ Dehydration
    - ○ Hypokalemia
    - ○ Metabolic alkalosis
    - ○ Hyperglycemia (in a diabetic)
  - ➢ Prokinetic drug

- Give the mechanism (s) of action of 6 prokinetic drugs used for the treatment symptoms of gastroparesis.
  - Metoclopramide
    - Central/peripheral dopamine receptor antagonist (D_2)
    - 5-HT3 receptor antagonist
    - 5-HT4 receptor agonist
  - Domperidone
    - Peripheral D_2 antagonist
  - Cisapride
    - Muscarinic (acetylcholine) receptor agonist
    - -5-HT3 receptor antagonist
    - -5-HT4 receptor agonist
  - Ondansetron
    - -5-HT3 receptor antagonist
  - Erythromycin
    - Motilin receptor agonist
  - Tegaserod
    - Cholinergic 5-HT4 partial agonist
  - Bethanechol
    - Muscarinic receptor agonist
  - Anticholinergic (buscopa
    - Muscarinic receptor antagonist

    for tachygastria)
  - α-adrenergic antagonists
    - α-adrenergic antagonist
  - Botulism toxin injection
    - Acetylcholine esterase inhibitor
  - Octreotide injection
    - Phosphodiesterase inhibitors
    - Somatostatin receptor agonist

Adapted from: Quigley EMM. *Sleisenger & Fordtran's gastrointestinal and li* *disease: Pathophysiology/Diagnosis/Management* 2006: pg. 1007; and 2010, pg. 81

- Endoscopic therapy
  - Decompression with enterostomy tube
- Gastric electrical stimulation ("humanitarian use device")
- Surgery
  - Decompression
    - Venting gastrostomy
    - Jejunostomy
  - Conversion
    - Previous partial gastrectomy → subtotal gastrectomy or near-total gastrectomy and Roux-en-Y gastrojejunostomy

Vomiting

- GI
  - The initial event leading to vomiting is the loss of intestinal slow-wave activity.
  - The loss of this slow wave activity is linked to propulsive peristaltic contractions.
  - The normal peristaltic contractions of the stomach and small intestine decline, and are replaced by retrograde contractions, beginning in the ileum and progressing upwards towards the stomach.
  - These retrograde contractions are accompanied by contraction of the external intercostal muscles and the diaphragm against a closed glottis.
  - The Valsalva-like action increases intra-abdominal pressure.
  - The diaphragmatic crural muscle and lower esophageal sphincter relax
  - The increase in intra-abdominal pressure forces the gastric contents up into the esophagus.
  - The larynx moves upward and forward, and UES (upper esophageal sphincter) relaxes.
  - Gastric contents are vomited.
  - The closed glottis protects the airway and prevents aspiration.

- Give 10 non-pharmaceutical maneuvers for the treatment of nausea and vomiting during pregnancy.

- Avoidance of precipitating factors
  - Meal Factors
    - Small, frequent, fluid, neutral pH and temperature, isotonic, low energy density, low fat meals
    - Certain amino acids (e.g. L-tryptophan – [cheese])
    - Avoid offending foods and beverages
    - Vitamin B6 (thiamine) (FDA A)
    - Ginger
    - Soda crackers (unproven benefit)
    - Avoid offending foods/beverages
    - Frequent, small meals, low in fat

- Stimulation of P6 acupuncture point

- Treat other factors
  - Underlying disease/ condition causing/ aggravating gastroparesis
  - Rectal/colonic distention
  - Pregnancy
  - Ascites
  - Hyperglycemia
  - Avoid circular vectoral motion
  - Avoid medications which may relax smooth muscle and thereby aggravate gastroparesis

- Gastric electrical stimulation

- Treat complications
  - GERD, esophagitis
  - Dehydration, electrolyte disturbances
  - Malnutrition

- Miscellaneous
  - Acupuncture – "P6" acupuncture point
  - Gastric electrical stimulation

Adapted from: Quigley EMM. *Sleisenger & Fordtran's gastrointestinal and liver disease: Pathophysiology/Diagnosis/Management* 2006: pg. 1007; Keller J, et al. *Nat Clin Pract Gastroenterol Hepatol* 2008; 5(8): 433.

H. Pylori Infection

- About 50% of the world's population have an H. pylori infection, but only about 15% develop peptic (gastric or duodenal) when disease, and ~1% develop gastric cancer or MALT lymphoma.
- So, there is a disconnect between H. pylori infection in the stomach, and H. pylori-associated diseases

SO YOU WANT TO BE A GASTROENTEROLOGIST!

SST (somatostatin and gastrin are produced with the gastric antrum, and gastric acid is secreted by the parietal cells in the gastric body.

- Give the explanation why gastric acid secretion falls with acute or chronic H. pylori pangastritis, yet why gastric acid secretion rises with antral gastritis.

➢ Acute or chronic H. pylori pangastritis
  - H. pylori
    - Directly
      - ↓ gene expression of parietal cell H^+ / K^+ -ATPase α-subunit)
      - ↓ gastrin release
      - ↓ duodenal HCO_3^-
    - Indirectly
      - ↑ anti-secretory cytokines IL-1β and TNF-α
      - ↑ CGRP sensory neurons → ↑ SST (somatostatin) → ↓ gastrin
      - ↓ HCl secretion from body parietal cells

➢ Chronic H. pylori antral gastritis
  - ↓ SST →
    - ↑ proinflammatory / prosecretory cytokines
    - ↑ prosecretory H_3 agonist (N^3-methyl histamine)
  - ↑ gastrin (from ↑ SST, and from ↑ IL-8 and PAF
  - ↑ gastrin → ↑ ECL cells in fundus / body → ECL hyperplasia
  - ECL hyperplasia → ↑ HCL secretion from parietal cell antibodies

- Give 10 bacterial factors and 10 host factors which are important in the H. pylori-associated development of peptic ulcer, lymphoma and gastric cancer.

➢ The H. pylori organism

- Adhesion / colonization
  - The genetically 'distinct strains'
  - Bacterial genes encoding proteins in the motility apparatus of H. pylori (movement of organism from lumen of stomach, into the mucus layer) and genes for urease (provides for 2 pH optimum values of 3.0 and 7.2).
  - Colonization occurs only in gastric epithelium or where there is gastric metaplasia.
  - O-glycans in deeper portions of the glandular mucosa present colonization
  - SLPI (secretory leucocyte protease inhibitor) produced by H. pylori reduces colonization.
  - F3 ab A, a bacterial gene product may be liquid for the host Lewis (Le) b receptor or MUC 5AC, enhancing colonization.
  - H. pylori urease binds to MHC (major histocompatibility) class II molecules to ↑ apoptosis.
  - TFF_1 (trefoil protein) on gastric epithelial cells and mucus bind H. pylori
  - TLRs (Toll-like receptors) in the PAMPS (pathogen-associated molecular receptors) family recognize H. pylori or their bacterial products which recognize LPS (lipopolysaccharide) or flagellin.
  - Cag PAI (cag pathogenicity island) is a segment of H. pylori DNA which provides cag E (type N secretion apparatus)
  - Cag PAI
    - Allow host gastric cellular membrane translocation
    - ↑ IL-8 expression
    - SRC kinase s phosphorylate tyrosine in Cag A protein
  - All H. pylori have the vac A gene, and half produce the protein, Vac A (vacuolating cytotoxin)
  - Vac A is a ligand for the cell membrane receptor, protein-tyrosine phosphatase.

- The Host
  - Intensity of host inflammatory response
    - Oip A (outer inflammatory protein A)
    - ↑ IL-8 → ↑ neutrophil infiltration
    - Peptidoglycan, acting by type IV secretion system
    - HP-NAP (H. pylori neutrophil-activating protein)
      - ↑ chemotaxis of neutrophils, monocytes
      - ↑ ROIs (reactive oxygen intermediates)
    - ↑ recruitment of neutrophils and macrophages → ↑ iNOS (inducible nitric oxide synthase)
  - Host immune response
    - Polymorphism for IL-1β → ↑ IL-1 → intense mucosal inflammation (gastritis)
    - IL-8, IL-10, TNF-α (tumor necrosis factor) also ↑ gastritis
    - Morphological changes in gastric epithelial cells
      - TJ (tight junctions) complexes become broken
      - ↑ epithelial cell proliferation and apoptosis
    - ↑ gene expression
    - Inflammatory cytokine
      - ↑ signaling mechanism for gene expression
    - ↑ MAP (mitogen-activated protein) kinases
    - ↑ redox factor-1
    - ↑ activity of NFk-B (nuclear factor kappa B)
    - ↑ activity of AP-1 (activator protein-1)
    - ↑ oxidative stress
      - ↑ oxidation of DNA
    - NOD_1 (nucleotide-binding oligomerization domain-1) in host
      - Sense H. pylori
      - ↑ NFk-B
    - GI physiology
      - SST (somatostatin), gastrin effects on secretion of gastric HCl and duodenal HCO_3^-
      - Mucus - ↓ volume
      - ↓ mucosal hydrophobicity
    - TNF-α and IFN-γ (interferon-gamma)
      - ↑ effects of HP-NAP (H. pylori neutrophil-activating protein)
      - Prime neutrophils
    - ↑ phagocytosis of H. pylori infected gastric epithelial cells

- ↑ chemokines (e.g. ENA-78 GRO-α activate neutrophils)
- ↑ cytokine induction
  - TNF-α
  - IL-6, IL-12, IL-17, IL-18
  - Heat shock protein 60
- T cell
  - ↓ IL-4 activation of STAT6 → ↑ Th1 and ↓ Th2 response
  - ↑ Th1 response
  - ↑ apoptosis
  - ↑inflammation
  - ↑ atrophy
  - ↑ dysplasia
- ↑ IL-1β, IFN-α, TNF-α
  - ↑ Fas antigen expression → Fas-FasL (ligand) interactions → ↑ gastric epithelial cell death by apoptosis
    ↓ NFAT (↓ nuclear translocation of a transcription factor) → ↓ IL-2
- Dysregulation of Treg (regulatory T cells)
- ↑ IgA, IgA, IgM and complement
- ↑ monoclonal antibodies to H. pylori cross-react with gastric epithelial cells
- Innate host responses impaired by catalase, urease

- Give the postulated mechanisms by which H. pylori causes DU (duodenal ulcer disease).
  - Antrum D cells ↓ - ↓ S - ↓ inhibition of G cells - ↑ G - ↑ HCl - ↑ metaplasia – H. pylori infection develops in metaplasia of the duodenum.
- Give the reason why the rate of symptom relief with Hp-triple therapy lower in functional dyspepsia where there has been an EGD,, than in uninvestigated dyspepsia (UD).
  - In UD, there are patients who have peptic ulcer disease (GU, DU) who respond well to eradication of H. pylori, plus those with only H. pylori-associated gastritis, any symptoms of which do not respond well to Hp eradication.
  - In patients who have had an EGD and an ulcer is found, their symptom response to H. pylori eradication is higher than in those infected with H. pylori but in whom there is no ulcer.

SO YOU WANT TO BE A GASTROENTEROLOGIST!

- H. pylori has been declared by the WHO (World Health Organization) to be carcinogen, with an attributable risk for gastric cancer (GCa) of ~60%.
- In addition to the dietary and life style factors, molecular factors represent a genetic basis for non-H.pylori GCa
  - Cytokines [IL-1β, polymorphisms of TNF-α and IL-10), and
  - TLR (toll-like receptors, especially TLR-2)

- Give 5 molecular factors which increase the risk of carcinogenesis from H. pylori.

There are a number of molecular factors which may increase the risk of developing GCa from an H. pylori infection:

- Motility
  - Proteins (Fla A, Fla B) providing for spiral movement of H. pylori

- Buffering of gastric acid (↓ H^+ secretion)
  - Urease gene cluster (Ure A, Ure B)

- Adhesion
  - Hop protein (outer membrane proteins)
  - Adhesion
    - Bab A (encoded by gene Bab A_2
    - Bab A binds to blood group antigen Lewis B

- Cag pathogenicity island
  - Greater (or 2 to 28) risk of GCa with Cag A^+ H. pylori

- Molecular needles
  - TFSS (type 4 secretion system) enhances movement of Cag A^+ bacterial protein into gastric epithelial cells

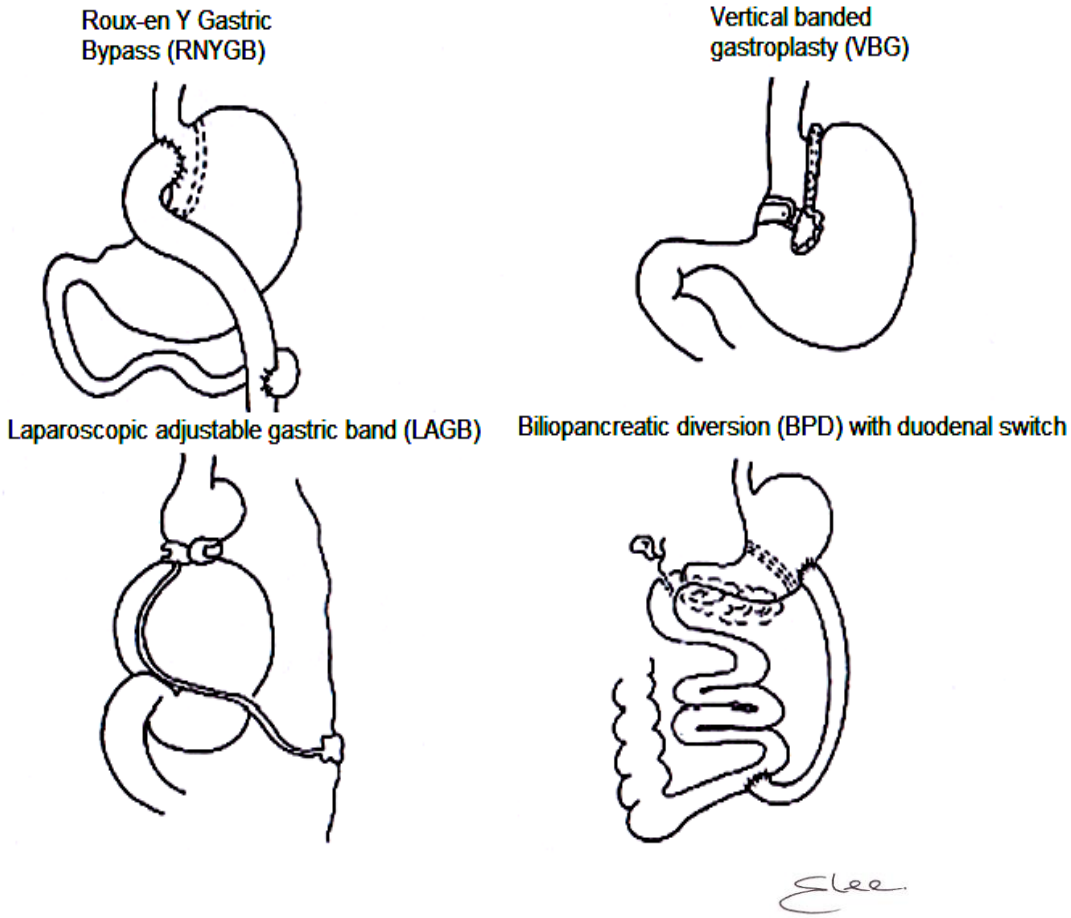
- Vacuolation
  - Vac A protein, a pore-forming vacuolating toxin, especially in the presence of Cag A^+ H. pylori strains, reduce the activation of T cells, and increase the risk of GCa.

Gastric Surgery

- Resection of a portion of the stomach is still performed in some persons with complicated peptic ulcer disease, for gastric cancer, or for weight reduction (bariatric surgery).

- While all types of gastric surgery are prone to the development of late complications, these are more prevalent, if there is an afferent loop, such as with a Billroth II procedure (antrectomy, vagotomy and afferent loop), or a Roux-en-Y hepaticojejunostomy.

- Draw the main types of bariatric surgery used for weight loss.



Adapted from: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* Figure 7-1, Page 116, Ninth Edition, 2010.

- Types of bariatric surgery
  - Restriction procedures
    - VBG (vertical banded gastroplasty)
    - LAGB (laparoscopic adjustable gastric banding)
    - SE (sleeve gastrectomy)
  - Malabsorption-producing procedures
    - BPD (biliopancreatic diversion)
    - BPD / DS (BPD plus duodenal switch)
  - Mixed procedures
    - RYGB (Roux-en-Y gastric bypass)
- Post-operative complications
  - The post-operative complications of bariatric surgery are often subdivided into 3 phases
    - 1 to 6 weeks
    - 7 to 12 weeks
    - 13 to 52 weeks

- Give the pathophysiological basis of 3 late complications of gastric surgery, and their suggested endoscopic treatment.

- Stoma
  - Aim for stomal diameter ~ 12 mm
  - Objective is to reduce / eliminate symptoms e.g. slowing of expected weight loss from surgery; during syndrome)
  - Narrowing
    - Symptomatic stomal stenosis
    - Bougie dilators or balloons, using guide wire TTS (through-the-scope placement)
    - Optimal aperture diameter 10 mm to 15 mm
    - If multiple dilators needed, dilate slowly (up to 3 mm [3 French sizes] at each session, every 1 to 2 weeks until symptoms resolve
  - Reduction in size of enlarged stomas and pouches
    - Use sclerosants, or suturing device
- Leaks
  - Fistulae, dehiscence of staple line, gastric leaks
    - Tissue apposition
      - Fibrin glue
      - Biomaterial (from pig intestine)
    - Stents

        - SEPS (self-expanding plastic stents)
        - SEMS (self-expanding esophageal metal stents)

- Band erosion
    - Nd:YAG laser
    - Scissors
    - Cutters (endoscopic)
- Marginal ulcer (MU) bleeding
    - Usually on jejunal side of gastrojejunal anastomosis
    - Early MU in 10%, late in 1%
    - Risk of MU associated with smoking, H. pylori infection, NSAIDs use
    - If bleeding occurs early after surgery, perform EGD in OR

- Name 3 bariatric procedures, and list 3 complications for each, and give 5 complications common to all bariatric surgical procedures.

- Specific procedures
    - Gastric bypass (Roux-en-Y)
        - Anastomotic leak with peritonitis
        - Stomal stenosis
        - Marginal ulcers (ischemia)
        - Staple line disruption
        - Internal and incisional hernias
        - Nutrient deficiencies (usually iron, calcium, folic acid, vitamin B12)
        - Dumping syndrome
    - Gastroplasty
        - GERD
        - Stomal stenosis
        - Staple line disruption
        - Band erosion
    - Gastric banding
        - Band slippage
        - Erosion
        - Esophageal dilation
        - Band infections
    - Biliopancreatic diversion
        - Anastomotic leak with peritonitis
        - Protein-energy malnutrition
        - Vitamin and mineral deficiencies
        - Dehydration

- Give 10 **late complications** common to all bariatric surgical procedures.
  - CNS
    - Psychiatric disturbance
  - Lung
    - Atelectasis and pneumonia
    - Deep vein thrombosis
    - Pulmonary embolism
  - GI
    - Anemia
    - Diarrhea
    - Ulceration
    - GI bleeding
    - Stenosis
    - Gallstones
  - Metabolic
    - Bone disease
    - Too rapid weight loss
  - Surgical
    - Wound infection
    - Failure to lose weight
    - Mortality (0.5-1%)

Abbreviation: CNS, central nervous system

Adapted from: Klein S. *2006 AGA Institute Post Graduate Course*: pg. 175.

CLINICAL PHYSIOLOGICAL CHALLENGE - Malabsorption

Case: A 65 year old man had a Billroth II gastric resection 25 years ago for failed ulcer healing when treated with an H_2 – receptor antagonist. He presents to you now with anemia and diarrhea. The stools are malodorous, and float.

- Give the mechanisms for the digestion of iron- and vitamin B12 – containing foods.
- Give how these normal processes are deranged after gastric surgery?
- Give the pathophysiology of the diarrhea and steatorrhea which may arises as a result of gastric surgery.

Gastric Cancer

- Diffuse-type gastric cancer (GCa) tends to be more closely related to gene factors, than is the case for intestinal-type GCa, which is linked more close to dietary and environmental factors.

See Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liv Disease.* 9th Edition. *Saunders/Elsevier*, Philadelphia, 2010, Table 54.3, page 89 for a detailed list of genetic abnormalities in gastric adenocarcinoma (GCa).

- Give 8 examples of the common genetic abnormalities in gastric cancer (GCa

The common (~ ≥ 40% gene frequency) genetic abnormalities in gast adenocarcinoma are

- Gene deletion / suppression
  - TP 53
  - FHIT (fragile histidine triad gene)
  - APC (adenomatous polyposis coli) gene LOH (loss of heterogeneity
  - DCC (deleted in colorectal cancer) gene LOH

- ↓ gene expression (due to epigenetic promoter hypermethylation)
  - p16 (marker for poor differentiation)
  - TFF_1 (human trefoil factor 1)
  - $RUNX_3$ (Runt-related transcription factor 3)
  - p27 (associated with a poor prognosis)
  - CDH_1
    - Encodes for E-cadherin (acts as a tumor suppressor gene)
    - Seen in HDGC (hereditary diffuse gastric cancer)
  - MLH_1 and MLH_2 (human mut L homolog 1, MSI [microsatellite instabil phenotype)

- ↑ gene expression (over amplification)
  - COX-2 (cyclooxygenase-2)
  - HGF (hepatocyte growth factor)
  - VEGF (vascular endothelial growth factor)
  - c-Met
  - AIB-1 9amplified in breast cancer)

- DNA aneuploidy
- Mutations
  - PIC3A (encodes for a catalytic subunit of PI3K (phosphatidylinositol

kinase)
- PTPRT (protein-tyrosine phosphatase receptor-type)
- Beta-catenin
- EGF / EGFR (epidermal growth factor / epidermal growth factor receptor)

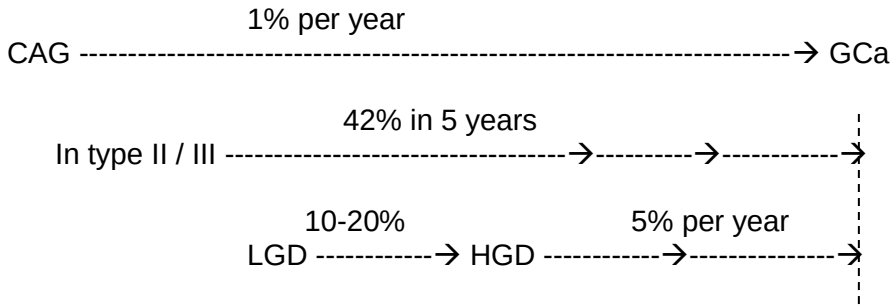
- Microsatellite instability

Note that there are other genetic abnormalities in GCa which are less common (approximately gene frequency, 15% to 25%):

Persons with gastric cancer have a 5 year survival rate of only about 25%. In a sense, H. pylori infection may be considered a premalignant condition, and treatment of this infection either early in life (before "point of no return") or in persons who have had EMR (endoscopic mucosal resection) for early gastric cancer may reduce the risk of development or redevelopment of GCa.

- Give 7 premalignant conditions for GCa.
  - Chronic atrophic gastritis (CAG)
  - Risk of progression of chronic atrophic gastritis to GCa
    - 1% per year
    - Depends on the extend of CAG
    - MAG → corporal atrophic gastritis

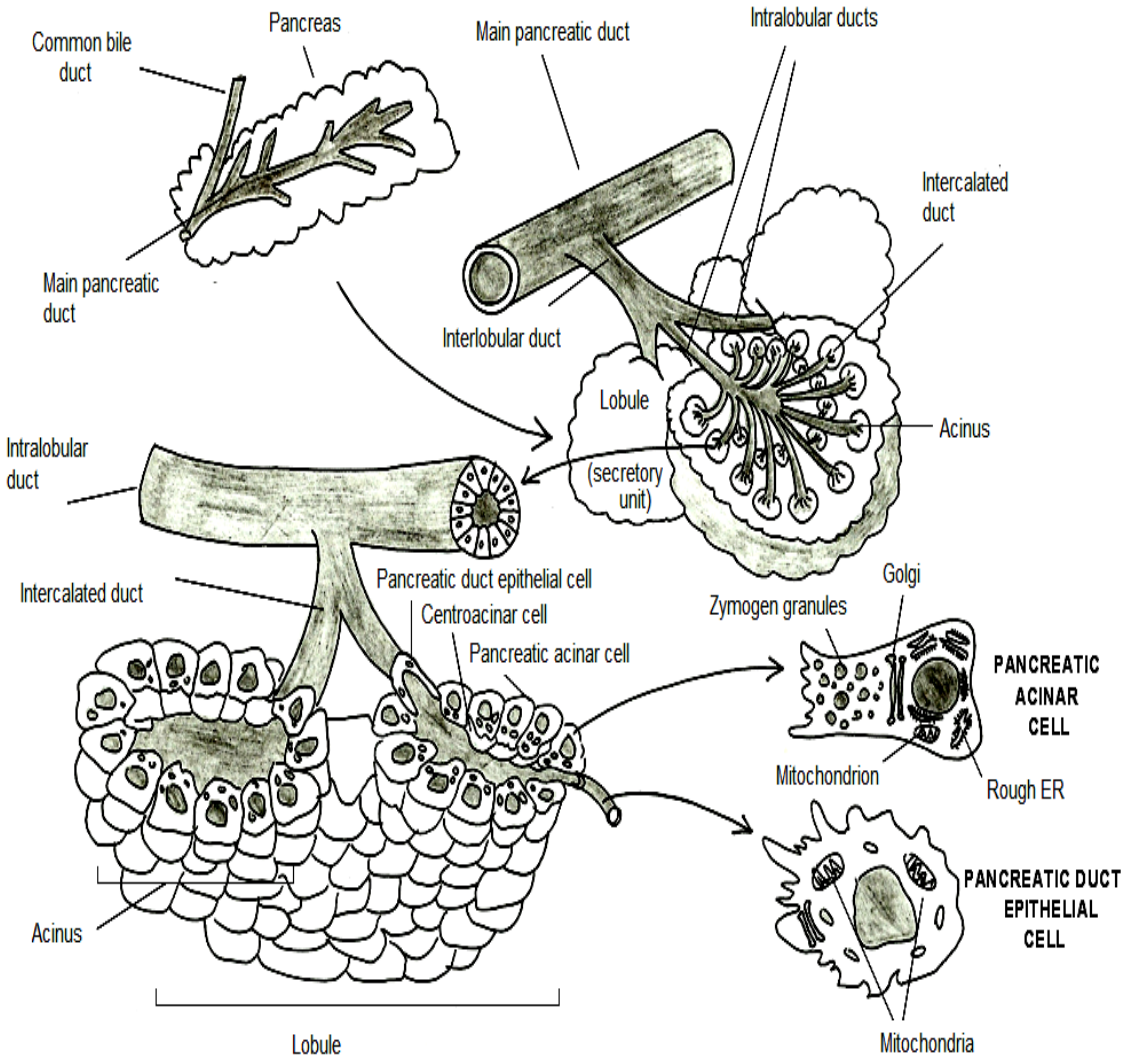
  Progression of stages of chronic active gastritis to gastric adenocarcinoma (GCa)



- Production of pepsinogens (PG) from oxyntic cells
  - ↓ PGI
  - PG II, normal
  - ↓ PG I / PG II
- Intestinal metaplasia (IM)
  - Type I
    - Complete intestinal metaplasia
      - Absorptive cells with brush border membrane
      - Paneth cells
      - Goblet cells (sialomucins)
  - Type II
    - Incomplete IM
      - Goblet cells
  - Type III
    - Intermediate IM
  - Risk of early GCA in type II / III IM, ~42% in 5 years
- Dysplasia
  - LGD (low grade dysplasia)
    - Regresses in 60%
    - Progresses to HGD (high grade dysplasia) in 10% to 20%
  - HGD
    - Rarely progresses
    - Progresses to GCa in 5% per year
- Fundic gland polyps (FGPs) (~ 50%)
  - > 1 cm, progression to GCa ~ 1% per year
  - FGP in familial adenomatous polyposis (FAP), dysplasia in 40%
- Adenomas (10%)
  - Progress to GCa (in situ), ~11% in 4 years
- Hyperplastic gastric polyps
  - Rare progression to GCa
- Ménétrier disease
  - Progress to GCa in ~15%
- Previous partial gastrectomy
  - Especially Billroth II, usually “stump cancer” begins at the anastomosis > 20 yr after surgery

PANCREAS

Functional Anatomy of the Pancreas



Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Figure 43-1, page 913.

- From 15 to 100 acinar cells form an acinar cluster.
- Acinar cluster connects the acinar lumen to an intercalated duct. Multiple intercalated ducts join the intralobular ducts.
- The intralobular ducts join the interlobular duct, and then the main pancreatic duct.

- The cells at the junction of the acinus and the intercalated ducts are cuboidal centroacinar cells. These as well as the proximal intercalated duct cells have many mitochondria to facilitate salt and water secretion.
- The more distal intercalated ducts are lined by larger cuboidal cells containing cytoplasmic vesicles and granules, which facilitate the secretion of both alkaline fluid and proteins.
- The average amount of pancreatic juice produced daily contains 15-100 g of protein (in fact, the pancreas has the body's highest rate of daily protein synthesis and secretion).
- Acinar cells secrete enzymes (protein), as well as isotonic NaCl-rich fluid.
- This acinar fluid represents about a quarter of the total fluid secreted by the pancreas gland.
- The other 75% of the fluid secreted is HCO_3^- rich fluid from the pancreatic duct cells.
- The HCO_3^- in the duodenal lumen helps to neutralize the acidic "squirts" of the mixture of the 2 mm particles of food as well as gastic acid which leaves the stomach as the result of antral contraction and the coordinated relaxation of the pylorus.

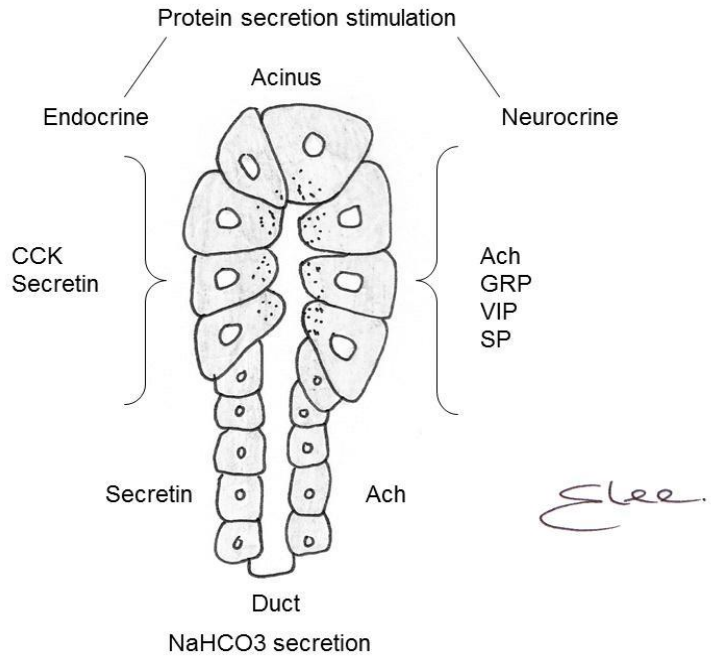
"Play a crucial role in finding your own humility and humanity"

Grandad

Basal Secretion

- With fasting (the interdigestive period), there is only a low of level basal (or constitutive secretion [CS]) of protein by the acinar cells).
- The basal secretion from the stomach, biliary tree and pancreas vary in relation to the four phases of the small intestinal MMC (migrating motor complexes). In phase I of the MMC, intestinal motility is minimal, and so also are the basal gastric, biliary and pancreatic secretions.
- Duodenal motility increases in phases II and III of the MMC, and this is associated with increased interdigestive period secretion.
- As the MMC motor activity falls in phase IV back to the inactive phase I, pancreatic secretion also falls.
- This cyclic pattern of fasting and fed-associated pancreatic secretion is partially affected by the parasympathetic and the sympathetic autonomic (α-adrenergic tone) systems, as well as through the hormonal effects of CCK during MMC phases I and II.

SITE OF ACTION OF GI PEPTIDES ON PANCREATIC ACINAR AND DUCT CELLS



Abbreviations: Ach, acetylcholine; CCK, cholecystokinin; GRP, gastrin-releasing peptide; SP, substance P; VIP, vasoactive intestinal peptide

Adapted from: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Figure 56-1, page 922, Ninth Edition, 2010

➢ ***Stimulated Secretion***

- Give the gastrointestinal peptide hormones acting on the pancreas a gallbladder.

Hormone	Source	Action
➢ Pancreas		
○ Cholecystokinin (CCK)	– I cells in duodenum a jejunum – Neurons in ileum a colon	↑ Enzyme secretion
○ Secretin	– S cells in small intestir	↑ HCO_3^- and fluid secretion pancreatic ducts
○ Gastric inhibit peptide (GIP)*	– K cells in duodenum a jejunum	Exocrine: ↓ fluid absorption ↓G, ↓H Endocrine: ↑ insulin release
○ VIP	– ENS neurons	↑ Secretion by pancreas
○ PYY	– Endocrine cells in ile and colon	↓ Enzyme and fluid secretion
○ Somatostatin (SST)	– D cells	↓ Endocrine/exocrine secretic
➢ Gallbladder		
○ CCK	– I cells in duodenum a jejunum – Neurons in ileum a colon	↑ Contraction of smooth mus

*GIP is also known as “glucose-dependent insulinotropic polypeptide”

Abbreviations: PPP, peptidergic postganglionic parasympathetic neurons

Printed with permission:*Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Table 41-1, page 889.

Hormone	Cell Secretion Acinar (enzymes)	Duct (HCO_3) and H_2O
CCK	↑	
Secretin		↑
VIP		↑
PYY	↓	↓
SST	↓	↓

Pancreatic Proenzymes and Enzymes

- Give 10 proteins secreted from the acinar cells of the pancreas.

  - Proenzymes*
    - Cationic trypsinogen
    - Anionic trypsinogen
    - Mesotrypsinogen
    - Chymotrypsinogen (A, B)
    - Kallireinogen
    - Procarboxypeptidase A (1, 2)
    - Procarboxypeptidase B (1, 2)
    - Prophospholipase
    - Proelastase
  - Enzymes
    - Amylase
    - Carboxylesterase
    - Sterol esterase
    - Lipase
    - DNase
    - RNase

Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, Table 56-1, page 923.

- Trypsinogen and Trypsin
  - Some acinar enzymes are proenzymes, others are secreted as active enzymes.
  - Amylase and lipase are synthesized stored and secreted from the pancreas as active enzymes.
  - The inactive enzymes (zymogens are trypsinogen, chymotrypsinogen, proelastase, procarboxypeptidase, and phospholipase A_2).
  - The proenzymes are secreted in an inactive form.
  - The inactive proenzyme trypsinogen is acted upon by enterokinase, a brush border membrane glycoprotein peptidase.
  - Enterokinase forms trypsin from
    - Cationic trypsinogen

        - Anionic trypsinogen
    - Trypsin converts the trypsinogens and other proenzymes to active enzymes.
    - The formation of trypsin by enterokinase facilitates removal (hydrolysis) of an N-terminal hexapeptide fragment.
    - Trypsin is autocatalytic
        - Trypsin activates
            - Trypsin (autocatalysis)
            - Chymotrypsin
            - Elastase
    - Trypsin, chymotrypsin and elastase are endopeptidases.
    - Endopeptidases are exopeptidases.
    - Exopeptidases cleaves "… peptide bonds at the carboxyl terminus of proteins.
    - Gastric pepsin and pancreatin proteases form oligopeptides and free amino acids.
    - AM (brush border membrane) enzymes further break down oligopeptides.
    - Oligopeptides and free amino acids are transported across the AM by Na^+- and H^+- coupled transporters.
- PST_1 (pancreatic secretory trypsin inhibitor)
    - Inhibitors to autocatalytic activity of trypsin
- Amylase
    - Salivary and pancreatic amylase hydrolyze the 1,4-glycoside linkage in starch and glycogen.
    - These 1,4-glycoside linkages occur at every other junction between carbon 1 and oxygen.
    - The 1,4-glycoside linkages acted upon produce maltose and maltotriose, as well as α-dextrins.
    - Maltose has two α-1,4 linked molecules, and maltotriose has 3 α-dextrins contain 1,6-glycosidic linkages.

➢ Lipid hydrolytic enzymes

- Lipases
    - In the stomach, lipids are emulsified, producing a lipid droplet.
    - Bile acids increase the surface area of the lipid droplets.

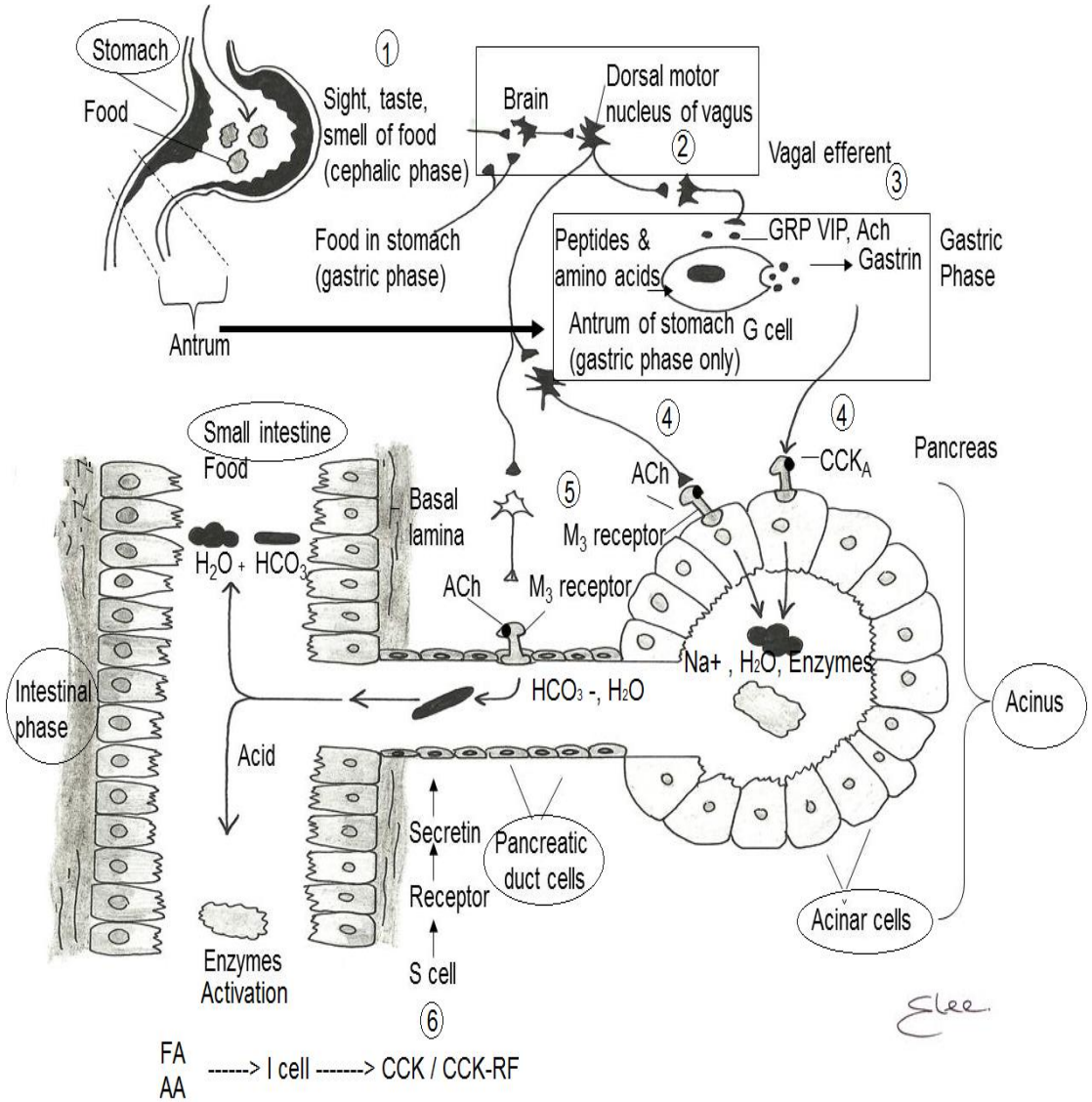
- Colipase forms a complex with lipase and bile acids.
- Lipase binds to the complex of bile acids and colipase.
- Lipase acts on the oil-water interface of the droplet.
- The 3 pancreatic lipases are lipase, phospholipase A_2, carboxylesterase.
- "In addition to the pancreatic lipase 9aka triglyceride lipase), there is a minor contribution from salivary and gastric lipases.
- Pancreatic lipase hydrolyzes (cleaves) the fatty acids (FA) from the 1 and 3 carbons on the triglycerides (aka triacylglycerol), leaving Fas and B-monoglyceride (a FA still esterified to glycerol at the 2 position).

- Phospholipase A_2 hydrolyze FA from carbon 2 of PC (phosphatidylcholine), leaving FA plus LPC (lysophosphatidylcholine)
- Carboxyesterase hydrolyzes
  - CE (cholesterol esters)
  - FSV (fat-soluble vitamin) esters
  - TG (triglycerides), DG (diglycerides) and MG (monoglycerides)

Protein Secretion at the Organ Level

- **Cephalic phase: vagal afferents and efferents**
  - This cephalic phase of pancreatic secretion contributes between 10 to 20% of the maximal daily pancreatic enzyme secretion.
  - The pancreatic secretion of protein, fluid and electrolytes is coordinated in both a stimulatory and inhibitory manner by the cephalic, gastric, and intestinal phases.
    - Vagovagal reflex
    - Enzyme-rich, water/HCO_3^- poor secretion
  - 1) The cephalic phase of pancreatic secretion begins with the sight, smell and taste of food.
  - 2) This vagal afferent sensory information is integrated in the dorsal vagal complex (DVC) of the hypothalamus, as well as on the mammillary bodies.
  - With this neuroluminal stimulation resulting from the intake of food, there is regulated secretion (RS; 5-10 times > CS [Constitutive secretion, aka basal secretion]).

CEPHALIC, GASTRIC AND INTESTINAL PHASES OF PANCREATIC SECRETION



Abbreviation: DVC, dorsal vagal complex

Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Figure 43-9, page 924.

- Once the DVC has integrated the vagal afferents, the vagal efferents are then activated.
- 3) The pancreatic ganglia release the neurotransmitters Ach, GRP (gastrin-releasing peptide) and VIP.
- These neurotransmitters bind to their individual receptors on the acinar cell.
- Ach, GRP and VIP increase acinar cell Ca^{2+}_{I} (intracellular concentration of Ca^{2+}).
- PTF-1 (pancreatic transcription factor) binds to the PCE (pancreatic consensus element) in the enhancer regions of the 5' flanking nucleotide sequences
- This regulates the transcription of the mRNAs for the synthesis of the pancreatic digestive enzymes and coenzymes.
- The endocrine regulation of pancreatic secretion by the acinus is mediated by CCK and secretin.
- The efferent pathways of the vagus nerve release Ach, GRP and secretin to begin both gastric and pancreatic secretion through the activation of M3 receptors on both the gastric parietal and pancreatic acinar cells to release digestive enzymes, and to lesser extent to stimulate ductular cells HCO_3^- and fluid.
- This pancreatic secretion of water and electrolytes (especially HCO_3) provides partial alkalization of gastric HCl entering the duodenal lumen.
- The effect of the cephalic phase on pancreatic fluid secretion is much less than on the enzyme secretion.
- As a result,the enzyme concentration in pancreatic juice is high during the cephalic phase (because of the relatively high enzyme content and low fluid volume).

- **Gastric phase**
  - The gastric phase of pancreatic secretion contributes between 50 to 80% of maximal daily pancreatic secretin enzyme secretion.

➢ Acetylcholine

- 4) The distention of the stomach by ingested food and fluid stimulates the stomach to release Ach through cholinergic vagal pathways.
- Acetylcholine (Ach), secretin, cholecystokinin (CCK), gastrin-releasing peptide (GRP), and vasoactive intestinal peptide (VIP) act as neural and humoral agonists.

- 5) Ach acts through an M3 receptor to activate the pancreatic and the ductular cells to activate the pancreatic acinar and ductular cells.

➢ Gastrin

- Semidigested food peptones and amino acids(especially valine, methionine and phenylalanine) release gastrin from the antral and duodenal G-cells.
- Gastrin is a relatively poor agonist of the CCK_A receptors on acinar cells.

- **Intestinal Phase**
  - The digestive capacity of the pancreatic enzymes for carbohydrates, proteins, nucleic acids and lipids becomes even greater with the alkalization of the duodenal contents by the ever-increasing pH (alkalinization) of the luminal contents achieved by the higher rates of HCO_3^- secreted into pancreatic fluid, thereby the neutralization of the squirts of gastric acid entering the duodenum.
  - This partial alkalinization of the duodenal lumen provides the necessary environment for the activation of the pancreatic proenzymes in the zymogen granules (trypinsogens, chymotrypepsinogen, proelastase, protease E, precarboxpeptidase A and B).
  - This duodenal alkalinization reduces the acid-associated stimulation of the duodenal S cells which release secretin; thereby, this becomes a negative feedback loop.
  - H^+ in duodenum releases secretin from
    - Stimulates water / HCO_3^- secretion
  - There is a dynamic interchange between the effect of gastric H^+ releasing secretin, and secretin stimulating, pancreatic HCO_3^- which neutralizes H^+ and ↓ secretin secretion.
  - Secretion secretes water / HCO_3^-
    - Potentiates the action of Ach to stimulate pancreatic enzymes.

➢ Secretin

- 6) With the arrival of acidic chyme in the duodenum, S cells release secretin, which stimulates the secretion of alkaline (HCO_3^- -containing) pancreatic fluid from the duct cells.
- Acidic chyme entering the duodenum from the stomach is the major stimulant of pancreatic secretion of protein-and HCO_3^- - rich fluid.
- Secretin (and VIP) increase cAMP in the acinar and ductular cells.
- The HCO_3^- partially neutralizes the gastric acid emptied into the duodenum.
- It is essential to achieve the pH optimum for activation and activity of pancreatic enzymes: this is the importance of secretin.

- Fatty acids in the duodenum also reduce gastric acid secretion, slow gastric emptying, and stimulate pancreatic HCO_3^- rich ductular secretion.

- CCK
  - 7) Peptides, amino acids (AA) and fatty acids (FA) in duodenal lumen release
    - CCK from the basolateral membrane of duodenal mucosal I neuroendocrine cells, and
    - CCK-RF (CCK-releasing factor protein)
  - Because CCK is released from the I cells and acts in a paracrine or endocrine manner, it is not subject to proteolytic destruction.
  - CCK activates vagal afferent neurons.
  - Protein and lipid breakdown products also stimulate vagal neurons.
  - Neuroendocrine: CCK activates vagal afferent neurons, the sensory information is integrated in the dorsal vagal complex, and the vagal efferents are activated.
  - The pancreatic ganglia release the neurotransmitters Ach, GPR, VIP, binding to their individual receptors on the acinar cell and activating pancreatic acinar exocrine secretion of enzymes and proenzymes.
  - CCK binds to its receptor on the acinar cell, and causes the release of pancreatic digestive enzymes and proenzymes from the acinar cells.
  - CCK, GRP and Ach increase acinar cell Ca^{2+}_I (intracellular calcium, Ca^{2+}) concentration, whereas VIP and secretin increase cAMP.
  - CCK stimulates acinar cell protein and fluid (NaCl-rich) secretion.
  - CCK, GRP and Ach increase acinar cell, Ca_i^{2+}, whereas VIP and secretin increase cAMP.
  - When H^+ enters the duodenal lumen, secretin is released from the basolateral membrane of the D (neuroendocrine) cells.
  - Secretin increases the cAMP in both acinar and ductular cells.
  - Because some of these CCK-releasing factors are proteins, and because these proteins are secreted into the duodenal lumen, they are subject to breakdown by the pancreatic proteolytic enzymes (e.g., trypsin).
  - Thus, releasing factors (RF) secreted from the intestine are responsible for negative feedback regulation of pancreatic secretion.
  - There is regulation of these mRNAs for pancreatic enzymes in response to enrichment of the dietary nutrient load: ↑ carbohydrates, ↑ amylase; ↑ fat, ↑ lipase.

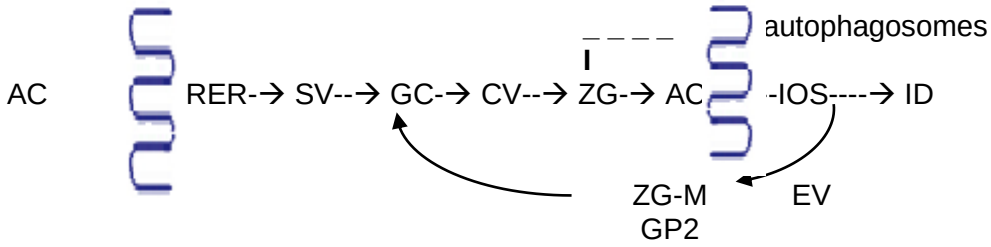
Pancreatic Secretion of Enzymes at the Cellular Level

Background: Acinar Cells

- The pancreatic acinar cells are the main source of secretion of enzymes.
- The pancreatic ductular cells are the main source of secretion of water and electrolytes.
- However, the acinar cells secrete small amounts of water and electrolytes, and the duct cells secrete small amounts of enzymes.

➢ Pancreatic Acinar Cell Secretion of Protein: organelles

- Give the organelles involved in the synthesis of enzymes and proenzymes, and trafficking of vesicles to the apical membrane of the pancreatic acinar cell.



Abbreviations: AC, acinar cell; AC-IDS, acinar cell – intralobular duct side; AC-PBS, acinar cell – portal blood side; CV, condensing vacuole; EV, empty vesicle; GC, Golgi complex; ID, intralobular duct; RER, rough endoplasmic reticulum; SV, small vesicle; ZG, zymogen granule; ZG-M, zymogen granule membrane

- **AA**: The acinar cells take up amino acids from the portal blood.
- **RER**: In the acinar cells, the rough endoplasmic reticula (RERs) synthesize nascent proteins.
- Protein synthesis and secretion in the pancreatic acinar cells
  - Acinar cell, a lot
  - Duct cell, a little

Please see Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, Table 56-1, page 923, Pancreatic Acinar Cell Secretory Products.

- Give the physiology of the secretion of digestive enzymes by the pancreatic acinar cells.
  - "Synthesis of digestive enzymes takes place in the internal space of the rough endoplasmic reticulum [RER].
  - "Ribosomal subunit attaches to [the cell's] mRNA and initiate synthesis of a hydrophobic 'signal' sequence on the NH_2-terminal of nascent protein.
  - This complex then attaches to the outer surface of the endoplasmic reticulum [ER]
  - "The signal sequence targets the protein…..into the lumen of the RER" (Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, page 924).
  - Post-translational modification in the ER includes
    - Forming disulfide bridges
    - Phosphorylation
    - Sulfation
    - Glycosylation
  - Further changes may occur in the conformational structure of the proteins
  - Proteins in the RER are transported to the Golgi complex.
  - Glycosylation of the protein occurs in the Golgi complex.
  - Golgi complex sorts and targets newly synthesized proteins into various cell compartments.
  - Specific conformational changes in the proteins is facilitated by chaperone and foldase proteins.
  - When there is a need for more protein enzyme synthesis, there is increased synthesis of chaperone and foldases
  - **SV**: The nascent proteins form small vesicles in RERs.
  - These small vesicles leave the RER's and traffick to the Golgi complex (**GC**).
  - The normal extracellular proteolysis is important for digestion of food protein or protein breakdown products in the duodenal lumen.
  - The proteolytic enzymes of the acinar cells have the potential to digest themselves (autodigestion).
    - pancreatic fluid secretion, and
    - by the mixing of the enzymes with food in the intestinal lumen
  - GC: In non-pancreatic cells, normally, the lysosomal enzymes of the Golgi complex would break down these newly synthesized proteins.

- The pancreatic proteins destined to be secreted as enzymes lack the mannose G phosphate receptor.
- Because these pancreatic proteins lack this mannose G phosphate receptor, they are not broken down by the lysosomal enzymes in the Golgi complex.
- "by keeping proteins in their appropriate conformation, the UPR [the unfolding protein response] maintains normal transport of proteins between compartments of the cell.
- From the ER Golgi complex (GC), the digestive enzymes are transported to the zymogen granules.
- For the digestive proteins in the GC which are destined for the lysosomal hydrolase pathway, mannose-6-phosphate (M-6-P) groups are added to the hydrolases.
- Vesicles are formed in the cis-GC and the lysosomal hydrolases return to the GC.
- **CV**: Proteins leave the Golgi complex in the cytoplasm of the acinar cells in large membrane-bound structures, the condensing vacuoles.
- The proteins in the condensing vacuoles become densely packed and further condensed, and are surrounded by membrane, pinched off and secreted as smaller "zymogen granules".
- **ZG**: The zymogen granules traffic to the inner surface of the brush border membrane of the acinar cell, where the membrane of the secretory zymogen granules temporarily fuses with an area of the apical plasma membrane of the acinar cells.
- **AC-IOS**: The inactive proenzymes in the secretory zymogen are released into the lumen of the intralobular ducts (**ID**) of the pancreas.
- **ZG-M**: The membrane of the zymogen granule detaches from the brush border membrane of the acinar cell.
- Digestive enzymes are secreted from the acinar cells by the process of exocytosis (receptor-mediated secretion).
  - Exocytosis is defined as the movement of the secretory granule to the brush border membrane (of the acinar cell).
  - Recognition of a plasma membrane site for fusion of the secretory granule
  - "… the fission of the granule membrane-plasma membrane site after fusion" (Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, page 925).
  - Recognition, fusion and fission are modified by

- Calmodulin-dependent
- Protein kinases
- Actin-myosin
- SNARE (soluble-N-ethylmaleimide-sensitizing factor attachment protein [SNAP] receptor) proteins
- GTP-binding proteins

- The acinar cell membrane shrinks, and becomes “resealed”.
- **EV**: The zymogen granule minus its membrane becomes “empty” zymogen granules.
- The acinar cell also secretes the protein **GP2**.
- GP2 binds to the inner leaflet of the membrane of the zymogen granule membrane.
- The GP2 bound to the inner membrane of the emptyzymogen granule aids the trafficking of these membranes back to the Golgi of the acinar cells.
- These “empty” zymogen granules now form new condensing vesicles which contain more inactive enzymes.
- The recyclining continues with the shutting of the zymogen granules and membranes back and forth between the Golgi and brush border membrane of the acinar cell.
- Under certain pathological conditions, GP2 combines with lithostatin (the pancreatitis-associated protein, which is secreted in pancreatic juice).
- The GP2 – lithostatin complex forms protein plugs in the pancreatic duct lumen.

“Setting goals is the first step in turning the invisible into the visible.

Tony Robbins

- Neurotransmitters
  - The neural pathways controlling the primary enzyme-rich acinar secretion, and the HCO_3^--rich pancreatic epithelial ductular cells, are the postganglionic parasympathetic and sympathetic nerves.
  - These nerves, when stimulated, release neurotransmitters (cholinergic, adrenergic and peptidergic).
  - The cholinergic, adrenergic and peptidergic neurotransmitters bind to their appropriate receptors on the acinar and ductular cells.
- Monophasic (VIP, GRP) and biphasic (Ach, CCK) dose responses
  - The major receptors stimulating the acinar cells are for Ach and CCK.
  - CCK (cholecystokinin), GRP (gastrin relating peptide), Ach (acetylcholine), substance P (cellular regulation)
    - ↑ cellular metabolism of membrane phosphoinositides
    - ↑ phospholipase c-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate to 1,2-diacylglycerol and IP_3 (inositol 1,4,5 triphosphate
    - ↑ IP_3 → ↑ Ca_i^{2+} from mobilization of ER stores
  - ↑ Ca_i^{2+} also from ryanodine receptors
  - cADP-ribose and NAADP (nicotinic acid adenine dinucleotide phosphate
  - 1,2-diacylglycerol and PKC (protein kinase C), arachidonic acid

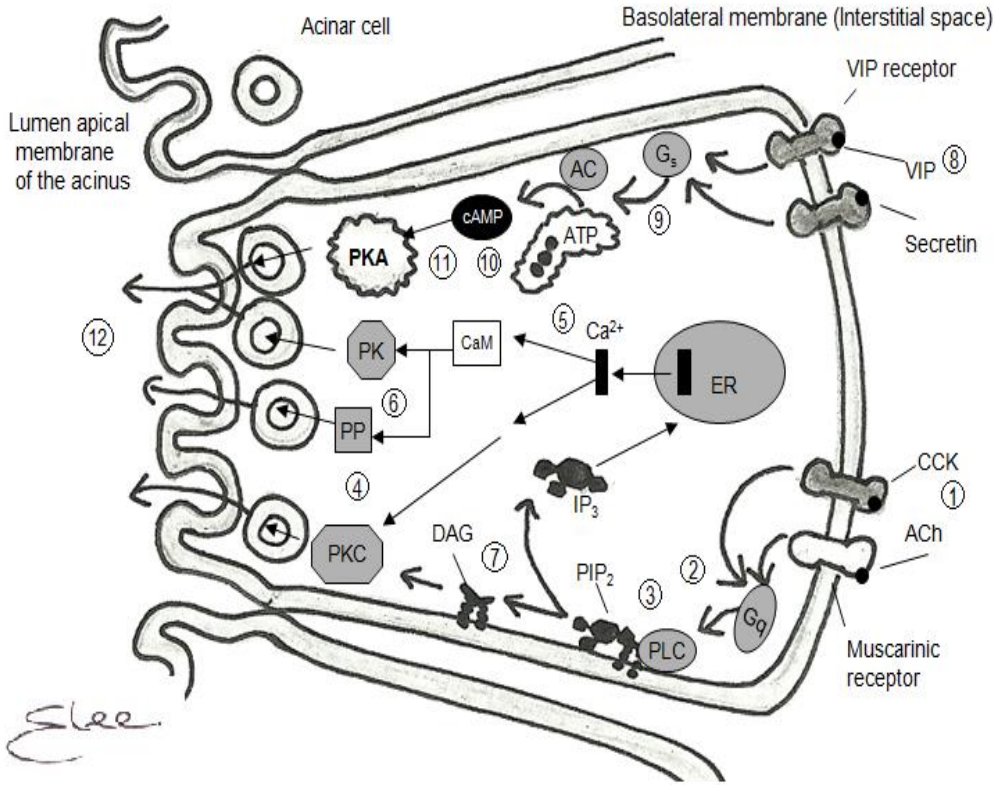
Adapted from: Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, Figure 56-6, page 925.

  - The inital rate of protein secretion rises, but as the doses of Ach or CCK are increased further, secretion of pancreatic enzymes falls.
  - This increase and then decrease in pancreatic enzyme secretion on response to increasing concentrations of Ach or CCK represents the biphasic dose response in enzyme secretion.
  - In contrast, with VIP or GRP, there is a monophasic dose-response: an increased protein secretion which reaches a plateau, (and does not fall as with carbachol or CCK in their biphase dose-response).

- PTF-1 and PCE
  - The enhancer regions in the 5' flanking nucleotide sequences are the PCE (pancreas consensus element).
  - The PTF_1 (pancreas transcription factor 1) binds to PCE.
  - PTF-1 (pancreatic transcription factor) binds to the PCE (pancreatic consensus element) in the enhancer regions of the 5' flanking nucleotide sequences which regulate the transcription of the mRNAs for the digestive enzymes and co-enzymes.
  - The PCE of the genes for amylase, chymotrypsin and elastase regulate the transcription of the mRNAs for these enzymes
  - There is upregulation of pancreatic enzymes in response to dietary nutrient load:

Dietary enrichment	Pancreatic enzymes mRNA expression
Carbohydrate	↑ amylase, ↓ chymotrypsinogen
Alcohol	↓ amylase
Fat	↑ lipase

- Give the cellular steps involved in the secretion of digestive proteins from pancreatic acinar cells.
- **Stimulated secretion**
  - (1) Ach binds to the muscarinic receptor on the basolateral membrane (BM) of the acinar cell, and stimulates PLC to release DAG.
  - Ach and CCK binds to its BM receptor on the acinar cell BM.
  - Both Ach and CCK receptors activate (2) Gq heterotrimeric G protein , stimulate (3) PLC and, (4) PKC as well as ↑ (5) intracellular Ca^{2+}.
  - 2) Gq activates protein lipase C(PLC), forming PIP_2, IP3 and DAG.
  - When acinar cells have been stimulated by peptides such as CCK or VIP, and the hormonal stimulus is removed and is reapplied, the receptors become desensitized.
  - Because of the desensitization of the CCK and VIP receptors, secretion becomes less than what would have been seen with the initial stimulation.



Abbreviation: CaM, calmodulin

Adapted from: Boron Walter F. and Boulpaepemile L. *Medical Physiology*, Second Edition,. 2009; Figure 43.4, page 916.

- 5) The intracellular concentration of Ca^{2+} ($[Ca^{2+}]i$) increases.
- 6) ↑$[Ca^{2+}]i$ activates calmodulin, protein kinases (PK) and protein phosphatases (PP).
- 7) DAG stimulates PKC and IP3, which release Ca^{2+} from intracellular stores.
- 8) VIP and secretin bind to their receptors on the BM of the acinar cells.
- 9) VIP and secretin activate Gs.
- 10) Gs stimulates Ach to produce more cAMP; Ach has only a modest effect on the signaling pathway, and to activate PKA (11).
- 12) The PKA, PK, PP and PKC alter the phosphorylation of structured and regulatory proteins to insert the zymogen granule membranes into the apical membrane of the acinar cell.

- Digestive (food stimulated) secretion
  - CCK released from I cell in duodenum.
  - CCK afferent neurons to dorsal vagal complex
  - Vagal efferent neurons release VIP, GRP, CCCK, encephalin from endings of pancreatic nerves.
- Nutrient load
  - FA > 8 carbon length
  - MG (monoglycerides)
  - Peptides
  - Amino acids
  - Glucose

SO YOU WANT TO BE A WHAT - GASTROENTEROLOGIST!

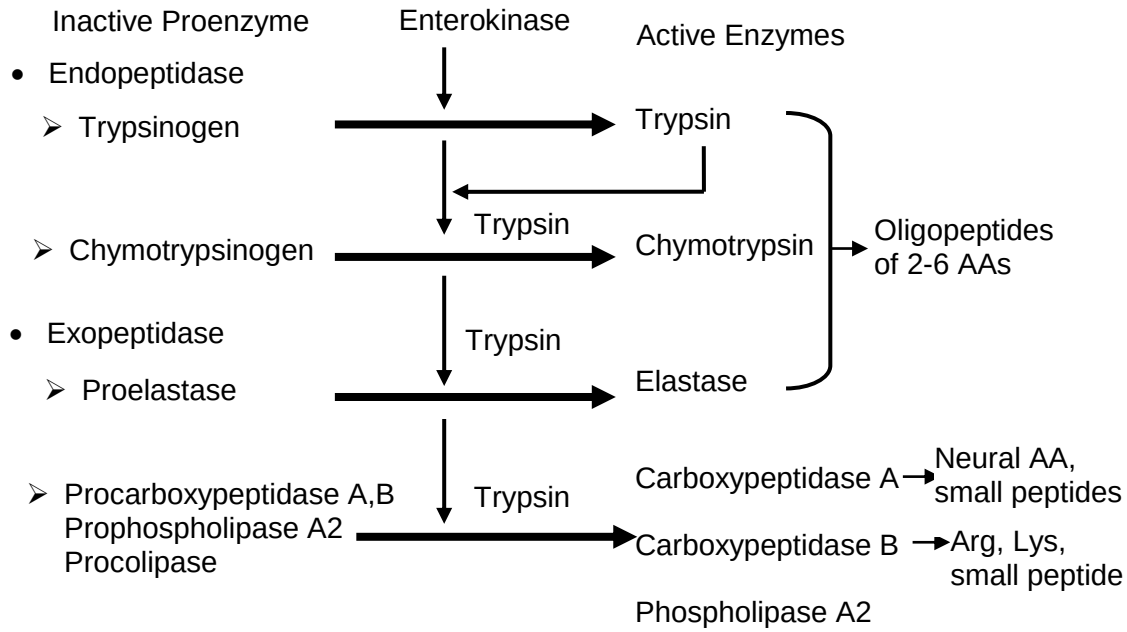
It is widely taught that CCK stimulates pancreatic acinar cells to secrete enzymes, but in humans these acinar cells have no CKK receptors.

- Give the mechanism by which CCK stimulates pancreatic acinar cell secretion of enzymes.
  - CCK acts on the cholinergic vagal afferent pathway, and its action (as well as that of secretin) is increased synergistically by 5-HT.

Control of Pancreatic Tryptic Activity

- Activation of trypsin ("turning on" the activity)
  - The trypsin plus TAP (trypsinogen activation peptide)peptides form the inact trypsinogen enzyme.
  - The TAP is cleared from trypsinogen to form the active trypsin.
  - The activation of the cleavage of TAP results from enterocyte brush bord membrane enterokinase, or by other molecules of trypsin.
  - This activation of trypsin by trypsin is called "autoactivation".
  - Trypsin is activated by ↑ Ca^{2+} (↑ Ca^{2+} blocks trypsin degradation), and inactivated (degraded) in the acinar cell by ↓ Ca^{2+} (↓ Ca^{2+} blocks activation).

ACTIVATION OF PANCREATIC ENZYMES



- The endopeptidases produce oligopeptides of 2-6 amino acids
- The exopeptidases produce single amino acids.
- Carboxypeptidase A – forms neutral amino acids and small peptides.
- Carboxypeptidase B – forms arginine, lysine, and small peptides
- Enterokinase plays an important role in activating trypsinogen to form trypsin.
- Trypsin activates more trypsinogen as well as other proteolytic enzyr proteases.

Adapted from: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Figu 100-14, page 1713, Ninth Edition, 2010

- The PKA, PK, PP and PKC alter the phosphorylation of structured a regulatory proteins to insert the zymogen granule membranes into the bru border membrane of the acinar cell.

➢ Dereactivation (autolysis) of trypsin

- Trypsin may autolyze itself ("trypsin-mediated autolysis") by acting at its ov connecting chain.
- CTRC (chymotrypsin C, aka enzyme Y) degrades the connecting chain

- Calcium
  - Ca_i^{2+} plus cAMP have a synergistic effect on digestive enzyme secretion
  - The local concentration of Ca^{2+} will activate or deactivate trypsin, either by exposing the activation site or the blocking site (autolysis).
  - The intra-acinar (autoreaction) concentration of Ca^{2+} is detected ("sensed") by the G-protein coupled receptor CASR (calcium-sensing receptor).
  - Low Ca^{2+} favors autolysis, and high Ca^{2+} favours autoreaction.
  - Intra acinar cell Ca^{2+} concentrations may increase as a result of numerous factors:
    - ↑ extracellular Ca^{2+}
    - ↑ Ca^{2+} entry
      - Opening of Ca^{2+}
        - Channels
        - Tunnels
        - Bile reflux
  - The receptor-mediated secretion of pancreatic enzymes occurs by
    - ↑ Ca_i^{2+} (intracellular Ca^{2+})
    - ↑ adenylate cyclase
      - ↑ cAMP (cyclic AMP)
      - ↑ cAMP-dependent protein kinase A
  - VIP (vasoactive intestinal peptide)
  - Secretin

- Whole Organ Regulation
  - Interdigestive (fasting/secretion)

    - Ach, motilin and PP (pancreatic polypeptide) regulate MMC (migrating myoelectric complex)
    - During MMC phase II / III there is a burst of secretion of
      - Pancreatic enzymes
      - Pancreatic HCO_3^-
      - Bile from small amount of gallbladder contraction
  - Cephalic phase
    - Acinar cells, fluid and enzyme secretion
    - Duct cells-HCO_3, fluid

- Gastric distention
  - Vagovagal reflex
  - Enzyme-rich, water / HCO_3^- poor secretion
- Digestive (food stimulated) secretion
  - CCK released from I cell in duodenum.
  - CCK afferent neurons to dorsal vagal complex
  - Vagal efferent neurons release VIP, GRP, CCCK, encephaline from endings of pancreatic nerves.
- Nutrient load
  - FA > 8 carbon length
  - MG (monoglycerides)
  - Peptides
  - Amino acids
  - Glucose
- Intestinal phase
  - H^+ in duodenum releases secretin
  - Secretin stimulates-water/ HCO_3^- secretion
  - Secretin may potentiate the action of Ach to stimulate enzymes.

➢ Turning "off" pancreatic secretion

- Luminal CCK-releasing factors (CCK-RF) may contribute to CCK release in the interdigestive phase, as well as after a meal (digestive phase).
- Under basal conditions, there is little trypsin to inactivate CCK-RF.
- With the ingestion of nutrients which are substrates for trypsin, trypsin digests the CCK-RF as well as ingested food.
- Less CCK-RF is broken down, so more CCK-RF is available to stimulate CCK secretion.
- Depending upon the balance between the proteins and lipids in the duodenal lumen, (both of which stimulate secretion of CCK and CCK-releasing factors), and the proteolytic enzymes breaking down the CCK-releasing factors, there may be more or less of these CCK-releasing factors.
- Thus, CCK-RF are trypsin-sensitive proteins.
- These CCK-releasing factors (CCK-RF) do not play as important a role as does CCK itself on pancreatic acinar cell secretion of pancreatic enzymes.

- The enteropancreatic feedback system
  - The intraluminal peptides monitor peptide and LCRF (luminal CCK-releasing factors) → ↑ CCK release from I cells.
  - Trypsin activates other digestive enzymes and autocatalytically digests more trypsinogen.
  - When trypsin no longer bound to meal protein, ↑ trypsin available to cell surface.
  - ↑ trypsin at cell surface → ↓ CCK-RF → ↓ CCK release
  - ↓ CCK → ↓ enzyme release
  - Intraluminal SRF (secretin-releasing peptide) → secretin release from S cells

➢ Loss of stimulated secretion

- Pancreatic secretion falls after a meal, because of the loss of the cephalic, gastric, and intestinal phases of stimulated secretion.
- Secondly, pancreatic secretion falls from the direct inhibitory effects of PYY (peptide YY, released from neuroendocrinic cells in the distal small intestine and colon), somatostatin (SST, especially SST-28, released from small intestinal D-cells), and glucagon (released from pancreatic islet [endocrine] α cells).
- Ca^{2+}, PL, and CHOL exit the hepatocytes by means of the MDR1, MDR-2, and ABCG5/ABCG8 transporters in the apical/canalicular membrane.

To the young physician

Your role is to serve, not to be served

Grandad

Protection of the Pancreas from Autodigestion

Above has been outlines the control of pancreatic secretion. Part of the process of protecting the pancreas from autodigestion is to decrease the "on" and to increase to "off" components of the control systems.

- Give 5 mechanisms that protect the acinar cells from autodigestion by its own proteases.

Note: This question relates to the protection at the cellular level; a later question explores the control of pancreatic tryptic activity in the lumen.

- Lysosomal enzymes
  - Prematurely released tryspin may be degraded lysosomal enzymes through
    - The process of fusion of secretory granules w lysosomes (crinophagy), or through
    - The process of the lysosomes engulfing t secretory granules (autophagia).
- Autophagosomes
  - Some trypsinogen-containing zymogen granules th are not secreted from the brush border membrane the acinar cell can be taken up by autophagosom for removal.
  - These autophagosomes fuse with lysosomes to fo autolysosomes in the autolysosomes in the contents the granules, including proteolytic pancreatic enzym are degraded by lysosomal enzymes, preventing th release.
  - In pancreatitis
    - Block in enzymes secretion leads to accumulation of intracellular zymogen granule which increases uptake of these structures autophagosomes.
    - Alternatively, or additively, granules improper released from the basolateral surface of the acir cell are taken back up by the cell and sequester by autophagosomes.
    - Following autophagosome-lysosome fusio content degradation fails to occur because decreased levels of cathepsin B and L.

- Zymogen granules
  - Inactive pro-enzymes (trypsinoge chlymotrypsinogen, proelastase) are packag in zymogen granules.
  - Zymogen granules are resistant to intracellu breakdown, and become active only in t intestinal lumen as a result of enterokina
- Trypsin inhibitor
  - The zymogen granules contain pancrea trypsin inhibitor, which will protect against sm amounts (10-20%) of trypsin.
  - This trypsin inhibitor becomes activated befc the granules reach the duodenal lumen.
- Digestive enzymes
  - Any prematurely released trypsin may itself digested.
  - Trypsin thereby becomes inactive by way other digestive enzymes in the zymog granule.
- Dilution of pancrea juice
  - Fluid secreted from the acinar cells and th from the duct cells dilutes and washes aw any prematurely released proeolytic enzyme.
- Mucins a prostaglandins
  - Mucins are hydrated, forming mucus.
  - Prostaglandins stimulate the secretion of t duodenal $HCO3^-$ from the surface crypt-vill cells, as well as possibly also from t submucosal cells.
  - The more pronounced defect in cathepsin (which inactivates trypsin), than in t activating enzyme cathepsin B leads to tryp generation within the autolysosome.
  - Active enzymes are released from the enlarged autolysosomes into the cytoplasm initiate pancreatic cellular injury.

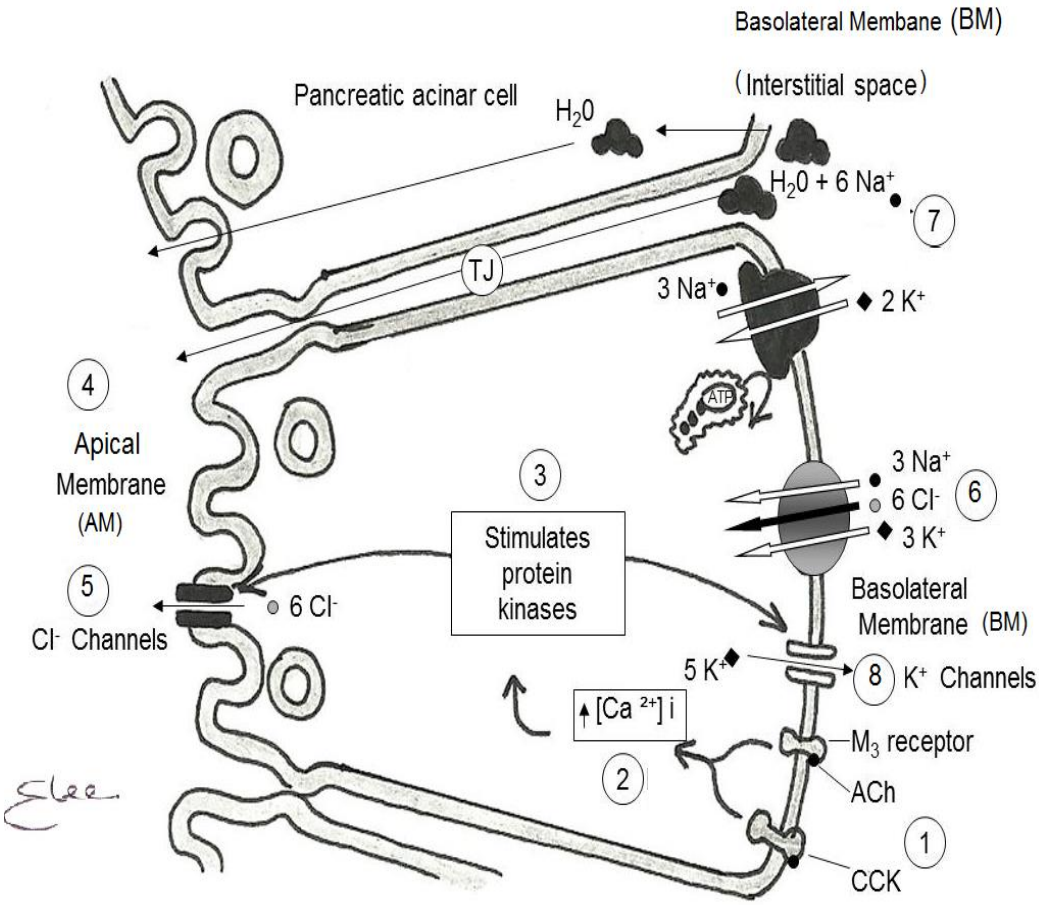
➢ ***Pancreatic Acinar Cell Secretion of Water and Electrolytes***

Useful background: Pancreatic secretion

- Rate – Interdigestive (fasting), 0.2 mL/min
  – Postprandial (stimulated), 4.0 mL/min
- Volume – ~ 2.5 L/day
- Fluid secretion – Acinar cell – a little
  – Ductal cell – a lot

- Give the cellular physiology of the pancreatic acinar cell secretion of water and NaCl.

➢ The pancreatic acinar cell secretion of water and NaCl



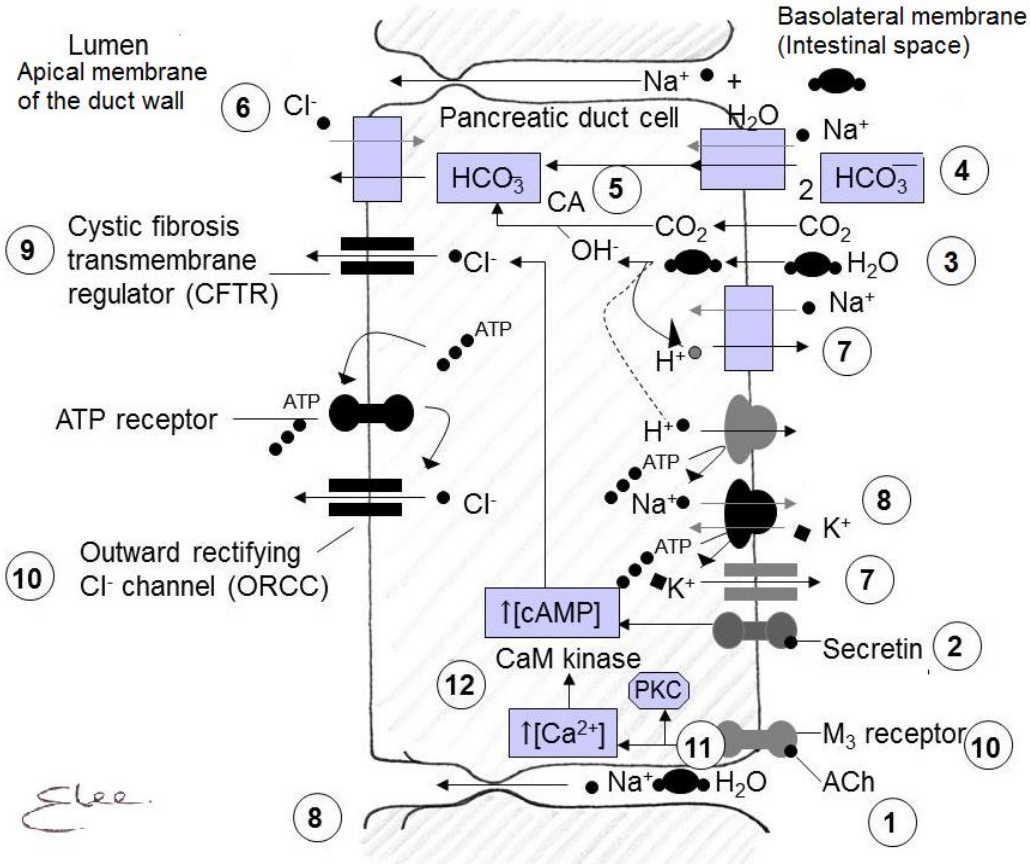
Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Figure 43-5, page 917.

The major secretion of water and electrolytes in pancreass is by the duct cells.

- The duct cells secrete a small amount of protein, and acinar cells secrete small amounts of water and electrolytes.
- Pancreatic acinar cells secrete a fluid rich in NaCl, whereas the duct cells secrete a fluid rich in HCO_3^-.
- 1) CCK and Ach are bound to high affinity receptors on BM of acinar cell
- Activation of CCK by secretin and Ach
- 2) Binding of Ach and CCK increases intracellular calcium (Ca^{2+}_i)
- 3) ↑ Ca^{2+}_i activates PKC and calmodulin (CaM)-dependant protein kinases
- The ↑ Cl^- channel permeability leads to the loss of Cl^- across the AM.
- 4) Na^+, K^+ and Cl^- cross the BM Na^+ / K^+ / Cl^- co-transporter.
- 5) Cytosolic Cl^- crosses into lumen through Cl^- channels in AM.
- 6) the ↑ flux of Cl^- draws H_2O plus Na^+ across the TJs (tight junctions), resulting in the secretion of NaCl plus H_2O.
- 7) Cytosolic Na^+ crosses BM Na^+/ K^+ exchange pump
- 8) K^+ which as entered cytosol with BM Na^+/ K^+/ Cl^- co-transporter and BM Na^+/ K^+ exchange pump crosses K^+ channels in BM.

➢ Pancreatic duct cell secretion of water and HCO_3^-

- 1) Secretin and Ach are the major regulators of pancreatic duct cell secretion of HCO_3^-.
- 2) H^+ in duodenal lumen
  - Release of secretin from duodenal S cells
- Secretin binds to secretin receptor (SR) on acinar cell
  - Activate Cl^- channel (CFTR) on AM (pical membrane) and K^+ channel on BM (basolateral membrane)
- SR activates adenylate cyclase → ↑ cAMP
- The release of secretin from the duodenal S cells causes the pancreatic duct cells to secrete alkaline fluid (water plus HCO_3^-) by the apical membrane
  - Cl^-/HCO_3^- exchanger
  - the Cl^- channels CFTR (cystic fibrosis transmembrane regulator)
  - ORCC (outward rectifying chloride channel).
- Secretin activates the cAMP signaling pathway and opens the CFTR Cl^- channels through phosphorylation.



Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Figure 43-6, page 919.

- 3) CO_2 and H_2O diffuse across basolateral membrane (BM) of the pancreatic duct cell.
- 4) HCO_3^- and Na^+ are co-transported across the BM by the Na^+ / HCO_3^- cotransporter.
- 5) CO_2 plus H_2O are converted by cytoplasmic carbonic anhydrase (CA) ir HCO_3^- and H^+ .
- 6) The intracellular HCO_3^- is pumped out of the cell across the brush borc membrane (AM) by the Cl^- / HCO_3^- exchanger.
- 7) Na^+ enters the cell by the BM Na^+ / H^+ exchanger (NHE).
- Na^+ also enters the cell by the BM Na^+ / HCO_3^- cotransporter (step 4).
- 8) This intracellular Na^+ exits the duct cell across the BM Na^+ / K^+ ATPase.
- Na^+ entering the cell by the BM Na^+- HCO_3^- cotransporter also exits by way the BM Na^+-K^+ pump.
- Fluid secretion by the acinar cells requires the Na^+-K^+ pump to drive t

$Na^+/K^+/Cl^-$ cotransporter in the BM.

- The $Na^+/K^+/Cl^-$ cotransporter moves Cl^- into the cell along with Na^+ and K^+.
- The cytosol now contains increased K^+ from the BM Na^+-K^+ pump and fro the BM Na^+/K^+/ Cl^- cotransporter.
- The BM Na^+-K^+ pump moves 3 Na^+ molecules out of the cell for every 2 molecules which enter.
- This imbalance of Na^+ over K^+ creates a gradient of Na^+ across the BM (high Na^+ in the interstitial space and lower Na^+ in the cytosol of the acinar cell).
- 7) The K^+ which was initially pumped across the BM and into the cytosol Na^+-K^+-ATPase is extruded back into the interstitial space through the BM channel.
- Fluid secretion also requires the K^+ channel in the BM as well as the channel in the AM.
- Water and Na^+ flow passively cross the intercellular junctions in response the osmotic gradient produced by the secreted Cl^-, forming the alkaline flu (high $NaHCO_3$) in the lumen of the duct.
- This lumen-negative charge drives Na^+ across the tight junctions (TJ) between t acinar cells.
- Water moves with Na^+ from the interstitial space across the TJs and into the lumen the acinus.
- Water also moves through aquaporin channels in the BM and the AM.
- 9) Cl^- in the cytoplasm is pumped across the AM and into the lumen of t duct by CFTR (the cystic fibrosis transmembrane regulator), and by
- 10) ORCC (rectifying chloride [Cl^-] channel)
- The lumen-negative voltage pulls Na^+ into the interstitial space and Na+ pl H2O cross the tight junctions (TJ) and into the lumen of the duct.
- While the HCO_3^- in the ductular fluid results from the Cl^- / HCO_3^- exchanger, it is t Cl^- movement in the duct cell which causes the BM to depolarize.
- The electrical gradient across AM caused by this depolarization results in t HCO_3^-/Cl^- exchange and production of $NaHCO_3$.
- Thus, it is the opening of the CFTR Cl^- channels which exit of Cl^- cause t HCO_3^- secretion (Cl^- / HCO_3^- exchanger) and thereby the formation of NaHC in the lumen of the duct.
- Stimulation of secretion of $NaHCO_3$ from duct cells
- Acetylcholine (Ach) acts on the BM M3 receptor
- 11) The CCK and Ach receptors cause the intracellular concentration of Ca (Ca_i^{+2}) to rise.

- Ach – activates Gq; Gq stimulates PLC, and PLC release DAG (diacylglycer
- DAG stimulates PKC and IP3 .
- IP3 release stores of Ca^{2+} in the cytosol, increasing the intracellular concentration Ca^{2+} ($[Ca^{2+}]i$). Because Ach acts on the M3 receptor to activate PKC, but becau PKC is a much less potent secrelague as compared to PKA, secretion is a mu more potent stimulus for HCO_3^- secretion.
- Ach has a lesser role in the stimulation of HCO_3^- secretion by activating G which stimulates PLC.
- The increased intracellular Ca^{+2} stimulates protein kinases, which, open the channels in the BM and the Cl^- channels in the brush border membrane.
- 12) Protein kinases also open the Cl^- channels in the AM, and Cl^- passes ir the lumen of the intercalated ducts.
- The movements of the Cl^- into the lumen makes the lumen more negative charge, as compared with the interstitial space.
- Secretin acts on its receptor on the BM, and the level of cAMP in the cytos increases.
- There are PKA and PKC phosphorylation sites on CFTR.
- PKC stimulates PKA on CFTR Cl^- transporter.
- The secretion of Cl^- by opening the AM CFTR and Cl^- channel provides the to exchange with the AM Cl^- / HCO_3^- exchanger, and result in the Ach drivi secretion of $NaHCO_3$ containing fluid.

Mixing of secretions from acinar and duct cells

- The fluid leaving the pancreatic ducts and entering the intestinal lumen is a mixture of secretions from the acinar and duct cells.
- The acinar cells secrete a NaCl-rich fluid, and pancreatic duct cells secret a fluid rich in HCO_3^-.
- When the acinal and ductular cells secrete during the cephalic, gastric and intestinal phases of pancreatic secretion, these NaCl and HCO_3^- rich fluids mix.
- Because the duct cells produce much more fluid than do the acinar cells (ratio of 3:1), as the rate of secretion of pancreatic juice increases (>0.6 mL/min), the concentration of HCO_3^- in the mixed fluid rises, whereas that of the Cl^- falls.
- This alkalinity of the pancreatic secretion prevents activation of the digestive acinar cell proenzymes until they leave the pancreatic duct and enter into the duodenal lumen.

Pancreatic Duct Cell Secretion of Protein

- Just as the acinar cells secrete small amounts of fluid, the duct cells secrete small amounts of some protein.
- The main pancreatic duct contains both duct cells as well as many goblet cells.
- Goblet cells in the pancreas as well as in other parts of the GI tract secrete large molecular weight glycoproteins called mucins.
- The glycoproteins produced in the duct cells are synthesized and secreted on a continuous basis in small cytoplasmic vesicles.
- Secretion stimulates the synthesis and thereby the secretion of the duct cell glycoprotein.

CLINICAL PHYSIOLOGICAL CHALLENGE – Test the Pancreas

Case: A 45 year-old alcoholic with recurrent episodes of acute pancreatitis develops weight loss and diarrhea. Pancreatic insufficiency is suspected, and the attending physician wishes to test the pancreatic function in this man.

- Give an outline of the process of normal pancreatic fluid secretion, and thereby explain the principle of the secretin test for pancreatic HCO_3^- secretion
- Describe the process of normal pancreatic secretion of enzymes and proenzymes, and thereby explain the principle of the Lundh test meal for pancreatic enzyme secretion
- Give the mechanisms by which the pancreas prevents autodigestion, including the normal post-prandial process of inhibition of the cephalic, gastric and intestinal phases of pancreatic secretion.

Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)

Useful background: Characteristics of CFTR (cystic fibrosis transmembrane conductance regulator)

- CFTR is a peptide which functions as an anion channel.
- Cl^- and HCO_3^- pass through this channel, causing alkaline pancreatic fluid secretion.
- In the pancreas, CFTR is in the apical membrane of the pancreatic duct cell.
- CFTR is regulated.
- The regulation of CFTR is by way of three regulatory domains
  - NBD (nucleoside binding domain) 1 and 2.
  - R (regulatory) domain
    - R domain binds to cytoplasmic glutamine to stimulate Cl^- secretion.
    - $NBD_{1/2}$ binds to cytoplasmic ATP to stimulate HCO_3^- secretion.
    - The conformation of the CFTR protein changes depending upon whether it is bound to $NBD_{1/2}$.
- The second messenger system regulates NBD_1, NBD_2 and the R domain of CFTR.
- For CFTR, second messenger system includes
  - ATP
  - PKA
  - Ca^{2+}
  - Glutamate intra-acinar cell)
- The second messenger system in turn is stimulated by secretin and VIP as well as Ach.
  - Secretin/VIP → receptors →↑ cAMP → ↑ PKA (protein kinase A)-mediated phosphorylation of R domains of CFTR.
  - Ach → ↑ Ca_i^{2+} (intracellular Ca^{2+})
- Numerous genetic mutations of CFTR have been described.
- The commonest genetic mutation in CFTR is ΔF508, a three-base pair deletion of the phenylalanine-coding codon 508.
- When there are major mutations in CFTR alleles, the function of CFTR is lost.
- When CFTR no longer functions normally as a regulated anion channel in the apical membrane of the pancreatic duct cell, mucus is not hydrated, ducts are blocked and tissue atrophies.

- The usual phenotype change in CFTR in the pancreas cause
  - Thick, inspissated macromolecules such as mucus.
  - This blockage causes
    - Fibrosis
    - Dilated ducts
    - Pseudocysts
    - Chronic inflammation
    - Atrophy
- There may be modifier genes, or environmental factors which influence the clinical features of CF.
- Many organs are affected in CF, and vary with the nature of the genetic changes e.g. alter the impact and environmental factor on causing pancreatitis, such as alcohol damage leading to pancreatitis.
- The clinical impact of CFR, a susceptibility gene, may also be affected by an unidentified modifier gene.
- A modifier gene interacts with a susceptibility gene to "increase the risk of developing a pathological outcome" (Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, page 937).

"Don't worry about the neurobiological mechanisms of motivated learning – just provide a safe, stimulating and welcoming environment for teaching and learning."

Grandad

Acute Pancreatitis

- Definitions
  - Acute pancreatitis is "……an event triggered by sudden pancreatic injury that is followed by sequential inflammatory responses" (Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, page 901).
    - Alternate definitions:….an acute inflammatory process of the pancreas with variable involvement of other regional tissue or remote organ systems" (Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, page 931).
    - "…best defined clinically by a patient with [or all of the following criteria: (1) symptoms such as epigastric pain, consistent with the disease; (2) a serum amylase or lipase greater than three times a year limit of normal; or (3) radiologic imaging consistent with the diagnosis, usually using computed tomography (CT) or magnetic resonance imaging (MRI) ((Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, page 960).
  - Acute pancreatitis may be caused by or associated with genetic defects which disrupt mechanisms which normally protects the process from trypsin associated injury, and therapy increases the susceptibility of the pancreas.

- Give 6 major factors involved in the **pathogenesis** of acute pancreatitis.
  - Failure of the normal mechanisms of protecting pancreas fro autodigestion by trypsin and the pancreatic proenzymes activated trypsin.
  - Activation of complement and kinin systems
  - Associated genetic abnormalities
    - $SPINK_1$ (aka PSTI, pancreatic secretory trypsin inhibitor) mutation resulting in loss of the normal effect of inactivating 20% of tryps activity.
    - $PRSS_1$ 9cationic trypsinogen gene)
  - Associated environmental abnormalities e.g.
    - ↑ serum triglyceride
    - ↑ serum Ca^{2+}
  - Disassociation
    - Between continued synthesis but blocked secretion of pancrea

enzymes
- Enzymes accumulate in acini, causing damage
- Possible colocalization of pancreatic enzymes in the lysosomes

- Disruption of tight junctions
  - Pancreas: loss of paracellular barrier of acinar cells and intralobu pancreatic duct cells
  - GI tract: ↑ translocation of gut bacteria
- Microcirculatory changes
  - Vasoconstriction
  - Release of VCAM-1 (an endothelial adhesion molecule)
    - PGs (prostaglandins)
    - PAF (platelet activating factor)
    - Leukotrienes
  - Ischemia
  - AV shunting in gut (plus ischemia → GI bleeding)
  - Possible reperfusion injury
- Immune changes
  - Activation of complement and release of C5a
  - Recruitments of PMNs and macrophages
  - ↑ proinflammatory cytokines
  - ↑ mediators of inflammation
    - Arachidonic acid metabolites
    - NO (nitric oxide)
    - Reactive oxygen metabolites

Success is peace of mind which is a direct result of self-satisfaction in knowing you did your best to become the best you are capable of becoming.

John R Wooden

Hereditary Pancreatitis

- Definition
  - Hereditary pancreatitis is "……recurrent acute or chronic pancreatitis in an individual from a family in which the pancreatitis phenotype appears to be inherited through a disease-causing gene mutation expressed in an autosomal dominant pattern."
    - Caused by mutations in $PRSS_1$ (cationic trypsinogen) gene
    - Clinical phenotype: recurrent acute pancreatitis
    - Penetrance of genotype to phenotype 80%
    - Familial pancreatitis is "….pancreatitis from any cause that occurs in a family with an incidence [of pancreatitis] that is greater than would be expected by chance alone, given the size of the family and incidence of pancreatitis within a defined population" (familial may or may not be caused by a genetic defect).
    - Usually caused by
      - SPINK1 mutations, or
      - CFTR – SPINK1 genotypes atypical (homozygous or atypical compound heterozygous.
    - Tropical pancreatitis is "……a form of early age-onset, non-alcoholic chronic pancreatitis occurring in tropical regions what is often clustered among family members, and that has a complex genetic basis."
      - SPINK1 N34S mutations are common

- Give examples of four **major susceptibility genes** which cause or predispose to pancreatic disease.
  - Cationic trypsinogen ($PRSS_1$) [protease serine 1]) gene
  - There are 3 forms of trypsinogen
  - $PRSS_1$ cationic trypsinogen (65%)
  - $PRSS_2$ anionic trypsinogen (30%)
  - $PRSS_3$ mesotrypsin
  - PST_1 (aka $SPINK_1$ [serine protease inhibitors Kazal type 1])
  - $PRSS_2$ anionic CFTR ("cystic fibrosis conductance regulator gene)
  - SBDS (Schwachman-Bodian-Diamond Syndrome) gene
  - CASR (Calcium-sensing receptor) gene polymorphism

- Some mutations of CFTR, SPINK1, and PRSS1 genes are associat with acute recurrent or chronic pancreatitis.
- All patients with such mutations do not develop acute recurrent chronic pancreatitis, especially those with CFTR and SPINK1 ge mutations.

Useful background: Pancreatic Gene Mutations

- Pancreatic Secretory Trypsin Inhibitor (PSTI) Gene Mutations (aka $SPINK_1$ [serine protease inhibitor, Kazal type 1])
  - Colocalized in zymogen granules in the pancreatic acinar cells.
  - Presents the premature activation of trypsinogen in the pancreatic acinar cells.
  - Mutations, or polygenic mutation of CFTR- $SPINK_1$ are associated with familial pancreatitis.
  - Secrete with trypsin
  - An acute phase reactant
  - Acts as the first line defense against prematurely activated trypsinogen
  - Co-mutations such as with CFTR or $PRSS_1$ increase the risk of fibrosis.
  - ↑ $SPINK_1$ with inflammation of pancreas
  - $SPINK_1$ N34S is the most common $SPINK_1$ mutation
  - $SPINK_1$ mutations lead to less trypsin inhibition.
  - These loss-of-function polymorphisms are important
    - When acute pancreatitis occurs.
    - When there is recurrent premature activation of trypsin (e.g. CFTR or PRSS, mutation).

Useful additional material: Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, Table 57-4 (Clinical manifestation of cystic fibrosis) and Table 57-5 (Frequency of gastrointestinal manifestations in cystic fibrosis), page 941).

Adapted from: Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, page 937).

- Cationic trypsinogen gene ($PRSS_1$) mutations
  - There are numerous gene mutations in $PRSS_1$.
  - These mutations may be Ca^{2+}-dependent or independent.
  - This is Ca^{2+} dependent
  - The main genetic mutations in $PRSS_1$ are $R_{122}H$ and $N_{29}I$ mutations.
  - $PRSS_1$ gene mutation is associated with hereditary pancreatitis.
  - The $R_{122}H$ is a "conversion mutation" which alters the high fidelity regulatory mechanism of $PRSS_1$.
  - The $R_{122}H$ and $N_{29}I$ mutations of $PRSS_1$ result in
    - ↑ activation, or
    - ↓ inactivation
  - The switch on/off represents a process to enhance trypsin activity ("gain-of-function").
- Ca^{2+}-independent-mutation
  - Process related to maintenance of intracellular pH.

- Give the pathophysiology of **acute alcohol-associated damage** to the pancreas.
  - Direct effect of alcohol and its "toxic" metabolites on pancrea acinar cells.
  - ↑ lithogenicity of pancreatic fluid (alcohol-related ↑ secretion a viscosity of pancreatic juice → precipitation of protein (such as G 2) → formation of pancreatic stone (crystals of calcium carbonat polysaccharides, fibrillar proteins, and a gel-like matrix)
  - Acute but, sometimes subclinical pancreatitis → scaring preductular area → stasis of ductules → progression to fibrosis
  - Acute pancreatitis activates trypsin → ↑ inflammation → ↑ cytokin → activates pancreatic stellate cells → fibrosis

SO YOU WANT TO BE A GASTROENTEROLOGIST!

- Give 6 systemic diseases associated with pancreatitis.

○ Endocrine	– Diabetic ketoacidosis – Hypercalcemia
○ Hematology	– HUS (hemolytic uremic syndrome)
○ MSK	– SLE (systemic lupus erythematosis)
○ Transplantation	– The procedure itself, or immune suppressant drugs

- Give the pathophysiology of the failure of 3 organs other than the pancreas other than the pancreas in necrotizing pancreatitis.

  - Heart
    - SIRS (systemic inflammatory response syndrome)
      - Activation of
        - Trypsin
        - Phospholipase
        - Elastase
      - Release into portal circulation of TNF, PAS
        - Activation of hepatic Kupffer cells
        - ↑ CRP (C-reactive protein)
        - ↑ IL-6
  - Lung
    - ARDS (acute respiratory distress syndrome)
      - Breakdown of surface from
        - ↑ activity of phospholipase A
  - Kidney
    - ↓ SBP (hypotension) ↓ blood volume (hypovolemia)
  - GI tract
    - Microcirculatory changes e.g. vasoconstriction → ischemia, AV shunting in gut

- Chronic pancreatitis "…..is a process that usually begins with recurrent pancreatitis and ends with immune-related destruction of the pancreas and widespread glandular fibrosis" ((Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, page 931).
- Chronic pancreatitis displays chronic inflammation as well as fibrosis.
- The fibrosis of chronic pancreatitis results from immune-mediated process (e.g. TGF-β and IL-10) which activate the pancreatic stellate cells.

- Give the pathophysiology of **chronic pancreatitis** due to alcohol abuse.

➢ Direct injury by alcol or a metabolite	○ Liver – Acetaldehyde ○ Pancreas – FAEEs (fatty acid esters) → ↑ Ca_i^{2+}
➢ Oxidative stress	○ Production of free radicals ○ ↑ peroxidation of membrane lipids
➢ CCK	○ Alcohol-associated ↑ sensitivity of acinar cells to CCK ○ Redirection of CCK-mediation zymogen granule exocyt from apical to basolateral membrane of acinar cell.
➢ Genes / enzymes	○ Alcohol-associated – Altered expression of genes responsive physiologic stress ○ Over – ↑ expression / activity of necrosis / apoptosis
➢ Stellate cells	○ ↑ activity of pancreatic acinar cells associat stellate (myofibroblastic) cells – ↑ extracellular matrix → ↑ fibrosis → ischemia – ↑ proliferation – Phagocytosis ○ Alcohol / alcohol metabolites ○ Cytokines (from necrosis of cells) ○ Growth factors (PDGF, TGF-B1) ○ Transcription factors

	○ Angiotensin II ○ Autocrine factors
➢ Protein precipitati (plugs)	○ Alcohol stimulates pancreatic secretion of flu with ↑ protein, ↓ volume and HCO_3^-
➢ Gain of function gene mutation, leading SAPE	○ CFTR (cystic fibrosis transmembra conductance regulator gene) ○ PRSS1 (cationic trypsinogen gene) ○ SPINK1 (a trypsin inhibitor) ○ These genetic mutations a hypothesized to lead to repeat episodes of acute pancreatitis, which s in motion a process that leads to chroi pancreatitis (SAPE, sentinel acu pancreatitis event)

Abbreviations: Ca_i^{2+}, intracellular Ca^{2+}; CCK, cholecystokinin; PDGF, platelet-derived growth factor; TGF, transforming growth factor

Abdominal pain is common in persons with pancreatitis. It has been suggested that the pain is due to ↑ pressure in the duct or parenchyma of the pancreas, resulting in tissue ischemia.

- Give evidence for **3 alternate theories** for the pain of chronic pancreatitis.

➢ Nerves (neuroimmune interaction)
  - ○ ↑ number and diameter of nerves in the pancreas
  - ○ ↑ inflammation around these nerves and ganglia
  - ○ Axons release pain mediators, such as
    - Substance P
    - CERP
    - Glutamate
  - ○ GAP-43 (growth associated protein 43)
  - ○ ↑ NGF (nerve growth factor) and TrkA (NGF receptor)
  - ○ ↑ endogenous proteases

○ CCK
  - ○ ↑ sensitivity of pancreas to stimulation by CCK
  - ○ ↑ duct pressure

- Redirecting pancreas enzymes to basolateral rather than apical portion of acinar cells

- Give the factors responsible for the development of **diabetes mellitus** in chronic pancreatitis.
  - Loss of islet cells
    - From chronic pancreatitis disease process
    - From resection of pancreas
    - ↑ amylase → ↑ insulin resistance
  - Hepatic insu receptors
    - ↓ number
    - ↓ function

➢ Enzymes (secreted in their acti form)
- Amylase
- Lipase
- Carboxylesterase
- Sterol esterase
- DNase
- RNase

➢ Proenzymes*
- Endopeptidases
  - Trypsinogen
    - Cationic
    - Anionic
    - Mesotrypsinogen
    - Chymotrypsinogen
  - Kallireinogen
  - Chymotrypsinogen
  - Proelastase
- Exopeptidase
  - Procarboxypeptidase A,B
  - Pyrophospholipase
  - Procolipase

*Inactive enzmes, requiring activation enterokinase and trypsin

- The pancreatic enzymes digest carbohydrates (amylase), proteins and lipids (lipase, phospholipase, colipase, carboxyl ester lipase), and nucleic acids

Pancreas Divisum (PD)

- Definition: A congenital abnormality caused by: “failure of the dorsal and ventral pancreatic ducts to fuse”.

- Most of the pancreatic secretions then drain “…….through the relatively small dorsal duct of Santorini and minor [accessory] papilla rather than the ventral duct of Wirsung and the major papilla” (Feldman M., et al. *Sleisenger and Fordtran’s Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, page 918).

- Sphincterotomy of minor papilla or placement of stent in minor (accessory) papilla may be helpful for symptoms of pancreatitis (acute or chronic), especially with pain characteristic of acute recurrent pancreatitis.

    - In patients with CFTR gene mutations, pancreas divisum is found in about half!

    - The frequency of pancreas divisum is the same in patients with idiopathic pancreatitis, chronic alcoholic pancreatitis, and in controls without any pancreatic diseases (5-7%).

    - CFTR gene (and at a lower level, SPINK1 and PRSS1) mutations and pancreas divisum might be cofactors associated with the occurrence of acute recurrent or chronic pancreatitis.

    - Pancreas divisum should no longer be considered as a cause of pancreatitis.

Source: Bertin C, et al. Am J Gastroenterol 2012; 107: 311-317.

There’s always a taller mountain.

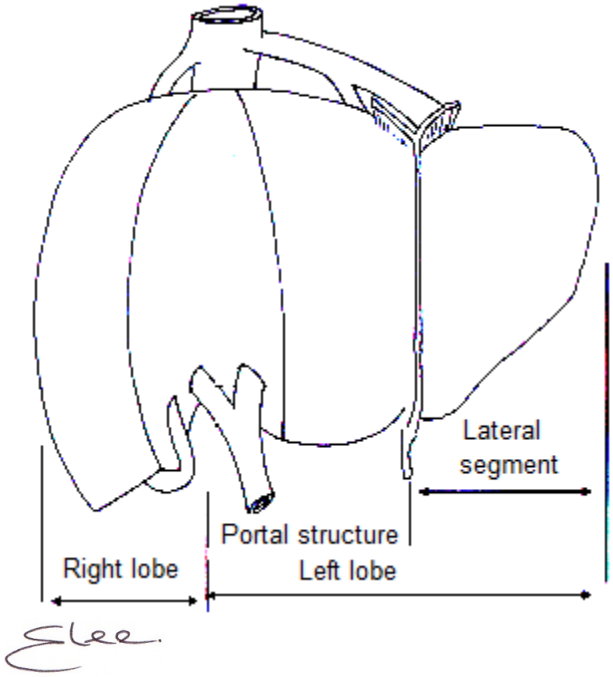
There is always someone better than you are.

Grandad

HEPATOBILIARY SYSTEM

Anatomy of the Liver

- Anterior view of the two functional halves of the liver

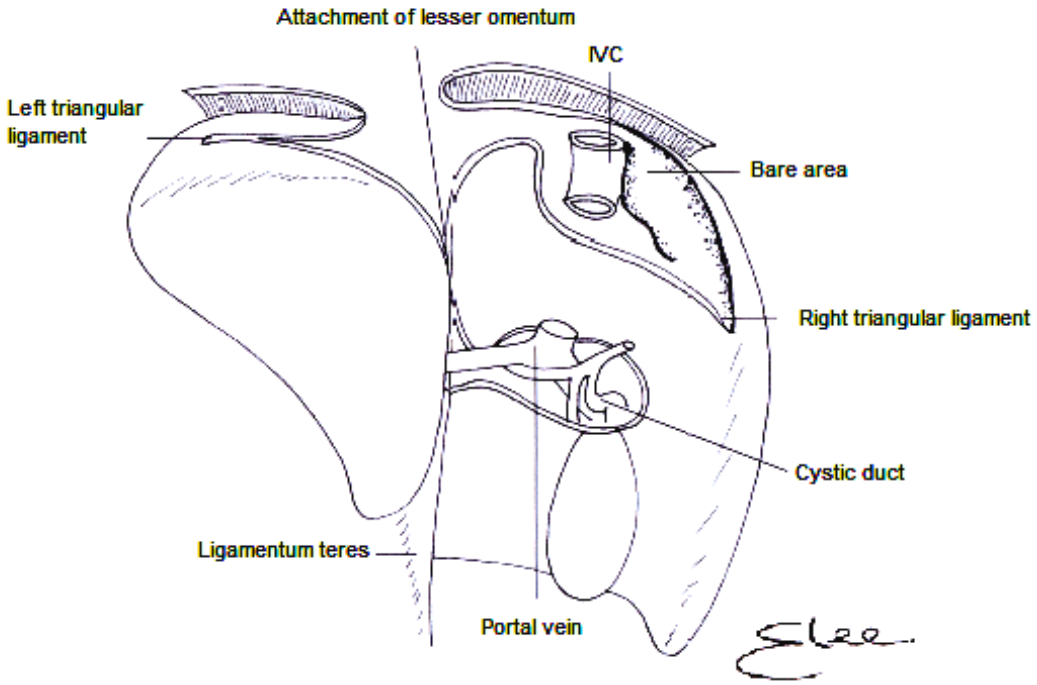


  - The three hilar structures are represented by a single tube with right and left branches.
  - The hepatic veins are shown with a long extrahepatic course for clarity.
  - The functional division into the right and left lobes is not related to the attachment of the falciform ligament.

"Belief in oneself is one of the most important bricks in building any successful venture"

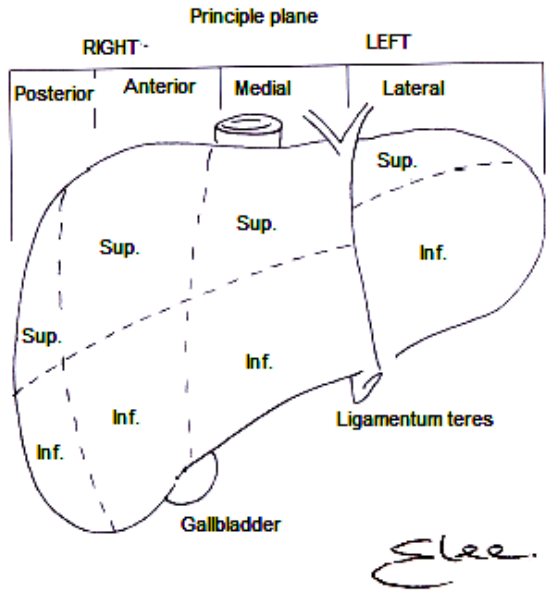
Lydia M. Child

- Posterior view of the liver, showing the right and left

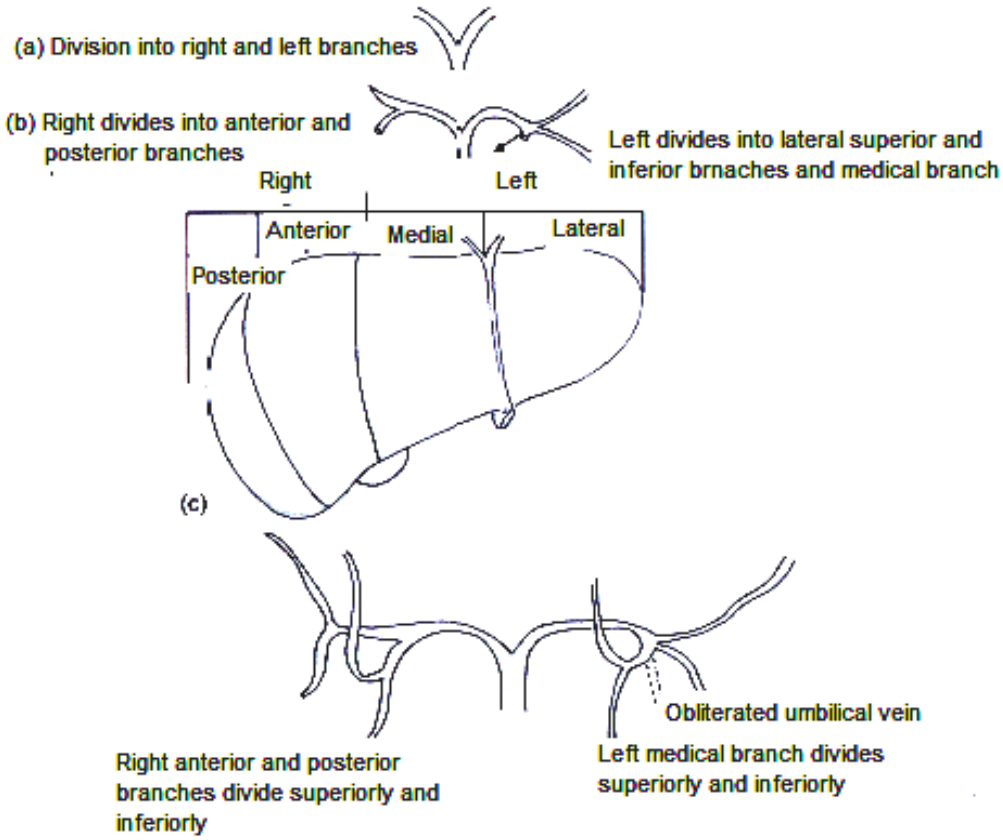


Triangular Ligaments

- Anterior view of the segments of the liver



- Intrahepatic branches of the portal vein

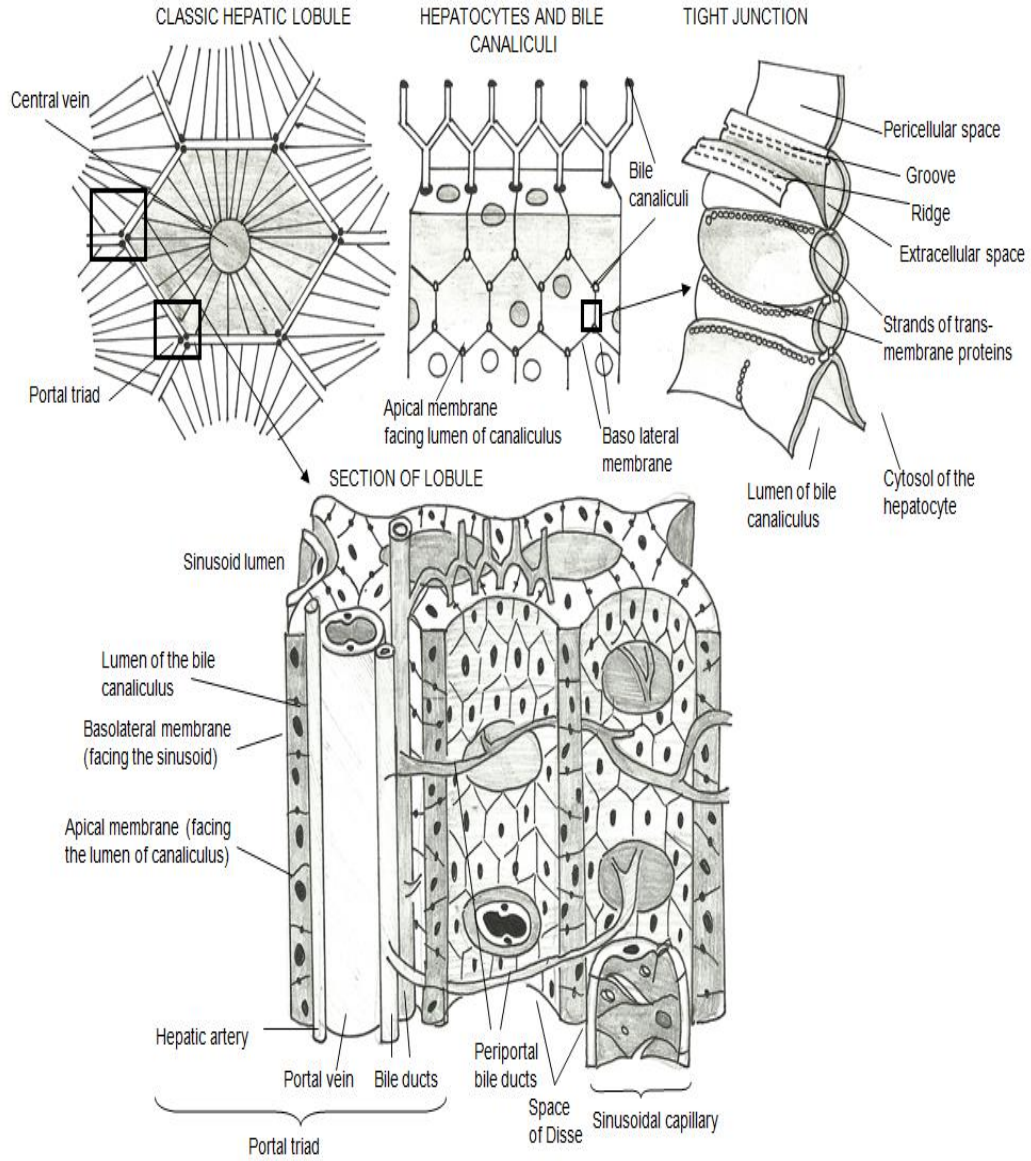


Elee.

"Let's change the interactions between alerting, orienting and executive functions, and control in a standard curing paradigm."
Grandad

Heptatocytes, Sinusoids, and the Intrahepatic Bile System

HEPATOCYTES, SINUSOIDS, AND THE INTRAHEPATIC BILE SYSTEM



Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Figure 46-1, page 981.

Structure

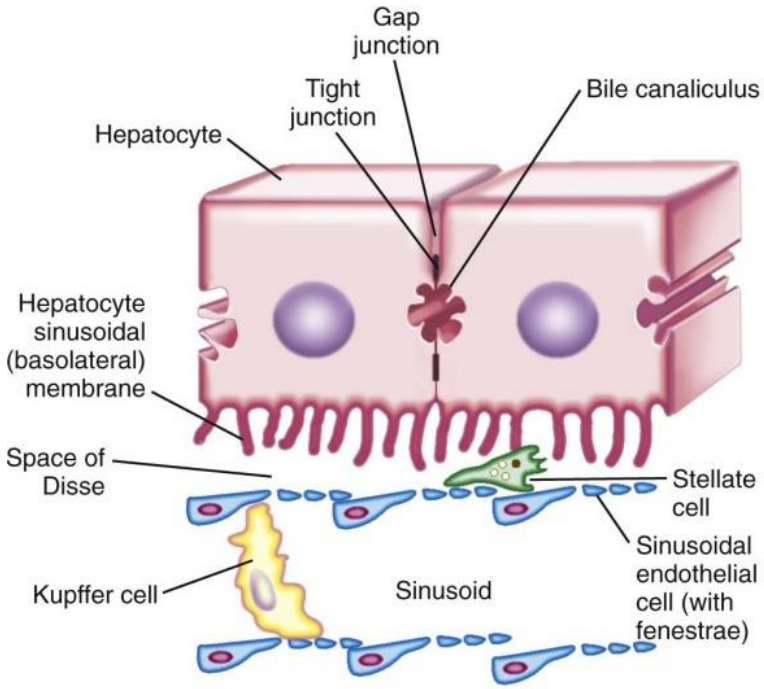
- Triad
  - The brush border membrane of the hepatocytes faces the lumen of the bile canaliculae.
  - Gap junctions allow some leakyness of the membrane, and thereby some passive movement of solutes.
  - The extracellular matrix in the sinusoidal spaces of Disse contains the support structure or scaffolding for the liver lobules.
  - Kupffer cells (fixed monocytes in the reticuloendothial system [RES]) are in the sinusoidal spaces (of Disse), where they are in direct contact with the sinusoidal endothelial cells and portal blood.
  - Stellate (fat-storing or “Ito”) cells also are in the space of Disse.
  - These Ito or stellate cells store fat, and also act both as myofibroblasts, and as the source of the fibrotic reaction which leads to cirrhosis.
  - The hepatic triad is formed by the hepatic artery (HA), the portal vein (PV), and the bile duct.
  - At each corner of the hexagonal hepatic lobule is a triad, and in the center is a central vein (CV).
  - The portal lobule represents the hepatocytes which drain into a bile duct in the hepatic triad.
  - Plasma in the sinusoidal space comes into direct contact with hepatocytes in the space of Disse.
  - The endothelial cells are fenestrated and lack a basement membrane. Thus, these endothelial cells are highly permeable.
  - Stellate cells are between the endothelial cells and the hepatocytes, where they are in direct contact.
  - Two adjacent hepatocytes form a canaliculus, with the cells joined by tight junctions and the communicating gap junctions.
  - The canalicular domain of the plasma membrane of two adjacent hepatocytes encloses and forms the bile canaliculus.

- The portal tracts normally arise at differing heights and angles from the conducting tract and not, as represented here, by simultaneous division.
- Thus, there may be as few as three or as many as six portal tracts seen around any one lobule.
- Three conducting portal tracts (Cpt) here each split into three terminal portal tracts (TPT).
- The parenchyma has been diagrammatically represened as three zones which do not have a morphologically visible counterpart in routine histology.
- Zone 1 (Z 1) is immediately around the terminal portal tract. Zone 2 (Z 2) surrounds zone 1, and zone 3 (Z 3), drawn in as hepatocytes and sinusoids, fills in the spaces between the zone 2s down to the central efferent vein (CV).
- Each terminal portal tract is surrounded by zoned parenchyma and constitutes an acinus.
- Each group of three acini with their conducting portal tract form a complex acinus.
- Nine potential acini are illustrated, of which six are more fully detailed to include zone 3 hepatocytes.
- No acinus is complete because each will dain to more than one central vein.
- Note also that one lobule is formed from between three and six acini with approximatedly half of any one acinua contributing to the lobule.

"The man who follows the crowd will usually get no further than the crowd. The man who walks alone is likely to find himself in places no one has ever been"

Alan Ashley-Pitt

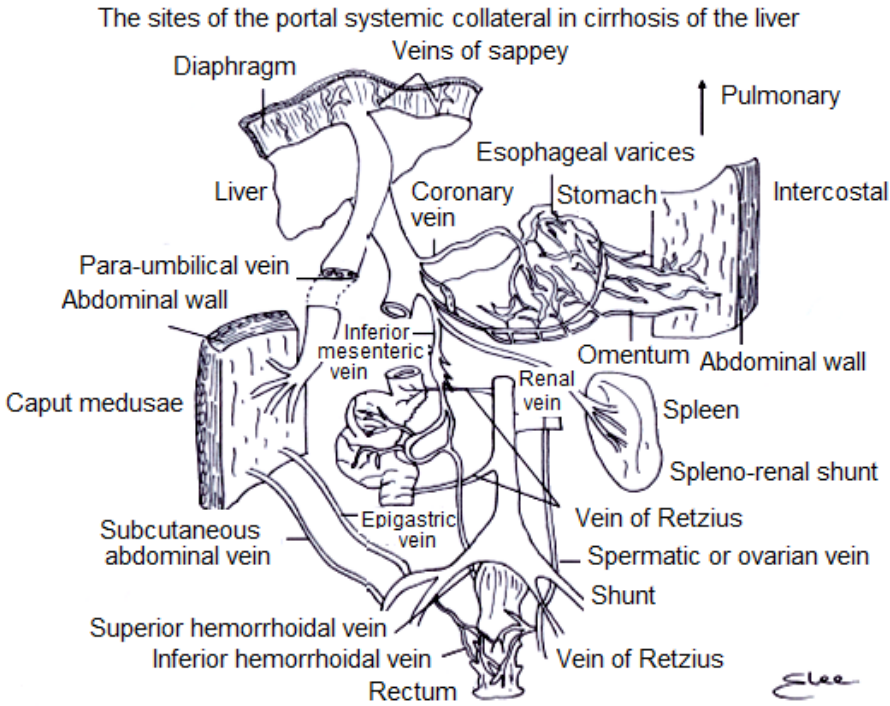
FUNCTIONAL RELATIONSHIPS



Adapted from: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Figure 72-1, page 1208, Ninth Edition, 2010.

- The portal circulation is important for
  - The delivery to the liver of nutrients and other substances absorbed into or secreted by the intestine.
  - The development of increased presence in the portal circulation leads to PHT (portal hypertension), which be associated with the development of serious conditions such as bleeding esophageal varices, ascites, renal dysfunction, and hepatic encephalopathy.
  - Blood vessels that constitute the portal circulation and hepatic outflow tracts become distented as a result of increased blood flow and/or increased resistance, causing portal hypertension.
  - Progressive branching division into the right and left branches of the intrahepatic portal vein and its distribution to the lobes of the liver.

Portal Circulation



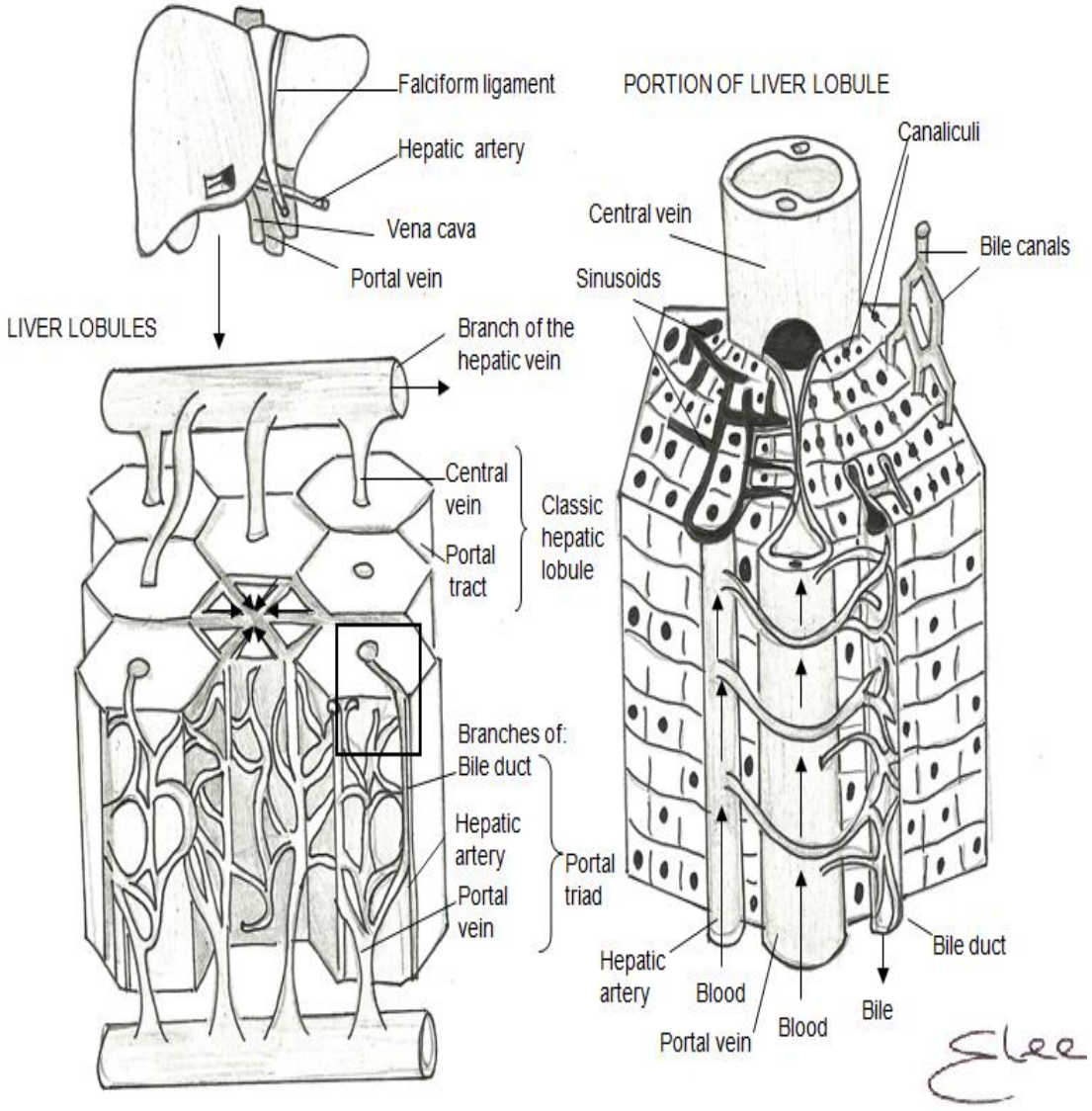
Adapted from: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* page 149, Ninth Edition, 2010; and Sherlock and Dooley, page 61, 11th Edition, 2002.

- Portal vein is posterior to the pancreas
- Division of the right branch into the anterior and posterior branches divide superiorly and inferiorly.
- Division of left branch into lateral and medial branches.
- Four segments of liver are supplied by the right anterior and posterior and the left lateral and medial branches
- Flows and pressures in hepatic vein (HV), portal vein (PV) and hepatic artery (HA):

	Flow, ml/min	Pressure, mm Hg
HV	1600	4
PV	1200	7
HA	400	100

- Inferior mesenteric vein
  - Tributaries from left colon and rectum
- Superior mesenteric vein
  - Tributaries from small intestine, right colon, and head of pancreas

Blood Supply and Flow to the Liver



Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Figure 46-2, page 983.

- Blood to the liver flows in the portal vein (PV) and the hepatic artery (HA) (75% and 25%, respectively).
- Blood from the PV mixes with blood from the HA, flows to the central vein (CV), and then to the hepatic vein (HV).
- Blood from the HA flows into the sinusoidal capillaries, through the space of Disse (parasinusoidal space), between the microvilli of the hepatocyte basolateral membrane facing the sinusoid, and then into the hexagonally-shaped hepatocytes.
- Hepatocytes which are close to the terminal portal vein (PV) and the terminal hepatic arteriole in the portal triad (zone I) have the richest blood supply.
- There is heterogeneity of hepatic function in zone I and III
- Zone I hepatocytes in the portal acinae are relatively resistant to toxic damage.
- The type and amount of subtrate or xenobiotic presented to zone I hepatocytes is different from zone III.
- Zone I hepatocytes specialize in oxidative metabolism, whereas zone III hepatocytes specialize in biotransformations and drug detoxification.
- Hepatocytes in zone III near the central nerve (CV) have a comparatively poor blood supply, and are more vulnerable to damage.
- The sinusoidal lumen is lined by fenestrated sinusoidal endothelial cells that allow the transport of macromolecules out of the space of Disse.
- Quiescent hepatic stellate cells reside within this space of Disse, adjacent to hepatocytes and endothelial cells.
- In cirrhosis, a number of changes occur in the hepatic microcirculation, including loss of fenestrae in endothelial cells (defenestration), constriction of sinusoids, and activation of hepatic stellate cells.
- Activation of the stellate cells stimulates the formation of fibrosis.

Portal Hypertension

CLINICAL PHYSIOLOGICAL CHALLENGE- Portal Hypertension

Case: A 45 year old man develops esophageal varices. He consumes over 20 ounces of alcohol a day, and is suspected to having portal hypertension (PHT) from cirrhosis.

- Give the portal circulation, define PHT, and explain the mechanism of development of PHT in prehepatic, intrahepatic and posthepatic disorders.

- Definition:
  - Hepatic sinusoidal pressure ≥ 6 mm Hg
- Background
  - After a meal, portal blood flow increases, with no ↑ portal pressure, because the hepatic sinusoids have
    - High
      - Compliance
      - Accommodation
    - Low
      - Resistance
  - Blood supply to the liver is 70% portal vein (PV) blood (from mesenteric circulation)
  - 30% hepatic artery (HA) from celiac artery
  - With the dual blood supply (portal venous inflow) to the liver, when blood flow increases in the PV, it decreases in the AA, and vice versa, through an autoregulatory mechanism
    - Recall that we are dealing with three distinct vascular beds
      - Intrahepatic
      - Splanchnic
      - Systemic

- Give the pathogenesis of the systemic and splanchnic (extrahepatic) vasodilation which leads to the hyperdynamic circulation in portal hypertension.
  - Basic factors
    - ↑ resistance
    - ↑ flow hyperdynamic circulatory state
    - ↑ collaterals (↑ angiogenesis)
    - ↓ fenestration
    - Ohm's law

$$\Delta P = FxR$$

F, flow; ΔP, change in pressure; R, resistance

- Portal flow and resistance increase
  - Mechanical factors
  - Regenerative nodules
  - Fibrotic bands
  - Defenestration and capillarization of sinusoids
  - Swelling of hepatocytes and kupffer cells
  - Vascular factors
- Intrahepatic vasoconstriction, and ↓ response vasodilations

○ ↑ systemic sympathetic activity

➢ Hepatic vasoconstriction and ↑ increased intrahepatic resistance

○ Law of poiseuille $R = 8 \eta L / \P r^4$

○ Resistance (R) is affected by the length and radius (r) of the vessel, and by the viscosity of the blood

○ ↑ sheer stress on sinusoids
- ↑ eNOS → NO (nitric oxide) → intrahepatic vasodilation
- ↑ ET-1 (endothelin-1) → binds to
  - ET-A receptors on HSC → ↑ HSC contraction → intrahepatic vasoconstriction
  - Also binds to ET-B receptors on endothelial cells → activates eNOS → vasodilation
- In cirrhosis, there may be ↑ NO production
- In splanchnic endothelial cells from ↑ sheer stress, ↑ cytokines, and ↑ phosphorylation of eNOS, leading to vasodilation in splanchnic and systemic vascular beds (note below that there is ↓ intrahepatic NO, ↓ intrahepatic vasodilation, ↑ intrahepatic vasoconstriction)

○ In cirrhosis, there is ↓ production of NO
- ↓ activation of eNOS protein in endothelial cells
- ↑ caveolin-1 (an eNOS inhibiting protein)
- ↓ AKT (protein kinase B)
- Phosphorylation of eNOS
- ↑ GRK (G protein-coupled receptor kinase, which inhibits eNOS)
- ↑ VEGF (vascular endothelial growth factor) from shear stress may ↑ eNOS (VEGF is a NO stimulatory growth factor)

○ The vasodilation in splanchnic and systemic (peripheral) vascular beds leads to
- ↑ cardiac output
- ↓ mean arterial pressure
- ↑ systemic blood flowing into splanchnic circulation

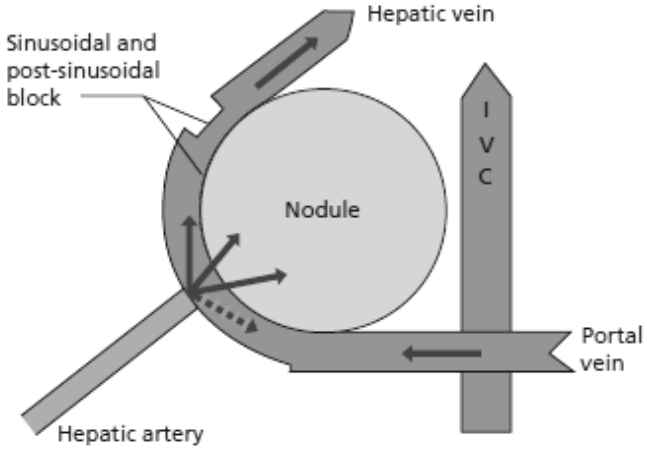
○ The overall effect is ↓ intrahepatic NO and ↓ intrahepatic vasodilation (effect on endothelial cells as well as possibly on HSCs)

- ↓ intrahepatic vasodilation results in ↑ intrahepatic vasoconstriction
- ↑ intrahepatic vasoconstriction leads to ↑↑ intrahepatic resistance (law of Poiseuille)
- Give NO in cirrhosis will restore the intrahepatic vasodilation arising from the endothelial cells, but the HSC in cirrhosis contribute to the vasoconstriction and are less responsive to NO. Replacement in cirrhosis, so benefit of restoring ↓ NO levels does not fully restore the vessel diameter to normal
- In cirrhosis, there is ↑ intestinal endotoxin (LPS) → ↑ TGF-β, ↑ ET-1, with binding to ET-A receptors on HSC, and intrahepatic vasoconstriction

Abbreviations: HSC, hepatic stellate cell; LPS, lipopolysaccharide; eNOS, endothelial NO synthase

- Sympathetic overactivity (norepinephrine) in cirrhosis will also act as an intrahepatic vasoconstrictor, as also will
  - Angiotensin
  - Leukotrienes
  - Thromboxane

THE CIRCULATION OF HEPATIC CIRRHOSIS



- A nodule obstructs the sinusoids and HV, causing ↑ HV pressure
- The nodule is suppled mainly by the HA

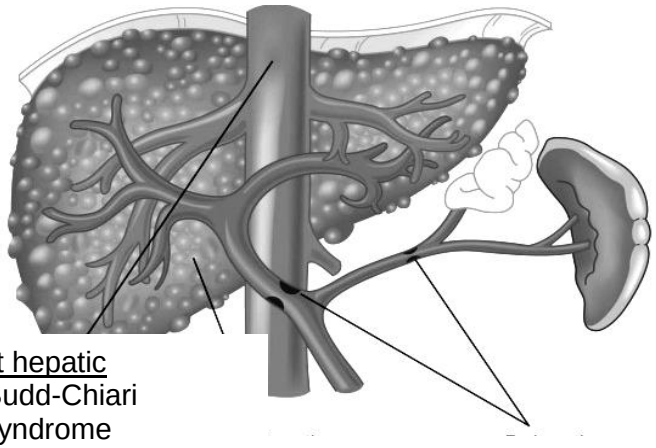
The dotted line (- - -) represents possible retrograde flow in the PV

Abbreviations: HA, hepatic artery; HV, hepatic vain; PBC, primary biliary cirrhosis; PV, portal vein; S, sinusoids

Adapted from: Sherlock and Dooley, 11th Edition, 2002, page 168, 170 and 169.

CLASSIFICATION OF CAUSES OF PORTAL HYPERTENSION

- The different sites of increased resistance to portal flow (posthepatic, intrahepatic, and prehepatic) and associated diseases are shown.
- Many diseases have a mixed pattern.



Increased Portal Blood Flow
- Arteriovenous fistula
- Idiopathy tropical splenomegaly

Post hepatic
- Budd-Chiari syndrome
- Constrictive pericarditis
- Inferior vena cava obstruction
- Right-sided heart failure
  - Webs
  - Tumour
  - Invasion
  - thrombosis
- Severe tricuspid regurgitation

Intrahepatic
- Pre-sinusoidal (non-cirrhotic)
  - Idiopathic portal hypertensior
  - Primary biliary cirrhosis
  - Sarcoidosis
  - Schistosomiasis
  - Chronic active hepatitis
  - Congenical hepatic fibrosis
  - Toxins: vinyl chloride arsenic
  - Idiopathic portal hypertensior
- Sinusoidal
  - Alcoholic cirrhosis
  - Alcoholic hepatitis
  - Cryptogenic cirrhosis
  - Post-necrotic cirrhosis
- Post-sinusoidal
  - Sinusoidal obstruction syndrome
  - Veno-occlusive disease
  - Central hyaline sclerosis (alcoholic liver disease)

Prehepatic
- Portal vein (PV) thrombosis
- Splenic vein (SV)thrombosis
- Wedge hepatic vein pressure may be high in patients with "presinusoidal" causes, especially as the disease progresses, indicating sinusoidal and/or collateral

Adapted from: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Figure 90-5, page 1493, 2010; and Sherlock and Dooley, Figure 10.36, page 166, 10.40, and 10.41, pages 168-169, 11th Edition 2002.

➢ Treatment

- Give the mechanism(s) of action of 5 **drugs** used to treat portal hypertension (PHT).

  - Drugs used for treatment of PHT generally are selected to act to
    - ↓ portal blood flow, or
    - ↓ intrahepatic resistance

  - ↓ Portal Blood Flow → ↓ PP (Portal Pressure)
    - Octreotide
      - ↓ glucagon
        - ↑ splanchnic vasoconstriction
        - ↓ portal and azygous blood flow
        - ↓ postprandial splanchnic blood blow
      - ↓ GT-1-dependent contraction of HSC (hepatic stellate cells)
    - β-adrenergic blocking agents (non-selective)
      - ↓ cardiac output (CO)
      - Vasodilation of mesenteric circulation
      - ↑ "unopposed action of α1-adrenergic receptors →
        - ↓ portal flow
        - ↓ intrahepatic resistance
    - Vasopressin
      - Splanchnic vasoconstriction
      - ↓ PV (portal vein) inflow
      - ↓ CO (inotropic)
      - ↓ HR (chronotropic)
  - ↓ Intrahepatic Resistance
  - Under study
    - A1-adrenergic antagonists
    - ARBs (angiotensin II receptor type I blockers)
    - Nitrates
  - Nitrates
    - Venodilation from ↓ Ca_i^{2+} in smooth muscle in venous wall

Esophageal Varices (EV)

- Give the 4 zones of venous drainage in EV.
  - Truncal zone
    - 10 cm in length, above perforating zone
    - 4 longitudinal veins in the lamina propria
  - Perforating zor
    - Above palisade zone
    - Network of esophageal submucosal veins anastomo with periesophageal external veins
    - Periesophageal veins drain into azygous system
  - Palisade zone
    - 2-3 cm above (GE) junction
    - 4 longitudinal veins anastomose with veins in t lamina propria
    - No anastomosis with periesophageal external (i.e., perforating veins) veins
    - Limited soft tissue support high risk of bleeding
  - Gastric zone
    - 2-3 cm below GE junctions
    - Longitudinal veins in submucosal and lamina prop these longitudinal veins gather at the gastric cardia a drain into short gastric and left gastric veins

- Give the pathogenesis of esophageal varices (EV).

When the pressure (P) in the esophageal varix rises (> 12 mm Hg), when the radius (r) of the varix increases (small → large varices), and when the thickness of the wall of the varix (w) becomes thin because of the enlarging and dilating vein, then according to the law of Laplace (T = Pr/w), the tension (t) on the wall of the varix becomes so great that a hole develops in the ruptured wall of the vein, and profuse bleeding occurs.

  - Prediction of bleeding from varices
    - Pressure
    - Radius
    - Wall thickness
  - Clinically useful to measure portal pressure
    - HVPG (hepatic venous pressure gradient) = WHVP (wedged hepatic venous pressure) – FDVP (free hepatic vein pressure)
    - Splenic pulp pressure gradient (SPPG)
    - In presinusoidal portal hypertension, HVPG is normal, but SPPG is increased
    - Portal vein pressure

- Endoscopic variceal pressure
  - Varices (miniature pneumatic pressure sensitive gauge on an endoscope
  - Endoscopic balloon
  - Represents the gradient between pressure in portal vein and the intra-abdominal inferior vena cava (IVC)
  - Not useful to measure presinusoidal causes portal hypertension (only good for sinusoidal and causes of PH)
  - Portal vein thrombosis (PVT) causes presinusoidal portal hypertension, which is not correctly assessed by HVPG (is normal in PVT)
  - HVPG cannot be obtained when the hepatic veins are blocked, e.g.
    - BCS (Budd-Chiai syndrome)
    - Intrahepatic, presinusoidal causes of portal hypertension (e.g., idiopathic portal hypertension
    - HVPG may be normal

Abbreviations: HVPG, hepatic venous pressure gradient; FHVP, free hepatic vein pressure; WHVP, wedge hepatic venous pressure

SO YOU WANT TO BE A HEPATOLOGIST!

Gastric vascular ectasia (GVE), including those that occur in the antrum (GAVE), are associate with thrombi in vessels, fibro-hyalinosis, are proliferation of spindle cells. There is no associated portal hypertension bleeding may occur

When patients are supplemented with iron but become transfusion dependent, thermoablative therapy may be given (e.g. argon plasma coagulation, or cryotherapy), or oral estrogen-progesterone combination therapy may be given. If this fails and bleeding continues, surgical approaches may need to be considered, such as antrectomy or liver transplantation.

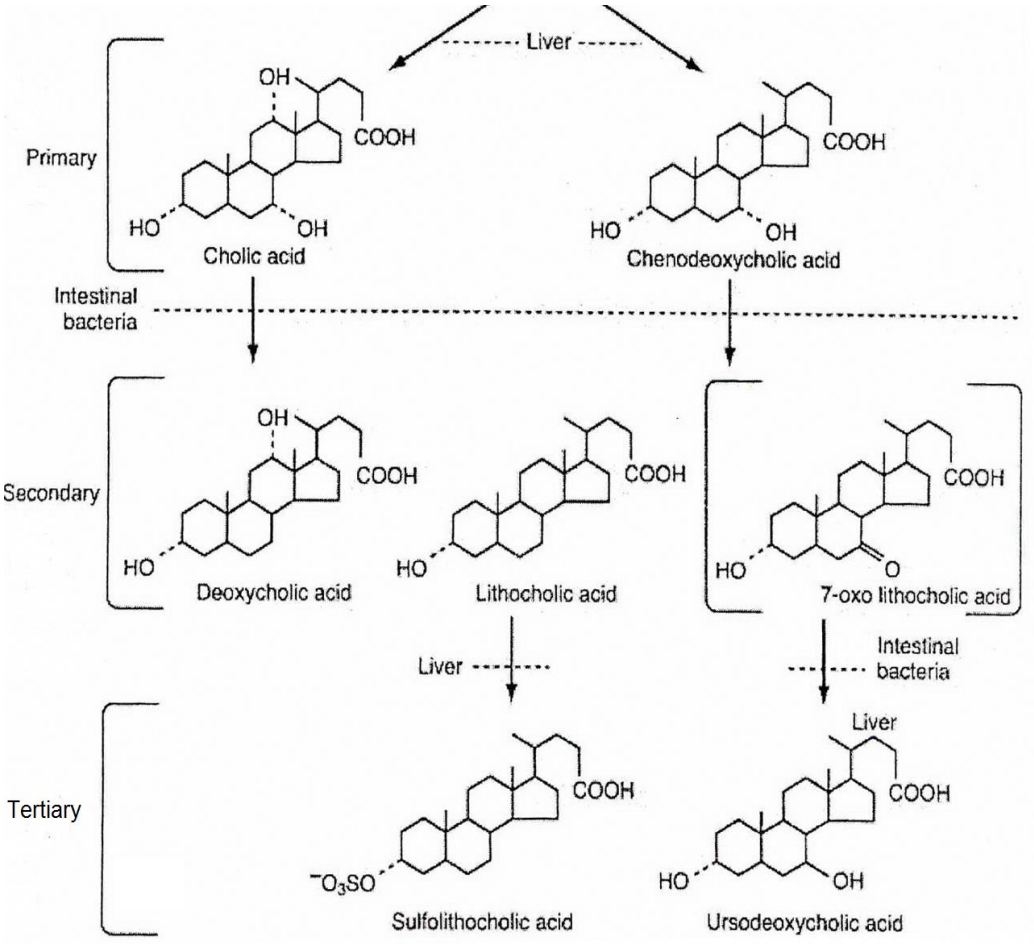
The puzzle is why is TIPS, or any other maneuver to reduce portal pressure, not successful therapy in GVE / GAVE.

- Give an explanation for why liver transplantation (LT) is effective therapy for both PHG (portal hypertensive gastropathy, as well as for GVE / GAVE).
    - The pathogenesis of PHG is portal hypertension, which is corrected by LT
    - The pathogenesis of GVE / GAVE is unknown, but is not related to PHT per se, and is presumably related to the process which causes thrombi in the microvasculature of the stomach
        - It is speculated that GVE / GAVE is caused by some product produced in the failing liver, and which is not appropriately cleared in the presence of this liver failure.
        - LT corrects the liver failure

The Enterohepatic Circulation (EHC) of Bile Acids

- Definition
    - The reabsorption of bile acids, their return to the liver, resecretion by the hepatocyte, and return to the lumen of the small intestine represents the EHC (enterohepatic absorption of bile acids).

STRUCTURE OF PRIMARY, SECONDARY AND TERTIARY BILE ACIDS



Source: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Figure 64-2, page 1077, Ninth Edition, 2010; and *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Figure 46-9, page 995.

Background: Overall Steps in EHC

- To understand the enterohepatic circulation (EHC) of bile acids, one has to appreciate the role of hepatocytes, cholangiocytes, gallbladder, small intestine (especially the terminal ileum) and colon (especially the colonic bacterial).
- Bile is comprised of bile acids, bilirubin, lipids (e.g., cholesterol, phospholipids), as well as electrolytes and water
  - The functons of the EHC
    - Maintain the desired CMC (critical micellar concentration of bile acids) in the lumen of the small intestine to optimize the absorption of lipids.
    - The EHC also provides a pathway for the secretion of cholesterol from the body, by way of the metabolism of CH to BAs.
  - The enterohepatic circulation (EHC) of bile requires
    - The meal-stimulated contraction of the gallbladder
    - The peristalsis moving bile acids along the length of the small intestine for the absorption of lipids
    - Bile acid uptake and exit from the ileocytes
    - The role of the ileum in the regulation of hepatic synthesis of bile acids
    - The uptake, synthesis and excretion of bile acids bile from the hepatocytes.
  - The liver has 3 steps to contribute to the EHC of bile:
    - Synthesis
    - Secretion across the canalicular membrane (aka apical membrane [AM]), and absorption from the portal blood across the sinusoidal membrane (SM) and back into the hepatocyte.

Useful background: BA metabolism

o Size of BA pool	– 2 g to 4 g (50 to 60 mmol per Kg bo weight)
o Cycles of BA pool	– Per meal, 2 to 3
	– Per day, 6 to 10
o Absorption of BA per day	– 10 to 30 g (pool size x number cycles)
o Colonic loss of BA per day	– 200 to 600 mg

- Hepatic synthesis of BA p
  day
- Efficiency of EHC
  - Matches daily fecal losses
  - >> 95%
  - Loss / pool size x number of cycles
    500 mg / 4g x 10 cycles per day → (
    g / 40 g x 100%)

- Give the transport proteins for bile acids in the EHC.

The vocabulary

	AM	BM	Cytosol
➢ Ileocyte	ASBT (apical sodium bile acid transporter)	OST αβ (organic solute transporter	➢ FXR (farnesoid receptor) – FXF (fibroblast grov factor 19)

	SM	CM
➢ Hepatocyte	NTCP (sodium-taurocholate cotransporting polypeptide)	BSEP (bile salt export pump)
➢ Cholangiocyt	ASBT	OST α/β
➢ Renal proximal tubular cell	ASBT	OST α/β

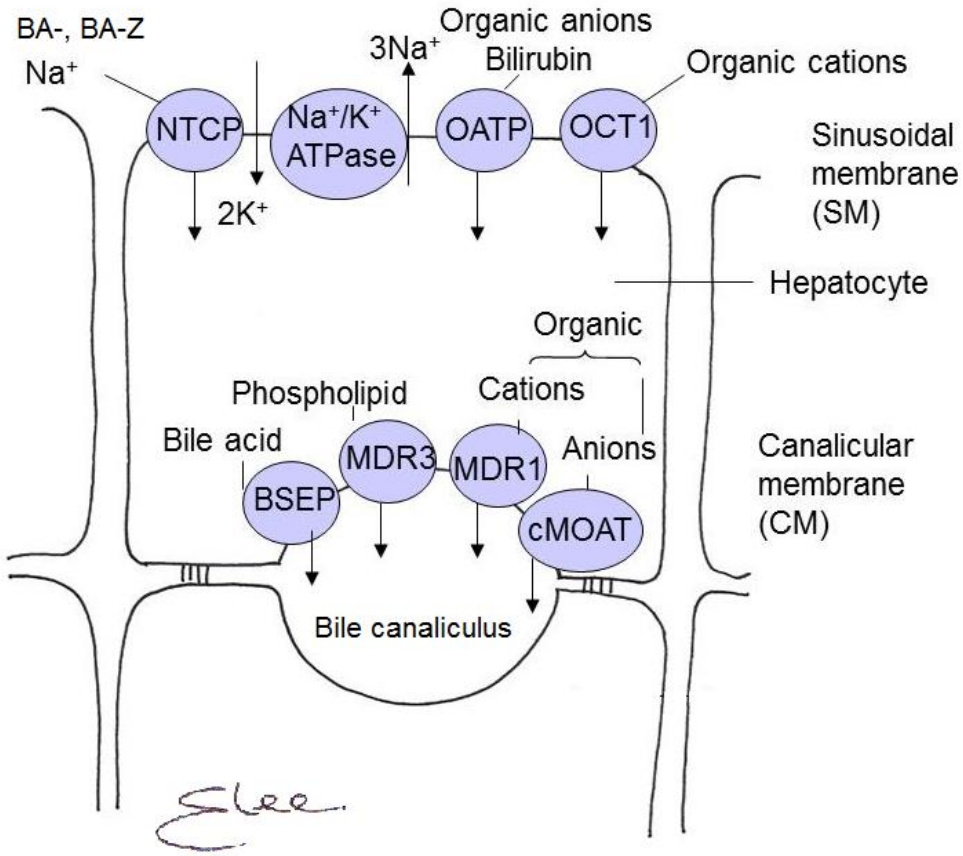
Abbreviations: AM, apical membrane of hepatocyte; BM, basolaterol membrane; CM, canalicular membrane; SM, sinusoidal memebrane

"A lot of things in medicine that make sense,
don't work out"

That's what evidence-based medicine is all about

Grandad

The Role of the Liver in the EHC of BA



First, the vocabulary

- SM (sinusoidal [basal/ basolateral] membrane)
  - NTCP, sodium (Na^+) taurocholate cotransport polypeptide
  - Na^+/ K^+ ATPase
  - OATP, OATP1B1 / OATPB3, organic anion-transporting polypeptides
  - OCT1, organic cation transporter

- CM (canalicular [apical] membrane)
  - BSEP, bile salt export pump

- MDRs, ATP-dependent phospholipid transporter (flippase)
- MDR1, ATP-dependent transporter of organic cations
- cMOAT, multispecific organic anion transporter
- NPC1L1, Niemann-Pick C1 Like 1 protein
- CC, Cl^- channels
- AE2, anion exchanger isoform 2 (Cl^- /HCO_3^- exchanger)
- Aquaporins

- **Hepatic Sinusoidal Membrane (SM) – Uptake of Bile Acids in Portal/ Sinusoidal Blood**
  - 95% of the bile acids in the lumen of the intestine return to the liver through the portal venous blood, and are taken up by the sinusoidal membrane (SM) of the hepatocyte.
  - The bile acids cycle in the EHC (liver-intestine and back to the liver) numerous times (4 to 6) with each meal.
  - Because the intestinal absorption of bile acids is so high, the EHC is very efficient, and the liver does not need to produce large amounts of bile acids each day.
  - The bile acid transporters in the SM include NTCP, Na^+ / K^+ ATPase, NAE, OATP and OCTI.
  - At the sodium - taurocholate cotransporting polypeptide (NTCP; gene symbols SLC 10A1) mediates the uptake of BA-Z and BA-, as well as neutral sterols and cyclic oligopeptides.
  - NTCP carries half of the unconjugated bile acids, and the other half enter by passive diffusion.
  - NTCP carries some drugs such as the diuretic furosemide, and the calcium channel blocker verapamil.
  - Sodium-dependent uptake of bile acids through NTCP is driven by an inwardly directed sodium gradient generated by Na^+, K^+ - ATPase, and the membrane potential is generated in part by a K^+ channel.
  - In the SM are also
    - Organic anion transporting polypeptides, OCT1 (organic cation transport 1)
    - NHE (Na^+ / H^+ exchanger)
    - Na^+-H^+ exchanger
    - Na^+-HCO_3^- cotransporter (symporter)

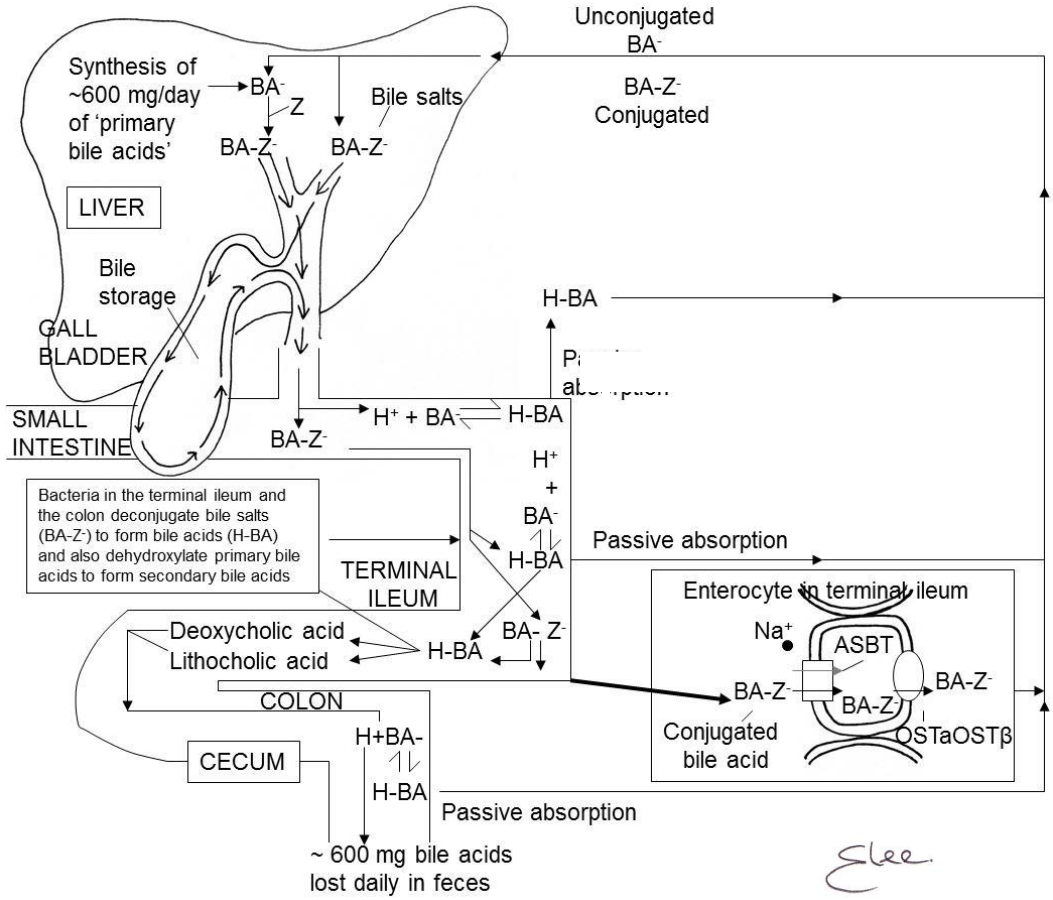
- CFTR

- The Na^+ - independent bile acid uptake is mediated by the organic anion-transporting polypeptides OATP1B1 (SLCO1B1) and OATP1B3 (SLCO1B3).
- The BAs which are taken by the hepatocyte sinusoidal membrane (SM) mix with any newly synthesized BAs.
- The BAs traffic to the canalicular membrane (CM) of the hepatocyte where they are transported into the bile ducts back into the duodenal lumen, to form BAMs.
- The SM bile acid transporter include
  - NTCP (sodium taurocholate cotransporting protein)
  - OCT1 (organic cation transporter)
  - NHE (Na+ / H+ exchange)
  - Na+ / K+ ATPase
  - OATP (organic anion transport protein)

➢ **Hepatic synthesis**

- About 600 mg per day of the primary bile acids (cholic and chrenic acids) are synthesized in the liver each day from cholesterol.
- The daily hepatic secretion of primary bile acids (BA) is balanced by the daily loss of a similar amount of bile acids in the stools.
- The rate limiting enzyme for the hepatic synthesis of bile acids (BA) from cholesterol is 7α- hydroxylase.
- The nature of the control of 7α-hydrolase by the ileocyte FXR /RXP and bile acid concentration will be considered a little later.
- Bile acids (BA) are conjugated mostly with the amino acids glycine and taurine (BA-Z), as well as to sulfate or gluconate (BA-Y).
- Thus, bile acids (BA) are either protonated (H-BA) or deprotonated (BA^-) at the pHs normally seen in the lumen of the small intestine.
- To use the correct terms, H-BA are protonated or neutral bile acids, and BA^- (deprotonated) BA^- are bile salts (BS).
- Because BA-Z and BA-Y are negatively charged, they are also bile salts.
- In the hepatic cytoplasm, BA-Z are attached to binding proteins which are also transported by NTCP (sodium taurocholate co-transport polypeptide)
- Bile acid binding proteins move by a vesicular pathway towards the AM (apical membrane, aka CM, canalicular membrane).

OVERVIEW OF ENTEROHEPATIC CIRCULATION OF BILE ACIDS



Abbreviations: BA, bile acids; BA- bile salts; BA-Y, bile acids conjugated to sulfate or gluconate; BA-Z, bile acids conjugated to glycine or taurine; OSTα,β, organic solute transporter

Printed with permission: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Figure 46-13, page 999 (adapted).

CLINICAL PHYSIOLOGICAL CHALLENGE: The Role of Liver in the Enterohepatic Circulation of Bile Acids

Case: A 50 year old women with proven primary biliary cirrhosis (PBC) develops pruritis.

- Give the process of the hepatocyte sinusoidal uptake, synthesis, and canalicular secretion of the bile acids (steps in the enterohepatic circulation of bile acids).

- **Hepatic Canalicular Membrane (CM) - Secretion of Bile Acids**

  - The CM bile transports unconjugated bile salts, protonated bile acids H^+-BA, conjugated bile salts (BA-Z-[Z= glycine or taurine]), as well as BA-[HCO_3^- and $SO4^{-2}$], HCO_3^-, and Cl^-.

  - The CM bile acid transporters include
    - BSEP, the bile salt export pump
    - MDR3, an ATP-dependent phospholipid transporter (flippase); and a HCO_3^- transporter.
    - MDR1, the ATP-dependent transporter of organic cations
    - cMOAT, the multi-specific organic anion transporter;

  - In the hepatocyte cytosole, the macromolecules may be degraded by cytosomal lysosomes or the macromolecules be transported cross the hepatocytes and across the canalicular membrane by exocytosis.

  - Transport of BA- out of the hepatocyte and across the AM is mediated by BSEP (bile salt export pump), a member (ABCB11) of the ABC (ATP-binding cassette) protein family.

  - The canalicular membrane also expresses ATP-dependent export pumps that transports phospholipid (multidrug resistance protein 3, MDR3; ABCB4), cholesterol and plant steroids (ABCG5/BCG8), and drug metabolites (MDR1; ABCB1) into bile.

- **The role of cholangiocytes in the EHC of BA**

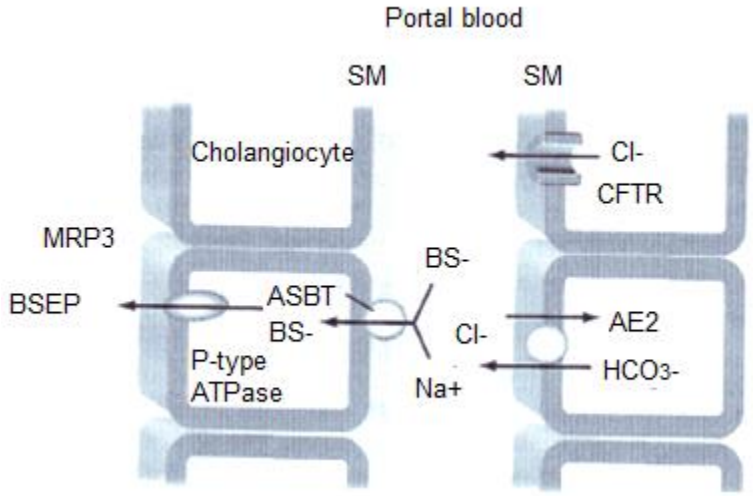
  - The CM also transports into the bile canaliculi
    - Bile pigments (eg, bilirubin)
    - Electrolytes and water (active secretion plus diffusion) [numerous aquaporin isoforms]
    - Vesicular transport of cholesterol
    - Phospholipids and
    - IgA protein

  - ASBT (apical sodium - dependent bile acid transporter, SLC10A2) of conjugated bile acids absorption by cholangiocytes of the larger bile ducts.

  - Cholangiocytes also express FIC1 (ATP8B1), a P-type ATPase mutated in progressive familial intrahepatic cholestasis.

- The affinity of BSEP for the negatively charged bile salts is TC>TUDCA>GC.
- The bile salts BA-Y (bile acids conjugated to sulfate or gluconate are transported by ATP-dependant MRP2.
- The concentration of bile salts (BA- and BA-Z) is higher in the canalicular cells as compared to the hepatocytes.
- Other compounds in the hepatocytes are secreted in a vectorial fashion across the AM and into the bile canaliculus.

CHOLANGIOGIOCYTE TRANSPORTERS IMPORTANT FOR BILE ACID SECRETION



Abbreviation: AE, anion exchanger (Cl^- / HCO_3^- exchanger), CFTR, cystic fibrosis transmembrane regulator; SM, sinusoidal membrane

Adapted from: Sherlock and Dooley, 11th Edition, 2002, Figure 64-4, page 1082.

Exchange transporters	Co-transporters	Channels
Na^+-K^+	Na+-HCO_3^-	Cl-
Na^+-AA	GLUT2	K-
Na^+-H^+		
H^+-Ca^{+2}		

Abbreviation: AA, amino acid

- Cholangiocytes express a variety of carriers important for modifying bile
  - Cystic fibrosis transmembrane regulator (CFTR)
  - the AEs (anion exchangers) for Cl^- and HCO_3^- secrete HCO_3^- and Cl^- for secretion of bicarbonate sodium-glucose cotransporter (SGLT1)
  - SL5A1
  - OA^- (organic anion)
  - OC^+ (organic cation)
  - PL (phospholipid)

Role of the gallbladder in the EHC of Bile Acids (BA) and Bile Flow

- The Na^+-H^+ exchanger (NHE) and the HCO_3^- - Cl^- exchanger (AE) in the brush border membrane of the gallbladder epithelium lead to the absorption of NaCl and water.
- Gallbladder absorption of water from the bile increases the concentration of bile acids, cholesterol (Chol), phospholipids (PL) and organic anions and cations in the gallbladder bile.
- The major difference between canalicular / hepatic bile versus gallbladder bile is the higher concentration of bile acids, cholesterol, phospholipids etc. in the stored gallbladder bile.
- If the concentration of bile acids, Chol and PL are normal, the lipids in the gallbladder bile remain soluble.
- The canalicular / hepatic bile passes through the extrahepatic biliary tree, after about half of the hepatic bile has become concentrated by water absorption in the gallbladder.
- This mixture of hepatic and gallbladder bile enters the duodenum, where the bile acids solubilize lipids and continue the process of lipid absorption.
- When this process is deranged, cholesterol gallstones may form.
- The development and complications of gallstone is considered in a later section.

Role of the Small Intestine in the EHC of BA

- Intestinal lumen
  - The content of bacteria in the intestinal lumen increases from the proximal to the distal small intestine (10^4 to ~ 10^7 per hpf [high power field]).

- Bacteria in the terminal ileum and colon deconjugate bile salts (BA-Z) to form bile acids (H-BA).
- Bacteria also dehydroxylate the primary bile acids secreted by liver (cholic and chenic acids, forming the secondary bile acids deoxycholic and lithcholic, respectively.
- The secondary bile acids may be dehydroxylated again by intestinal bacteria into the tertiary bile acids.
- The tertiary bile acids may be absorbed and enter the enterohepatocyte circulation together with the primary and secondary bile acids.
- In the terminal ileum and colon, bacteria deconjugate a small amount of these bile salts to form unconjugated bile acids ($H - BA \leftrightarrow H^+ + BA^-$).
- This deconjugation by luminal bacteria allows the unconjugated bile acids (BA^-) to be passively absorbed by non-ionic diffusion.
- Secondary metabolism of bile acids by the intestinal bacteria microbiotica includes
  - 7α-dehydroxylation
  - Deconjugation
  - Epimerization of 3α- and 7α- hydroxyl groups
  - Hepatic reduction of the 7-oxo derivative of chenodeoxycholic acid to 7-oxo lithocolic acid
  - Hepatic re-epimerization of 3β-hydroxy bile acids
  - Sulfation at the 3 or 7 positions by the liver and kidney
- Like the unconjugated primary bile acids, the unconjugated secondary bile acids are absorbed in the ileum, pass in the portal blood to the hepatocyte sinusoidal membrane, where they are taken up by the hepatocytes.and reconjugated in the hepatocyte cytosol by the addition of a glycine or taurine amino acid.

➢ Duodenum and Jejunum

- When bile acids (BA) are conjugated in the liver to the amino acids glycine and taurine, the conjugated BA become more hydrophilic and less hydrophobic, so then passive absorption of the conjugated BA in the proximal intestine is less.
- Less passive absorption of conjugated BA in the proximal intestine maintains an adequate concentration of BA in the intestinal lumen to provide for continued solubilization (critical micellar concentration, CMC), digestion and absorption of lipids.

- As the BA pass along the length of the small intestine, there becomes less and less exogenous or endogenous lipids to be absorbed, and the BA needs to be reabsorbed.
- Only small amounts of bile acid are absorbed by diffusion in the jejunum.
- Non-ionic diffusion of the protonated unconjugated bile acids (H - BA) is much higher than is ionic (BA^-)diffusion.
- This low absorption of bile provides the means to continue to have sufficient bile acids in the lumen to solubilize and to facilitate the absorption of about 95% exogenous and endogenous lipids.
- The complex and integrated process of lipid absorption is considered in a later section; here we only briefly consider lipid absorption against the overall perspective of understanding the enterohepatic circulation of bile acids.
- The endogenous microbacteria in the lumen of the terminal ileum and colon deconjugates the primary and secondary bile acids.
- Enteric bacteria dehydroxylate Ba (primary [1^o] → secondary [2^o] BA), and deconjugate $1^o/2^o$ BA; enteric bacteria also epimerize the 7α-hydroxy group of CDCA (chenic acid), forming UDCA (ursodeoxycholic acid, 3α, 7β – dihydroxy BA).

Abbreviations: BA, bile acid; CA, cholic acid; CDCA, chenodeoxycholic acid (aka chenic acid); DCA, deoxycholic acid; LCA, lithocholic acid; UDCA, ursodeoxycholic acid

- Most lipids are absorbed in the proximal intestine.
- The MM (mixed micelle) becomes a SM (simple micelle), and shuttles back to the intestinal lumen.
- Once again back in the lumen, the SM solubilizes lipids, becomes a MM, and diffuses back to the brush border membrane (BBM) of the small intestinal enterocytes.
- Once the lipids have been absorbed from the MM, the bile acids pass along the length of the jejunum to the ileum, where they are absorbed by transporters.
- The enterohepatic circulation of bile salts (EHC) functions to maintain the CMC in the lumen of the proximal intestine, and thereby facilitate the absorption of lipids.
- Once the concentration of bile in the duodenal lumen reaches a critical concentration (about 5 mM), the bile acid monomers form micelles.

- This critical concentration of bile acids is called the “critical micellar concentration” (CMC).
- When the CMC is reached, the negatively charged spheres (“micelles”) are formed.
- These simple micelles solubilize the lipid digestive products in the intestinal lumen (fatty acids, B-glycerol, phospholipids, free cholesterol).
- These micelles that have solubilized the lipid digestive products are called mixed micelles (MM).
- MM diffuse across the unstirred water layer on the luminal side of the enterocyte BBM.
- In the aqueous phase in the intestinal lumen, the water-soluble lipids, glycerol, as well as short- and medium- chain length fatty acids diffuse across the BBM, through the cytosol of the enterocyte, and across the basolateral (BLM) and into the portal circulation
- Some of the lipids solubilized in the MM partition into the AM, and diffuse into the cytosol of the enterocyte.
- Some fatty acids are transported across the BBM, by FAT (fatty acid transporter).
- Cholesterol is transported across the BBM by the Niemann-Pick C1 like1 protein transporter (NPC1L1).
- When the MM are depleted of their solubilized lipids, the now simple micelles move back into intestinal lumen where they solubilize more lipids, and once again shuttle across the UWL and AM.

➢ Ileum – Absorption, and control of the hepatic synthesis of BBM

- Conjugated bile acids are actively transported across the ileocyte AM as conjugated bile acids (BA-Z) by ASBT (apical sodium bile acid transporter; (gene symbol, SLC10A2).
- A small amount of BS^- in the cytosol are pumped back across the AM by ASBT.
- Transcriptional regulator of the nuclear bile acid receptor is present in the ileocyte (see later section on control of EHC).
- Bile acids in the cytosol of the ileocytes are actively transported across the BLM by BSEP (bile salt export protein; gene symbol ABC B11).
- The unconjugated primary, secondary and tertiary bile acids are also cycled in the portal blood circulation back to the liver.

- Colon
  - The bile acids which are not absorbed by the small intestine spill into the colon.
  - The usual loss of unabsorbed bile acids in the feces (about 600 mg per day) is matched by hepatic synthesis of bile acids.
  - Bile acids in high concentration in the lumen of the colon stimulate cGMP and cause fluid secretion (choleretic diarrheal).

- **Control of the enterohepatic circulation (EHC) of BA**
  - ↓ BA uptake by ileal ASBT - ↓ BA in ileocyte cytosol - ↓ FXR - ↓ FGF19 - ↓ FGF4 / B-klotho - ↓ transcriptional inhibition of 7α-hydroxylase - ↑ conversion of cholesterol → 1° BA.
  - Transcriptional regulator of the nuclear bile acid receptor:
    - 1° BA are synthesized by the hepatocytes
    - 1° BA increase FXR
      - FGF 19 passes to the liver in the portal venous blood.
      - FGF 19 interacts with the dimeric receptor FGFR4/β-klotho present on the SM of the hepatocyte.
      - The activated FGFR4/β-klotho receptor initiates a phosphorylation cascade.
      - This phosphorylation cascade causes transcriptional repression of the gene which encodes cholesterol 7α-hydroxylase, the rate-limiting enzyme in bile acid biosynthesis.
      - ↓ hepatic cholesterol 7α-hydroxylase, ↓ 1° BA are synthesized in the hepatocytes.
  - In patients with ileal dysfunction or resection, there will be ↓ absorption of the bile acids across the BBM of the ileal enterocytes.
  - When the concentration of bile acids in the ileal enterocyte is lower, there will be
    - ↓FXR and ↓FGF 19 formation and release.
    - ↑ BSEP, so ↑ BA is transported out of the ileocytes.
    - ↓ NTCP, thereby decreasing the uptake of conjugated (BA-Z) and unconjugated bile salts (BA) into the hepatocytes across the CM.

- ↓ FGF 19 means ↓ hepatic activation of FGFR4/β-klotho, ↓ transcriptional inhibition of 7α-hydroxylase → ↑ conversion of cholesterol → 1º BA.

Disease Correlates

- With the < 100 cm of resection, there will be sufficient ileal malabsorption of bile acids in the ileum that an excess of bile salts will spill into the ileum, stimulate cAMP in the colonocytes, and cause a secretory bile salt-induced diarrhea (choleretic enteropathy).

- With a major ileal resection (> 100 cm of terminal ileum), the capacity of the 7α-hydroxylase enzyme to be upregulated is exceeded, and not enough primary bile acids can be produced to maintain the upper intestinal luminal bile acid concentration above the CMC.

- Defective FGF19 release from the ileum is reported in patients with IBS-D whose symptoms improve with the BA sequestrant cholestyramine, suggesting that excessive hepatic BA synthesis due to defective FGF19 signalling is associated with BA diarrhea.

- In patients with idiopathic bile acid malabsorption, ileal bile acid transport is normal.

CLINICAL PHYSIOLOGICAL CORRELATION – Bile Acid Wastage

Background: When a person undergoes an ileal resection of < 100 cm, the liver attempts to upregulate the synthesis of primary bile acids from cholesterol in an attempt to maintain a normal proximal intestinal concentration of bile acids, and thereby maintain normal fat absorption.

Problem: Give the effect of reduced ileal uptake of bile acids on the mechanisms controlling the hepatic synthesis of bile acids. Explain the pathophysiology for a short ileal resection (< 100 cm) causing choleraic diarrhea, whereas a longer ileal resection (> 100 cm) results in steatorrhea.

Choleretic Diarrhea

- Background

  - About 95% of BA in the lumen of the intestine are reabsorbed and recycled to the liver, representing the high efficiency of the EHC.

  - The ~5% of the luminal bile acids that are not reabsorbed and recycled spill into the colon bacterial 7α-dehydroxylate in the colon produce 2º from 1º bile acids:
    CA → DCA (50% absorbed by colon)
    CDCA → LCA → UDCA (a 3º [tertiary] bile acid)

- Give the pathophysiology of why a resection of < 100 cm of ileum results in choleretic diarrhea, whereas a > 100 cm resection results in steatorrhea.

  - The neutral and the acidic pathways for the production of bile acids fr cholesterol are capable of increasing their usual secretion rates by 25%

  - When there is a small reduction in the EHC (enterohepatic circulation bile acids [BA]), such as might occur from an ileal resection of < 100 c the hepatic conversion of BA increases, and sufficient BA may secreted across the AM and into the bile ducts and lumen of t duodenum to maintain a normal CMC (critical micellar concentration bile acids]).

  - Bile acid micelles and vesicles form, and lipids are absorbed.

  - Because of the loss of some of the ileum. Some BA esca reabsorption and spill in increased amounts into the colon.

  - In the colon the increased [BA] stimulated cGMP and secretion of C leading to a watery bile-acid induced (choleretic) diarrhea

- Give the change in pathophysiology which occurs with a loss of > 100 cm of ileum, leading to steatorrhea.

  - When there is a major loss of ileum (> 100 cm), the mount of malabsorbed BA is so great that liver cannot compensate for the large loss of BA by increasing the hepatic conversion of cholesterol to bile acids, the CMC is not achieved, and fat malabsorption (steatorrhea) occurs.

Secretion of HCO_3^- and Water by Cholangiocytes

- Cholangiocytes express a variety of transporters:

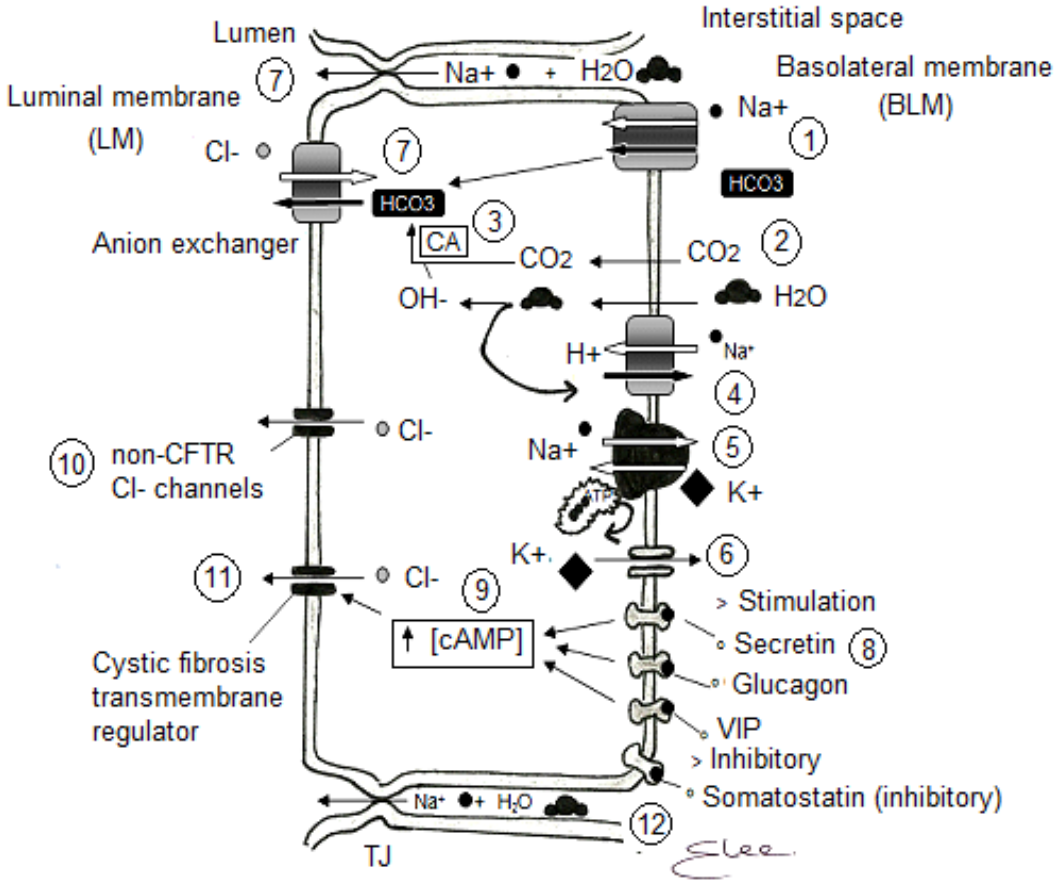
LM (luminal membrane)	BLM (basolateral membrane)
○ CFTR (cystic fibro transmembrane regulator) ○ Non-CFTR Cl- channels ○ AE2 Cl^- / HCO_3^- exchanger ○ ASBT absorption of Na^+ / BA^- ○ SGLT1 (SLC5A, sodiu glucose cotransporter) ○ Numerous aquaporin isoforms	○ Na^+ / HCO_3^- cotransporter ○ NHE (Na^+ / H^+ exchanger) ○ K^+ channel ○ IRP3 / OST tra

- 1) Na^+ enters the cholangiocyte with HCO_3^- across the BLM by the way of Na^+ / HCO_3^- cotransporter.
- The net effect is that intrahepatic and extrahepatic bile duct cholangiocytic epithelial cells secrete HCO_3^- and water.
- 2) H_2O and CO_2 diffuse across the BLM and into the cytosol.
- 3) Intracellular H^+ formed from CO_2 and H_2O by CA leaves the cholangiocytes across the BLM by NHE, the Na^+ / H^+ exchanger.
- The BLM Na^+/-K^+ ATPase maintains low intracellular [Na^+] and high [K^+].
- In the cytosol of the cholangiocyte, carbonic anhydrase (CA) forms HCO_3^- and H^+.
- The H^+/Ca^{2+} exchanger maintains low intracellular [Ca^{+2}] (not shown in figure)

"The only disability in life is a bad attitude"

Scott Hamilton

SECRETION OF HCO_3^- AND WATER BY CHOLANGIOCYTES



Abbreviations: CA, carbonic anhydrase; CFTR, cystic fibrosis transmembrane regulator; TJ, tight junction

Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Figure 46-11, page 996.

- 5) Na^+ which has entered the cholangiocyte through BLM Na^+ / HCO_3^- (1), cotransporter exits the cell across BM Na^+ / K^+ ATPase.
- 6) K^+ which entered the cholangiocyte across BLM Na^+ / K^+ ATPase (5) leaves the cell through K^+ channel in the BLM.
- 7) HCO_3^- formed from CA (3) and transport into cytosol by Na^+ HCO_3^- cotransporter (1) is transported across the LM by AE, the Cl^- / HCO_3^- anion exchanger.

- 8) Secretin, glucagon and VIP increase cAMP in the cytosol, whereas somatostatin decreases cAMP levels.
- AQP8 acts as a water channel, increasing water permeability across (transcellular movement of water).
- Under basal condition, AQP8 (aquaporin 8) is in intracellular vesicles.
- In the presence of ↑ cAMP
  - AQP8 inserts into the canalicular membrane
  - 9) ↑ cAMP is to enhance the secretion of bile (a choleretic effect).
- 10) Some Cl- also leaves the cell through non-CFTR channels.
- 11) cAMP opens CFTR with Cl- leaving the cholangiocyte.
- 12) Na^+ and H_2O cross the tight junctions (TJs) between the cholangiocytes and into the lumen.

CLINICAL PHYSIOLOGICAL CORRELATION – Cholangiocyte Function

Background: The cholangiocyte sinusoidal membrane (SM) contains the AE2 (anion exchanger, aka HCO_3^-/Cl^- exchanger, as well as CFTR and non-CFTR Cl^- channels. The cholangiocyte basolateral membrane (BM) contains the Na^+/K^+ ATPase, Na^+/H^+ exchanger (NHE), and the $NaHCO_3$ transporter.

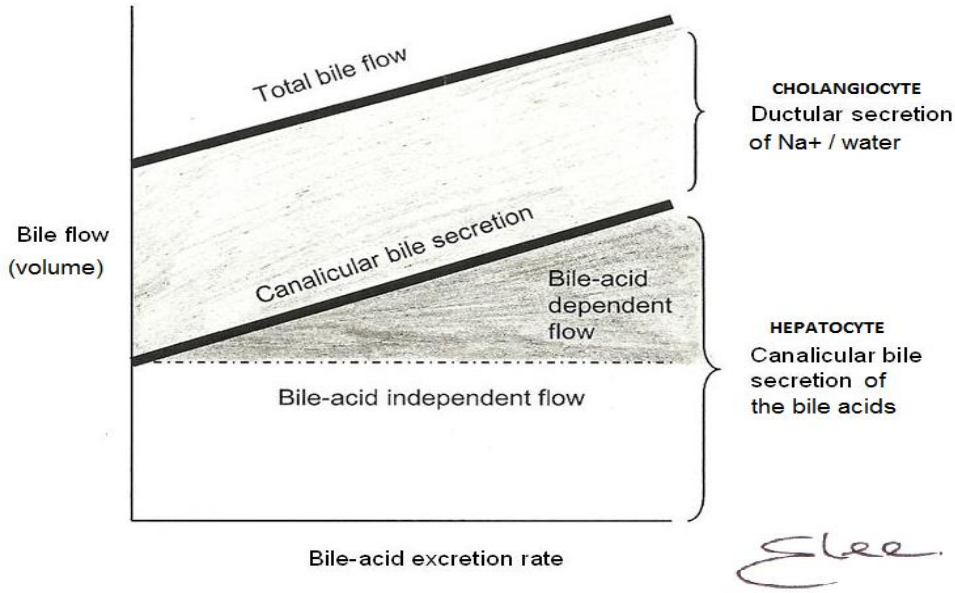
Problem: Draw a diagram which depicts the steps in the cholangiocyte secretion of $NaHCO_3$ and water.

"Optimism is the faith that leads to achievement.
Nothing can be done without hope and
confidence"

Helen Keller

Bile Secretion and Flow

- Give the physiology of bile acid-dependent and independent canaliculated secretion of water (bile flow / secretion).
- There is bile acid-dependent and BA-independent bile flow, as well as Na^+-dependent bile acid clearance.



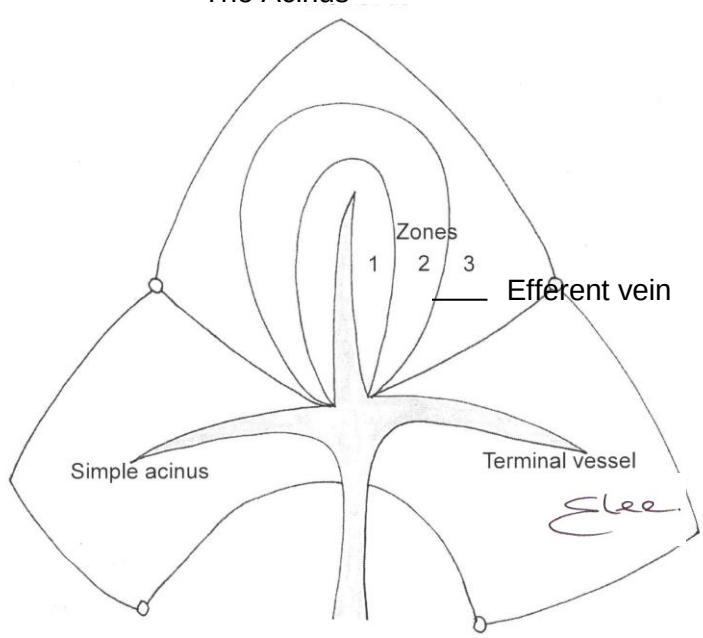
- The composition of bile is modified by the absorption of water in the gallbladder, concentrating 20-fold the hepatic bile.
- Bile is made up of hepatocyte canalicular and cholangiocyte ductular secretion.
  - Hepatocyte: Bile acid dependent flow
  - Cholangiocyte: Bile acid independent flow
- When there is no excretion of bile acids (bile-acid excretion rate is zero), the bile flow is from H_2O secretion by cholangiocytes (bile acid independent flow) is still flow of bile containing water and electrolytes.
- As the rate of bile acid excretion increases cholangiocyte ductular secretion also rises.
- Hepatic bile flows down the common hepatic duct (CHD).
- About half of the hepatic bile continues down the CHD into the common bile duct (CBD) through the sphicter of Oddi, and into the lumen of the duodenum.
- The other half of the hepatic bile is diverted from the common hepatic duct (CHD) into the cystic duct, and then into the gallbladder.

- As a result of the absorption of large amounts of water from the hepatic bile which enters the gallbladder, the relative composition of substance in hepatic bile is different from gallbladder bile.

Metabolic Function of Liver

- Heterogeneity of hepatocyte metabolic function in the three hepatic zones

The Acinus



Preterminal vessel

- Rappaport zone 1 is adjacent to the entry (portal venous) system
- Zone 3 is adjacent to the exit (hepatic venous) system

Adapted from: Sherlock and Dooley , 2002, 11th Edition, Figure 1.12, page 8.

- This means that toxic damage more often occurs in the vulnerable contrilobular zone III hepatocytes than in zone I. This toxic damage is also known as DILI, drug induced liver injury.
- The 150 (or more) isoforms of the cytochrome P- (CYP)450 enzyme are endoplasmic reticulum (ER) proteins.

- CYP 450 enzymes catalyze the phase I biotransformation reactions, metabolizing about half of all drugs.
- The CYP-450 mono-oxygenases insert an atom of oxygen into the substrates, rendering the substrate more water-soluble, allowing the substate to be reactive with the phase III enzymes.
- The reactions of the important CYP 3A isoform of the CYP-450 enzyme, include hydroxylation, dealkylation, and dehalogenation.
- The phase III enzymes further increase the water solubility of the substrate by conjugating the substrate to gluconate, glutathione (GSH) or sulfate (cytosolic sulfotranferases).
- Other phase III enzymes which enhance the water solubility of the substrate include the sulfotransferases and glutathione-S-transferases.
- Two nuclear receptors are present in the liver and intestine, and function as transcription factors responsible for drug metabolism.
- SXR (steroid and xenobiotic receptor) and CAR (constitutive androstane receptor) interact with xenobiotics and drugs, bind to DNA response units and thereby, alter the expression of drug metabolizing enzymes, as well as MDR1 (metabolizing resistance transporter).

Oxidative Energy	(Oxidation)	(Ketogenesis)
➢ Amino Acids	○ Catabolism of AA	○ Glutamine Synthesis
➢ Carbohydates	○ Gluconeogenesis	○ Glycogen Synthesis
	○ Glycogen degradation	○ Glycolysis
		○ Ketogenesis, glycolys glycogen and glutami synthesis
➢ Lipids	○ Oxidation of FAs	○ Liponeogenesis
	○ HMG CoA reductase	○ Cholesterol synthesis
➢ Bile	○ Bile acid depende canalicular flow	○ 7α-hydroxylase
		○ Bile acid independe canalicular flow
➢ Urea	○ Ureagenesis	○ Biotransformation

Abbreviations: AA, amino acids; FA, fatty acids

Source: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Table 46-1, page 984.

Drug Induced Liver Injury (DILI)

- Give 8 clinicopathological presentations of DILI, and for each give an example of a causative drug.

Phenotype	Features	Examples of causative drugs
○ Acute fatty liver with lactic acidosis	- Microvesicular steatosis with systemic mitochondrial dysfunction	▪ Valproate, fialuridine, nucleoside analogues
○ Acute hepatic necrosis	- Collapse and necrosis of liver parenchyma	▪ Many drugs, including isoniazid
○ Acute liver failure	- Collapse and necrosis of liver parenchyma INR > 1.5 and encephalopathy	▪ Many drug, including isoniazid, bromfenac, nitrofutantoin
○ Acute viral hepatits-like liver injury	- Prodome of malaise, fatigue and lassitude	▪ Isoniazid, halothane
○ Autoimmune-like hepatitis	- Detectable autoantibodies and/or autoimmune features on liver biopsy specimen	▪ Minocycline, nitrofurantoin, methyldopa
○ Without inflammation	- Pruritis with hyperbilirubinemia	▪ Anabolic steroids, estrogen
○ Cholestatic hepatitis	- Elevated serum levels of alkaline phosphatase and bilirubin	▪ Amoxicillin-clavulanate
○ Cirrhosis	- Variable degrees of collagenization	▪ Methotrexate

○ Immunoallergic hepatitis	- Skin rash - Eosinophillia and fever (drug allergy)	▪ Cotrimazole, phenytoin
○ Nodular regeneration	- Microscopic or macroscopic liver nodules	▪ Azathioprine
○ Non-alcoholic fatty liver	- Microvesicular or macrovesicular steatosis - With or without ballooning and steatohepatitis	▪ Amiodarone, tamoxifen

Phenotype	Features	Examples of causative drugs
➢ Sinusoidal obstruction syndrome	○ Obliteration of central veir	– Cyclophosphamide, busulfan, pyrrolizidine alkaloids
➢ Vanishing bile duct syndrome	○ Paucity of interlobular bile ducts with cholestasis	– β lactam antibiotics

Abbreviations: DILI, drug induced liver injury; INR, international normalized ratio

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- Give 10 possible risk factors involved in the pathogenesis of DILI.

Drug Factors	Environment	Host
○ Class	- Diet	▪ Age
○ Dose	- Toxins	▪ Sex
○ Duration	- Exposure tobacco	▪ Weight
○ Drug-drug interactions	- Alcohol	▪ Genetic factors ▪ Metabolism

- Coffee
- Chemicals
- Pollutant
- Oxidants
- Probiotics

- Immune factors
- Other disease

- Drug factors
    - Drug factors have not been clearly associated with liver injury in pre-clinical toxicology test systems or in patients with DILI.
    - Drug-drug interactions could alter the concentration of a drug or a reactive metabolite at a cellular location involved in the initiation, maintenance or concentration of resolution of liver injury.
    - DILI is difficult to exclude when multiple drugs or herbal products are co-administered
- Environment
    - The microenvironment and macroenvironment are known to vary greatly within and among individuals but are difficult to study and quantify.
    - Metabolomic approaches and studies of the human microbiome may provide important insights into the role of environment factors in the pathogenesis of DILI.
- Host
    - Genome-wide association studies have demonstrated reproducible associations between several host genetic polymorphisms and DILI, but in general host factors have only rarely been implicated in the pathogenesis of DILI

Abbreviation: DILI, drug induced liver injury

Adapted from: Tujlos S and Fontana RJ. *Nat. Rev Gastroenteroll Hepatol* 2011;8:202-211

Useful Background: Factors to consider in the **diagnosis of DILI**

- Time to onset
    - Variable range but usually < 12 months
- Clinical features at presentation
    - Can be asymtomatic
    - Rash and eosinophilia are found infrequently
    - Classification into hepatocellular versus mixed/and or cholestatic liver injury on the basis of R value

R = (initial ALT/ULN) / (AP/ULN): patient with an R value > 5 have hepatocellular liver injury, and R<2 is indicative of cholestatic liver injury.

The R value helps the clinician to identify other etiologies of acute liver injury to consider and exclude (i.e. differential diagnosis)

- Risk factors for hepatotoxicity
    - Few clinical risk factors established with certainty
    - Possible risk factors involved in the pathogenesis of DILI have been proposed
- Course of liver injury following drug cessation
    - Drug withdrawal (dechallenge) is problematic in patients with fulminanat liver failure and those who develop chronic injury
- Exclusion of competing causes of liver injury
    - Laboratory and radiological testing for hepatitis A, hepatitis B, hepatitis C, alcohol, autoimmune disorders and pancreaticobiliary diseases
- Prior reports of hepatotoxicity
    - Problematic with newly approved drugs and herbal products
    - Currently no centralised source of information
- Drug rechallenge

- Rarely done and gives variable results

- Liver histology

  - DILI can mimic nearly all known forms of acute and chronic liver injury

Abbreviations: ALT, alanine aminotransferase; AP, alkaline phosphatase; DILI, drug induced liver injury; ULN, upper limit of normal

Acetaminophen hepatotoxicity as a common example of DILI

- Role of host metabolic system

  - Most of ingested acetaminophen is usually transformed by glucuronyl transferases and sulfotransferases to stable metabolites.

  - These stable metabolites of the ingested acetaminophen are excreted into the urine and bile.

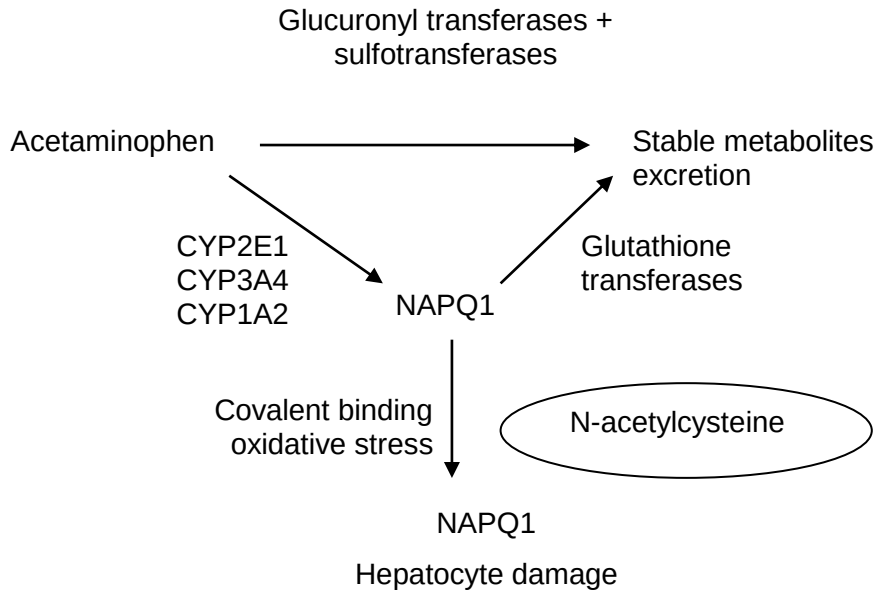
  - If this usual system is overwhelmed, a larger proportion of acetaminophen is oxidatively metabolized by CYP isoenzymes to the highly reactive intermediate metabolite, NAPQ1.

  - NAPQ1 covalently binds to hepatocyte proteins and can disrupt mitochondrial function, resulting in hepatocyte damage.

  - Administration of N-acetylcysteine restores the depleted stores of hepatic glutathione and reduces cellular damage.

"The best way to predict the future is to create it"

Unknown



- Role of host immune system
  - Normal circumstances – common variant of MHC II
  - The release of MMP9 from infiltrating macrophages facilitates liver regeneration via its binding to CD44 receptors on lymphocytes.
  - The MHC susceptible variant is usually a minor polymorphism.
  - By itself this polymorphism does not account for all of intraindividual variation in DILI susceptibility, and the generally low incidence of DILI caused by a particular drug.
  - Drugs are small molecules that may become bound by proteins (haptenized), forming drug-protein complexes during physiological circumstances or following metabolic activation *in vivo.*
  - Usually, drug and protein form a drug – protein complex.
  - The drug-protein complexes are phagocytosed by APCs (antigen presenting cells).
  - The APCs present the drug or drug-protein/peptide complexes to Helper T-cells, by way of common variant of MHC II.
  - Common MHC II s resistant to the formation of hepatocyte damage.

- Macrophages infiltrating the liver recognize damaged hepatocytes, and release proinflammatory cytokine (IL-1β, TNF), as well as proregenerative cytokines (IL-10, IL-6).
- No hepatocyte damage, no DILI – because of common MHC II.
- Susceptibility – susceptible variant of MHC II.
- Differences in human leukocyte antigen genotypes can lead to variation in MHC peptide-binding grooves.
- ↓ expression of MMP4 causes ↓ CD44 binding on lymphocytes, less regeneration and more inflammation and hepatic damage.

Abbreviations: APC, antigen presenting cell; drug induced liver injury; MHC, major histocompatibility complex

Printed with permission: Tujos S and Fontana RJ. *Nat Rev Gastroenterol Hepatol* 2011;8:202-211.

CLINICAL PHYSIOLOGICAL CHALLENGE – Drug-induced Liver Injury

Case: An 18 year-old distraught first year medical student consumes 10 grams of acetaminophen plus 250 ml of vodka over a two hour interval. S(he) is brought to the ER when mental confusion was noted.

- Give the possible drug, environment and host factors involved in the pathogenesis of DILI (drug-induced liver injury).
- Give the role of the metabolic and host immune systems in the pathogenesis of acetaminophen hepatotoxicity.

- Give the hepatic components protecting against drug-induced liver injury (DILI).

➢ Glutathione system
  - Pro-oxidants signal Nrf (the redox-sensitive transcription factor)
  - Nrf → ↑ CYP 2E1 expression → ↑ hepatic glutathione synthesis (from cysteine) → ↑ antioxidant effects
  - Cystolic glutathione

- In the reduced state, resulting from the effect of NADPH and glutathione reductase
- Glutathione deficiency injures mitochondria, releases cytochrome C and MPT (mitochondrial membrane permeability transition), leading to activation of caspases and apoptosis

- Biochemical pathways of cellular damage
  - Covalent binding of drug to cellular proteins
  - Oxidation of proteins
  - Post-translational modification of proteins
  - Lipid peroxidation
  - Cleavage of DNA
  - ↑ Ca_i^{2+} (intracellular concentration of Ca^{2+})

- Hepatic non-hepatocyte cells
  - Kupffer cells
    - Act as macrophages and antigen-presenting cel
    - Activated Kupffer cell may release TNF, ROS a as-L, which may lead to hepatocyte apoptosis necrosis
  - Endothelial cells
    - Their low glutathione context make th susceptibility to vascular injury
  - Stellate cells
    - When activated, will deposit matrix and lead fibrosis

"Be an advocate, seek something good for everyone:
seek justice, love and peace"

Grandad

SO YOU WANT TO BE A HEPATOLOGIST!

Drugs are metabolized by:

- Phase I reactions
  - Microsomal drug oxidases and the CYP gene superfamily
    - Involves the hemoprotein f the CYP gene
    - Drugs are converted to a toxic metabolite (e.g. acetaminophen → NAPQ)

- Phase II
  - Conjugation
    - Through glucuronic acid or inorganic sulfate by formation of ester links with the drug

- Phase III
  - Secretion of drugs or their metabolites by transporters
    - ATP-binding cassette (ABC) protein
    - MDR1 (multidrug resistance protein C1)
    - MRP1 (multidrug resistance-associated proteins)
      - MRP-3 sinusoidal membrane of hepatocytes
      - MRP2 canalicular membrane (CM) of hepatocytes

- Give the reason why DILI resulting from drugs metabolized by phase I reactions (e.g. acetaminophen) is localized in zone 3 (around the terminal hepatic venules [THV]).

  - Phase 1 reactions are catalyzed by microsomal drug oxidase, the key component of when is a hemoprotein of the CYP gene superfamily.
  - CYP2E1 is located in hepatocytes which form a 1 to 2 hepatocyte thick rim around the THV.

- Immunologic mechanisms
  - Formation of ligands with death receptors
  - Porin-mediated introduction of granzyme
  - "altered antigen" drug metabolite interacts with cellular proteins to form drug-protein adducts (haptens)
  - Drug-induced autoimmunity

- Give 5 major toxic mechanisms of drug-induced liver injury, leading to hepatocyte apoptosis and necrosis.
  - Direct hepatotoxicity
    - Injury to
      - Mitochondria
      - Plasma membrane
  - ROS (reactive oxygen species)
    - "the liver is exposed to oxidative stress by the propensity of hepatocytes to reduce oxygen…."
    - Some drugs (e.g. acetaminophen) may be converted by CYP into pre-oxidant reactive metabolites
    - CYP-mediated metabolism → formation of reactive metabolites → ↓ glutathione → injury to mitochondria → 1) release of cytochrome C and 2) operation of MPT (mitochondrial permeability transition) → activation of caspase → apoptosis
    - Formed from injured hepatocytes and kupffer cells
    - Reactive metabolites undergo covalent binding to proteins
    - Protein-drug adducts
      - Inactive important enzymes
      - May be acted upon by immunodestructive processes

Alcoholic Liver Disease (ALD)

- Definition: ALD is a disorder associated with the intake of ecessive amounts of alcohol over prolonged periods of time, leading to acute and / or chronic compensated / decompensated liver disease.

- Alcohol intake
  - Metabolizing enzymes
    - ADH (alcohol dehydrogenase)

- Main enzyme at < 10 mM alcohol

- CYP2E1 (cytochrome P450 2E1)
  - Main enzyme at > 10 mM alcohol self-inducible (alcohol increases CYP 2E1)
- Catalase
- ALDH (aldehyde dehydrogenase)

Alcohol -------→ Acetaldehyde

Acetaldehyde ---ALDH---> Acetate

- Role of acetaldehyde
  - Acetaldehyde forms adduct with reactive residues 9oxidatively modified proteins) on proteins or small molecules
  - Antibodies from against these adducts (e.g. MAA [malondialdehyde and acetaldehyde])
- Methionine metabolism
  - Methionine is related upon by MAT (methionine adenosyltransforate) to SAM (S-adenosyl-methionine)
    - SAM usually is acted upon in the transmethylation pathway, forming SAH (S-adenosyl homocysteine)
    - In ALD, there is ↓ MAT and ↓ SAM
    - With SAM and ↓ transmethylation
    - ↓ transmethylation - there is ↓ DNA RNA, histones, biogenic amines, phospholipids
  - SAH (s-adenosyl homocysteine) and SAM (s-adenosyl methionine)
  - ↑ SAH and ↓ SAM / SAH ratio --→ ↓ transmethylation reactions → ↓ SAM
  - ↓ SAM →
    - ↓ glutathione in mitochondria --→ mitochondria dysfunction
    - ↑ LPS (lipopolysaccharide)
      - Stimulated release of TNF
      - ↑ IL-10 from monocytic cells
    - ↑ homocysteine, ↑ SAH
  - ↑ SAH and ↑ homocysteine
  - ↑ hepatocyte sensitivity to TNF-mediated destruction
  - ↑ hepatocyte sensitization from
    - ↓ glutathione in mitochondria
    - ↑ SAH

        - ↓ proteasomes
        - ↓ THF, ↓ methionine
    - Alcohol ↓ folic and absorption and metabolism, including
        - ↓ 5-mTHF (5-methyltetrahydrofolate [THF])
- Hypoxia
    - Alcohol
    - ↑ O_2 uptake by liver
    - ↑ lobular O2 gradient
    - ↑ NADH / NAD+ --→ ↑ HIF (hypoxia-inducible factor) 1 and 2 genes
    - When alcohol ↓, HIF ↓
    - Reperfusion injury, especially in centrilobular area
- Mitochondria dysfunction
    - ↑ TNF impairs function of mitochondria
    - ↑ $NADH/NAD^+$ ---→ ↑ generation of superoxidase
    - In ALD, ↓ transport of glutathione into mitochondria
    - Glutathione-depleted mitochondria -→ ↑ sensitive to damage by TNF
    - ↑ depolarization leads to
        - ↑ membrane permeability
        - ↑ apoptosis of hepatocytes
- Redox state (oxidative-reduction state)
    - Alcohol --→ ↑ CYP 2E1 --→ leaking of electrons --→ ↑ oxidative stress
    - ↓ ATP
        - ↓ metabolism of
            - Carbohydrate
            - Fat (e.g. steatosis)
    - ↑ oxidants: ↑ ROS (reactive oxygen species), ↑ RNS (reactive nitrogen species), ↑ oxidative stress
    - ↓ antioxidants
    - ↑ cell injury
        - ↑ membrane lipid peroxidation
        - ↑protein and DNA oxidation
    - ↑ NF-kB (nuclear factor kappa B) --→ ↑ TNF
- Dysregulated proteasome system
    - ↓ removal of proteins damaged by oxidative stress
- Dysregulated cytokines production
    - Hepatocytes

        - IL-8, IL-18
        - Neutrophil chemotactic peptide
        - Angiogenesis factor
    - Kupffer cells
        - NF-kB → TNF-α
        - IL-10
    - Sinusoidal endothelial cells
        - Adhesion molecules
    - Stellate cells
        - TGF-β → fibrosis
    - ↑ intestinal permeability → ↑ LPS
    - ↑ apoptosis of hepatocytes → ↑ release of IL-8 (neutrophil chemoattractant), IL-18 (primes TNFα release, and type 1 helper T cell response)
        - ↑ TNF-α inducible IL-6, IL-8, Il-18, MCP-1 (monocyte chemoattractant protein 1)
    - As well as ↑ pro-inflammatory cytokines, there is ↓ anti-inflammatory cytokines

"hepatocyte apoptosis may sustain proinflammatory cytokine production and cell injury or death" (Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, page 1387).

- Autoimmune changes
    - Mutation in CTLA-4G (cytotoxic T lymphocyte-associated antigen 4G) allele actives T cell function
    - Hydroxyethyl radical from breakdown of alcohol modifies CYP 2E1
    - Modifies CYP2E1 plus CTTA-4G mutation → ↑ anti-CYP2E1 autoantibodies
    - Activation of HSC and Hepatic Fibrosis

<u>Non-Alcoholic Fatty Liver Diseases (simple steatosis and non-alcoholic steatohepatitis)</u>

"Obesity is a disorder of the GI tract"

- Give the **pathogenesis** of NAFLD (non-alcoholic fatty liver disease) and NASH (non-alcoholic steatohepatitis).

- GI Tract
  - ↑ intake / absorptio - ↑ fat to liver
  - Fat, CHO - CHO to liver
    - ↑ lipogenesis (↑ TG liver)
    - ↑ insulin resistance
  - Bacterial toxins - ↑ ROS
    - ↑ oxidative stress

- Adipocyte
  - ↑ FFA / ROS - Activation of macrophages / immune system → lipoptosis
    - ↑ insulin-resistant adipocytes → ↑ FFAs

- Liver
  - Metabolism
    - ↑ insulin, ↑ insulin-resistance
    - ↑ leptin, ↑ leptin resistance
    - ↓ adiponectin
  - Mitochondria
    - ↓ β oxidation of FFA
    - ↑ synthesis of FFA
      - ↓ respiratory chain complexes
      - ↓ ATP
      - ↑ ROS, ↑ PPAR γ, ↑ TNF-α
      - ↑ DNA damage
    - ↑ SREBP1 / ↑ CHREBP → ↑ TG synthesis
    - ↓ Apo B-100 (also ↓ β oxidation) → ↓ fat transp out of liver → ↑ TG in liver
  - ↑ CYP 2E1
    - ↑ leak of electrons → ↑ ROS, ↑ RNS → ↑ NF- → ↑ TNF-α
    - ↓ ATP
    - ↓ antioxidant
    - ↑ membrane lipid peroxidation
    - ↑ protein / DNA oxidation
    - ↑ hepatic insulin
    - ↑ synthesis of PL, CE

- Dysregulated cytokine production (↑ pro- / ↓ anti-inflammatory cytokines)
  - Hepatocytes
    - IL-8, IL-18

    - Neutrophil chemotactic peptide
    - Angiogenesis factor
  - Kupffers cells
    - NF-kB → TNF-α
    - IL-10
  - Sinusoidal endothelial cells
    - Adhesion molecules
  - Stellate cells
    - TGF-β → fibrosis
  - ↑ intestinal permeability → ↑ LPS
  - ↑ LPS
  - ↑ apoptosis of hepatocytes → ↑ release of IL-8 (neutrophil chemoattractant), IL-18 (primes TNFα release, and type 1 helper T cell response)
    - ↑ TNF-α inducible IL-6, IL-8, Il-18, MCP-1 (monocyte chemoattractant protein 1)
  - As well as ↑ pro-inflammatory cytokines, there is ↓ anti-inflammatory cytokines

"hepatocyte apoptosis may sustain proinflammatory cytokine production and cell injury or death" (Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, page 1387).

- Inflammasomes (proinflammatory mulprotein complexes: redox state)
  - Nod-like receptor (NLR) protein or a PYHIN protein – sense pathogen-associated molecular pattern (PAMPs) or damage-associated molecular patterns (DAMPs)
  - Are keys to the production of the proinflammatory cytokines IL-1β IL-18.
  - Reactive oxygen species (ROS) activate the NLRP3 inflammasome and their generation is triggered by many PAMPs.
  - Increased ROS generation is a proinflammatory insult in the progression of NAFLD.
  - Inflammasome-dependent processing of IL-1β and IL-18 could be important in NAFLD / NASH
- Stellate cell activation
  - Activation of hepatic stellate cell (HSC)
    - Pro-inflammatory cytokines
    - Angiotension
    - Oxidative stress

- Growth factors
- Extracellular matrix
- PDGF (platelet-derived growth factor
- Connective tissue growth factor
- Connective tissue growth factor
- Leptin-associated production of TGF-β by kupffer cells

➢ Signaling pathways regulating HSC activation
  - PDGF
    - PDF signals partially through ERK activation and also through AKT via mTOR mediated protein synthesis regulation.
    - PDGF activation also allows for influx of Ca2+ ions, which contributes to gene regulation
    - In addition to PDGF, several other growth factors can activate tyrosine receptors which lead to Akt activation and then either mTOR or JNK activation.
  - TGFβ
    - TGFB recruits Smad 2/3, leading to their phosphorylation and stimulation of fibrogenic gene expression.
  - Others
    - Emerging pathways of importance of fibrosis include contributions from
      - Adipokines (leptin)
      - Neuroendocrine signals (2-AG)
      - TLR signaling
      - Angiogenic signals (VEGF), among other

  - SREBP-1, the sterol regulatory element binding protein-1c, CHREBP, the carbohydrate regulatory element

  - Increased levels of free fatty acid (FFA), uncompensated oxidative stress, lipid peroxidation, cytokine dysregulation, mitochondrial dysfunction, and other environmental and genetic factors may contribute to the development of hepatocellular injury and fibrosis in susceptible persons.

Abbreviations: NFkB, nuclear factor kappa B; ROS, reactive oxygen species; RNS, reactive nitrogen species

Adapted from: Pellicoro A, and Faber KN. *APT* 2007; 26 (2): pg. 149-160 and Wood NJ. *Nat Rev Gastroenterol Hepatol* 2012; 9(3):123.

- Environmental factors
  - Excessive dietary carbohydrates and fats
  - Dyslipidemia
  - Drugs
  - Toxins
  - Nutritional deficiencies
- GI tract
  - Abnormal control of food intake
  - ↑ dietary fat, carbohydrate
  - ↑ VLDL, apo B-100
  - ↑ FFA
  - Gut lipopolysaccharides (LPS)
    - Endo-/ exotoxin-mediated release of proinflammatory cytokines and inflammasomes
  - Altered intestinal microbiome
- Liver
  - ↑ Insulin resistance
  - ↑ HGP
  - ↑ VLDL production
- Adipose tissue
  - ↑ Visceral fat (non-peripheral fat)
  - ↑ Portal FFA
  - ↑ Cytokine production
    - ↑ NF-kB
    - ↑ IL-6
    - Lysosomal destabilization (↑ TNF-α)
  - ↑ ROS, ↑ CYP 2E1
  - ↓ Adiponectin
  - ↑ leptin, ↑ leptin resistance

- ↑ FFA
  - Mitochondrial dysfunction → ↓ ATP, ↑ mitochondrial DNA damage
  - ↓ adiponectin

➢ Inflammasomes

- Proinflammatory multiprotein complexes consisting of a Nod-like receptor (NLR) protein or a PYHIN protein
- Sense pathogen-associated molecular pattern (PAMPs)
- Damage-associated molecular patterns (DAMPs) and are key to the production of the proinflammatory cytokines IL-1β IL-18.
- Reactive oxygen species (ROS) activate the NLRP3 inflammasome and their generation is triggered by many DAMPs.
- Increased ROS generation is a proinflammatory insult in the progression of NAFLD.

➢ Imbalance (↓ lipolysis / ↑ lipogenesis)

- ↑ FFA → ↓ Lipolysis of adipocyte TG
- ↓ FFA β-oxidation by mitochondria
- ↑ esterification into TG
- Synthesis of
  - Phospholipids
  - Cholesteryl esters
- ↑ CYP 2E1
  - ↑ ROS
  - Hepatic insulin resistance
- ↑ Lipogenesis
- Accumulation of triglyceride-derived toxic lipid metabolites activates intracellular inflammatory pathways within hepatocytes and Kupffer and other immune cells, in resemblance to defects within adipocytes.
- PPAR-α
  - FA oxidation
  - Oxidative stress
- Regulation by
  - Insulin
  - Catecholamines
  - Glucagon
  - Growth hormone

  - Leptin
  - Adiponectin
- Activation of hepatic stellate cells (HSC) leads to collagen deposition and the potential for cirrhosis.
- The HSC is the central effector in hepatic fibrosis
- HSC undergoes activation through a two-phase process
  - Initiation
  - Maintenance
- Initial liver injury results in hepatocyte cell apoptosis with generation of apoptotic bodies, reactive oxygen species (ROS), and paracrine stimulation of HSCs.
- LPS from the gut also stimulate HSCs.
- I**nitiation of activation** of HSCs.
  - Growth factor signaling
  - Fibrogenic signaling pathways
    - TGFB1
    - CTGF/CCN2
  - Adipokine signaling pathways
    - Leptin
    - Jak, PPARγ
  - Neuroendocrine pathways
    - Cannabinoid receptor signaling
    - Opioid signaling
    - Serotonin
    - Thyroid hormones
- In the initial phase, the hepatocyte becomes sensitized to additional activation by upregulating various receptors.
- The hepatocyte begins to secrete autocrine growth factors, chemokines, and ECM.
- Activation → down-regulation of inhibitory MHC class I molecules
- ↓ lipid droplets from HSC
- ↑ PDGFR
- PDGF signals partially through ERK activation and also through AKT via mTOR mediated protein synthesis regulation.
- PDGF activation also allows for influx of Ca2+ ions, which contributes to gene regulation

- In addition to PDGF, several other growth factors can activate tyrosine receptors which lead to Akt activation and then either mTOR or JNK activation.
- TGFβ recruits Smad 2/3, leading to their phosphorylation and stimulation of fibrogenic gene expression.
- Emerging pathways of importance of fibrosis include contributions from adipokines (leptin), neuroendocrine signals (2-AG), TLR signaling, and angiogenic signals (VEGF), among other.

Adapted from Lee UE, et al., Best Pract Res 2011; 25: Figure 2, page 197

- **Maintenance** of HSC activation
  - The perpetuation phase is the maintenance of HSC activation.
  - This maintained HSC activation involves changes in HSC proliferation, chemotaxis, fibrogenesis, and contractility.
  - Fibrocytes derived from the bone marrow transdifferentiate into myofibroblasts, and may increase ECM production.
  - Increased ECM enhances mechanical stiffness of the ECM can be sensed by and activate HSCs.
  - The contribution of dendritic cells to fibrosis is not well understood, but may include NK cell-mediated HSC killing.

Abbreviations: FFA, free fatty acids; HSC, hepatic stellate cell; ROS, reactive oxygen species

Adapted from: Lee UE, et al. *Best Practice and Research Clinical Gastroenterology 2011;* 25: 195-206, Figure 1.

- Give 6 factors which activate HSC (hepatic stellate).
  - Pro-inflammatory cytokines
  - Angiotensin
  - Oxidative stress (ROS)
  - Growth factors
  - Extracellular matrix
  - PDGF (platelet-derived growth factor)
  - Connective tissue growth factor
  - Connective tissue growth factor

- Leptin-associated production of TGF-β by kupffer cells
- Signals from Kupffer and endothelial cells
- ↑ Apoptosis, ROS → ROS paracrine stimulation of HSC
- Altered ATP homeostasis
- Mechanical stiffness of extracellular matrix (ECM)

- Give the regulated changes in gene expression which occur during activation of HSCs.

➢ Transcription factors and targets (modulation of the activity of transcription factors)
  - Transcription factors which activate the transcriptional targets of HSCs: Ets-1, Mef 2, CREB, EGR-1, vitamin D receptor, Foxf1, Jun D, and C/ERPB
  - HSC activation: α_1 and α_2 chains of type I collagen – α – SMA, TGFB1, TGF receptors MMP-2, and TIMPs I and 2
  - HSC inactivation (anti-fibrotic) – Lhx2 (LIM homeobox gene 2) - FoxO1 (forkhead box gene, group O)
  - Development and differentiation (factors expressed by HSCs)
  - Dominant – positive
    - b HLH (basic helix-loop-helix transcription factors)
    - E box DNA hexanucleotide sequence for b HLH binding
    - Myo D, SREBP-1c, C-Myb and C-Myc
  - Dominant – negative (antifibrotic)
    - I d proteins
    - C/ERPα

➢ Nuclear receptors
  - FXR (farnesoid X receptor)
    - RXR and FXR dimerize after “sensing” bile acids.
    - The dimerized RXR and FXR activate SHP.
    - SHP suppresses the expression of the collagen gene.
  - PXR (pregnane X receptor)
    - PXR is activated by steroids, certain drugs, xenobiotics and antibiotics.
    - PXR dimerizes with RXR and induces cytochrome P450 enzymes.
  - PPARs (peroxisome proliferators-activated nuclear receptors

- ↓ HSC activation – expression of collagen gene.

- Retinoid responsive RXR and RAR.

- Epigenetic regulation (methylation of promoters)
  - "Epigenetics refers to changes in DNA methylation and associated histone modifications that influence the chromatin states and impact gene expression patterns, without affecting the sequence of the target DNA."
  - Activation of HSC leads to "global" reduction in methylation.
  - ↑ CBF1 and MeCP2 result in ↓ IKB by way of promoter methylation.
  - IKB repression results in de-repression of NFKB activity.
  - ↑ NFKB increases the survival of HSCs.

- Micro RNA
  - "micro RNAs (mi RNAs) represent a family of small non-coding RNAs controlling translation and transcription of many genes"
  - TGFB, LPS and NF-kB down-regulate the miR-29 family of miRNAs
  - ↓ mi-R-29 enhances hepatic fibrosis (i.e. mi-R-29 is anti-fibrotic)
  - Post-translational regulation of transcription factors
    - Phosphorylation
    - Sumoylation
    - Prenylation
    - Acetylation
    - Glycosylation

Source: Lee UE et al., *Best Practice & Research Clinical Gastroenterology* 2011;25:195-206.

- Pancreas
  - Insulin resistance
    - Obesity
      - Hyperinsulinemia
      - Increased serum leptin levels
      - ↑ β cell apoptosis
      - ↓ Insulin secretion
      - ↑ T2DM

- Muscle tissue
  - ↓ Mitochondrial function
  - ↓ VO_2 max

- ↑ Insulin resistance

➢ Macrophage immune system

- In obesity, activation of macrophages/immune system contributes to the development of dysfunctional, insulin-resistant adipocytes that release excessive amounts of FFA and cause insulin resistance and lipoapoptosis in distant tissues (liver, muscle, pancreatic beta cells, vascular bed, other).

Abbreviations: ATP, adenosine triphosphate; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-α; TGF-β, transforming growth factor-β.

Printed with permission: Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, Figure 85.1, page 1404.

➢ Activation of HSC

- See previous sections please

CLINICAL PHYSIOLOGICAL CHALLENGE – Fatty liver

Case: A 58 year old "Cuddly" grandmother suffering from type II diabetes (insulin resistance) is noted to have an elevated serum AST and ALT. Abdominal ultrasound confirms the clinical suspicion of fatty liver.

- Give the pathophysiological steps leading to non-alcoholic fatty liver disease (NAFLD)
- Outline the initiation and perpetuation steps leading to steatohepatitis and fibrosis
- "Go for Purple": List the regulated changes in gene expression which occur during activation of HSC (hepatic stellate cells)

Useful background: Additional aspects of pathogenesis of NAFLD

- Diet
  - ↑ n-6 PUFAs
    - Linoleic acid
    - Arachidonic acid
  - ↓ N-3 DUFAs
  - ↑ CHO / fructose →
    - ↑ hepatic lipogenesis
    - ↑ inflammatory pathways
    - Δ gut microbiotica

- Gut microbiotica

- Genetics
  - Genetic polymorphism in PNPLA3 (patanin-like phospholipase domain containing)
  - Germline-encoded mechanisms of pattern recognition, affecting innate immunity
    - PAMPs (pathogen-associated molecular patterns)
      - TLR (toll-like receptors
      - NOD (nucleotide-binding oligomerization domain)
    - DAMPs (damage-associated molecular patterns)
      - NLR leucine rich repeat containing receptors
      - Others

- Toll-like-receptors (TLRs)
  - TLR2
    - Ligands for bacterial, fungi and protozoal products
    - Saturated fats in diet ↓ protective role of TLR2s
  - TLR4
    - ↑ activity in response to LPS (lipoprotein polysaccharides)
  - TLR9
    - Endosomial TLR9 recognizes unmethylated Cp6 motifs-a direct role not identified in NAFLD
  - DNA viruses
  - LPS (lipopolysaccharides) speculated to be of importance

- NLRs
  - Form cytoplasmic multiprotein complexes, known as inflammasomes
  - Inflammasomes (adaptor proteins) contain apoptosis-associated speck-line protein containing
    - CARB
    - Serine protease caspase-1

- NKT (natural killer T) cells
  - Altered proportion of NKT cells in periphery and parenchymal tissue may ↑ risk of NASH

- Types of hepatocyte injury and death
  - Apoptosis

        - FFAs cause
            - Lysosomal permeabilization / destabilization → activation / release of cathepsin B
            - Mitochondrial dysfunction
            - Lipotoxicity
            - ↑ Fas protein expression
            - ↑ TNF / TNF -1R expression
        - Programmed cell death
    - Necrosis
        - Accidental cell death in response to
            - ↓ ATP
            - ↑ ROS
    - Necroptosis
        - Receptor-induced, caspase-independent cell death
        - CYP2E1-dependent receptor-interacting protein (RIP) expression
        - RIP-1 – RIP-3 dependent TRAIL-induced receptors

- Terminal events
    - May play a role in NAFLD via NLRP3 and IL-1 signaling
    - Lytic release of cellular compounds
        - Necrosis
        - Pyroptosis
    - Breakdown of cellular components with instant plasma membranes
        - Apoptosis
        - Autophagia
    - Pyroptosis caspase-1 dependent cell death

Source: Wree et al., *Nat Rev Gastroenterol Hepatol* 2013; 10: 627-636.

Autoimmune Hepatitis (AIH)

- Give the pathophysiology of autoimmune hepatitis (AIH).
    - Unknown, and possible environment antigenic peptide (molecular mimicry) --→ inciting antigen fits in the antigen-binding groove of the class II DR molecule of the MHC antigen presenting cells (in USA / Canada, DRB1 gene, susceptibility alleles DRB1*0301 and DRB1*0401)
    - The DR molecule-antigen complex of the APC (antigen-presenting cell) interacts with the antigen receptor of CD4+ T-helper cells (1st signal).

- The CD28 molecule on the surface of CD4+ t-helper cell binds to the B7 ligand on the surface of the APC" (2nd signal) (Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, page 1464).
- CD4+ T-helper cells differentiate into Th1 or Th2 T-cells
- The cytokine milieu of Th1 and Th2 cytokines is regulated
- This balance between Th1 and Th2 may be disturbed by
  - ↓ NKT (intrahepatic natural killer T cells)
  - ↓ regulation of CD8+ T cell proliferation by
  - T-reg (T-regulatory CD4+ CD25+) cells
- Hepatocyte damage is caused by
  - Cell-mediated cytotoxicity (regulated by type 1 cytokines), or
  - Antibody-dependent cell-mediated cytotoxicity (regulated by type 2 cytokines), or by
  - Both mechanisms
- Th1 (type 1 cytokine) pathway of activated CD4+ t-helper cells is stimulated by polymorphism of
  - TNF A*2 (TNF gene)
  - TNF receptor superfamily gene (FAS)
  - CTLA4 (the cytotoxic T lymphocyte antigen 4 gene)
  - TNFRSF6 (FAS gene phenotype at position – 670)
- These polymorphisms and Th1 T-cells
  - Sensitize cytotoxic T cells
  - ↑ cell-mediated cytotoxicity
  - ↑ apoptosis of hepatocytes
- The cytokine pathways are increase by ↓ activity of T-reg (T-regulatory) cells
- Th2 (type 2 cytokine) pathway of activated CD4+ T-helper cells causes differentiation of B cells into plasma cells
  - Plasma cells → ↑ production of immunoglobulin
  - Immunoglobulins → antibody-mediated cellular toxicity

Primary Biliary Cirrhosis (PBC)

- Epidemiology
  - F >> M, 9:1
  - Age of diagnosis usually 30 to 60 years
  - Incidence $3/10^5$
  - Prevalence $40 / 10^5$

- Give the pathophysiology of primary biliary cirrhosis (PBC).
  - Immune-mediated response to an unknown antigen (molecular mimicry)
  - Activation of CD4+ and CD8+ T lymphocytes, B cells, and NK cells
  - Aberrant polyclonal activation of B cells (↑ IgG, IgM)
  - Breakdown of tolerance against mitochondrial antigens, leading to the production of autoantibodies against proteins on the inner membrane of mitochondria
    - AMA (anti-mitochondrial antibody, present in 95% of PBC patients) directed against the E2 component of
      - PDC E2 (pyruvate dehydrogenase complex)
      - PDC E1a subunit of PDC
      - E3 BP subunit of PDC
      - BCO-ADC-E2 (branched-chain of 2-oxo-acid dehydrogenase complex)
      - Molecular target is gene gp120 or nucleoprotein p62
      - Anti-gp 210
        - AMA+PBC, 25%
        - Sensitivity AMA-PBC, 50%
        - Specificity, AMA+PBC, 99%, AMA-PBC, 25%
      - Gp 210 and p62 associated with poor prognosis
      - Gp 201 protein and AMA persist in serum after liver transplantation for PBC
  - PDC-E2-specific CD4+ lymphocytes interact with duct cells in the small intrahepatic bile ducts
  - This interaction of CD4+ and CD8+ T cells is facilitated by ICAM-1 (intracellular adhesion molecule 1)
  - Other antibodies:
    - ANA (antinuclear antibodies)
      - In 50% of AMA+PBC, and 85% of AMA-PSC
      - Anti-MNA (anti-multiple nuclear dot)antibodies
      - Molecular target, sp 100 (soluble protein)
    - Anti-centromere antibodies
    - Antinuclear envelop antibodies
  - Other autoantibodies
    - Rheumatoid factor, 70%

  - Anti-smooth muscle, 16%
  - Anti-thyroid, 41%

- Immunoblotting and ELISA (enzyme-linked immunosorbent) assays have excellent performance characteristics for AMA: > 9% for both sensitivity and specificity.

- These methods may detect AMA in persons who were AMA negative when tested by direct immunofluorescence testing

- HLA class II molecular phenotype may be associated with PBC

Hepatitis B (HBV)

- Give the molecular biology of hepatitis B virus (HBV).

  - HBV is a DNA virus which goes through an RNA intermediate, and requires an active viral reverse transcriptase / polymerase enzyme.

  - High daily point mutation rate, low replication fidelity

  - The 5 gene encodes HBsAg, a viral surface, envelop protein

  - The core gene has
    - Precore region → HbeAg
    - Core region → core proteins

  - HBV DNA polymerase reverse transcriptase pregenomic RNA into a negative-strand HBV DNA ("needed for encapsidation of viral RNA into a negative strand of viral DNA (reverse transcription) and conversion of this first HBV DNA strand into a second DNA strand of positive polarity (Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, page 1291).

  - The negative-strand HBV DNA is a template for the synthesis of a positive-strand.

  - The positive strand is incorporated into the maturing nucleocapsid.

  - The nucleocapsids are coated with the surface protein (HBsAg) in the ER.

- From the ER, the enveloped nucleocapsid bud off from the hepatocyte membrane, and enter the circulation.

Hereditary Hemochromatosis (HH)

HH is an autosomal recessive disorder in the HFE gene on chromosome 6, leading to either the C282Y or the H63D mutation, low expression of hepcidin (the hepatic iron-regulatory protein), and iron loading of the hepatocytes.

- Give the role of **hepcidin** in the control of iron balance.
  - Hepcidin binds to ferroportin on BM (basolateral membrane) of duodenocyte and on macrophages, and causes the ferroportin to be internalized and inactive, with ↓ exit of iron from enterocyte, as well as decreased iron from macrophages.
  - ↑ hepcidin → ↓ iron absorption
  - ↑ ferroportin in BM causes ↑ Fe transfer out of enterocyte, and overall ↑ iron absorption
  - ↑ HJV - ↓ hepcidin - ↑ iron absorption
  - ↑ hepcidin - ↑ ferroportin internalization - ↓ Fe transport across enterocyte BM
  - BMP binding to its receptor on the hepatocyte increases SMAD protein-dependent activation of hepatocyte expression

- Give the speculated mechanism of HFE in **sensing of body iron stores** (BIS).
  - HFE may bind to TFR1 and TFR2
  - The HFE-TFR1 complex on the surface of the hepatocyte senses TS (transferrin saturation)
  - When TS↑, diferric transferrin displaces HFE from the HFE-TFR1 complex
  - HFE now binds to TFR2
  - HFE-TFR2 complex causes the hepatocyte to sense ↓ TS (and ↓ BIS), so expression of hepcidin falls, and iron absorption increases

- Give the non-HFE forms of HH.
  - Neonatal hemochromatosis
  - Juvenile hemochromatosis
    - Hepcidin (HAMP), or
    - HJV (hemojuvelin)
  - TFR2 (transferrin receptor 2)
    - Hemojuvelin BMP (bone morphogenetic protein) binding to its recep on the hepatocyte surface.
    - Loss-of-function mutation or gain-of-function

- Give the changings in iron binding, sensing; and transport proteins in HFE and non-HFE HH.
  - Mutations of HFE, HJV, hepcidin, TFR2, ↑ ROS, ↑ IL-6, HFE – TFR2 complex → ↓ hepcidin expression → ↑ active ferroportin (and ↑ AMT1) → ↑ Fe absorption → ↑ BIS (body iron stores).
  - As BIS increase → ↑ BMP binding to hepatocyte receptors → ↑ SMAD protein-dependent increased expression of hepcidin → ↑ inactive ferroportin → ↓ iron absorption
  - In HH, the mutation in HFE provides abnormal "sensing" of the body iron stores the intestinal crypts "sense" that the body iron stores (BIS) are low, despite these BIS actually being high in HH.
  - The sensing of ↓ BIS leads to ↓ hepcidin, ↑ active ferroportin, and ↑ iron absorption
  - The ↑ iron absorption continues, unresponsive to the ↑ BIS because of the mutation of HFE
  - Hepcidin decreases iron "release" from enterocytes (↓ transport of FE across the enterocyte BM), and hepcidin also decreases iron release from macrophages.
  - The ↓ hepcidin in HH causes ↑ iron release from macrophages

- Give the changes in iron binding, sensing, and transport proteins in **inflammation**, which lead to iron overload.
  - Iron metabolism is altered in inflammatory disorders (e.g., anemia of chronic disease)
  - In inflammatory disorders the ↑ proinflammatory IL-6 increases STAT3 9signak transducer and activator of transcription-3) signaling
  - ↑ STAT3 signaling increases hepcidin

- This inflammation associated ↑ hepcidin causes
  - Retention of iron in duodenocytes → ↓ iron absorption
  - Retention of iron in macrophages, loading of iron in RES (reticuloendothelial system) with less iron being available for synthesis of heme, and anemia

- Give the mechanism of iron overload in ALD and HCV.
  - In ALD (alcoholic disease) and HCV, ROS (reactive oxygen species) are formed
  - ↑ ROS
    - Acts on a C/EBP (CCAAT / enhancer binding protein → ↓ hepcidin expression
    - ↑ Fe in hepatocytes
  - This leads to ↑ iron absorption and release of iron in macrophages, the iron overload in the conditions

- Give the speculated role of ↑ Fe in hepatocytes and the development of fibrosis.
  - ↑ hepatocyte Fe → lipid peroxidation of hepatocytes
  - Injured hepatocytes release profibrogenic cytokines
  - Profibrogenic cytokines activate stellate cells
  - Activate stellate cells lead to hepatic fibrosis

"Abandon all hope, Ye who enter here

Lasciate ogne speranza voi ch'entrate

Dante Alighieri

Wilson Disease (WD)

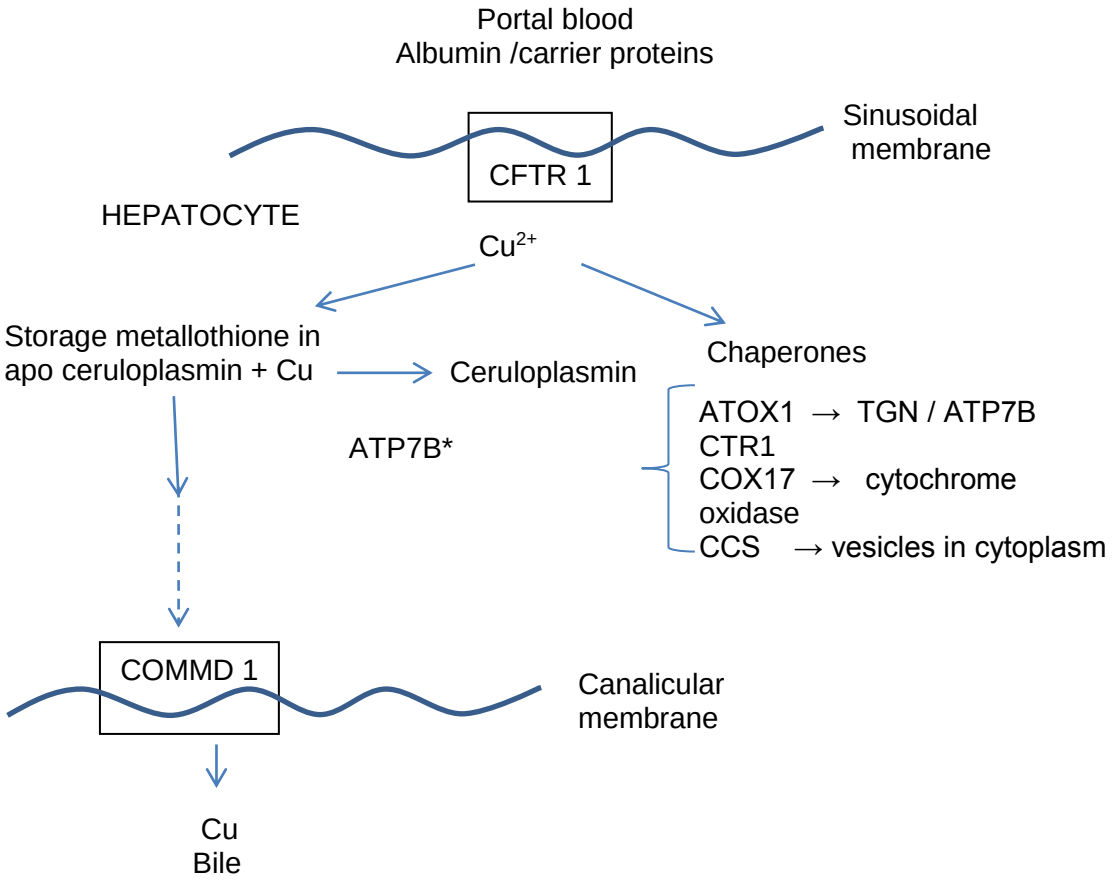
- Definition

  - WD is an autosomal recessive condition caused by a mutation in ATP1B WD gene on chromosome 13, resulting in reduced secretion of copper from the liver into the bile, and accumulation of excessive iron in numerous tissues such as CNS, eye, liver, red blood cells, kidney.
  - Dietary copper (Cu) is absorbed, and bound to plasma albumin,

    and
  - Plasma Cu crosses the hepatic sinusoidal membrane (SM).
  - The hepatic cytosolic Cu attaches to chaperones ATOX1, COX17, CCS cytoplasmic
  - These 3 chaperones target the Cu to 3 different proteins
    - ATOX1 → ATP7B → trans-golgi network (TGN) → vesicles → COMMD4 transporter in cannicular membrane (CM)
    - COX17 → SCO1
    - CCS → mitochondrial membrane
    - Cu / Zn superoxide dismutase (SOD)
    - Cytochrome oxidase
  - ATP7B is the Wilson ATPase which is mutated in Wilson disease, leading to:
    - Impaired incorporation of copper (Cu) into apoceruloplasmin (apoceruloplasmin + Cu → ceruloplasmin), and
    - The retention of copper tissues such as the liver because of
    - ↓ trafficking of Cu-ATP7B from TGN to COMMD, and across CM

"Harsh words are heavy and often fall with a big thud,
but a kind word will bounce on and on…"

Anonymous



- Exam alert
  - Be sure to associate WD and liver disease plus neuropsychiatric symptoms and especially Parkinsonian symptoms, Kayser-Fleischer rings from a copper-sulfur granular complex in Descemet membrane of the cornea, and sunflower cataracts from copper deposited in the lens of the eye.
  - Caution: NAFLD is common and WD is rare; fatty liver is common in WD, so when you see a person with NAFLD, always ask yourself-could this be WD?
  - Curiosity and Caution: WD is very rare, but is frequently tested (or over-tested) in written and clinical examinations.

➢ Pathophysiology

- Give the pathophysiology of Wilson disease.
  - Copper (Cu) from albumin and other carriers in blood is taken up into the hepatocyte by the copper transporter CTR1.
  - Copper (Cu) enters the liver and binds to Cu chaperones CTR1, COX17, and ATOX1.
  - Copper in the liver binds to apoceruloplasmin, forming ceruloplasmin.
  - Ceruloplasmin is an α2-glycoprotein.
  - Under basal conditions, the Wilson ATPase is located in the trans-Golgi network (TGN), where it receives Cu from the Cu chaperone ATOX1.
  - At low Cu concentrations, the Wilson ATPase participates in the mechanism for incorporating Cu into apo-ceruloplasmin to generate holo-ceruloplasmin (blue collar plus Cu).
  - High copper concentrations it the Wilson ATPase lead to reduced biliary excretion of Cu.
  - Cu transfer to the transmembrane domain is accompanied by ATP hydrolysis and formation of transiently phosphorylated intermediates of the Wilson ATPase ('phosphorylation').
  - Structural phenotypic defects include
    - Absence of any intact Wilson ATPase
    - Misfolding of Wilson ATPase leading to its retention in the endoplasmic reticulum
    - Failure to bind to ATOX1 (not shown)
    - Ineffective interaction with other proteins, which directs the intracellular movement of the Wilson ATPase.
  - Functional phenotypic defects include
    - Abnormal phosphorylation or copper transfer
    - Disruption of intracellular copper trafficking.
  - Either type of phenotypic defect can result in copper accumulation and toxicity in hepatocytes.

Abbreviations: BC, bile canaliculus; Mt, metallothionein; RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum; TGN, trans-Golgi network.

Printed with permission: Ferenci P, et al. Defining Wilson disease phenotypes: from the patient to the bench and back again. Gastroenterology 2012;142(4):692-6.

➢ Clinical

- Give 20 **clinical features** that suggest the diagnosis of WD.
  - Hepatic
    - Acute liver failure
    - Acute hepatitis
    - Chronic hepatitis
    - Autoimmune-like hepatitis Asymptomatic hepatomegaly
    - Isolated splenomegaly
    - Persistently elevated serum aminotransferase activity (AST, ALT)
    - Fatty liver
    - Cirrhosis: compensated or decompensated
  - Young patient
  - Neuropsychiatric symptoms
    - Depression
    - Neurotic impulsive behavior
    - Basal ganglia involvement
      - Disorder of gait
      - Rigidity
  - Neurological
    - Movement disorders (tremor, involuntary movements)
    - Drooling, dysarthria
    - Rigid dystonia
    - Pseudobulbar palsy
    - Dysautonomia
    - Migraine headaches
    - Insomnia
    - Seizures
    - Depression
    - Neurotic behaviours
    - Personality changes
    - Psychosis
  - Eye
    - Sunflower cataracts
    - Kayser-Fleisher rings

- Hemolytic anemia
- Hyperphonia (soft voice)
- Kidney
  - Aminoaciduria
  - Nephrolithiasis
  - Fanconi syndrome
  - Nephrolithiasis
- Gallbladder
  - Cholelithiasis
- Pancreas
  - Pancreatitis
- MSK
  - Arthritis of larger joints
  - Osteoporosis
  - Osteochondritis
  - Rickets
  - Rhabdomyolysis
- Heart
  - Cardiomyopathy
  - Arrhythmias
  - SCD (sudden cardiac death)
- Endocrine
  - Hypoparathyroidism
  - Amenorrhea
  - Infertility
  - Spontaneous abortion
  - Menstrual irregularities
  - Repeated miscarriages
- Skin
  - Lunulae ceruleae

Modified in part from: Roberts EA, Schilsky ML; American Association for Study of Liver Diseases (AASLD). Diagnosis and treatment of Wilson disease: an update. Hepatology. 2008 ;47(6):2089-111.

More useful background

- A young person with liver disease in WD who develops neurological symptoms usually has cirrhosis.
- Hemolytic anemia in WD occurs in
  - Acute hepatitis
  - ALF acute liver failure
- Basal ganglia symptoms / signs
  - Plus KF rings = WD
  - No KF rings ≠ WD
- Watch out : NAFLD may occur in WD, which you should suspect from
  - Neurological symptoms / signs
  - ↓ AP, ↓ uric acid
  - Hemolytic anemia
  - KF rings

- Give the **histopathological changes** in the liver in WD.
  - Lysosomal deposits of Cu-metallothionon (orcein stains), especially Kupffer cells
  - Inflammation, including interstitial hepatitis
  - Mallory bodies
  - Patchy involvement of cystic dilation of mitochondrial cristae

Findings	Score	Findings	Score
Coombs-negative hemolytic anemia		Mutation analysis	
- Present	1	- On both chromosome detected	4
- Absent	0	- On 1 chromosome detected	1
		- No mutations detected	0

TOTAL SCORE	Evaluation
≥ 4	Diagnosis of WD established
3	Diagnosis possible, more tests needed
≤ 2	Diagnosis very unlikely

Printed with permission: European Association for Study of Liver. EASL Clinical Practice Guidelines: Wilson's disease. *J Hepatol.* 2012;56(3):671-85, Table 5.

Scoring system developed at the 8th International Meeting on Wilson disease.

Findings	Score
KF rings	
- Present	2
- Absent	0
Neurologic symptoms**	
- Severe	2
- Mild	1
- Absent	0
Serum ceruloplasmin	
- Normal (> 2 g/L)	0
- 0.1- 0.2 g/L	1
- < 0.1 g/L	2

Findings	Score
Liver copper	
- > 5x ULN (>4 µmol/g)	2
- 0.8-4 µmol/kg	1
- Normal (< 0.8 µmol/g)	-1
- Rhodamine-positive granules	1
Urinary copper (in the absence)	
- Normal	0
- 1.2x ULN	1
- > 2x ULN	2
- Normal but > 5x ULN	3

SO YOU WANT TO BE A HEPATOLOGIST!

The serum concentration of ceruloplasmin is decreased in WD, but the reduction is not diagnostic for the condition.

- Give a classification of the other causes of reduced ceruloplasmin levels.
  - Chronic liver disease
  - Malnutrition
  - Malabsorption
  - Nephrotic syndrome
  - Hereditary aceruloplasmin
- Give the reasons why measurement of liver copper may be normal or non-diagnostic in early WD.
  - In early WD the lysosomal Cu may be distributed unevenly in the liver, so there is a possible sampling error.
- Give the mechanism for the iron deficiency which may occur the WD.
  - Fe2+ stored in ferritin must be oxidized for the Fe2+ to become Fe3+ and to be bound to transferrin to be transported to bone marrow for production of RBC.
  - Ceruloplasmin is a feroxidase (oxidase activity).
  - ↓↓ ceruloplasmin, as occurs in WD, this results in less oxidation of Fe2+ in ferritin, so ↓ Fe^{3+} to be bound to transferrin and used for RBC

➢ Diagnosis

- Give 7 **laboratory features** that suggest the diagnosis of WD.
  - Transaminases < 1500 U/L (may be normal)
  - AST / ALT > 2.2
  - ↓ AP (alkaline phosphatase)
  - ↑↑ bilirubin (B) (from liver disease plus RBC hemolysis)
  - AP/B < 4
  - ↓ serum ceruloplasmin
  - ↓ serum copper
  - ↑ non-ceruloplasmin-bound copper
  - ↑ copper in the urine (reflects ↑ non-ceruloplasmin-bound copper)
  - ↑ liver copper (> 250 µg per g dry weight of liver)

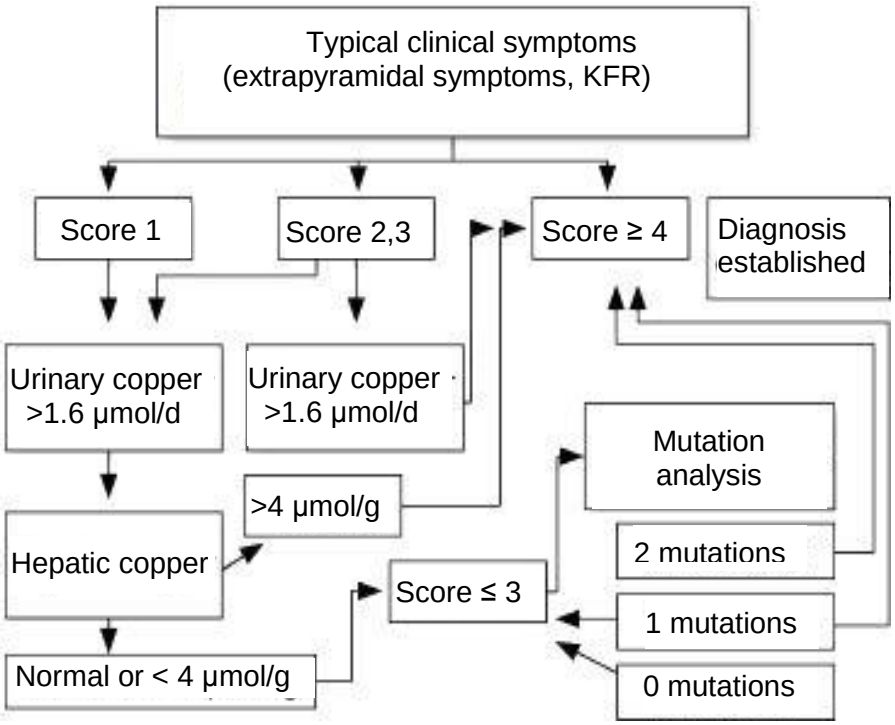
Routine tests for **diagnosis** of Wilson disease.

Test	Typical finding	False negative*	False positive*
Serum ceruloplasmin	Decreased by 50% of lower normal value	Normal levels in patients with marked hepatic inflammation Overestimation by immunologic assay Pregnancy, estrogen therapy	Low levels in: - Malabsorption - Aceruloplasminemia - Heterozygotes
24-hour urinary copper	> 1.6 µmol / 24 h > 0.64 µmol / 24 h in children	Normal - Incorrect collection - Children without liver disease	Increased - Hepatocellular necrosis - Cholestasis - Contamination
Serum "free" copper	> 1.6 µmol / L	Normal if ceruloplasmin overestimated by immunologic assay	
Hepatic copper	> 4 µmol/g dry weight	Due to regional variation - In patients with active liver disease - In patients with regenerative	Cholestatic syndrome

nodules

Test	Typical finding	False negative*	False positive*
Kayser-Fleische rings by slit lar examination	Present	Absent - In up to 50% patients with hepa Wilson - In most asymptoma siblings	Primary biliary cirrhosis

Printed with permission: European Association for Study of Liver. EASL Clinical Practice Guidelines: Wilson's disease. *J Hepatol.* 2012;56(3):671-85, Table 4.



Printed with permission: European Association for Study of Liver. EASL Clinical Practice Guidelines: Wilson's disease. *J Hepatol.* 2012;56(3):671-85, Figure 1.

Approach to diagnosis of Wilson disease (WD) in a patient with unexplained liver disease.

- The gold standard for diagnosis of WD
  - Hepatic copper > 250 µg / g dry tissue – Yes
  - ↓ serum ceruloplasmin
  - Free copper, ↑ urine copper – No (seen in heterozygotes, and other chronic cholestatic conditions)
- WD is caused by autosomal recessive ↓ / absent function of the gene ATP7B; who needs to be screened for WD in the patient's family.
  - First-degree relatives
- Suggested algorithm for Wilson's disease based on the Leipzig Score. ∗In children the cut off can be lowered to 0.64 µmol/d.

➢ Treatment

➢ Clinical Alert in Treatment of WD: Stopping Rules – don't
- Do not suddenly stop chelating treatment in WD
- Suddenly stopping chelating therapy may
  - ↑ neurological defects
  - ↑ risk of fulminant hepatic failure
  - ↑ risk of being refractory to treatment when Cu-chelating therapy is introduced

- Why is it important to appreciate that it takes ~ 6 months for D-penicillamine and trientine to improve neurological symptoms and liver enzymes in WD.
  - So that therapy is not considered to be unsuccessful and incorrectly and prematurely stopped.

"Your actions [in the practice of medicine] will be judged by your peers"

Jacque Guilbert, MD

- Give adverse effects of therapy of WD (Wilson disease).

➢ D-penicillamine
  - CNS
    - ↑ Neurological symptoms
  - Hypersensitivity reactior
    - Fever
    - Rash
    - Lymphadenopathy
    - Neutropenia
    - Thrombocytopenia
  - Endocrine
    - Hypothyroid
  - Skin lesions
    - Pemphigoid lesions
    - Lichen planus
  - Hematology
    - ↓ RBC
    - ↓ WBC
    - ↓ platelets
    - Lymphadenopathy
  - MSK
    - Lupus-like syndrome
    - Arthralgias
  - Lung
    - Goodpasture syndrome
  - Kidney
    - Proteinuria
    - Iron deficiency
    - Suppression of bone marrow

➢ Trientine
  - Gastritis
  - Iron deficiency
  - Suppression

➢ Ammonium tetrathiomolybdate
  - Suppression of bone marrow
  - Hepatotoxicity

Please see: Swan MG. Chapter 58. In: Therapeutic Choices. Grey J, Ed. 6th Edition, *Canadian Pharmacists Association*: Ottawa, ON, 2011, Table 7: Wilson disease, page 780.

α1- Trypsin (α 1-AT) Deficiency

- Genetics
  - Autosomal recessive disorder associated autosomal co-dominant mutation in A1AT (serine protease inhibitors) on chromosome 14
  - Leading to chronic disease of lungs (emphysemia) and liver (cirrhosis)
- Clinical
  - Pulmonary emphysema
  - Hepatic cirrhosis
  - Panniculitis, ulcerative, neutrophilic
  - Aneurysm of aorta, cervical artery

Note; recombinant plasma A1AT replacement therapy useful for lung but not liver A1AT changes

- Pathophysiology

- Give the pathogenesis of α1-AT deficiency.
  - Replacement of glutamine with lysine from a mutation at position 342 of the α1-AT (SERPINA 1) gene leads to degradation of the serine proteases in the serum and tissues
  - Loss of serum α1-AT activity results in
    - Loss of normal inhibition of the activity of the elastase protease
    - ↑ neutrophil elastase activity
    - ↑ proinflammatory effect of ATZ polymers on neutrophils
  - The AT protein is misfiled and polymerized and malprocessed.
  - This abnormal structure of the AT2 protein lead to its retention in the ER, and ↓ intracellular degeneration (abnormal "quality control" of the protein)
  - Loss of normal inhibition of the activity of the elastase protease
  - The gain-in-function alter intracellular signaling pathways, with the changes in processes include
    - ↑ autophagy

- Mitochondrial injury
- ↑ apoptosis

➢ Histopathology

- Give the **histopathology** of α1-AT deficiency.
  - ↓ bile ducts
  - Intracellular cholestasis
  - Inflammation
  - Steatosis
  - PAS (periodic acid-Schiff) – positive, diastase-resistant globular inclusions of polymers of α1-ATZ protein in periportal hepatocytes and Kupffer cells
  - IHC (immunohistochemistry)
    - Staining with monoclonal antibody to α1-ATZ confirms α1-ATZ proteins in the globular inclusions.

➢ Treatment
  - Liver transplantation corrects the metabolic disorder
  - 5-year survival rate for α1-AT
    - Children, 83%
    - Adults, 90%

- List 7 liver diseases/conditions/presentations in HIV-infected persons even in the **post-HAART** era.
  - HCV (coinfection in 25%) - faster development of fibrosis, poorer response to HCV treatment
  - HBV, HBV-associated ↑ mortality
  - Alcohol liver disease (associated life style)
  - NAFLD (fat redistribution from HAART [lipodystrophy]))
  - Cholangiopathy (intra- and extra-hepatic)
  - Asymptomatic ↑ in transaminases, alkaline phosphatase
  - Kaposi's sarcoma
  - Opportunistic infection:
    - Cholangiopathy: mycobacterium
    - Fungal: cryptological, histological, coccidiomycosis, extra pulmonary pneumacystitis carcinoma
  - Nodular regenerative hyperplasia (vasculopathy)

Adapted from: Wilcox, C. Mel. Gastrointestinal Consequences of Infection with Human Immunodeficiency Viurs. *Sleisenger & Fordtran's Gastrointestinal and Liver Disease: Pathophysiology/ Diagnosis/Management* 2006; pg.676-79.

Liver and Pregnancy

- Give 4 physiological mechanisms which contribute to the altered metabolism of drugs during pregnancy.
  - ↑ maternal blood volume (50%)
  - ↓ maternal serum albumin concentration (20%)
  - Progesterone → proliferation of SER
  - Estrogen → proliferation of RER
  - ↑ cytochrome P-450 gene products

- Give the maternal and fetal factors implicated in the pathogenesis of HELLP.

➢ Mother

- ↑ sFlt1 (soluble forms-like tyrosine kinase 1 (an antagonist of VEGF [vascular endothelial growth factor] and an antagonist of PIGF [placental growth factor])
- ↑ sEng (soluble endoglin, which reduces the formation of capillaries)
- ↑ procoagulant (e.g., factor V Leiden, anticardiolipin antibody)
- ↑ systemic vascular resistance
- ↓ plasma volume
- ↓ LCHAD (long-chain 3-hydroxyacyl – CoA dehydrogenase), which when reduced leads to ↓ mitochondrial oxidation of fatty acids)

➢ Fetus

- ↓ blood flow of fetus from placenta
  - ↓ trophoblast invasion of wall of uterus
  - Dilation of spiral arteries
  - ↓ uteroplacental perfusion
- Both HELLP and AFLP may be associated with maternal defects in the mitochondrial oxidation of fatty acids arising from deficiency of LCHAD (long-chain 3- hydroxyacyl- CoA dehydrogenase).
- LCHAD in fetus: "AFLP may develop regardless of materr genotype if the fetus is deficient in LCHAD and carries at lea one allele for the G 1528C LCHAD mutation" In cases of AFLP t mother, father and child should be tested for the G1528C LCHA

mutation

- ↓ carnitine palmitoyltransferase is also associated with AFLP

Complications of Cirrhosis

- **Hepatic Encephalopathy (HE)**

- Outline the contribution of the small and large intestine, liver, skeletal muscle, kidney and brain to the pathogenesis of hepatic encephalopathy (HE) in patients with liver failure and HE (hepatic encephalopathy).

  - Small bowel and large intestine

    - Dietary amino acids and urease-positive bacteria → glutamine

    - Uptake of glutamine

glutaminase (deamination)
glutamine → glutamate + NH_3
←
glutamine synthetase (amination)

    - ↑ activity of gut glutaminase is increased in liver disease.

    - ↑ production of ammonia by urease-producing bacteria in GI tract

    - ↑ production of ammonia and glutamate from increased action of intestinal glutaminase

  - Liver

    - Portosystemic shunting, by-passing portovenous system with less hepatic detoxification of ammonia via the urea cycle

    - NH_3 → urea, periportal hepatocytes → glutamine, perivenous hepatocytes

    - In presence of hyponatremia, myoinositol falls, with less compensation for ↑ intracellular glutamine

  - Skeletal muscle

- Normally responsible for uptake of 50% of NH_3
- Atrophy of skeletal muscles, with ↓ muscle glutamine synthetase → ↓ synthesis of glutamine → ↑ NH_3

➢ Kidney

- increased NH_3 production in presence of hypokalemia
- In presence of hypokalemia and metabolic alkalosis,

  NH_4 → NH_3, which crosses BBB
- Plasma NH_3 > 150 µmol is associated with brain herniation from brain edema.

➢ Brain

- In health,

  NH_3 + glutamate ----------→ glutamine
  glutamine synthase in astrocytes
- In cirrhosis, ↑brain blood flow and ↑blood brain barrier permeability → ↑ NH_3 uptake into cerebellum and basal ganglia
  - ↑ brain edema
  - ↑ swelling of astrocytes - ↑ neurosteroids → ↑ activity of GABA-benzodiazepine system
  - ↑ benzodiazepine system
  - ↑ production of glutamine by astrocytes
- Abnormal form and function of astrocytes, with ↓glutamine synthetase and peripheral type benzodiazepine receptors (PTBR)
- ↑brain glutamine → ↑mitochondrial permeability → brain edema.
- ↓ glutamate uptake by synaptic excitatory amino acid transporters ↑extracellular brain levels of glutamate, ↓glutamatergic neurotransmitter system.

- Hyperammonemia activates N-methyl-D-aspartate-nitric oxide-C-granylate cyclase (NMDA-NO-C6MP) signal transduction pathway, impairing memory, learning and sleep.

- On the basis of your knowledge of the pathogenesis of hepatic encephalopathy (HE), give the treatment of episodic and persistent HE, and provide the rationale for each treatment.

➢ Treat precipitants

- Increased ammonia production - excessive protein intake, constipation, GI bleed (20%), azotemia (30%), hypokalemia
- Increased protein catabolism - surgery, diuretics, arterial hypotension/hypovolemia,
- Malnutrition - skeletal muscle wasting, less muscle metabolism of NH_3
- Increased diffusion across BBB – alkalosis
- Synergistic effects of cytokines – infection (SBP) (10%)
- Altered brain function – sedative drugs, psychotropics, analgesics, benzodiazepines; hyponatremia; astrocyte swelling
- Dehydration – fluid restriction, diuretics, excessive paracentesis, vomiting, diarrhea (mechanism unknown)
- Hypoxia, anemia, fever, sepsis
- Metabolic-:K+↓ (50%), ↑BS, alkalosis; ↓hypoxemia, thyroid, dehydration
- Drugs (30%) - benzodiazepines, analgesics, interferon, alcohol, NSAIDs, acetaminophen
- Surgical shunting, anesthetic, TIPS
- Liver decompensation, HCC, PVT
- Surgery e.g., L-Tx (liver transplant)

➢ Lactulose (beta-galactosidofructose), lacitol beta-galactosidosorbitol (traps NH_3) - enter colon, broken down by colonic bacteria to lactic acid, acetic acid acidification of stool pH < 5

$$NH_3 \xrightarrow{pH < 5} NH_4^+ \text{ (non-absorbable)}$$

- Hyperosmolar purgation, ↑ stool volume, loss of nitrogen compounds

➢ Neurotransmitters: flumazenil (a competitive GABA-benzodiazepine receptor antagonist) or bromocriptine

- Branched chain amino acids (no longer widely used)
- Measure and manage cerebral blood flow (CSF)
  - Intracranial pressure (ICP) monitoring, transcranial Doppler, jugular venous oximetry
  - Manage lactic acidosis and sepsis
  - 45˚ elevation of head of bed
  - Moderate hypothermia to reduce ICP and CBF, reduce arterial NH_3 and cerebral NH_3 uptake
- Manage circulatory effects
  - Fluid management, consider CVP monitoring
  - Perform short synacthen test, and give GCS if adrenal insufficiency is present
  - Inotropes: terlipressin (a vasopressin analog) or norepinephrine
  - Albumin
- 'Biotics (pre-, pro- and synbiotics)
  - ↑ bacterial NH_3 utilization
  - ↓ pro-inflammatory response
  - ↓ gut permeability
  - ↓ bacterial translocation
- Extracorporeal liver assist devices (ELADs)
  - MARS (molecular absorbent recirculating system): providing counter-current hemodiolysis against albumin and biocarbonate circuits
  - SPAD (single-pass albumin dialysis): counter-current albumin dialysis against high blood flow in a fibre hemodin filter, and continuous veno-venous hemofiltration
  - Prometheus R system, direct albumin adsorption through a specific polysulfur filter
  - Enteral feeding/TPN
- Orthoptic liver transplantation
  - ↑ lactobacillus spp., ↓ urease-containing bacteria, ↓ NH_3 production by replacing urease-positive bacteria
  - ↓ production of potentially toxic SCFA (proprionate, butyrate, violerate)
  - Removes shunted (non-detoxified blood)

- Driving license – psychometric testing

Adapted from: Fitz GJ. *Sleisenger & Fordtran's Gastrointestinal and Liver Disease: Pathophysiology/Diagnosis/Management* 2006 pg. 1971-1972.

- **Cirrhosis**

- Give the coagulation disorders in cirrhosis.

- Mechanism of ↓ synthesis of procoagulant factors
  - Vitamin K-dependent factors II, VII, IX, X
  - Factor V
  - Factor XI

- Mechanism of thrombocytopenia in cirrhosis
  - Hypersplenism
  - ↓ hepatic synthesis of thrombopoietin
  - Bone marrow toxicity
    - HCV
    - Alcohol
  - ↓ thrombin produced by platelets

- Mechanisms of dysfibrogenemia
  - Altered production of activators / inhibitors of fibrolysis
  - Endotoximemia-associated activation of coagulation cascade
  - ↓ clearance of fibrinolytic proteins
  - ↑ D-dimer
  - ↑ fibrinogen degradation products
  - ↑ clot lysis time

- Hepatic transplantation

Once hepatic cirrhosis develops, the complications chronic liver disease (hepatic encephalopathy, esophageal varices, ascites / hepatorenal syndrome [HRS],

hepatocellular cancer [HCC], acute liver failure [ALF] may require transplantation of healthy donor liver, with the removal of the cirrhotic liver.

Acute Cellular Rejection (ACR) Following Liver Transplantation (LT)

- Occurs in ~20% LT patients, usually in the first month, and more with DLT (decreased liver transplant) than with LRD (living related donor).

- Early post-LT
  - ↑ALT/↑AP
  - Jaundice, fever → suspect ACR → liver biopsy or diagnosis
    - Portal tract
    - Inflammation (mixed mononuclear cells)
    - Bile ductules*
    - Non-supurative cholangitis
    - Vessels endothelitis

- Treatment
  - Methylprednisolone
    - 1000 mg IV bolus for 1 day, then tapered doses from day 2 to day 7, 200 mg IV od → 20 mg od
  - ~75% respond within 3 days to 5 days
  - For steroid-resistant (non-responsive) ACR
    - Use other immune suppressive, i.e. tacrolimus, sirolimus, MMF,ATG, ALG, OKT3, basiliximab
  - When all else fails, retransplantation of liver!

CLINICAL CAUTION:

- Post-LT for HCV infection
  - May look like ACR (acute cellular rejection) clinically, as well as on biopsy
  - Avoid boluses of steroids which would accelerate the HCV hepatitis
  - ↑ dose of CNI or add MMF

- Give the immunopathological steps involved in **liver rejection** after orthoptic liver transplantation (LT), and the pharmaceutical agents used to block each step required for T-cell maturation.
    - The pharmacological agents which block the processes leading to acute rejection post-LT are **shown in boxes**.

➢ Alloantigenic recognition

- The alloantigen is bound to a MHC molecule (major histocompatibility complex)
- Alloantigen becomes bound to an APC (antigen-presenting cell)
- The antigen binds to a receptor on the T-cells

➢ Antilymphocyte antibodies

➢ Lymphocyte activation

- Ligands on the APC also bind to receptors on the T-cells
- These T-cell receptors include CD27, CD11a, CD28, CD54 and CD154
- As a result of this co-stimulation, the T-cell receptor complex is internalized.
- The internalized t-cell receptor complex binds to immunophilin

➢ Calcineurin inhibitors (CNIs); cyclosporine and tacrolimus

Targets FKBP (FK-binding protein) — Targets intracellular cyclophilin

Target immunophilin

- Overall effect is ↓ T cell response to class I and II antigens

- Immunophilin stimulates calcineurin
- Calcineurin removes pyrophosphate from NFAT (nuclear factor of activation of T-cell)
- NFAT traffics to the nucleus
- In the nucleus, NFAT drives the transcription of IL-2 in T-cells

- Clonal expansion
  - IL-2 is secreted from T-cells
  - IL-2 acts in an autocrine manner to bind to the IL-2R (IL-2 receptor) on the surface of hepatocytes

> - Monoclonal antibodies to IL-2R basiliximab
> - mTor (mammalian target of rapamycin) bound by sirolimus

  - IL-2R acts as a transduction signal to stimulate DNA synthesis and proliferation of T and B cells

> Azathioprine and mycophenolate inhibit DNA synthesis, and thus ↓ proliferation of T and B lymphocytes.

- Inflammation
  - T-cell proliferation associated with secretion of
    - Cytokines
    - Chemokines
    - Adhesion molecules
  - Cytokines, chemokines, adhesion molecules cause recruitment of inflammatory cells

> - Antilymphocyte antibodies of glucocorticosteroids act against this cell-mediated cytotoxicity.

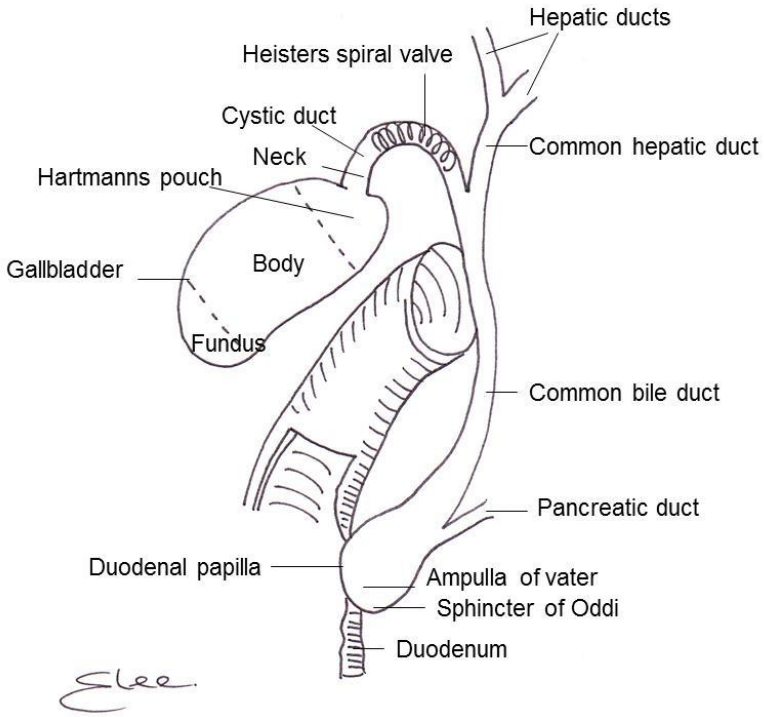
  - Cell-mediated cytotoxicity damages the hepatocytes

> - Steroids also
>   - ↓ antibody binding
>   - ↓complement binding
>   - ↓ T cell synthesis of IL-2, IL-6, IFN-γ (interferon gamma)
>   - ↑ IL-10

GALLBLADDER

- The function of the gallbladder is to concentrate and store bile, and to release the bile at appropriate stimulated intervals.

ANATOMY OF HEPATOBILIARY TREE AND GALLBLADDER



Adapted from: Sherlock and Dooley, Figure 1.6, page 3, 11th Edition, 2002

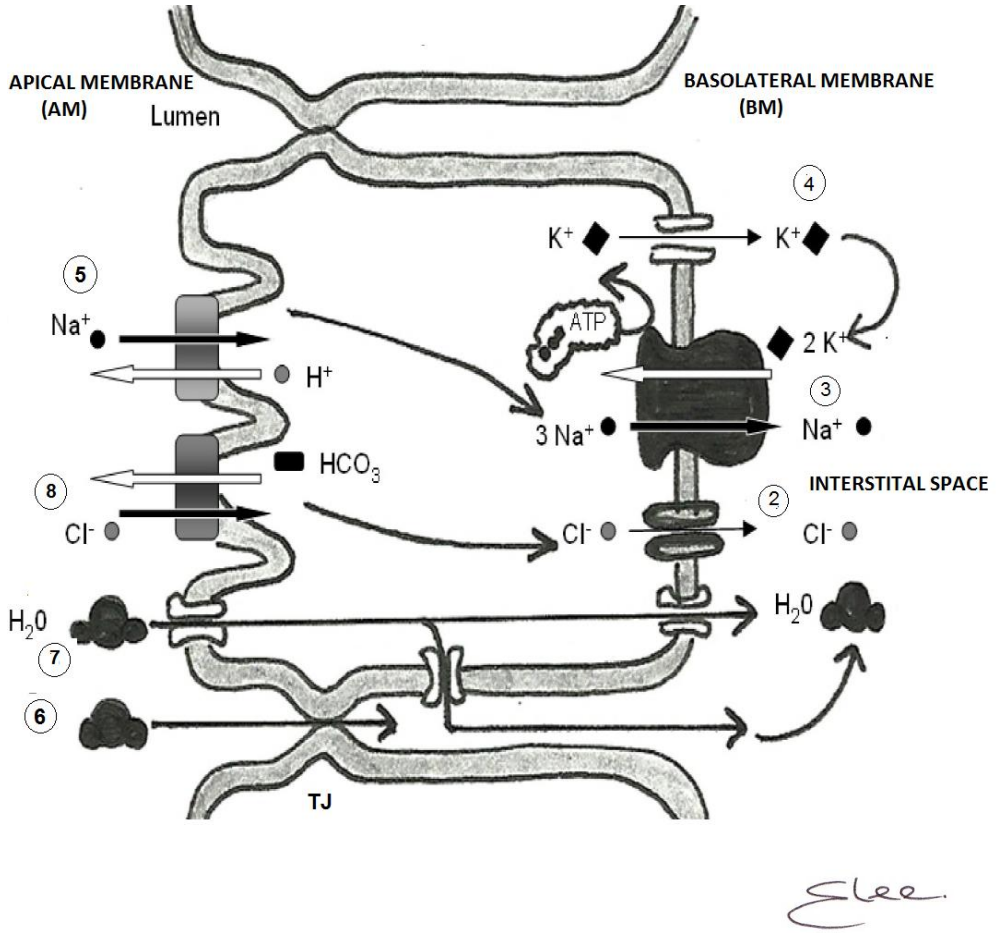
CLINICAL PHYSIOLOGICAL CORRELATION – Gallbladder Absorption

Background:

- The basolateral membrane (BLM) epithelium contains Na^+/K^+ATPase, as well as K^+, Cl^- and H_2O channels.
- The apical / luminal membrane (LM) of the gallbladder contains both Na^+/H^+ exchanger (NHE) and HCO_3^-/Cl^- exchanger, as well as H_2O channels.

- Draw a diagram which depicts the absorption of H_2O by the gallbladder epithelium.

FLUID ABSORPTION BY THE GALLBLADDER EPITHELIUM



Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009, Figure 46-12,page 997.

"Let's see if there is mechanistic information that we can tease out."

Grandad

Absorption of Electrolytes and Water by Wall of Gallbladder

- The gallbladder concentrates gallbladder bile from dilute hepatic bile

- Give the mechanism of fluid absorption by the epithelium of the gallbladder.
    - 1) Cl^- is taken up by the apical Cl^- / HCO_3^- exchanger of the gallbladder epithelium.
    - 2) This Cl^- leaves the gallbladder epithelial cell through the BM Cl^- channel.
    - 3) The BM ATP-dependant Na^+-K^+ pump provides a Na^+ gradient.
    - 4) K^+ transported into the gallbladder epithelial cell across the BM exits the cell through the BM K^+ channel.
    - 5) The NHE (Na^+ / H^+ exchanger) in the AM secrets H+ from the cell, and absorbs Na+ from the hepatic bile.
    - 6) H_2O flows across the tight junctions (TJ) between the epithelial cells in the gallbladder.
    - 7) H_2O also flows through pores in the AM and BM, removing H_2O from the hepatic bile and from the more concentrated gallbladder bile.
    - 8) Because the exchange of Na^+ and H^+ is greater than for HCO_3^- and Cl^-, the gallbladder bile becomes more acidic than the hepatic bile.
    - This acidity of gallbladder bile enhances the solubility of bile acids in the more concentrated gallbladder bile.

"The difference between how a person treats the powerless versus the powerful is as good a measure of human character as I know."

Robert Sutton

Gallstones (Cholelithiasis)

➢ Types of Gallstones

- ➢ Composition
  - o Cholesterol
    - – Crystals of cholesterol monohydrate
    - – Calcium bilirubinate
    - – Calcium carbonate / phosphate
  - o Pigment
    - – Calcium bilirubinate
  - o Mixed
  - o Rare
    - – Calcium carbonate
    - – Calcium-fatty acids
- ➢ Site
  - o Intrahepatic
    - – Brown pigment
  - o Gallbladder
    - – Cholesterol >> black pigment
  - o Bile duct
    - – Mixed
- ➢ Physical state cholesterol
  - o Low concentrations
    - – Monomers of bile acids (BA)
  - o CMC (critic micellar concentration)
    - – Simple micelles are spontaneously formed with BA monomers
      - ▪ Small (~ 3 nm diameter)
      - ▪ Disk-like macromolecular aggregates
      - ▪ Solubilize and incorporate
        - – Cholesterol
        - – Phospholipids

    (mostly lecithin, with some phosphatidylethanolamines and sphingomyelin) to form mixed micelles (~ 4-8 nm diameter)

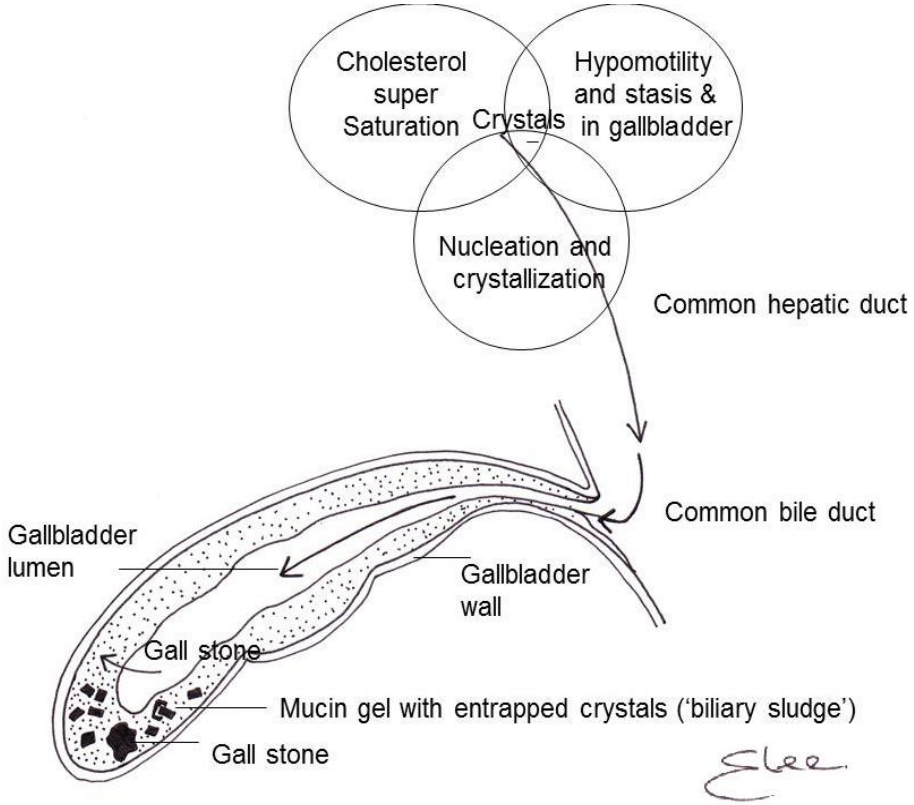
- Biliary vesicles
  - Secreted by hepatocytes
  - Composed of cholesterol monohydrate crystals and phospholipids
  - Large (40-100 nm diameter)
  - Multilamellar spherical structures
  - As the gallbladder (GB) absorbs H_2O, the GB bile becomes concentrated
  - The stability of the biliary vesicles determines the stability of bile molecular rearrangements such that
  - In high concentrations of BA, there are the biliary vesicles may transform into mixed micelles

➢ **Pathophysiology**

- Give the pathophysiology of the formation of gallstones.
  - Gallstones are usually formed from cholesterol, bilirubin, or a mixture of the two.
  - The steps leading to bilirubin gallstones will be covered in the next section on bilirubin metabolism.

➢ Formation of cholesterol gallstones

  - If the concentrations of bile acids, cholesterol, phospholipids or bilirubin are abnormal in the bile, the lipids may be insoluble.
  - The bile becomes “lithogenic”; lipid becomes insoluble in bile, and gallstones are formed.
  - Conditions required for cholesterol stones to form, may be too much cholesterol in the bile, or too little bile salt to solubilize the cholesterol.



- Give the steps in pathogenesis of cholesterol gallstones.
  - Lithogenic bile
    - ↑ hepatic secretion of cholesterol (lithogenicity and supersaturation)
    - ↓ cholesterol solubility
    - ↑ cholesterol nucleation and crystallization
    - Intestinal factors (EHC), e.g. altered, ↓ luminal bile acid concentration
    - ↓ gallbladder motility ("hypomotility")
    - Genetic factors, including LITH (lithogenic) genes

The lithogenicity of bile is finally determined by the molar % of cholesterol (CH), phospholipids and bile acids. The amount of CH available for transport across the AM of the hepatocyte is determined by a balance between entry of CH across SM, exit across the SM (the sinusoidal membrane), metabolism in hepatocyte cytosol, and exit across the AM (apical membrane).

- A number of observations suggest that there may be **genetic factors** leading to an ↑ risk of gallstones. These include studies in:
  - First Nations group persons
  - Siblings with gallstones
  - Monozygotic twins with gallstones
  - Relatives of index person with gallstones

- Give 5 **genetic abnormalities** associated with the formation of cholesterol gallstones. Consider your understanding of hepatocyte uptake, synthesis, catabolism and secretion of cholesterol.

➢ ↑ hepatic uptake of HDL-C	○ CETP (cholesterol ester trans protein)
➢ ↑intestinal CH absorption a ↑ hepatic VLDL synthesis	○ Apolipoprotein (APO) E and APC genes polymorphisms
➢ 7α-hydroxylase (CYP7A gene variant	○ ↓ conversion of CH (cholesterol) BA (bile acids) in the "neur pathway
➢ ↓ Phospholipid secretion	○ ABC B4 (aka MDR3 [multidr resistant gene 3]) missense mutati of phosphatidylcholine (P transporter in hepatocyte CM ○ ↓ CYP7A1 ○ ↑ HMG-CoA reductase ○ ↓ bile PC (phosphatidylcholine) ○ ↑ bile cholesterol
➢ ↑ CH secretion	○ Variant of ABCG5/-G8 on CM of t hepatocytes
➢ Aberrant splicing of CCK- (cholecystokinin-1 receptor)	○ ↓ gallbladder motility

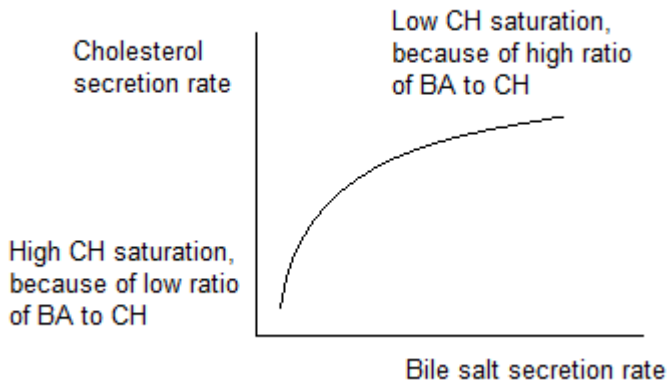
There are over a dozen genes and gene predicts that have been identified (please refer to Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, Table 65.1, page 1103, for a lot of Human Cholesterol Gallstone [LIH] gene and gene Products).

➢ Gallbladder

- Give 5 mechanisms leading to "hypomotility" by which the gallbladc contributes to the formation of gallstones.
  - Fasting period
    - ↑ volume
    - ↑cholesterol absorption ("loss of capacity selective absorption of biliary cholesterol a phospholipids") by GB
    - → ↑ stiff sarcoplasmic membranes
    - → ↓ activation of G-proteins in muscularis propria
    - → ↑ storage of CE
    - → ↓ motility
    - → ↑ inflammation and proliferation

➢ Cholesterol supersaturation and Lithogenicity of bile.

- Give the explanation for the formation of bile saturation with cholesterol whi may occur normally with fasting.



Abbreviations: BA, bile acids; BS-S, bile salt secretion rate; CH, cholesterol; CH cholesterol secretion rate

  - With fasting, the secretion rates of bile salts (BS) and CH (cholesterol) are both low, but there is proportionally more CH (ratio of BA /CH is slow, so there is CH saturation → "sludge" from lithogenic bile)
  - As bile salt secretion rate and flow increase with feeding, there is proportionally more BA than CH ratio of BA / CH is high, and the CH is solubilized.

- Postprandial
  - ↓ binding of CCK to CCK-1 receptors
  - ↓ emptying continued BG bile supersaturation
  - ↑ post-emptying (residual) volume
- Interdigestive
  - ↓ filling
  - ↑ lithogenic hepatic bile enters duodenum
  - ↓ cholesterol solubilisation
- ↑ mucin glycoproteins
  - Binds chol, PL, BA
  - ↑ nucleation factors
  - ↓ antinucleation factors
  - ↑ vesicles fusion / aggregation
  - ↑ mucin in lithogenic bile
- ↓ motility
  - Contractile of gallbladder (GB)
    - ↑ parasympathetic
    - ↓ sympathetic
  - ↑ fasting residual GB volumes
  - ↓ response to CKK
  - Likely important in CF, pancreatic insufficienc
- Sludge
  - Cholesterol monohydrate crystals, (bilirubinate, mucin
    - Radiolucent cholesterol stones
  - Black stones – hypersecretion of bilirul conjugates into bile
    - Hemolysis
    - Cirrhosis
  - Pigmental gallstones are seen in patients w chronic hemolytic anemia, liver disease, and w TPN.

- Give the role of liver and the small intestine in the development of lithogenic bile and cholesterol gallstones.

- Role of the liver in the control of cholesterol homeostasis

  - HMG-CoA reductase controls the rate of synthesis of cholesterol in t hepatocytes.

  - Cholesterol is also taken up across the hepatocyte sinusoidal membrar mixed with synthesized hepatic cholesterol, and exits the hepatocyte acro the canalicular membrane and into the ductules, as part of the EHC.

  - The major means of eliminating cholesterol from the body is by their conversi to bile acids, acting through the activity of 7α-hydroxylase.

  - Other routes of elimination of small amounts of cholesterol from the bo includes
    - Synthesis of steroid hormones
    - Sloughing of skin and intestinal epithelial cells
    - Loss in the bile
    - Loss in the feces.

- Nucleation and crystallization of cholesterol in bile

  - An imbalance of pronucleating and anti-nucleating factors in the gallbladc leads to cholesterol nucleatic and nidation. Cholesterol nucleation a crystallization are initiated in vesicles, so that the stability of vesicl determines their stability in bile.

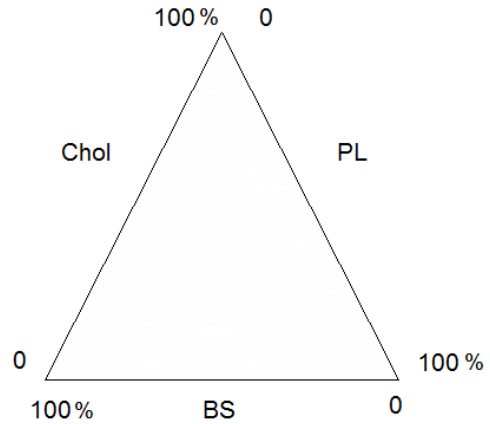
- Give 8 protein and non-protein factors that accelerate the nucleation a crystallization of cholesterol in bile, leading to the supersaturation of choleste and the formation of biliary sludge or cholesterol gallstones.

  - Mucins
    - Hypersecretion of mucins by gallbladder mucosal cells
    - Gel-forming mucins: MUC2, MUC 5AC, MUC 5B
    - Form disulfide-stabilized aggregates of mucin gels which bind lipids, bilirubin, Ca^{2+}
    - Create a scaffolding to hold crystals of cholesterol
    - Mucin gel of glycoproteins and calcium bilirubinate provide surface for nucleation
    - Nucleation proceeds with cholesterol monohydrate crystals pl the mucin matrix

  - ↑ bile lipid > 3 g/dl

- ↑ hydrophobicity of bile salt pool
- ↑ bile salt-to-phospholipid (lecithin) ratio
- ↑ cholesterol-to-lecithin ratio in vesicles
- ↑ Ca^{2+}
- ↑ deoxycholate in bile
- ↑ gallbladder mucin, mucin glycoprotein complexes (nidation)
- ↓ apolipoproteins A I and A II
- ↓unnamed slightly acidic glycoprotein (acidification of bile)
- Imbalance between promoters and inhibitors of nucleation

- Equilibrium phase diagram and triangular co-ordinates are used to determine the solubility of the cholesterol in the bile with measured concentration of BS and PL.



Abbreviations: BS, bile salt; Chol. cholesterol; PL, phospholipids

- The components are expressed as moles percent (%)
- If cholesterol saturation index (CSI) > 1, bile is saturated, and cholesterol can precipitate and form crystals
- The number of phase in the cholesterol (Chol)-, phospholipid (PL, lecithin)-, mixed bile salt (BS) system is shown.
- If cholesterol saturation index (CSI) is > 1, bile is saturated, and cholesterol precipitates and form crystals
- The maximal solubility of cholesterol is determined by the relative molar percentages of cholesterol, phospholipids and bile acids
- When the bile is supersaturated with cholesterol, unstable unilamellar vesicles form.

- Multilamellar vesicles then form from the unstable unilamellar vesicles.
- This allows for the quantification of the degree of saturation of bile with cholesterol
- This quantification of cholesterol saturation is the CSI (cholesterol saturation index), aka the lithogenic index
- Examples of CSI
  - < 1, unsaturated
  - 1, saturated
  - > 1, supersaturated (↑ risk of forming gallstones)

- In the presence of nidation factors, nucleation and crystalization occurs, and over time, gallstones form.
- In normal persons, gallbladder bile may become supersaturated with cholesterol for a short interval during fasting.
- When bile does not reach the intestine because there is for example an obstruction of bile from a gallstone in the common bile duct, no bilirubin reaches the colon, no tetrapyrrroles, urobilinogen or sterobilinogen are formed, and the stools become pale in colour and the urine becomes dark (tea-coloured).

CLINICAL PHYSIOLOGICAL CORRELATION – Cholesterol Gallstones

Case:

- Interruption of the enterohepatic circulation, increased hepatic secretion of cholesterol, or decreased synthesis of bile acids, or impaired gallbladder motility may result in the formation of cholesterol gallstones.
- These may cause blockage of the cystic duct and cholecystitis blockage of the common bile duct, jaundice as well as the pancreatic duct causing acute pancreatitis.

- Give the pathophysiology of the formation of cholesterol gallstones

➢ Liver

- Give the role of the hepatocyte in the metabolism of CH (cholesterol).

Cholesterol handling by the hepatocyte is determined by the following:

- CH uptake across the SM from LDL, HDL
- Chylomicron remnant (CMR) secretion of
  - CH across SM in VLDL back into portal blood

    - As CH in N-HDL (nascent HDL), using ABCA1
  - Metabolism
    - De Novo synthesis of CH from acetate, through HMG-CoA reductase
    - Esterification of CH to CE (cholesterol ester) by way of ACAT
    - 7α-hydroxylation of CH to BA (bile acids) by
      - CYP7A1
      - CYP27A1
  - Transfer of CH across AM by ABCG5/-G8

Abbreviations:
ACAT, acyl-coenzyme A: cholesterol acyltransferase
CM, canalicular membrane of hepatocytes
CMR, chylomicron remnants
CYP27A, 27-hydroxylase ("acidic" pathway)
CYP7A1, cholesterol 7α-hydroxylase ("neutral" pathway)
HDL-L, high-density lipoprotein
HMG CoA, 3-hydroxyl-3-methy glutaryl-coenzyme A
LDL, low-density lipoprotein
SM, sinusoidal membrane of hepatocytes
VLDL, very-low-density lipoprotein

➢ Small Intestine

- Give the role of small intestine to the development of gallstones.

  - Cholesterol
    - ↑ intake
    - ↑ efficiency of absorption
  - Bile acids (↓ EHC)
    - ↓ transit
      - ↑ gram + anaerobic bacteria → ↑ 7 dehydroxylase activity
      - ↑ conversion of 1° cholic acid to deoxycholic acid
      - ↑deoxycholic → ↑ lithogenicity of bile
    - ↓ ileal absorption
    - ↓ hepatic synthesis / secretion of bile acids

Pregnancy and Gallstones

- Give 5 changes in bile acid metabolism which occur during pregnancy, and which increase the risk of cholelithiasis.
  - ↑ cholesterol
  - ↑ supersaturation
  - ↑ bile acid pool
  - ↑ cholic acid
  - ↓ chenic acid (chenodeoxycholic acid)
  - ↑ volume of gallbladder (fasting/ fed states)
  - ↓ gallbladder contraction

- Give the genetic basis for cholestatisis of pregnancy seen in about 15% of cases.
  - About 15% of women with cholestasis of pregnancy have mutatio in the MDR3 (ABCB4) gene (a phospholipid flippase that moves P (phosphatidylcholine) from the inner to the outer surface of t hepatocyte canalicular membrane.
  - More common in women from Chile and Scandinavian countries

- Give the potential role of SAM (S-adenosyl – L- methionine) in the treatment of cholestasis of pregnancy.

SAM may enhance the benefit of UDCA (ursodeoxycholic acid) in cholestasis of pregnancy.

- Give 4 benefits of UDCA in the management of cholestasis of pregnancy.
  - ↓ concentration of bile acids in maternal serum and amniotic fluid
  - ↑ transport of bile acids in placenta
  - ↓ cholestatic potential of progesterone during pregnancy
  - ↓ pruritus
  - ↓ fetal distress, premature delivery, stillbirth

Drugs and Gallstones

Contraceptive estrogens and conjugated estrogens, the lipid-lowering clofibrate, the somatostatin analog octreotide, and the cephalosporin ceftriaxone are drugs which are associated with an increased risk of gallstones.

- Give the mechanisms for each of these four classes of drugs which lead to their increasing the risk of the development of cholelithiasis.

➢ Conjugated estrogens and contraceptive steroids

- Cholesterol synthesis
  - Estrogens activate the hepatic estrogen receptor α
  - The activated hepatic estrogen receptor α stimulate SREBP-responsive genes
  - Activated SREBP-2-responsive genes leads to
    - ↑ cholesterol synthesis and secretion into bile
    - Supersaturation of bile with cholesterol
- Lipoprotein
  - Estrogens cause ↑ expression of hepatic LDL receptor
  - ↑ hepatic LDL receptor leads to ↑ clearance of LDL
  - ↑ clearance of LDL causes
    - ↓ plasma LDL
    - ↑ plasma HDL
    - ↑ liver LDL
  - ↑ uptake of LDL into the liver causes
    - ↑ cholesterol synthesis
    - ↑ secretion into bile
- Smooth muscle
  - Estrogens slow smooth muscle motility, leading to gallbladc stasis

➢ Clofibrate

- Clofibrate cause ↓ activity of 7α-hydroxylase

- ↓ 7α-hydroxylase leads to
  - ↓ synthesis and secretion of bile acids from cholesterol
  - ↓ cholesterol solubility in bile

➢ Octreotide

- Octreotide cause
  - ↓ gallbladder emptying
  - ↓small intestine transit
  - ↑ "dwell" time for cholic acid to be dehydroxylated into the 2 b acid, deoxycholic acid
  - ↑ deoxycholic acid absorption in EHC and return to the liver
  - ↑ deoxycholic acid secretion into bile canaliculus
  - ↓ solubilization of cholic and chenic acids

➢ Ceftriaxone

- When the saturation of ceftriaxone (cef) in bile is exceeded, cef forms insoluble salts with calcium
- Calcium-cef forms biliary sludge → gallstones

Medicine is not a popularity contest.

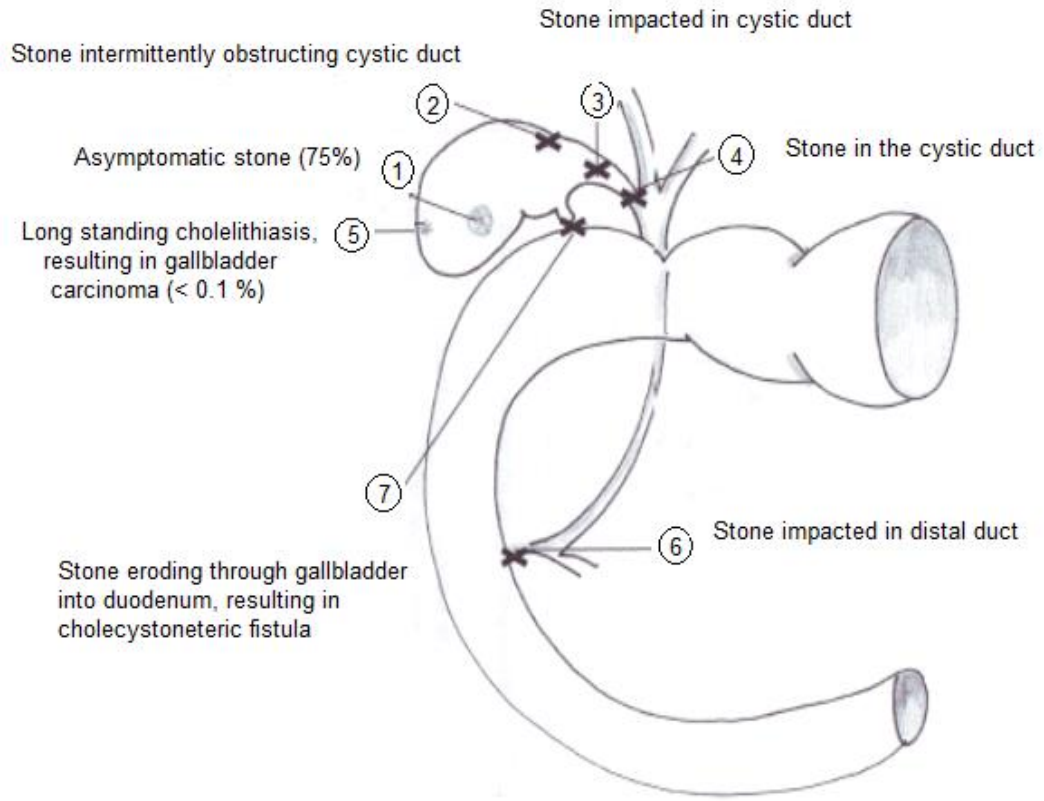
Know the evidence and do the right thing

for the patient

Grandad

Complications of Gallstones

The complications are similar for gallstones formed from cholesterol, bilirubin, or both chemical.



Adapted from: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Figure 62-5, page 1398, Eighth Edition, 2006

Clinical correlations

- Gallbladder and Cystic Duct
  - 1) Asymptomatic stones (75%)
  - 2) Stone intermittently obstructing cystic duct, causing intermittent biliary pain (20%).
  - 3) Stone impacted in cystic duct, causing acute cholecystitis (10%).
  - 4) Stone in cystic duct compressing or fistulizing into the bile, causing Mirizzi's syndrome (< 0.1%).

- 5) Long stainding cholelithiasis, resulting in gallbladder carcinoma (< 0.1%).

➢ Common Bile Duct

- 6) Stone mpacted in distal bile duct, causing jaundice, biliary type pain, and risk of ascending cholangitis or acute biliary pancreatitis (5%).
- 7) Stone eroding through gallbladder into duodenum, resulting in cholecystoenteric fistula (prerequisite for gallstone ileus).
- Stone may erode into stomach to cause gastric outlet obstruction (Bouveret's syndrome).
- Stone impacted in cystic duct causing acute cholecystitis (10%).

Adapted from: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Figure 65-7, page 1106, Ninth Edition, 2010.

> Causes of Biliary obstruction

- Bilirubin from destruction of red cells circulates bound to albumin
- Hepatic conjugation into water - soluble bilirubin glucuronide, formed by glucuronyl transferase
- Excretion into bile
- Gut bacterial action → Glucuronide
- stercobilinogen → stool pigment (excreted)
- Conjugated bilirubin from gut into blood → Urobilinogen
- Urobilinogen excreted in urine

> Causes of Jaundice

- Pre-hepatic; ↑ Hemoglobin destruction (usually due to hemolysis → ↑unconjugated bilirubin in blood)
- Hepatic disease –(e.g., viral hepatitis) interferes with bilirubin uptake; cholestasis reduces conjugation and excretion into common hepatic duct
- Post-hepatic – impaired bilirubin excretion into bile (= cholestasis)
  - Intrahepatic (drug, PBC, viruses)
  - Extrahepatic (biliary stones, cancer, pancreatic disease)

Adapted from: Davey P. Medicine at a glance. Second Edition, *Blackwell Publishing*, Figure 22, page 46, 2002

CLINICAL PHYSIOLOGICAL CHALLENGE - Gallstones

Case: A 28 year old mother of four healthy children develops RUQ pain and tenderness. Acute cholecystitis is diagnosed.

- Give the steps in the metabolism of bilirubin
- Give the pathophysiology of the development of bilirubin gallstones.
- Explain the colour of the urine and stools in persons with obstruction of the common bile duct (CBD)

CLINICAL PHYSIOLOGICAL CHALLENGE

Case: A 45 year old woman with primary biliary cirrhosis (PBC) has hypercholesterolemia and xanthelasma.

- Give the role of the liver in maintaining normal concentrations of cholesterol in the blood.

<u>Useful Quotes</u>

"…… for the average patient, early operation [for acute cholecystitis] is preferable because

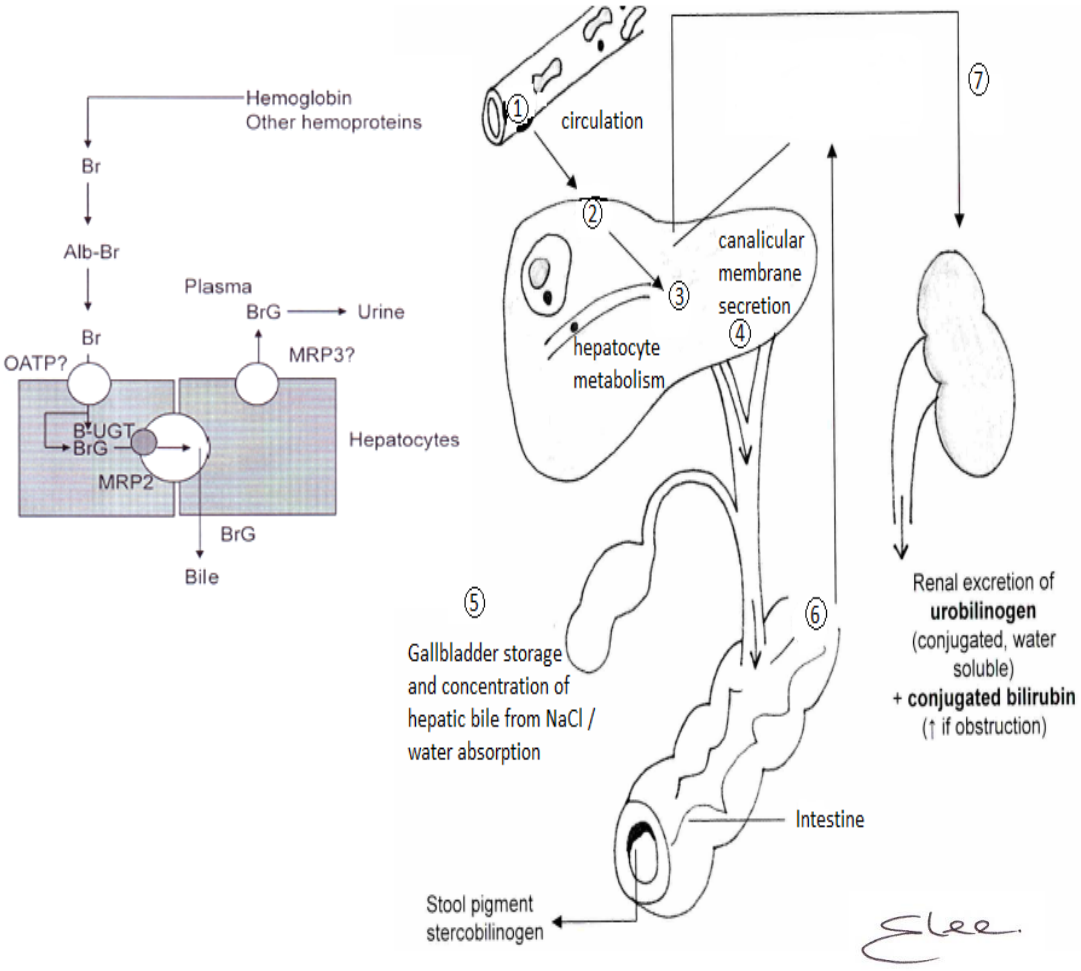
- The total length of hospitalization [is] reduced
- Costs are reduced
- Morbidity is less, and
- Deaths related to progressive acute cholecystectomy are prevented"

Feldman M, et al. Sleisenger and Fordtran's Gastrointestinal and Liver Disease. 9th Edition. *Saunders/Elsevier* 2010, page 1130.

"Always do your best. What you plant now, you will harvest later"

Og Mandino

Bilirubin Metabolism and Jaundice



Adapted from: Davey P. Wiley-Blackwell, 2006, Figure 22, page 46; and from *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* Figure 20-1, Page 324, Ninth Edition, 2010.

- 1) Blood Circulation
  - When RBCs (red blood cells) reach the end of their lifespan of approximately 120 days, these senescent cells are taken up by the macrophages in the hepatic reticuloendothelial system (RES), largely in the spleen.
  - In the RES, the hemoglobin in the RBCs is broken down by heme oxygenase to biliverdin and bilirubin (Br)

- Lipid soluble unconjugated bilirubin (UB) is made water soluble by the binding of UB to albumin (albumin – UB complex).
- The albumin-UB complex passes through the fenestrations in the endothelium of the hepatic sinusoids and into the space of Disse.
- In the space of Disse the UB dissociates from the albumin.

➢ 2) Hepatocyte Sinusoidal Membrane (SM) Uptake

- Electrochemical and electrogenic processes
- A Br-Cl^- - exchanger, possibly, by one of the OATPs.
- Small organic cations (OC+) are transported by OCT1 and OTC2 in the hepatic sinusoidal membrane.
- Large organic cations are transported by OATP-A (organic anion transport protin), and some enter the hepatocytes by way of the H-CA exchanger.

➢ 3) Hepatocyte Metabolism

- The Br in the cytosol of the hepatocyte is in phospholipids vesicles.
- Br in the phospholipid vesicles in the hepatocyte cytosol diffuses into the ER.
- In the cytosol of the hepatocytes, UB binds to glutathione S- transferase.
- UDP – GT (uridine – 5' – diphosphate glucuronyl transferase) in the ER.
  - Binds to UB to prevent its back flux across the SM and into portal blood
  - Conjugates UB to glucuronic acid
- The CB (conjugated bilirubin) is a mono- and diglucuronide

➢ 4) Hepatocyte Canalicular Membrane Secretion

- The CB is transported across the CM (canalicular membrane) of the hepatocyte by ATP-dependent MRP2 (multiple rug resistance-associated protein 2)

➢ 5) Gallbladder Storage and Concentration of Hepatic Bile

- Br in the hepatic bile enters the gallbladder, or trickles through the sphincter of Oddi.

- Upon the release of CCK in the upper intestine in response to fat and protein in the upper portion of the small intestine, the Br is excreted in gallbladder bile by way of the contraction of the wall of the gallbladder

➢ 6) Intestinal Metabolism

- he bacteria in the liver of the distal ileum and colon contain B-glucuronidases bacterial B-glucuronidases hydrolyse CB to UB.
- UB is reduced further by intestinal bacteria to urobilinogen.
- The fate of urobilinogen includes
  - Passed in stool
  - Oxidized to urobilin and passed in urine
  - Absorbed in the distal, ileum and colon, to be re-secreted
    - By liver into bile
    - By renal glomerulus into urine
  - CB is water soluble, and when the bilirubin level is high in serum and bile, not all CB is hydrolyzed to UB.
  - CB tea-coloured urine occurs only in conjugated hyperbilirubinemia urine. Urine turns to colour of tea.
  - In unconjugated hyperbilirubinemia, all UB is bound to albumin and does not enter the urine so the urine is not tea-coloured.
- Some CB is converted by colonic bacteria to urobilinogen (brown sterobilin which gives stool its characteristic brown colour), and is passed into the stools.
- Some CB is converted by colonic bacteria to urobilinogen.
- Sterobilin gives stool its characteristic brown colour); the brown stool is passed from the body.
- Some of the CB is deconjugated back into UB (unconjugated bilirubin) in the intestinal lumen.

➢ 7) Renal Excretion

- Some urobilinogen is absorbed in the small intestine, and converted to the yellow pigment urobilin, which is excreted by the kidney into the urine.

- When bile does not reach the intestine because for example an obstruction of bile from a gallstone in the common bile duct, no bilirubin reaches the colon, no urobilinogen or strobilin are formed, and the stools become pale in colour.

- When there is increased breakdown of red blood cells, or obstruction of some parts of the hepatobiliary tree, bilirubin gallstones may form.

CLINICAL PHYSIOLOGICAL CORRELATION – Bilirubin Metabolism

Case: A 32 year old women develops malaise, abdominal discomfort and jaundice.

- Draw a picture of bilirubin metabolism to distinguish between prehepatic, hepatic and posthepatic causes of jaundice.

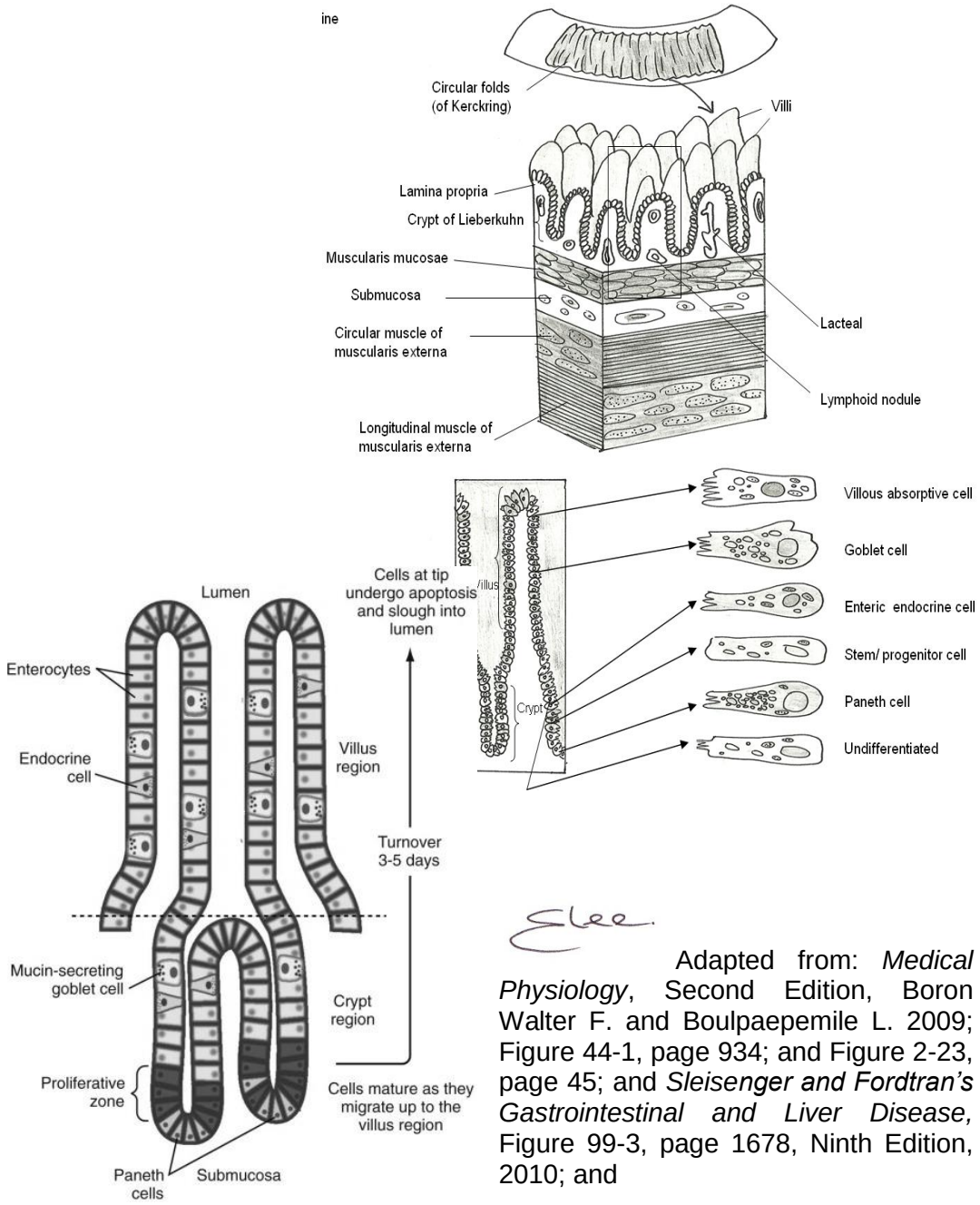
- Give the pathophysiology of the development of hyperbilirubinemia and jaundice.

"The darkest places in hell are reserved for those who maintain their neutrality in times of moral crisis"

Dante Alighieri

SMALL INTESTINE

MICROSCOPIC VIEW OF THE WALL OF THE SMALL INTESTINE



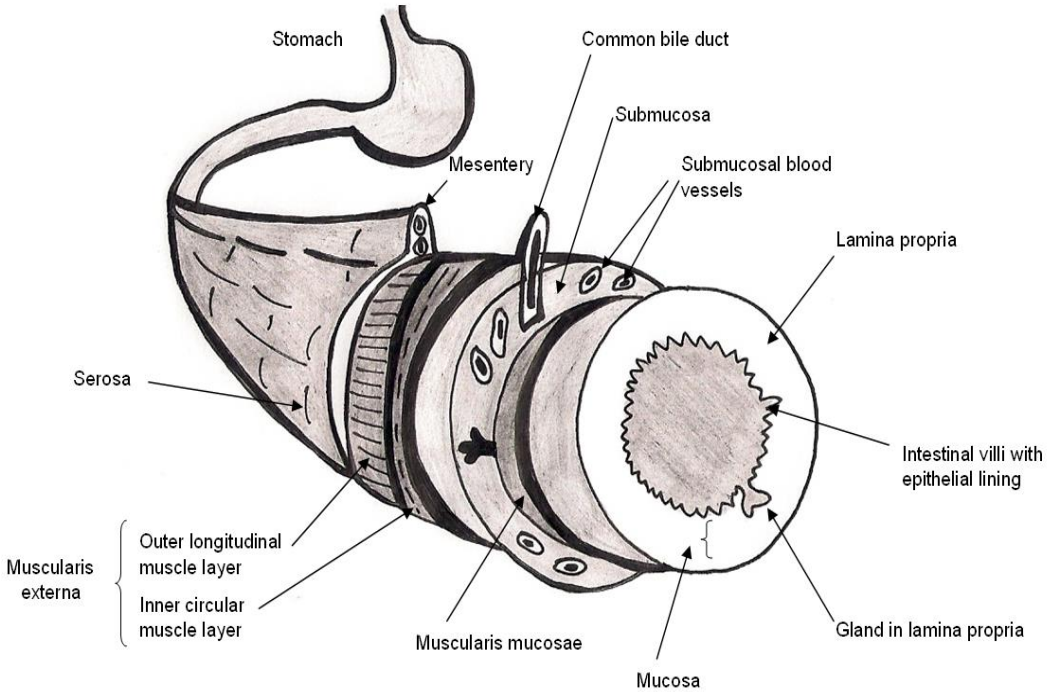
Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Figure 44-1, page 934; and Figure 2-23, page 45; and *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Figure 99-3, page 1678, Ninth Edition, 2010; and *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Figure 99-3, page 1678; and Figure 99-2, page 1676, Ninth Edition, 2010

The Types of Epithelial Cells of the Intestinal Mucosa: Enterocytes

Structure

- The main functions of the small and large intestine are the absorption of nutrients, electrolytes and water, as well as the secretion of water and electrolytes.
- The colon is about 5 feet long, and the small bowel is about 20 feet long.
- The mucosal surface area of the small and large intestine is increase macroscopically by the folds of Kerckring (valuvlae connivente) in the small intestine, and by the semilunar folds (Haustral folds) in the colon.
- The surface area of the absorptive cells at both sites is enlarged by the microvilli and by the crypts.

MACROSCOPIC VIEW OF THE WALL OF THE SMALL INTESTINE



Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009;, Figure 41-2, page 885.

- Only the small intestine has a crypt-villus unit.
- Both the small and large intestine have five cell types in common:
  - absorptive cell (villus, in the small intestine, and surface cell in the colon),
  - goblet cell,
  - enteric endocrine cell (enterochromatin-like cell; ECL),
  - stem/progenitor cell,
  - undifferentiated crypt cell.
- Only the small intestine has Paneth cells at the base of the crypts.
- Paneth cells remain at the base of the crypt and make defensins, which are important in host defense against pathogen in the intestinal lumen.
- All of these cell types originate from the proliferative zone near the base of the intestinal crypt.
- The small intestine has relatively more villous epithelial cells, and the colon have relatively more goblet cells.
- Because of the folds, villi, crypts, and microvilli, the luminal surface areas of the small and large intestine are greatly increase to approximately 200 m^2 (said to be the approximate size of a tennis court) and 25 m^2 (about the size of four ping pong tabbles), respectively.
- The other cells migrate up the villus axis, mature during this process, and eventually undergo apoptosis and slough after three to five days at the tip of the villus.
- Absorption occurs predominantly in the small intestinal villous cells (enterocytes), and in the colonic surface absorptive cells (colonocytes).
- There is a spatial geometry of transport proteins along the crypt-villus axis of the small intestine, as well as along the small intestine from jejunum to ileum.
- In addition to the crypt-villus surface heterogeneity, there is segmental heterogeneity along the length of the intestine:
  - the proximal intestine absorbs Fe^{+2}, Ca^{+2}, as well as
  - most dietary carbohydrates, lipids, amino acids, and
  - non-electrogenic and solute-coupled Na^{+} -absorption.

- The ileum absorbs
  - Vitamin B_{12}
  - Bile acids,
  - Is a reserve site for the absorption of nutrients not absorbed by the jejunum.
- Intestinal epithelial cells are structurally and functionally geared for vectorial transport.
- Some transport molecules are distributed at relatively constant concentrations along the axis,
  - Some exhibit a greater density in the base of the crypt.
  - Some exhibit a greater density towards the tip of the villus or surface.
- Only nonpolar (lipophillic) solutes freely cross a lipid membrane domain by simple diffusion.
- The transfer of ions and charged molecules necessitates specific transmembrane proteins to modulate entry into and exit from the cell.
- Ion-specific channels mediate membrane transport by facilitated diffusion.
- Carriers in the membrane undergo a conformational change.
- Active transport occurs against an electrochemical gradient.
- Active transport can be driven by adenosine triphosphate (ATP) (primary active transport, E1), or an ionic gradient (secondary active transport, E2).
- The Na^+ pump on the BM maintains an electrochemical profile.

Small Intestinal Motility

- Functions
  - Mixing (churning), storage (reservoir), propulsion (peristalsis)
  - Preceding waves of relaxation followed by contraction of smooth muscle (“the law of the intestine”).
- The submucosal (or Meissner's) plexus is located in the submucosal between the muscularis mucosae and the circular muscle of the muscularis externa.
- The myenteric (or Auerbach's) plexus is located between the circular and longitudinal layers of the muscularis externa.

- Meissner's and Auerbach's plexuses have ganglia.

- Three other plexuses are also present (in addition to Meissner's and Anerbach plexus): mucosal, deep muscular, and tertiary plexus

➢ Sensory afferent neurons

Spinal afferents (pain) → perivascular nerves → prevertebral ganglia → postganglionic sympathetic motor neurons → splanchnic nerves thoracic dorsal postganglia → dorsal roots → thoracic spinal nerves

- Mucosal "sensing" of nutrients provides feedback control on both gastric acid and small intestinal motor function.

- Mucosal afferents respond to absorbed nutrients, as well as the transmitters released from both the epithelial enterochromaffin-like (ECL) cells mechanical deformation of the mucosa, and from immunocompetent cells.
- Serosal afferents respond to distortion of the mesentery or serosa.

- Muscular afferents have low thresholds to respond to contraction or distension.

- The intramuscular arrays (IMAs) are nerve terminals in the longitudinal and circular muscle layers, and act as tension receptors.

- The intraganglionic laminar endings (IGLEs) are in the vagal afferent terminals in the myenteric plexus.

- Single IGLEs may have mechanosensitivity, whereas multiple IGLEs detect shearing forces between the circular and longitudinal muscles.

➢ Autonomic nervous system

- Parasympathetic

  - The parasympathetic supply to the intestine is cholinergic, cranial and stimulatory to smooth muscle in the intestine.

  - Vagal afferents are involved in physiological regulation.

  - The cell bodies of the parasympathetic supply are in the dorsal motor nuclei of the vagus.

- Sympathetic

- The sympathetic supply is adrenergic, and inhibitory to smooth muscle in the intestine.

- The cell bodies of the sympathetic supply are inhibitory except at the sphincters where the sympathetic supply is stimulatory.

- The primary sympathetic motor neurons are in the intermediate horn of the synapse, and the second order neurons are in the prevertebral ganglia.

- These synapse with ENS motor neurons in the intramural plexus.

- There is likely some connection between the vagal/parasympathetic and the spinal/sympathetic control systems.

➢ Enteric Nervous System (ENS) and Intestinal Cells of Cajal (ICC)

  - The ganglia of the ENS are clusters of cell bodies which are in the submucosal or myenteric plexus.

  - The interganglion fascicles (axons of motor neurons and interneurons) connect the ganglia of the submucosal and myenteric plexuses.

  - The myenteric plexus itself has three networks: the primary, secondary and tertiary plexus.

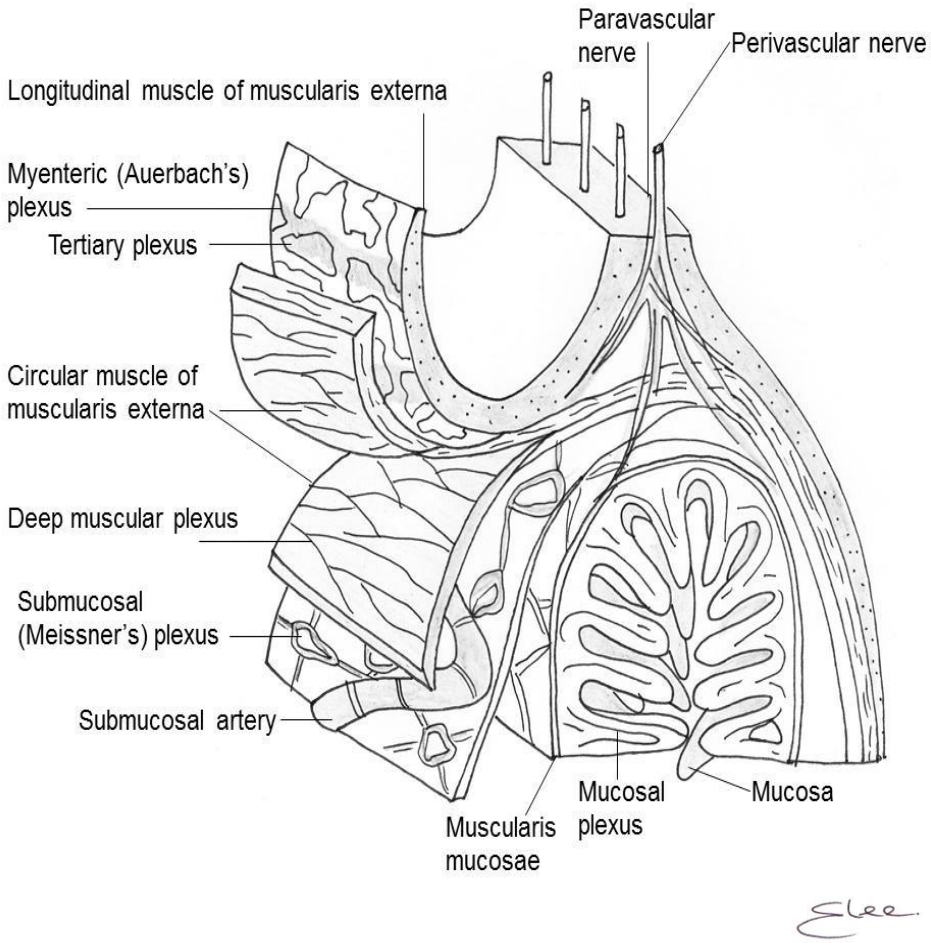
  - The afferent neurons in the myenteric plexus respond to chemical and mechanical stimulation of the muscle layer.

  - Spinal afferents mediate pain.

  - The spinal afferents are close to the perivascular nerves, and pass to the prevertebral ganglia, where they synapse on postganglionic sympathetic motor neurons.

  - At the prevertebral ganglia, the perivascular nerves in turn pass along the splanchic nerves in the thoracic spinal cord.

ENTERIC NERVOUS SYSTEM (ENS) OF THE SMALL INTESTINE



- Because of the extensive afferent interneuronal connections and constant sensory feedback, the ENS may control long segments of the intestine without any extrinsic input.
- The efferent output from the ANS (autonomic nervous system) may modulate the ENS.
- Enteric motor neurons
  - Excitatory
    - M2, M3 acetylcholine receptors
    - SP (substance P) receptors for NK1
  - Inhibitory
    - NO (nitric oxide), and

- VIP (vasoactive intestinal peptide) activate receptor on non-receptor mechanisms to
  - Open K^+ channels
  - Class Ca^{2+} channels
  - Cause relaxation

- Neurotransmitters
  - Nitric oxide (NO), synthesized from arginine by nitric oxide synthase (NOS), diffuses across the plasma membrane into the smooth muscle cells
  - NO binds to and activates guanylyl cyclase, which converts GTP to cGMP
  - cGMP causes smooth muscle relaxation.
  - The main excitatory neurotransmitters for small intestinal motility are
    - Excitatory acetylcholine (Ach; fast)
    - Substance P (SP; slow)
    - NO (fast)
    - ATP (fast)
    - VIP (slow inhibitory)

- Interstitial cells of Cajal
  - The plexuses contain the intestinal cells of Cajal (ICC), the electrical pacemaker system of the intestinal muscle cells.
  - The ICCs in the myenteric plexus (ICC_{MY}) between the inner circular and outer longitudinal muscles act as pacemakers.
  - Electrical slow waves are generated, and spread through gap junctions to the ICCs within muscle (ICC_{IM}) as well as in the deep muscular plexus (ICC_{DMP}).
  - The conduction is from nerve to ICC, and then through the electrical gap junctions to the myocytes.
  - Function of ICC
    - Pacemakers
    - Transductions of excitatory and inhibitory neural signals to myocytes
  - There are three groups of ICCs in the small intestine

- ICC – DMP (deep muscle plexus)
- ICC – MY (myenteric plexus; pacemaker cells)
- ICC – IM (intramuscular plexus)

- ICC – MY
  - Produce slow waves in myocytes → activity of other ICCS is entrained
  - Propagation of slow waves through gap junctions of myocytes
- Nerves from ENS (enteric nervous system)
  - Target ICC – MY, with response to myocytes
- Traditionally, the ICCs have been thought to generate the electrical slow wave and to transmit to the myocytes the neural inhibitory or excitatory signals.
- The intestinal cells of Cajal (ICC) lie close to the myocytes and to the nerve axons forming electrical gap junctions.
- When the actin (thin filaments) and myosin (thick filaments) in the myocytes contract, the smooth muscle cell shortens.
- The overall mechanical connection between large groups of myocytes is provided by a connective tissue stroma.
- The two patterns of intestinal motility include peristalsis, and the interdigestive motor cycle (IDMC).
- The IDMC produce the MMCs during the postprandial interval.
- Clinically muscle contractions may be assessed from changes in the pressure in the intestinal lumen.
- Wall motion of the intestine may be assessed by ultrasound, MRI, or by multichannel intraluminal impedance (MII) measurements.
- Small intestinal transit may be assessed by breath tests, drug pharmacokinetics, and by scintigraphic studies.

➢ Myocyte

- Excitatory motor neurons release acetylcholine (Ach), which acts on M2 and M3 cholinergic receptors to produce an inward current.
- This current depolarises and opens the L-type Ca^{2+} channels.

- NO and VIP from inhibitory neurons activate receptor as well as non-receptor mechanisms in the ICC_{IM}, opening K^+ channels and closing Ca^{2+} channels.
- This opening of K^+ and closing of Ca^{2+} channels counters the excitatory effect of Ach on muscle contraction.
- Smooth muscle cells within each muscle layer (inner circular layer encircling the lumen, outer longitudinal layer extending axially, and the muscularis mucosae) form a syncytium.
- In the syncytium, each myocyte is connected electronically with each other through electrical gap junctions.
- The mechanical connection between myocytes is provided by the intermediate junctions.
- Smooth muscle contractions reduce the diameter and/or shorten the length of the small intestine, thereby causing propulsion of the contents in the lumen.
- Actin and myosin contractile filaments are attached to dense bands within the myocytes
- There is a need for the Ca_i^{2+} (intracellular calcium) to increase in order for the myocyte to shorten
- This Ca_i^{2+} comes from
  - L-type Ca^{2+} channels
  - Release of Ca^{2+} from sarcoplasmic reticulum membrane via IP3 receptor-operated Ca^{2+} channels
- Ca^{2+} binds to calmodulin
- Calmodulin activates myosin light chain kinase
- Myosin light chain kinase phosphorylates MLC20 (20 kDa light chain of myosin)
- MLC20 has three functions to facilitate
  - Actin binding to myosin
  - Cross-bridge cycling
  - Development of mechanical force
- MLC phosphatase reduces the phosphorylate of MLC20

- Reduced phosphorylation of MLC20 reduces MLC20-associated functions, noted above, and thereby causes muscle relaxation (Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, page 1660)

- Small intestinal motility

  - Motor function in the small intestine provides time for mixing, digestion and absorption, as well as for the movement of chyme along the small intestine.

  - This movement provides exposure of the chyme to the generous surface area of the intestine, thereby allowing for ~80% to 90% absorption of nutrients and electrolytes and water.

  - Non-propulsive mixing (churning) in the antroduodenal cluster unit and small intestine arise from contraction of the inner circular smooth muscle.

  - This churning facilitates digestion and absorption after the intake of food and fluid.

  - After the intake of food (fed state), vagal stimulation and food itself (through myogenic, neurogenic and hormonal factors) stimulate peristalsis (propulsion).

  - The peristaltic wave arises from proximal inner circular muscle contraction and outer longitudinal muscle relaxation, in conjugation with more distal relaxation from circular muscle relaxation and longitudinal muscle contraction.

  - Rhythmic contractions of the small intestinal electrical and motor activity form the migrating motor complex (MMC).

  - There are four phases of MMC: I) quiet; II) increasing electrical and motor activity; III) peak electrical and motor activity and IV) decreasing electrical and motor activity.

  - MMCs occur every 90-120 minutes, and stop when a person eats.

  - 15% of MMCs arise from the stomach, and the remainder begin in the proximal small intestine.

  - During fasting, the stomach is cleared of particles bigger than 2 mm, and the small intestine is cleared during the fasting interval of MMC activity.

- The prokinetic (peristalsis enhancing) duodenal mucosal hormone motilin is released just before MMC phase III.

- Give the pattern of small and colonic motility which occur with feeding and with fasting.
  - Feeding pattern
    - "continuous low, varying-amplitude, ungrouped phas contractions, the activity of which depends on the quantity a composition of the ingested food" (Feldman M., et *Sleisenger and Fordtran's Gastrointestinal and Liver Diseas* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, pa 2121).
  - Fasting pattern
    - Four phases of the MMC (migrating motor complex) whi occurs every 1 to 2 hours between meals, and continues push food and fluid distally

I no contractions, but smooth muscle oscillations

II smooth muscle contractions, intermittent

III smooth muscle contractions, continuous, 11 / min proximally, a pushing luminal contents distally

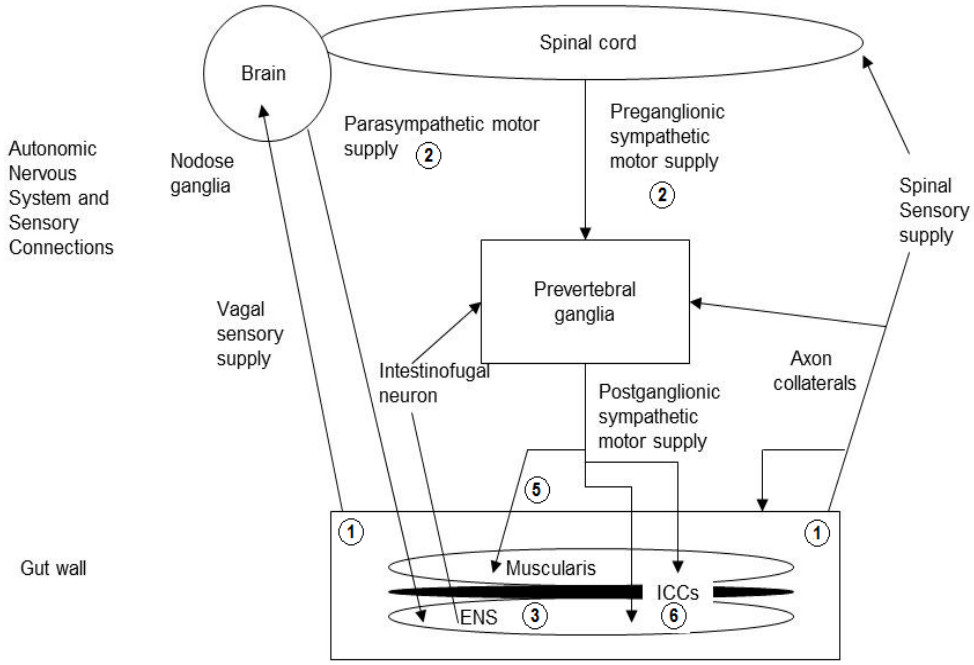
IV no contractions

➢ Control

"We are inherently critical as scientists, and inherently kind as physicians."

Grandad

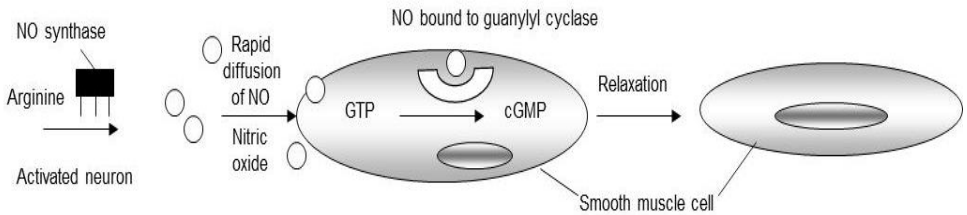
SMALL INTESTINE MOTOR CONTROL SYSTEM



Abbreviations: CNS, central nervous system; ENS, enteric nervousv system; ICC, intestinal cells of Cajal

Adapted from: *Sleisenger and Fordtran's Gastrointestinal and Liver Disea*s Figure 97.3, page 1646, Ninth Edition, 2010.

- Smooth muscle relaxation signaled by nitric oxide (NO)



Abbreviations: NO, nitric oxide

Adapted from: *Sleisenger and Fordtran's Gastrointestinal and Liver Disea*s Figure 1.6, page 12, Ninth Edition, 2010

SO YOU WANT TO BE A GASTROENTEROLOGIST!

- Give the physiological explanation for “the law of the intestine”.
    - Stimulus in the lumen of the intestine acts on the enterochrommaffin cells to release 5-HT
    - $5\text{-}HT_{1P}$ and $5\text{-}HT_3$ act on IPANs (intrinsic primary afferent neurons) in the submucosal plexus
    - IPANs release $5\text{-}HT_4$ which act on the interneurons in the myenteric plexus
    - These interneurons contain receptors for opiod, SS (somatostatin) and GABA γ-amino butyric acid)
    - The portion of the intestine just proximal to the stimulus releases interneuron Ach (acetylcholine) and SP (substance P)
    - The portion of the intestine just distal to the stimulus releases interneuron VIP (vasoactive intestinal peptide) and NO (nitric oxide)
    - The net result is the “law the intestine”: proximal contraction, distal relaxation

- Sphincters
    - Examples include
        - The upper and lower esophageal sphincter [UES, LES]
        - Pylorus
        - Sphincter of Oddi
        - Ileocecal sphincter
        - Internal anal sphincter (the external anal sphincter is comprised of skeletal muscle, not smooth muscle).

- There are sustained tonic contractions, as well as rhythmic alternating contractions and relaxations.

- There is normally tonic contraction of circular and longitudinal smooth muscle of the small intesine, with preceding relaxation stimulated from proximal food bolus or neurohormonal factors, and coordinated with proximal and distal contractions.

- Relaxations are due to membrane potential of smooth muscle (Vm) being low, and creating slow wave activity.

- When Vm exceeds the threshold to produce an action potential, there is a muscular contraction.

➢ Neurons

- The ENS is comprised of neurons with cell bodies in the wall of the intestine. The ENS consists of sensory neurons, interneurons, and motor neurons. Some sensory signals travel centrally from the ENS.

- Both the parasympathetic and the sympathetic divisions of the autonomic nervous system (ANS) modulate the ENS.

- The motor neurons of the ENS connect to the autonomic nervous system (ANS) which in turn connects to the central nervous system (CNS).

- Small intestinal sensory neurons pass through spinal or vagal routes to the CNS. More than 80% of the vagal fibers are efferent (motor).

- The smooth muscle acts as a syncytium, rather than as discrete motor units.
- Intrinsic
  - Afferent
    - Submucosal plexus – mucosal and submucosal plexus, also joined by interganglionic fascicles
    - Myenteric plexus – primary, secondary, tertiary plexus
    - Deep plexus – neurons responding to mechanical stimulation and mucosal chemical stimulation
  - Efferent
    - Excitatory transmitters
      - Fast Acetylcholine
      - Slow Substance P
    - Inhibitory
      - Fast VIP
      - B-NAD (B-nicotinamide adenine dinucleotide)
- Extrinsic
  - Afferent

- Give 8 pathophysiological causes of **postoperative ileus**.

➢ Neural pathways and neural inhibitors
  - ↑ sympathetic inhibitory activity
  - ↓ cholinergic activity (possibly due to ↑ VIP, ↑ neuropeptide Y, ↑ NO CRF (corticotropin-releasing factor)
  - Reflex motor inhibition from splanchnic afferents

➢ Inflammation
  - Handing bowel
    - ↑ activity of iNOS (inducible nitric oxide synthase) and ↑ CO (cyclooxygenase)-2
    - ↑ release of submucosal mast cells, monocytes, neutrophils
    - ↑ proinflammatory cytokines (IL-1β, IL-6, TNF-α)
    - ↑ MCP-1 (monocyte chemotactic protein-1)
    - ↑ ICAM-1 (intercellular adhesion molecule-1)
    - ↑ activity of α2- adrenoceptors

➢ Fluid overload

➢ Drugs
  - Anaesthetics (epidural, versus general)
  - Opioids

- On the basis of small intestinal manometric changes, indicate which of the following clinical conditions is caused predominantly by myopathic or neuropathic changes:

➢ Myopathic
  - DMD
  - DMPM
  - MD
  - Scleoderma

➢ Neuropathic
  - Parkinson disease
  - Spinal cord injury
  - Neurofibromatosis
  - IMG

Abbreviations: DMD, Duchenne muscular dystrophy; DMPM, dermatomyositis / polymyositis; IMG, idiopathic myenteric ganglionitis; MD, myotonia dystrophy

A patient is diagnosed with pseudo-obstruction of the small intestine. Small intestinal manometry is performed to establish whether the condition is primarily myopathic, neuropathic or mixed.

The study showed –

- Giant, propagated as well as non-propagated contractions
- Contractions lasting > 10 seconds, as well as occasional short (< 5 seconds) clusters of contractions with no following contractions for 1 minute.

- Give the most likely cause of the intestinal obstruction.
  - This motility pattern is characteristic of a mechanical obstruction.

(Let's hope that this is never the way in which you make the diagnosis of a mechanical small bowel obstruction!)

Small Intestinal Bacterial Overgrowth

- Give the usual bacterial presence (10^x/ml) of different sites along the gastrointestinal tract.
  - Stomach – 10^3/mL
  - Jejunum – 10^4/mL
  - Ileum – 10^{6-8}/mL (gram-positive)
  - Colon – 10^{10-12}/mL (gram-negative, anaerobic, facultative aerobic)

*note that > 10^5/mL in the proximal small intestine is considered to be abnormal, and is compatible with the diagnosis of SBBO.

- Secretory IgA is the predominant immunoglobulin found in intestinal secretions.

➢ List two functions of Secretory IgA.
  - Binds bacteria and dietary antigens (thus limiting absorption/immune response)
  - Phagocytosis

- What immunologic characteristic makes this immunoglobulin ideal for its function in the GI tract?
  - Resistant to digestion
  - Secreted
  - Does not activate complement cascade
  - Does not participate in antibody-dependent cytotoxicity.

- GI mucosal barrier

- Give 8 components of the GI mucosal barrier, and for each give their function to protect against enteric infection.

Components	Function
○ Epithelium: glycocalyx, villi	- Innate immune response - Antigen presentation - Block penetration of ingested antigens (tig junctions)
○ Defensins	- Antimicrobial peptides
○ Trefoil factors	- Protection from a variety of deleterious agents (bacterial toxins, chemicals and drugs); provide restitution after mucosal injury
○ Mucus/mucins	- Block penetration of ingested antigens
○ Proteases: pepsins, pancreatic enzymes	- Breakdown of ingested antigens
○ Gastric acid (pH)	- Breakdown of ingested antigens
○ Bile acids	- Breakdown of ingested antigens
○ Intestinal peristalsis	- Block penetration of ingested antigens
○ Secretory-IgA (s-IgA)	- Binds bacteria and dietary antigens - Phagocytosis
○ Indigenous microflora (microbiotica)	- Competitive inhibition - *Direct:* competition for essential nutrients and bacterial receptor sites; creation of restrictive physiological environments; secretion of

	antibiotic-like substances - *Indirect:* chemical modification of bile salts and dietary fats, induction of protective Ig responses, stimulation of peristalsis
○ GALT-associated IgA, IgG^a, IgM^a (serum)	- Clear antigens penetrating gastrointestinal barrier/systemic immunity - Assist in opsonization and phagocytosis of antigens
○ Lymphoid follicles in lamina propria	- Clear antigens penetrating gastrointestinal barrier
○ Intraepithelial lymphocytes (IEL)	- Innate and acquired immune responses
○ Mesenteric lymph nodes	- Phagocytosis and antigen presentation

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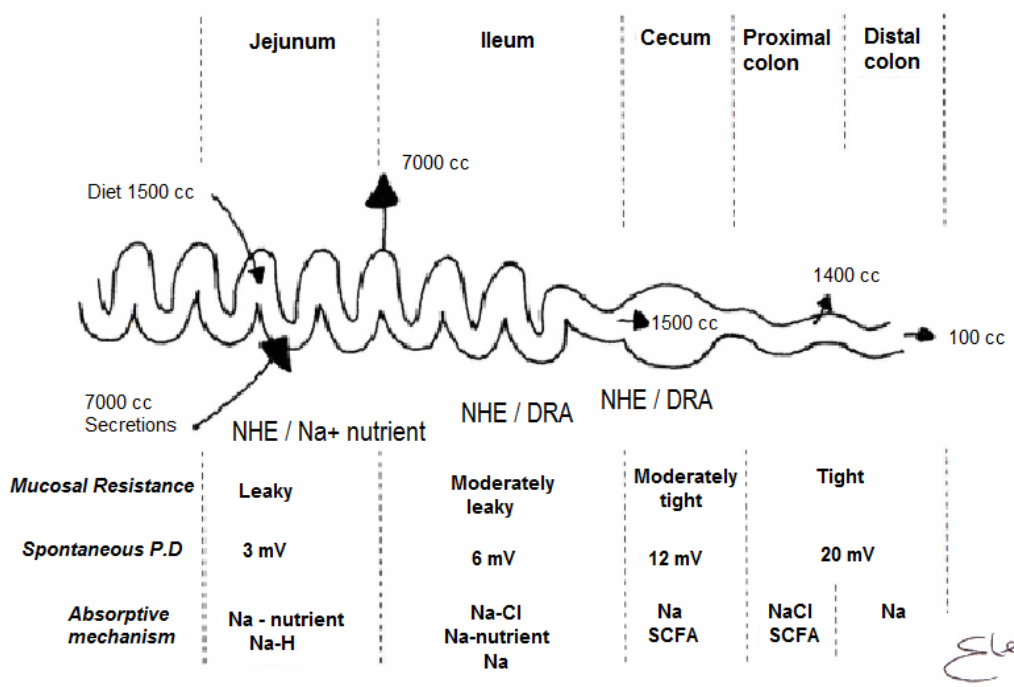
Absorption of Water

- Give the physiology of the intestinal absorption of water.

Intestinal Fluid Balance

- Water absorption is linked to the transport of sodium (Na^+) and nutrients, as well as to the permeability of the epithelial surface, as influenced by tight junctions (TJs).
- 8 to 9 litres of fluid flow through the small intestine each day.
- Salivary, gastric, biliary, pancreatic, and intestinal secretions make up the bulk of this amount, with about 1.5 L arising from oral intake.
- Most intestinal fluid is absorbed in the small bowel, with approximately 1000 to 1500 ml of fluid crossing the ileocecal valve.
- The colon extracts most of this fluid, leaving 100 to 200 ml of unabsorbed water daily in the stool each day.
- The small intestine receives about 8.5 L of fluid per day from the following sources: food, 1.5-2.0 L; saliva, 1.5 L, stomach, 1.5-2.0 L, pancreas, 1.0-1.5 L, bile, 0.5 L, small intestinal secretion, 1.0 L.



Adapted from *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009, Figure 44-2, page 936; and *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Figure 99-1, page 1678, Ninth Edition, 2010

- Of this approximately 8.0 L/day, 1.5-2.0 L enters the colon, thanks to the small intestinal fluid reabsorptve efficiency of about 75%.
- Of the 1.5- 2.0 L/day entering the colon, only about 0.1-0.2 L/day escapes absorption and is passed in the stool, giving an absorptive efficiency of 90%-95%.
- This means that the average daily stool output is only about 200 ml fluid and 50 g of food debris, desquamated mucosal cells, and bacteria.
- A diseased process in the small bowel is likely to result in a large volume of diarrhea ("big gushes"), whereas a disease process in the colon is more likely to result in a small volume of stool ("little squirts").
- Fluid and electrolyte secretion occur largely in the crypts of Lieberkuhn in the crypt-villus unit, and in the colonic crypts.
- The absorptive mechanisms in each segment of the gut differ, whereas chloride (Cl^-) secretion is found throughout the intestine.

SO YOU WANT TO BE A GASTROENTEROLOGIST!

- Give the "standing-gradient hypothesis in the context of understanding how a glass of water is absorbed.
    - The movement of solutes such as Na^+, glucose and amino acids by the enterocyte BBM (brush border membrane) produce small increases in the osmolarity in the intercellular and subepithelial spaces, causing water to move across the BBM (such as with glucose transport by SGLT1), as well as through TJs (tight junctions).

The movement of Na^+ and H_2O with glucose, as mediated by BBM SGLT1, is the basis of the benefit of ORS (oral rehydration solutions).

- Give the amount of Na^+ and H_2O which moves through this water channel.
    - For each molecule of glucose transported by SGLT1, there is cotransport of 2 molecules of Na^+ and 210 molecules of H_2O.

Water Absorption

- Overview
    - Water transport may be paracellular (through tight junctions), or transcellular.
    - The intestinal BBM (brush border membrane) of the villi of the enterocytes absorb solutes such as glucose, and water by transcellular aquaporins in the membrane, and by paracellular movement across TJs.
    - Transcellular transport of water molecules occurs via channel proteins (aquaporins) or carrier proteins.
    - For example, the Na^+ - dependent sugar transporter in the intestinal brush border (apical) membrane (AM) by SGLT1 co-transports H_2O.

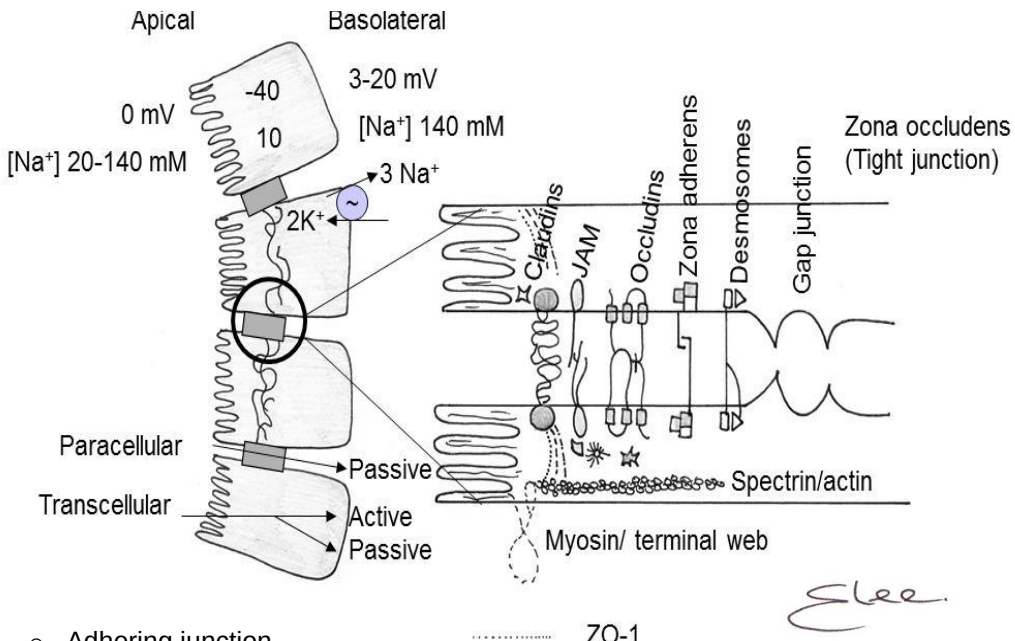
- **Paracellular transport of water (H_2O)**
    - The transport nutrients and electrolytes across the BBM and then across the BLM (basolateral membrane) produce the osmotic and electrochemical gradients to provide for the passive absorption of H_2O.
    - The electrochemical gradient is achieved by the BLM Na^+ / K^+ ATPase.

- The paracellular pathway is characterized by a series of structures which are defined by specific molecular distributions components of the tight junctions.
- In the enterocyte the tight junctions (TJs) separate the cell membrane into the BBM and BLM.
- This leads to an asymmetrical distribution of transporters.
- These two membrane domains have very different functional properties.
- The tight junction (aka zona occludens [ZO]) is a network of strands and grooves that consist of membrane proteins (e.g., occludins, claudins, and junctional adhesion molecules [JAMs]).
- These protein membranes attach to a group of scaffolding proteins (zonula occludens proteins [ZO-1, ZO-2, ZO-3] as well as multi-PDZ domain protein-1 [MUPP1]).
- These scaffolding proteins are linked to the cytoskeleton, which participate in vesicular transport (via monomeric guanosine triphosphatase [GTPase] of the Ras superfamily (Rab3b).
- The scaffolding proteins also participate and in the activation of signaling molecules that regulate junction assembly (partition-defective protein, PAR-3 and -6, and atypical protein kinase C, [aPKC]).
- As one progresses down the intestine, the junctions between cells become progressively ‘tighter’, so that their permeability to water becomes lower.
- When the intestinal villus enterocytes absorb solutes such as glucose, water is also absorbed by transcellular aquaporins and by paracellular processes (across TJs).

“Everybody is a genius. But if you judge a fish by its ability to climb a tree, it will live its whole life believing that it is stupid”

Albert Einstein

Architecture of intestinal epithelial junctions



- Adhering junction
  - Cadherins
  - Actin filaments
- Gap junction
  - Connexons
- Tight junction (TJ)
  - Groove
  - Ridge
  - Claudins (transmembrane proteins)
- Desmosomes
  - Cadherons
  - Plaque

Printed with permission: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Figure 99-2, page 1676, Ninth Edition, 2010.

- Cadherins span the paracellular pathway across the zona adherens (ZA).
- Cadherins are responsible for cell-cell attachment and maintenance of cell polarity.
- Desmosomes are cadherin-like molecules that are linked to intermediate filaments.
- Molecules associated with the zona adherens (including rab, SRC, and yes) are involved in intracellular signaling through second messengers.

- Cadherins bind to catenins, which are linked to the actin cytoskeleton by way of an additional family of molecules, including radixin, vinculin and α-actinin.
- Gap junctions are an assembly of membrane spanning proteins called connexins.
- Connexins allow the exchange of small molecules between neighboring enterocytes.
- When solute exits across the BM of the enterocyte and into the restricted basal space of the lateral intercellular spaces, the fluid in the interstitial space becomes slightly hypertonic.
- These osmotic gradients are sufficient to draw water from the intestinal lumen across the TJs, or through water pores in the AM and BM.
- The TJs are a network of strands and grooves (occludins) attached to a group of membrane proteins, which are then linked to the cytoskeleton of the enterocyte.

➢ Cotransport of Na^+

- Na^+transport plays a major role in the transcellular H_2O absorption.
- The negative cytoplasmic potential (~ -40 mV) acts as a driving force for Na^+ entry across the BBM.
  - Na^+ / nutrient- coupled transporters in the jejunum and ileum,
  - Na^+ / H^+ exchangers (NHE) in the jejunum and ileum,
  - Cl^- / HCO_3^- exchangers in the ileum and proximal colon (DRA),
  - ENaC (epithelia/ Na^+ channel) in the distal colon.

"Don't judge each day by the harvest you reap but by the seeds you plant"

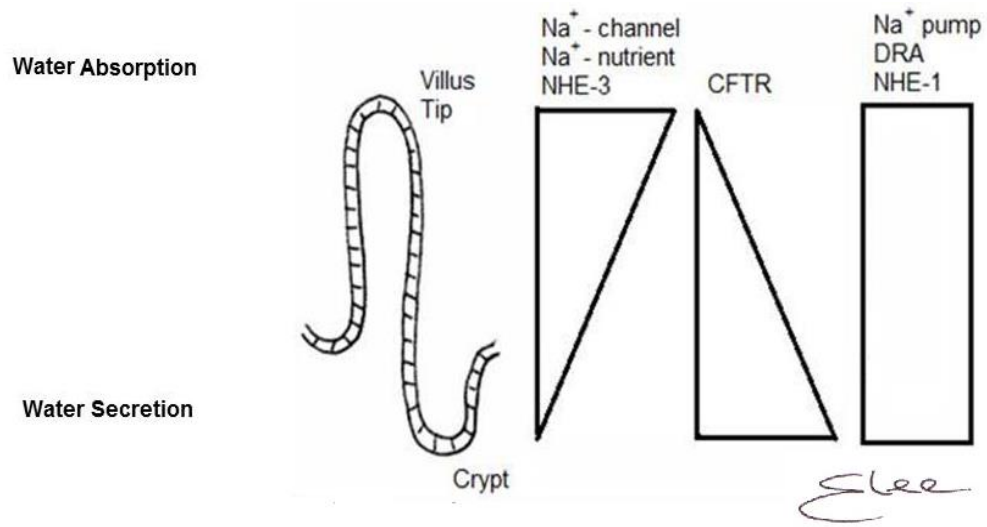
Robert Louis Stevenson

CLINICAL PHYSIOLOGICAL CHALLENGE - Diarrhea

Case: A 25 year old woman returns from a holiday in a Central American Country, complaining of watery (non-fatty and non-bloody) diarrhea. She is unable to tolerate drinking milk because of bloating, excess flatus and worsening diarrhea; but taking a mixture of sugar and salt improves her dehydration.

- Give the normal process of absorption of salt and water.
- Explain how the intestine normally protects itself from luminal bacteria.
- Give the scientific basis for the tests used to diagnose SIBO (small intestinal bacterial overgrowth).
- Explain the basis for her lactose intolerance.
- Give the scientific basis for the use of oral rehydration solutions.

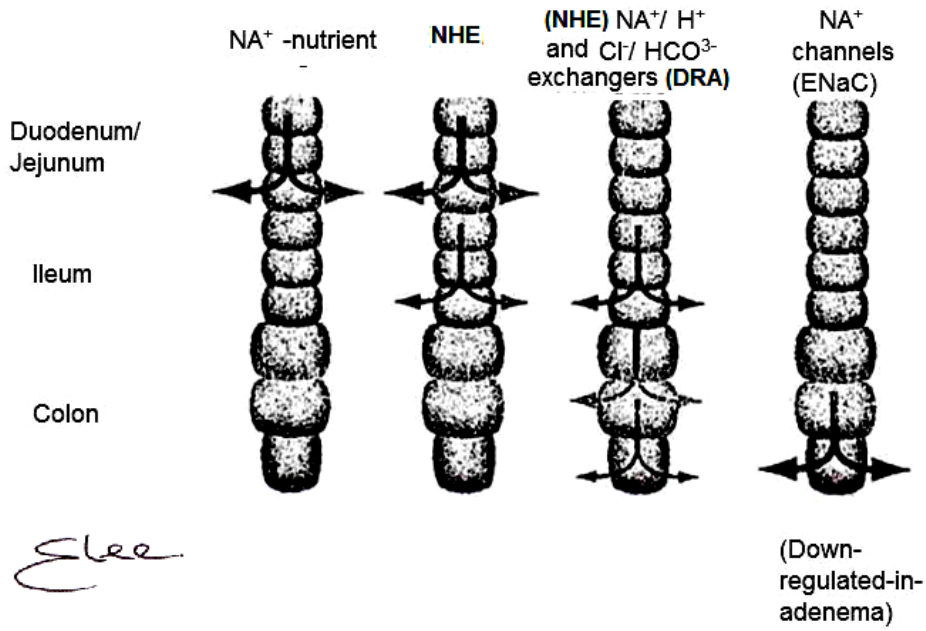
➢ **Gradient of Na^+ transporters along crypt-villus axis**



Abbreviations:CFTR, cystic fibrosis transmembrane receptors; DRA, Cl^- -$HCO3^-$ exchangers; NHE, Na^+ / H^+ exchangers

Adapted From: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Figure 99-4, page 1678, Ninth Edition, 2010

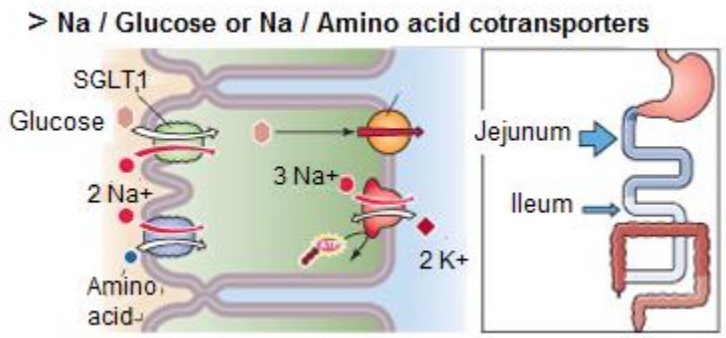
- **Gradient of Na^+ absorption from proximal to distal intestine**



Abbreviations: NHE plus DRA, parallel Na^+ - H^+ (NHE) exchanger plus Cl^- - HCO_3^- exchanger; DRA, down-regulated-in-adenoma [SLC 26A3])
Note: water absorption follows villus Na^+ absorption ; water secretion follows crypt Cl^- secretion

SMALL INTESTINAL AND COLONIC Na^+ TRANSPORT

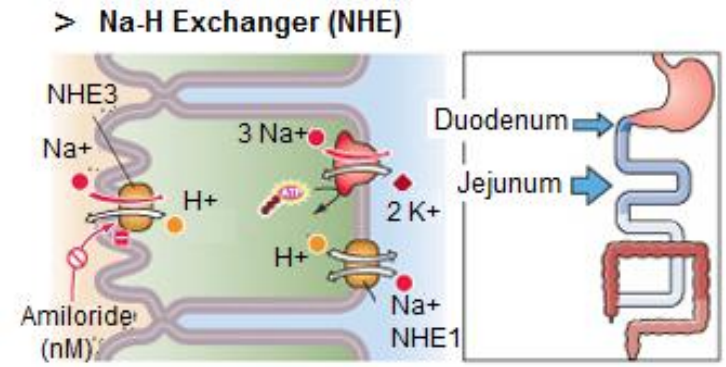
- NHE and Na^+nutrient cotransport in small intestine



Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Figure 44-3, page 938.

- The Na^+-K^+ ATPase in the BLM of the entire small intestine and colon exchanges 3 Na^+ molecules into the interstitial space for 2 K^+ transported into the cell.
- The lower intracellular Na^+ (about 15 mM or lower) generates an intracellular-negative membrane potential.
- This electrochemical drives the downhill entry of Na^+ from either the apical or basolateral side of the enterocyte (i.e., across the BBM or BLM).
- The Na^+ nutrient coupled transporters are not inhibited by increases in intracellular cAMP or Ca^{+2}.
- This forms the basis for the use of ORS (oral rehydration solutions) for persons with diarrhea (glucose plus Na^+ in water greatly accelerates the absorption of water).
- When the intestinal villus enterocytes absorb solutes such as glucose, water is also absorbed by transcellular aquaporins and by paracellular processes (across TJs).
- With food intake, the nutrients in the duodenum and jejunum increase Na^+ and H_2O uptake across the BBM.
- The increased uptake of Na^+ and H_2O is coupled to the movement of glucose or amino acids.
- If some glucose and/or amino acids reach the ileum, the solute-coupled Na^+ uptake occurs here as well.
- For each molecule of glucose transported with 2 molecules of Na^+ (and two accompanying anions) by AM SGLT1, 1100 H_2O molecules are drawn across the epithelium (Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, page 1710).
- In the short term, the number of SGLT1 transporters is increased by PKA- and PKC-dependent processes.
- PKC and MAP kinase (integrin-activated protein kinase) signaling pathways are induced by glucose to cause the recruitment of cytosolic GLUT2 to the BBM.
- GLUT2 recruited to the BBM facilitates the transport of glucose and fructose across the BBM, especially when the luminal concentration of these monosaccharides is high.

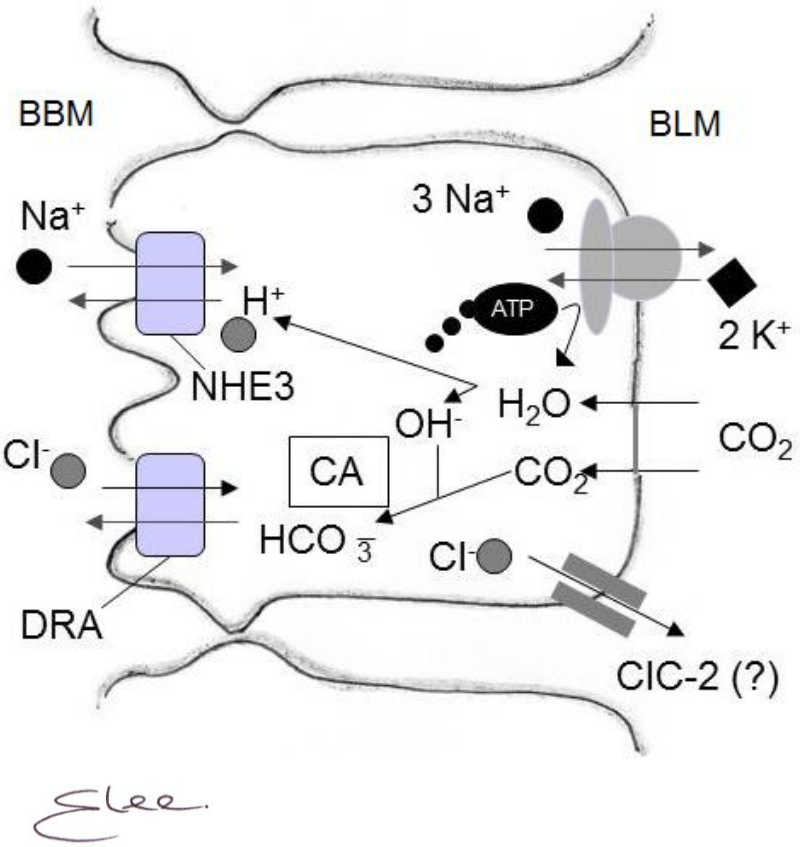
- NHE



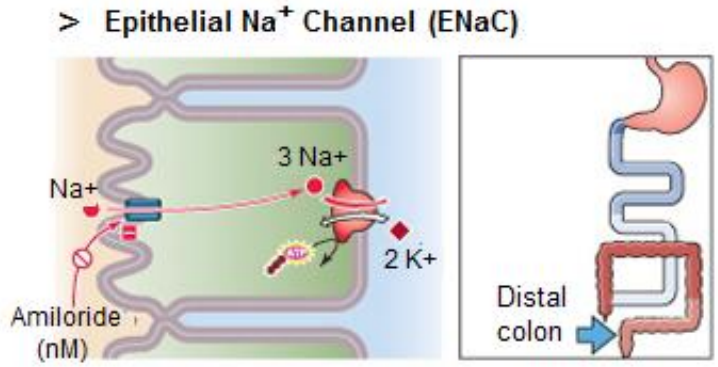
Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Figure 44-3, page 938.

- Also with food intake, the acid-stimulated secretin release from the duodenal S cells stimulates the pancreatic duct cells to secrete HCO_3^- - rich fluid.
- This excess of HCO_3^- in the lumen of the proximal small bowel as well as the increased intracellular H^+ stimulate the BBM NHE (Na^+-H^+ exchangers, NHE_2 and NHE_3).
- The energy for NHE_2/NHE_3 arises from the intracellular Na^+ gradient created by the BM Na^+ / K^+ pump.
- The nutrient - Na^+ pumps are energized by the Na^+ / K^+ pump in the BLM of the enterocytes.
- Na^+ crosses the BBM of the enterocyte, down an electrochemical gradient.
- The exit pathway across the BLM is the – Na^+, K^+- ATPase (aka the Na^+ / K^+ pump) sodium pump.
- Na^+ / K^+ pumps move Na^+ into two restricted spaces.
- K^+ channels help maintain the electrochemical gradient.
- The NHE in the BLM regulates the intracellular H^+ (pH_i) and has a much lesser role in creating a Na^+ gradient.

- Parallel NHE and DRA exchangers

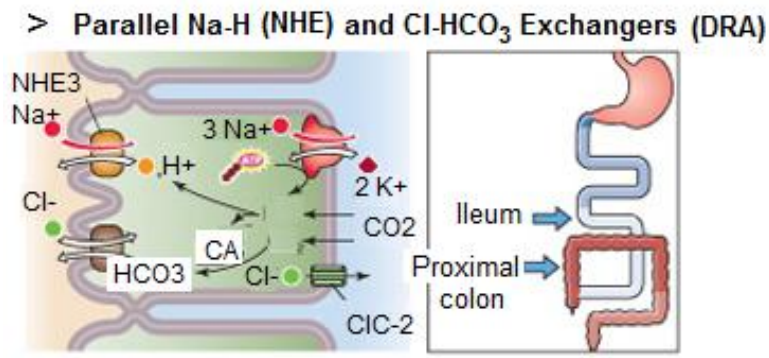


Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Figure 44-4, page 940.



Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Figure 44-3, page 938.

➢ ENaCs



Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Figure 44-3, page 938.

- In the distal colon, Na^+ is efficiently absorbed by membrane ENaCs (electrogenic Na^+ channels).
- The common exit pathway of Na^+ across the BLM is the Na^+ / K^+ pump (Na^+ / K^+ ATPase).

Regulation of Na^+/H_2O Absorption

➢ Hormone

- Give the effects of aldosterone, glucocorticosteroids and catecholamines on Na^+ / H_2O absorption by the small bowel and colon.

- Aldosterone – colon
  - ↑ ENac
  - ↑ Na+/K+ ATPase
  - ↑ SGKi
  - ↑ K^+ absorption and secretion
- Glucocorticosteroids small and large intestin
  - Low concentration Na+ absorption
    - ↑electroneutral
    - ↓ electrogenic
  - High concentration Na+ absorption
    - ↑electroneutral
    - ↓ electrogenic

- Catecholamines, (dopamine, epinephrin encepalins, somatostati
  - ↑ G α1 cascade
  - ↓ cAMP cascade
  - ↑ electroneutral Na+ absorption
  - ↓ secretion

Please see : Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, Table 99.1, page 1687, "Agents that Stimulate Intestinal Absorption of Fluid and Electrolytes".

- Neural regulation of ion transport
  - Sensory neurons responsive to intraluminal mechanical / chemical stimuli
  - Interneurons in either the myenteric or submucosal plexi
  - Sensory neurons releasing the vasoactive intestinal peptide (VIP) and acetylcholine (Ach) that act on epithelial cells
  - Additional neural input from somatostatin (SST), opiate, and noradrenergic neurons that modulate the function of the secretory neurons
  - Interaction between secretory neurons and blood vessels, immune cells, and paracrine cells

Adapted from: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Figure 87-8, page 1703, Seventh Edition, 2002.

- Give the effect of systemic pH on Na^+ / H_2O transport.
  - The Cl^- /HCO_3^- exchanger is also known as DRA (down-regulated-in-adenoma) or SLC $26A_3$.
  - These parallel BBM Na^+ / H^+ (NHE) and Cl^- / HCO_3^- (DRA) exchangers are influenced by small changes in the intracellular H^+ (pH_i).
  - Metabolic acidosis
    - ↑ electroneutral NaCl absorption
  - Metabolic alkalosis
  - ↓ electroneutral NaCl absorption
  - Intracellular cAMP, cGMP and Ca^{+2} act as intracellular messengers to reduce overall electroneutral NaCl absorption in the small and large intestines.
  - Any Na^+ and H_2O which are not absorbed in the proximal small intestine are absorbed in the distal small intestine and the right side of the colon.

- In the lower small intestine and proximal colon, Na^+ absorption occurs through two coupled transporters, (Na^+ - H^+ [NHE] and Cl^- - HCO_3^- [DRA]).

Cyclic AMP, cyclic GMP and intracellular calcium ($Ca^{2+}{}_I$) are involved in the secretion of chloride by intestinal crypt cells.

- Give the steps which are involved in the **transduction** of an external signal into a charge in cellular function, such as occurs with **secretagogues**.

    - An agonist (stimulatory or inhibitory) binds to the membra receptor for adenylate cyclase or for guanylate cyclase

    - Guanylate cyclase is activated, through inhibitory or stimulatc G proteins (Gi and Gs, respectively) (Guanine nucleoti regulatory proteins),

    - For example, VIP and prostaglandins activate receptors whi bind to Gs, whereas somatostatin activates Gi.

    - An increase in intracellular, cGMP activates PKG II (prote kinase II), and ↑ cAMPi activates PKA (protein kinase A)

    - PKG II acts through GKAP (G kinase-anchoring proteins) through AKAP (A kinase-anchoring proteins) to activa phosphorylation of specific target proteins to activa membrane channels or transporters.

Adapted from: Feldman M., et al. *Sleisenger and Fordtrai Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevi Philadelphia, 2010, page 1682.

"The ends justify the means"

The modern dilemma – Does it?

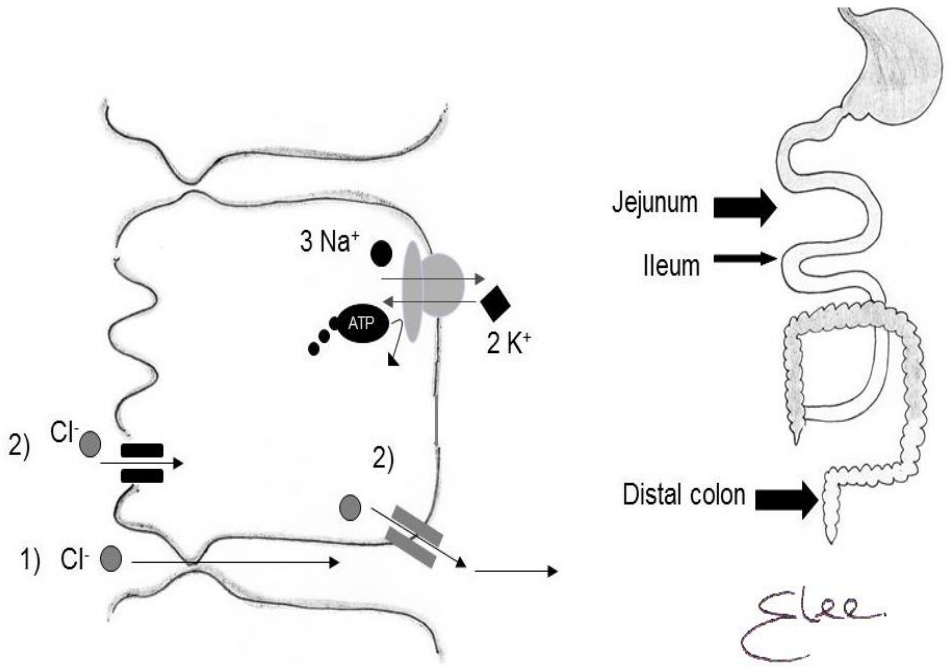
Machiovelli

Chloride (Cl^-) Absorption and Secretion

- The process of Cl^- absorption by the villus cells must be understood within the context of Cl^- secretion by the crypt cells in imbalance leads to diarrhea.
- Electroneutral NaCl absorption can mediate Cl- absorption in the interdigestive period. pHi couples the two exchangers. CA, carbonic anhydrase.
- Cl^- moves passively through the paracellular pathway or via cellular transporters.

- Absorption by villus enterocyte

➢ Passive Cl^- absorption – Tight Junctions and Chloride Channels



Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Figure 44-4, page 940.

- 1) In voltage-dependent Cl^- absorption, Cl^- may passively diffuse from the intestinal lumen into the blood across the tight junctions, driven by the lumen-negative transepithelial voltage (paracellular route).
- The electrochemical gradient for Cl^- across the Cl^- channels or TJs is derived from the electrochemical gradient for Na^+.
- The electrochemical gradient for this passive movement of Cl^- is the lumen negative (PD) potential difference of -15 to -25 mV.

- This lumen negative -15 to -25 mV PD is produced
  - In the small intestine
    - By the electrogenic transport of nutrient-coupled Na+ movement, and
    - By the BM Na^+ / K^+ ATPase
  - In the colon
    - ENaCa in the distal colon
- 2) Alternatively, Cl^- may diffuse through AM and BM Cl^- channels.

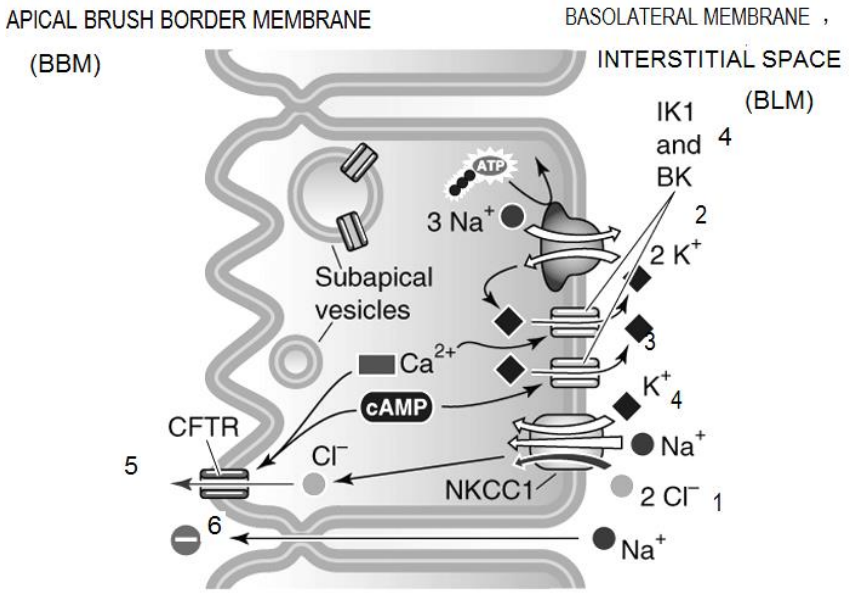
➢ Cl^-/ HCO_3^- exchanger (DRA), and parallel NHE exchangers

- The contribution of the Cl^-/HCO_3^- exchanger (in the absence of Na^+/H^+ exchange) at these sites is PC (proximal colon) > DC (distal colon) = IL (ileum).
- Cl^-/HCO_3^- exchange in the PC and IL may be accompanied by electroneutral Na^+ / H^+exchange (NHE).
- In the absence of a parallel Na^+ / H^+ exchanger, electroneutral Cl^- / HCO_3^- exchange at AM results in Cl^- absorption and secretion.
- In the upper small intestine, the Na^+ H^+ exchangers (NHE) are uncoupled (i.e. not coupled to another exchanger such as Cl^- - HCO_3^-) and are electroneutral (because no net transfer of charge occurs).
- NaCl absorption by the electroneutral processes of NHE_2 / NHE_3 and Cl^- /HCO_3^- exchange are increased when the Ca^{2+} concentration in the cell falls.
- The K^+ entering the cell by way of the Na^+-K^+- Cl^- cotransporter leaves the cell across the BM IK1 and BK K^+ channels.
- The colon achieves electrogenic Na^+ absorption and K^+ secretion.

- Secretion by crypt cells

➢ Electrogenic Cl^- Secretion by Intestinal Crypt Cells.

- Cl^- secretion is the basis of secretory diarrhea
- Under basal (unstimulated) conditions, very little Cl^- is actively secreted by the crypt cells in the small and large intestine.
- The thickness of the arrows in the inset indicates that the magnitude of the Cl^- secretory flux through this pathway is the same throughout the intestine.
- 1) The BM $Na^+/K^+/Cl^-$ cotransporter (NKCCl) in the BLM of the crypt cells transports Cl^- into the enterocytes or colonocytes.
- The Na^+ - K^+ - $2Cl^-$ carrier couples the movement of Na^+, K^+, and Cl^- in a 1:1:2 electrochemical equilibrium.
- The second messengers (cAMP, cGMP, Ca+2) increase Cl- secretion by the crypt enterocytes, and decrease NaCl absorption by the villous enterocytes.



Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Figure 44-5, page 941.

- 2) Na^+ crossing into the cell BLM by way of NKCCl is pumped out of the cell across the BLM the Na^+ / K^+ ATPase.

- 3) K^+ entering the cell across the BLM by NKCCl and Na^+ / K^+ ATPase leaves the cell across the BLM K^+ channels.
- 4) Secretion of Cl- requires two active processes (the BLM Na^+-K^+ pump and the Na^+-K^+- Cl^- transporter [NKCCl], as well as the BLM IK1 channels and the BK channels on the AM CFTR.
- The BM entry and BBM exit steps are integral to Cl^- secretion.
- 5) The Cl^- in the cell diffuses to the BBM and exits the cell across CFTR (cystic fibrosis transmembrane receptor, a Cl^- channel)
- The high Cl^- in the intestinal lumen makes the transepithelial voltage negative (lumen vs. cell).
- 6) This voltage-negative lumen provides the electrochemical gradient for the passive movement of Na^+ across the paracellular TJs.
- As CFTR pumps Cl^- out of the cell, and Cl^- falls, more Cl^- , Na+ and K^+ pass through NKCCI.
- In the jejunum, Cl^- diffuses across the TJs (paracellular route), driven by the lumen-negative transepithelial resistance.
- The paracellular pathway allows Na^+ movement from blood to lumen, driven by the lumen-negative transepithelial voltage.
- The Na^+ entering the cell from the blood through NKCCI exits across the BLM Na^+/ K^+ pump.
- Na^+ from the blood also crosses the TJ (paracellular pathway) and into the lumen.
- This results in the lumen negative transepithelial voltage.
- The transepithelial resistance is maintained lumen-negative by the BLM Na^+/ K^+-ATPase.
- In the jejunum, Cl^- may also be absorbed passively through the channels in the BBM and BLM.
- In the colon, the electrochemical gradient is produced by ENaC.
- The BBM ENaC in the proximal colon produces the electrochemical gradient for passive Cl^- absorption in the distal colon.
- Electroneutral Cl^- absorption may occur in the ileum and proximal colon by BBM DRA (Cl^- HCO_3^- exchange), as well as by both BBM Cl^- /-HCO_3^- and BBM Na^+ / H^+ exchange (DRA and NHE).
- Thus, the Cl^- / HCO_3^- and Na^+ / H^+ double exchange process is important in both the fasting or interdigestive period

- Give three classes of Cl^- channels.
  - CFTR1 (cystic fibrosis transmembrane conductance regulator)

- ClC2, salvage pathway for Cl^- transport in CF, and target for tl laxative, lubiprostome
- CLCA (Ca^{2+}-activated Cl^- channel), involved in rotavira associated diarrhea

- Give the mechanism by which K^+ channels modulate the hyperpolarization of the cell interior resulting from Na^+, K^+ -ATPase on the basal membrane (BM) of the enterocyte or H^+, K^+ -ATPase on the apical membrane (BBM) of the parietal cell, and promote Cl^- secretion.
  - BLM, small and large intestine
    - cAMP-activated KCNE3/KCNQ1 channels
    - Ca^{2+}-calmodulin-activated KCNN4 channels
  - BBM, colon
    - KCN MA1 channels

➢ Gastrointestinal Peptide Hormones Acting on Small Intestine and Colon

Hormone	Source	Action
○ Somatostatin	– D cells of stomach and duodenum, islet cells of pancreatic islets	▪ ↑ Fluid absorption/↓ secretion ↑ Smooth muscle contraction
○ Guanylin	– Ileum and colon	▪ ↑ Fluid absorption
○ Vasoactive intestinal polypeptide (VIP)	– ENS neurons	▪ ↓ Smooth muscle relaxation ↑ Secretion by small intestine
○ Neurotensin	– Endocrine cells, widespread in GI tract	▪ ↑ Histamine release
○ Substance P (SP)	– ENS neurons	▪ Neurotransmitter

Printed with permission: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Table 41-1, page 889.

- **Stimulate H_2O secretion**

➢ Secretagogues

- Open preexisting Cl^- channels, or
- Cause subapical vesicles to fuse with the AM, thus creating new Cl^- channels.
- Increased $[Ca^{2+}]_i$ also stimulates PKC, as well as Ca^{2+}- calmodulin-dependent protein kinase (CaM kinase).

Category	Secretagogue	Second messenger
○ Bacterial enterotoxin	– Cholera	– cAMP
	– Escherichia coli heat labile	
	– Heat stable	– cAMP
	▪ Yersinia	
	▪ Clostridium difficile	
○ Hormones a neurotransmitters	– VIP	– Ca^{2+}
	– Guanylin	
	– Acetylcholine	
	– Bradykinin	
	– Serotonin (5-HT)	
○ Immune cell product	– Histamine	– cAMP
	– Prostaglandins	
○ Laxatives	– Bile acids	– Ca^{2+}
	– Ricinoleic acid	

- **Stimulate H_2O Absorption**

➢ Absorptagogues*

Inhibit cAMP	Coupled Transport	Other Pathways
○ Norepinephrine	Glucose, amino acids	Aldosterone, angiotesin
○ Epinephrine	Dipeptides/tripeptides	Glucocorticoids
○ Dopamine	Short-chain fatty acids	Somatostatin (blocks Ca channels)
○ Enkephalins		GLP-2
○ NPY		
○ Somatostatin		

*Several agents activate multiple pathways.

Abbreviations: Ach, acetylcholine; ATP, Adenosine triphosphate; C intracellular calcium [concentration]; cAMP, cyclic adenosi monophosphate; cGMP, cyclic guanosine monophosphate; EG

epidermal growth factor; GLP-2, glucagon-like peptide 2; MAP mitogen-activated protein kinase; NO, nitric oxide; NPY, neuropeptide TNF, tumour necrosis factor; VIP, vasoactive intestinal polypeptide

- In the distal colon, the mineralocorticoid aldosterone increases the activity of both ENaC and the Na^+ -K^+ pump in the distal colon, thereby enhancing the electrogenic Na^+ transport in the distal colon.
- The Na^+ which has been taken up by ENaC is pumped across the BLM of the distal coloncytes by the Na^+ /-K^+ pump.
- Glucocorticoids have a small effect on the mineralcorticoid receptor, as well as having a major effect on Na^+/water absorption by way of its own receptor.
- The glucocorticoid receptor stimulates electroneutral Na^+ absorption in the small and large intestine.
- This paracrine effect is also seen with the paracrine effect of serotonin (5-HT) released by the enteric endocrine cells.

➢ Tyrosine kinase

- The active protein kinases (PKs) increase the AM conductance to Cl^-
- The secretagogues activate PKs.
- Three PKs are involved in Cl- secretion, PKA, PKC and PKG.

- Give the role of the PK2 involved in secretory diarrhea.

Protein kinase (PKs) involved in Cl^- secretion

PK	Activation	Second messenger
➢ PKA	○ Adenylate cyclase (AC)	– ↑cAMP
➢ PKC	○ Phospholipase C (PLC) ○ IP3 ○ ↑ Ca_i^{2+}	– Diacylglycerol (↑ DG)
➢ PKG	○ Guanylyl cyclase (GC)	– ↑cGMP

Intestinal Immune Cells

- The immunocompetent cells in the LP (lamina propria) forming the GALT (gut-associated lymphoid tissue) are comprised of
  - T lymphocytes, 60%
  - B cells and plasma cell, 25%
  - Macrophages, 10%
  - Mast cells and PMNs (polymorphonuclear leukocytes especially eosinophils), 5%
- Stimulation of these immunocytes causes release of inflammatory mediators.
- When here are ↑ PMNs as with inflammation, the PMNs respond to chemoattractants, pass in an integrin-dependent process from blood vessels into the intercellular space between colonocytes, and cause adenosine-mediated secretion

- Give the role of the **intestinal immune cells** in the physiology of Cl^- secretion.

➢ Intestinal Immune Cells

- Both secretory and absorptive factors are released by intestinal immune cells, enteric endocrine cells, and by the ENS (enteric nuron system). These include:
  - Adenosine,
  - Arachidonic acid
  - Histamine
  - Bradykinin
  - Leukotrienes
  - Metabolites
  - Nitric oxide(NO)
  - Platelet activating factor (PAF)
  - Prostaglandin (PGs)
  - Reactive O_2
- These mediators also have an indirect effect on the epithelial cells by way of the enteric nervous system or a paracrine effect on another cell type such as myofibroblasts or intestinal smooth muscle.

- Immune as well as other cells in the lamina propria such on monphages, fibroblasts, mast cells, as well as neutrophils, release chemical mediators (such as histamine, prostaglandins, PAF, bradykinen).
- These mediators have a direct effect on the epithelial cells.
- Intestinal cells release an array of secretory factors, which can act either
  - Directly on the epithelium, or
  - Indirectly by stimulating the mesenchymal cells or enteric neurons to release prostaglandins (PAGEs) or acetylcholine (Ach).
- Activation of mast cells in the lamina propria triggers the release of histamine.
- Histamine either
  - Directly affects epithelial cells, or
  - First stimulates an enteric neuron, and thereby indirectly affects epithelial cells.
- There are interactions among secretory neurons and blood vessels, immune cells, and paracrine cells.

Immune Cell	Products
– Macrophages	• Prostaglandins • O_2 radicals
– Mast cells	• Histamine
– Neutrophils	• Eicosanoids
–	• Platelet-activating factor
– Fibroblasts	• Eicosanoids • Bradykinin

Source: *Medical Physiology*, Table 44-3, page 945, Second Edition, 2009.

- The enteric neuron system (ENS) which has been stimulated by histamine modulates the
  - Epithelium (secretion),
  - Intestinal smooth muscle (motility), or
  - Vascular smooth muscle (blood flow)

CLINICAL PHYSIOLOGICAL CORRELATION – More Diarrhea

Background: Diarrhea may result from an imbalance between intestinal secretagogues and absorptagogues. The treatment of diarrhea may include measures to normalize this imbalance.

- Give the role of the intestinal immune cells, endocrine cells and enteric nervous system in the control of fluid and electrolyte balance by the intestinal tract.

CLINICAL PHYSIOLOGICAL CORRELATION – Chloride Flux

Background:

- One process by which diarrhea occurs in through the decreased absorption of chloride (Cl^-) in the upper portion of the villus, and the increased secretion of Cl^- is the lower portion of the crypt-villus unit.
- The movement of Cl^- may be electroneutral or electrogenic and involves transporters and channels in the brush border membrane (AM) and the basolateral membrane (BM).
- Secretion of Cl^- is fundamental to the process of secretory diarrhea, such as occurs in cholera.
- Draw a picture of Cl^- absorption and secretion from along the length of the small intestinal villus and in the colon.

HCO_3^-

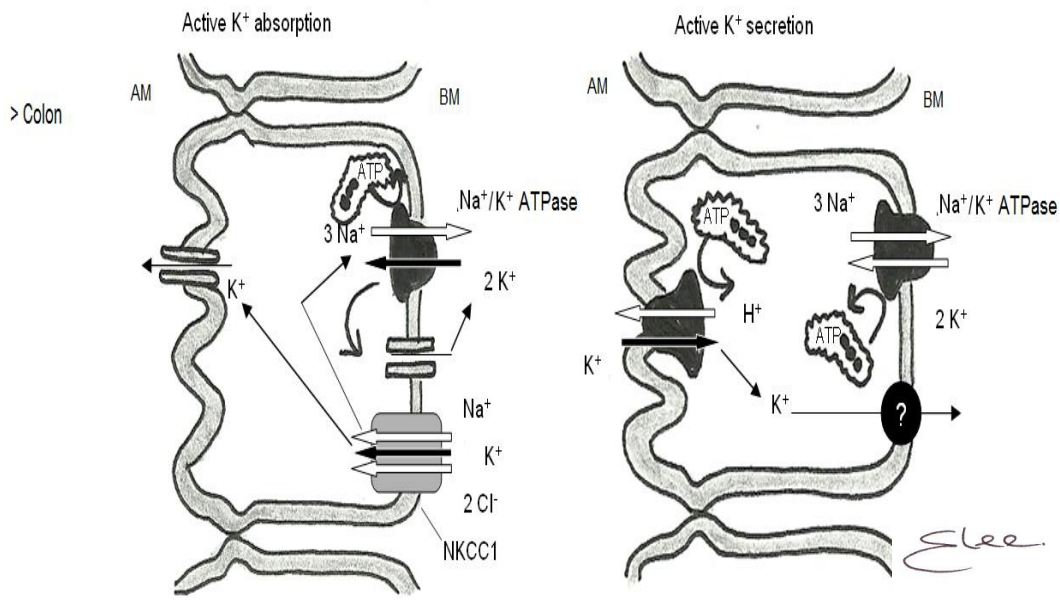
- When a patient has diarrhea, HCO_3^- is the major anion lost in the stool, being secreted by electrogenic and electroneutral processes, linked to the inward movement of Cl^- through NHE distributed along the length of the small and large intestine
- SLCs (solute carriers) for HCO_3^- include
  - SCL4 for HCO_3^-, and
  - SCL26, the multifunctional anion exchange family (e.g., Cl^-, HCO_3^-, sulfate, oxalate)
- Examples of SLC26 include
  - SLC26A3 (aka Cl^-/HCO_3^- exchanger, or DRA [down-regulated adenoma]) in the AM (apical membrane) of colonocytes, and
  - SLC26A6 (aka PAT1 [putative anion transporter 1], expressed in BBM of enterocytes > colonocytes]
- Mutations in SLC26A3 result in congenital Cl^- diarrhea

"The decisions of our own present are the architects of our present" [and future]

Dan Brown, Inferno

Potassium (K^+) Absorption and Secretion

- Absorption
  - AM (apical membrane), K^+ is exchanged for H^+ by the exchanger.
  - At the BM (basolateral membrane), K^+ is transported in exchange for NA+ by Na^+ / K^+ ATPase
  - The cytosolic K^+ crosses the BM possibly through a K^+ channel.
  - In the colon,
    - K^+ is absorbed into the colonocyte by the AM H^+-K^+ exchanger.



Abbreviations: AM, apical membrane; BM, basolateral membrane; NKCC1, Na^+ - K^+ - Cl^- cotransporter

Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Figure 44-6, page 943.

- Secretion in the small intestine
  - K^+ crosses the bm by way of Na^+ / K^+ ATPase, as well as NKCC1 (Na^+ - K^+ - Cl^- cotransporter)
  - K^+ is secreted across the AM through K^+ channel.
  - In the colon, K^+ is secreted by BM NKCCI (Na^+-K^+- Cl^- cotransporter)
- Control of K^+ absorption and secretion by the colon
  - Aldosterone increases K^+ secretion both passively and actively;
  - cAMP and Ca^{+2} stimulate K+ secretion in the colonic crypts (cAMP/Ca^{+2} increase the activity of AM K^+ channels more than BM K^+ channels, resulting in net K^+ loss from the colonocyte).

Digestion and Absorption of Proteins,Peptides, Oligopeptides and Amino Acids

- Proteins are absorbed intact in minute amounts, or as peptides, oligopeptides and amino acids.

- Source of Proteins

  - About half of the proteins in the intestinal lumen are from sloughed GI tract cells as well as from secretions.

- Dietary protein digestion begins with gastric pepsin (pepsinogen activated by gastric HCL), and continues when the pancreatic proenzymes become active proteolytic enzymes.
- Animal protein is better digested than is vegetable protein.
- Protein, which contains abundant hydroxyproline is poorly digested.
- Food preparation and storage may reduce the digestibility of protein.
- Some AAs are not synthesized in the body, and as such are considered to be essential and must be supplied by the diet.
- Some AAs are synthesized in the body, but inadequate amounts are produced when the demand is high, and under these circumstances the AAs are said to be conditional.

➢ Daily throughput

- Exogenous protein
  - 0.75 to 1 g/kg body weight per day
- Endogenous protein
  - Secretions
    - Saliva; secretions from stomach, gallbladder, pancreas, intestine; plasma crossing into intestine ~ 25 g/day
  - Sloughed mucosal cells

Peptides, Oligopeptides and Amino Acids

➢ Digestion – Lumen: Pancreatic Proteolytic Enzymes

- Gastric pepsin
  - Pepsinogen is secreted from gastric chief cells
  - Chief cells secrete pepsin 1 and 2, whereas mucus cells in t oxyntic and pyloric areas of the stomach as well as the Brunr glands of the duodenum secrete pepsin 2
  - The protein digestion products in the stomach

- ↑ H+ secretion
- ↑ pepsinogen secretion
- Modify the rate of gastric emptying

- Recall from the previous chapter on "The Pancreas", the importance the luminal steps of protein digestion, by the way of pancrea proteolytic enzymes.
  - The initial digestion of nutrients in the mouth and stoma continues in the lumen of the upper small intestine, where it furthered by the pancreatic and hepatobiliary secretions.
  - There are five proteolytic enzymes which break protein down oligopeptides and to amino acids: (trypsinoge chymotrypsinogen, proelastase, procarboxypeptidase A and B.
  - All these proenzymes are activated by trypsin ("autoactivatior which itself comes from trypsinogen.
  - Trypsinogen is also activated by jejunal enterokinase.
  - The three endopeptidases (trypsinogen, chymotrypsinogen a proelastase) produce oligopeptides of 2-6 amino acids (AAs).
  - The two exopeptidases carboxypeptidase A and B form single AAs
- Pancreatic Proteolytic Enzymes

- Give the names of 4 major pancreatic proteolytic enzymes, their cleavage sites, and their products.

There are up-sides, down-sides, in-sides, and out-sides; all kinds of sides (!)

Peptidase	Cleavage Sites	Products Peptidases
➢ Trypsinogen	o Internal bonds at lysine or arginine residues o Other pancreatic proenzymes	– Oligopeptides and proteolytic enzymes
➢ Chymotrypsin	o Bonds at aromatic or neutral amino acid residues	– Oligopeptides
➢ Elastase	o Bonds at aliphatic amino acid residues	– Oligopeptides
➢ Carboxy-peptidase A	o Aromatic amino acids from C-terminal end of proteins and peptides	– Aromatic amino acids and peptides
➢ Carboxy-peptidase B	o Arginine or lysine from C-terminal end of proteins and peptides	– Arginine, lysine, and peptides

Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Table 45-2, page 956.

- Pancreatic proteases (trypsinogen, chymotrypsinogen, procarboxypeptidase A, B and proelastase are secreted as inactive proenzymes
- Enterokinase (aka enteropeptidase) is attached to the BBM (brush border membrane) of enterocytes
- Bile acids in the intestinal lumen release enterokinase from the BBM
- Enterokinase accelerates the conversion of trypsinogen to trypsin
- Trypsin activates further trypsinogen, as well as chymotrypsinogen

SO YOU WANT TO BE A GASTROENTEROLOGIST!

As a result of the combined effect of pepsin and trypsin as well as enterocyte cytosolic and brush borer membrane oligopeptidases, exogenous and endogenous protein is broken down.

- Give the name of the proteins which are resistant to proteolysis in the lumen of the small intestine.
    - Intrinsic factor (IP) is strongly bound to cobalamin at pH > 3

Secretory IgA and intrinsic factor are two proteins which resist luminal proteolysis.

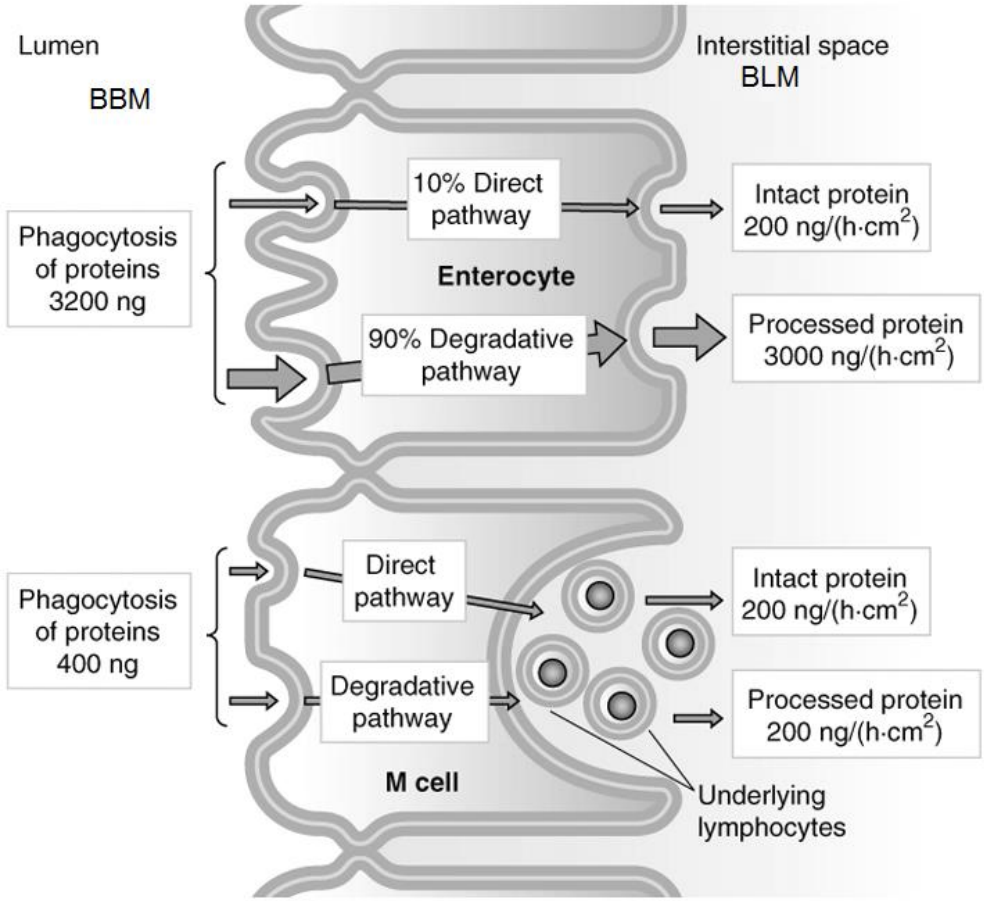
Pancreatic proteases break down exogenous and endogenous protein.

- Give 3 other of their functions.

Pancreatic protease aids in the digestion of protein, and as well

- Duodenal lumen
    - Remove R factor (haptocorrin) from cobalamin (vitamin B12), thereby allowing IF (intrinsic factor) to bind at pH > 3
    - BBM
        - Participate in the final process of activating the brush border membrane (BBM) enzyme sucrase-isomaltase
        - ↑ turnover of BBM hydrolytic enzymes
- Bacteria
    - ↑ inactivation of some luminal organisms

- Endocytosis of intact proteins



Abbreviation: AM, apical membrane; BM, basolatteral membrane

Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Figure 45-7, page 957.

- Only very minute amounts of protein are absorbed intact, approximately 3600 ng/h-cm^2 of this 90% is phagocytosed by the enterocyte degrative pathway, and 10% by the direct pathway.

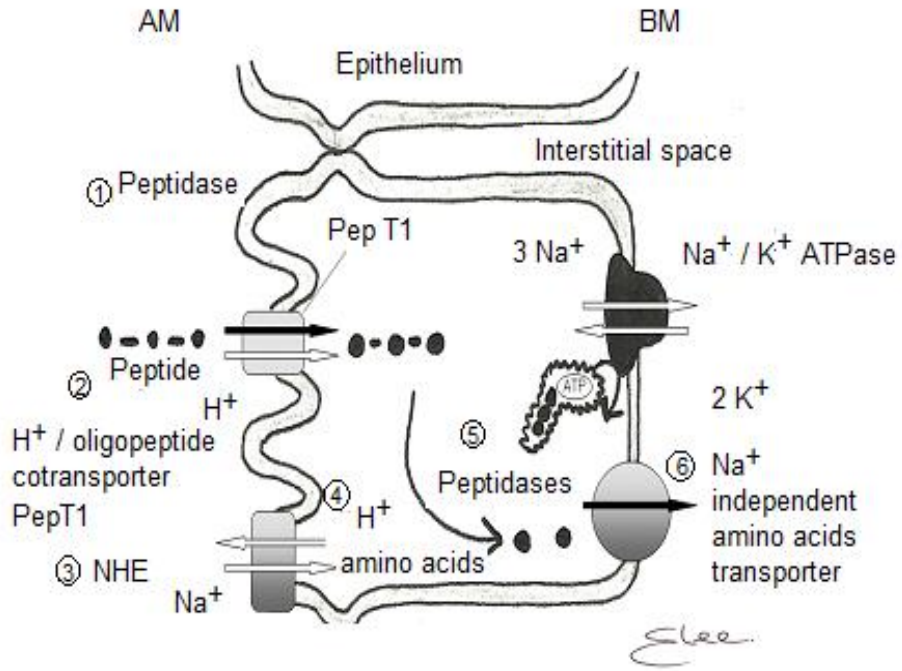
- Both enterocytes and specialized M cells can take up small amounts of intact protein across the BBM (brush border membrane) by the process of endocytosis.

- The more abundant enterocytes endocytose much more total protein than do the less abundant M cells.

- The less abundant M cells take up relatively little intact protein, but approximately half of this emerges intact at the basolateral membrane.

- The M cells on top of the Peyer's patches (lymphoid follicles in the lamina propria) take up 400 mg/h-cm^2 of protein, 10% by the degrative pathway, and 90% by the direct pathway.
- The lysosomal proteases in the enterocytes degrade ~90% of this endocytosed protein.
- The processed protein does not result in an allergic reaction, while the intact protein does.
- The intact protein is processed by immunocompetent cells, which are transformed into lymphocytes, and an immune response begins.

➢ Absorption of oligopeptides



Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009;Figure 45-8, page 958.

- 1) Peptidases in the BBM break down some oligopeptides to tripeptides, dipeptides, and amino acids (AAs).
- 2) Oligopeptides (dipeptides, tripeptides and tetrapeptides) are actively transported across the enterocyte BBM by the H^+/oligopeptide cotransporter, PepT1.
- AAs are absorbed faster by PepTi than by the Na+/AA transporter.
- PepT1 is an active transport process driven by a H^+ gradient.
- 3) The H^+ entering the enterocyte is removed by the BBM NHE.
- 4) The Na^+ entering the cell by NHE is pumped across the BLM by the Na^+ / K^+ ATPase.
- 5) Cytoplasmic peptidases break down the di- and tri- peptides to single AAs.

Dipeptides	Tripeptides
– L Form of amino acids peptide	– D Form of amino acids peptide
– Neutral amino acids in peptid	– Acidic or basic amino acids
– Long-side-chain amino acids peptides	– Short-side-chain amino aci in peptides

- 6) The AAs exit the enterocyte and pass into the portal circulation by way of one of the three Na^+ - independent BLM transporters.
- Digestion of oligopeptides continues by peptidases at the enterocyte BBM and in the cytosol.
- There are relative specificities of the intestinal peptide transporters for dipeptides and tripeptides.

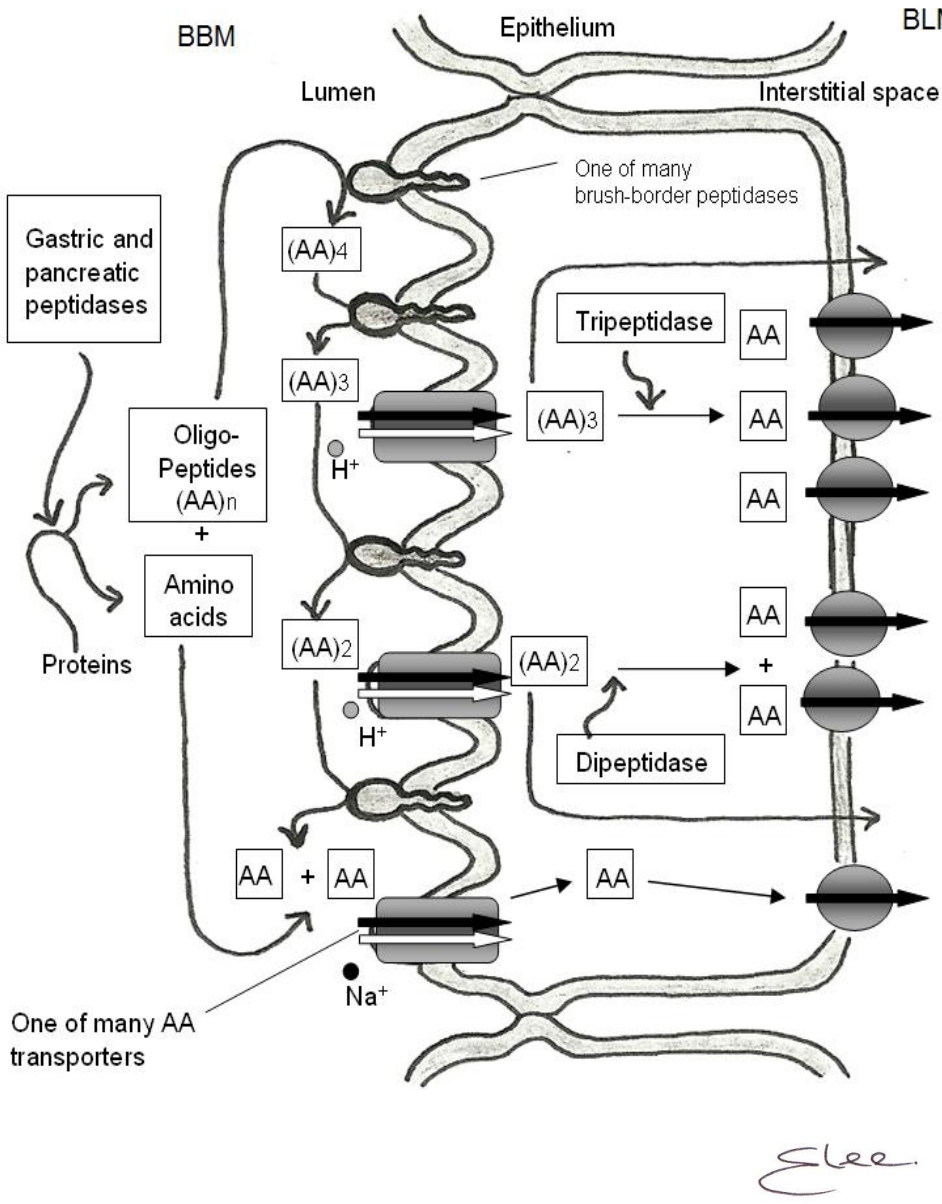
➢ Absorption of amino acids (AAs)

Peptidase		Cleavage Site	Products Peptidases
• Brush Border Membrane (BBM; aka AM, apical membrane) Peptidases			
○ Amino-oligopeptidases (at least two types)	–	Amino acids from C terminal end of 3 to 8 amino acid peptides	▪ Amino acids and dipeptides
○ Aminopeptidase A	–	Dipeptides with acidic amino acids at N terminal end	▪ Amino acids
○ Dipeptidase I	–	Dipeptides containing methionine	▪ Amino acids
○ Dipeptidase III	–	Glycine-containing dipeptides	▪ Amino acids
○ Dipeptidyl aminopeptidase IV	–	Proline-containing peptides with free α-amino groups	▪ Peptides and amino acids
○ Carboxypeptidase P	–	Proline-containing peptides with free C terminal end	▪ Peptides and amino acids
○ Gamma glutamyl transpeptidase	–	Gamma-glutamyl bonds and transfers glutamine to amino acid or peptide acceptors	▪ Gamma-glutamyl amino acid or peptide
○ Folate conjugase	–	Pteroyl polyglutamates	▪ Monoglutamate
• Cytoplasmic Peptidases			
○ Dipeptidases (several types)	–	Most dipeptides	▪ Amino acids
○ Aminotripeptidase	–	Tripeptides	▪ Amino acids
○ Proline dipeptidase	–	Proline-containing dipeptides	▪ Proline and amino acids

Abbreviation: AM, brush border membrane

Printed with permission: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Table 100-6, page 1714, Ninth Edition, 2010.

ABSORPTION OF AMINO ACIDS



Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. a Boulpaepemile L. 2009, Figure 45-6, page 955.

Major Amino Acid Transport Systems Detected in Enterocytes

Transport System	Substrates
➢ Brush Border (Apical) Membrane (BBM)	
o Neutral amino acids	
– NBB (SLC6A19)	Broad specificity for neutral ami acids
– PHE	Phenylalamine and methionine
– IMINO (SLC36A1)	Imino acids; prolir hydroxyproline
o Basic amino acids	Lysine, cysteine, basic ami acids
o Acidic amino acids	Glutamate, aspartate
– X-GA (SLC1A1)	
➢ Basolateral Membrane (BLM)	
o L	Broad selectivity
o A	Broad selectivity
o ASC (SLC1A5)	Neutral amino acids, alanir serine, cysteine
o N	Glutamine, histidine, asparagine

Printed with permission: *Sleisenger and Fordtran's Gastrointestinal and Li Disease,* Table 100-9, page 1716, Ninth Edition, 2010

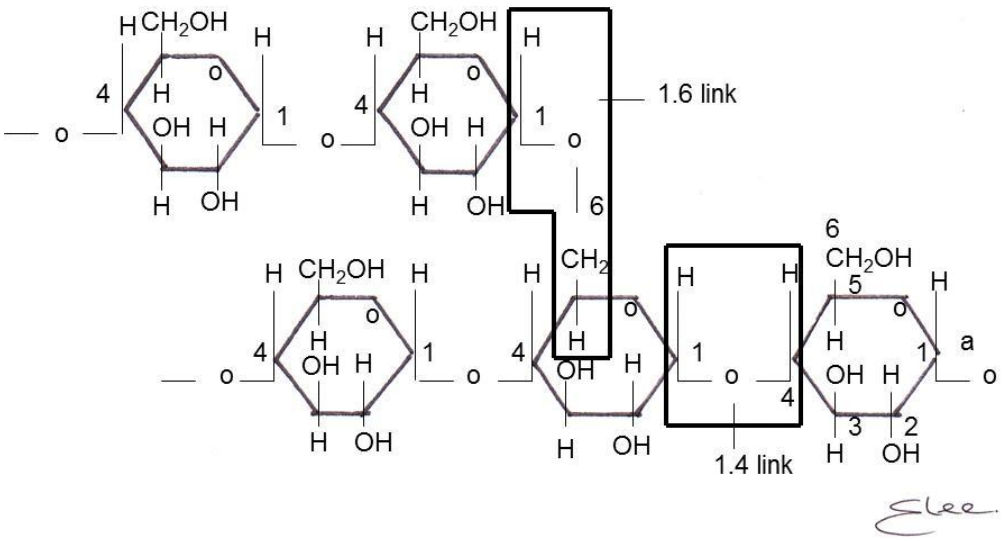
"When we are no longer able to change a situation,
we are challenged to change ourselves"

Viktor Frankl

Carbohydrate Digestion and Absorption

- Source
  - Ingested dietary disaccharides are split into monosaccharides by disaccharidases in the AM of the enterocyte..
  - Carbohydrate polymers may be short (oligosaccharides) or long (polysaccharides).
  - The polymers which cannot be digested are called non-digestable polymers, (aka “fiber”).
  - Polymers which are digestable are broken down to monomers, such as the monosaccharides glucose, galactose and fructose.
  - Pectins, cellulose and hemicellulose are both fiber and carbohydrates.
  - Some dietary fibers such as lignins are not carbohydrates.
  - Fiber may be soluble or insoluble. Some fiber can only be broken down by bacterial enzymes in the colon.
  - The polysaccharide starch is a major dietary source of digestible carbohydrate.
  - Starch is the storage form of carbohydrate in plants.
  - Glycogen is the storage form of carbohydrate in animal tissue (“animal starch”).

STRUCTURE OF STARCH



Digestion and Absorption of Carbohydrate

- Give the process of digestion and absorption of dietary carbohydrates.

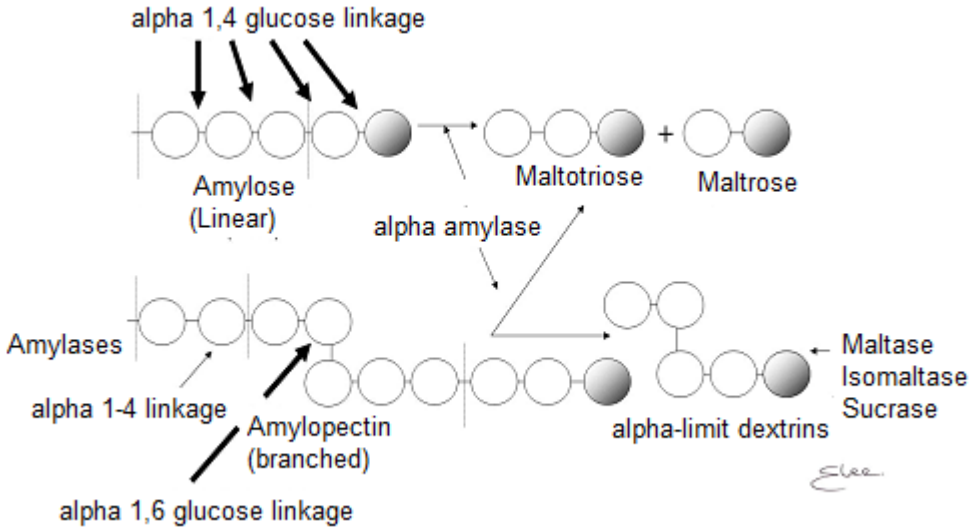
➢ Duodenal lumen

- Small amounts of amylase are present in saliva and in milk
- Most amylase is derived from pancreatic secretion.
- Pancreatic amylase is in
  - The lumen of the upper small intestine
  - Attached to the AM of enterocytes
- The major source of dietary carbohydrate is starch (animal "starch" is called glycogen).
- Starch is comprised of
  - Amylopectin
    - Branched chain of glucose molecule joined by α-1,6 linkage
  - Amylose
    - Linear chain of glucose molecule joined by α-1,4 linkages
  - Amylase results in α-limit dextrins, maltotriose and maltose from amylopectin
    - Amylase results in maltotriose and maltose from amylose

➢ Hydrolysis of complex carbohydrate by amylases

Hydrolysis of complex CHO by pancreatic amylases → Further hydrolysis of oligosaccharides by brush border membrane (BBM) disaccharides enzymes → Active transport of monosaccharides across the BBM → Facilitated transport of monosaccharides across basolateral membrane (BLM) and into portal blood

HYDROLYSIS OF AMYLOSE AND AMYLOPECTIN



- The entire process of digestion and absorption of nutrients from the lumen, and across both the BBM and BLM, is known as “assimilation”.
- The initial digestion of nutrients in the mouth and stomach continues in the lumen of the upper small intestine, where it is furthered by the pancreatic and hepatobiliary secretions.
- The plant and animal starches contain the straight-chain polymer amylose, as well as the branch-chain amylopectin.
- In amylose, α-1,4 linkages join the glucose monosaccharides together to form the straight chain polymer.
- In amylopectin, there are α-1,4 linkages as well as α-1,6 linkages, which lead to the branching in this polymer.
- Amylopectins are more abundant than amylose, and there are more α -1,6 linkages in glycogens (“animal starch”) than in plant starch.
- Salivary and pancreatic amylases in the intestinal lumen digest polymeric carbohydrates to disaccharides (intraluminal hydrolysis).
- Salivary and especially the pancreatic ∝-amylase hydrolyse only the internal α1,4 linkage in starch.
- These amylases do not hydrolyse the terminal α-1,4 linkages, the α-1,6 linkages which form the branch points, or the α-1,4 linkages which are adjacent to the α-1,6 linkages.
- The products of the α-amylase are maltose (glucose-glucose, i.e., 2 glucose molecules), maltotriose (glucose-glucose-glucose, i.e., 3 glucose

molecules), and α-limit dextrins (multiple glucose molecules which cannot be hydrolyzed by amylase because of their α-1,6 - linkages).

- Note that α-amylase does not hydrolyze the β-1,4 linkages in cellulose. This is why humans can't digest grass.
- Amylase produces maltose, maltotriose and α-limit detrins, but no free glucose.

- The nascent polypeptide (N) of these BBM digestive enzymes undergoes ribosomal mRNA translation, and then is translocated to the rough endoplasmic reticulum membrane (RER).
- Oligosaccharide side chains join the polypeptide to be transferred to Golgi apparatus for further processing
- After incorporation in the plasma membrane, luminal proteases cleave the molecule into its active subunits.

➢ BBM hydrolases (carboxylases)

- The catalytic sites of BBM sucrase-isomaltase, lactase and glucoamylase (maltase) project into the intestinal lumen, with the rest of the disaccharidase protein anchored in the BBM.
- Isomaltase hydrolyzes the α-1,6 linkage of the small α-limit dextrins (from amylopectin)
- α-limit dextrinase
  - α-1,6 linkage of 5- and 5-α-limit dextrins
- Maltase-glycoamylase
  - A-1,4 linkage of oligosaccharides of up to 9 glucose molecules
- Sucrase-isomaltase (SI)
  - Sucrose-α-dextrinase
- The regulation of SI and LPH is by differential activation by transcription factors.
  - GATA-type zinc-finger transcription factor family members
  - HNF1 (hepatocyte nuclear factor 1)
  - Cdx (caudal-related homeodomain proteins)
- Only a very small amount of dietary disaccharides are absorbed.
- Sucrase-isomaltase (SI) hydrolyzes sucrose into fructose and glucose, as well as hydrolyzing some maltose into glucose.
- SI is further processed by pancreatic proteases to cleave the molecule into its active subunits.
- SI/LPH are in BBM of crypt and villus enterocytes, but are expressed only in the villus.

- SI diurnal variation in the activity of a carbohydrase (fasting > feeding) is due to feeding releasing pancreatic enzymes which reduce the T1/2 of the disaccharidases.
- Maltase (glucoamylase) hydrolyzes maltose and maltoriase.
- Sucrase-isomaltase and maltase also hydrolyze the terminal α-1.4 linkages of maltase and α-limit dextrins.
- The rate of hydrolysis of sucrase is high, so that the rate of uptake of the sugars released by the hydrolyzing of the disaccharidases (fructose and glucose) is limited by the activity of the BBM transporters, and not by the activity of sucrase.
- The activity of the disaccharidases is higher in the proximal than in the distal small intestine, and is higher in the enterocytes in the upper as compared to the lower portions of the villus.

➢ Synthesis of disaccharidases (Dis)

- ER glycoprotein proenzymes of Dis → Golgi complex processing → insertion of LPH and SI into BBM.

Adapted from: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Figure 97-12, page 2163, Eighth Edition, 2006

AM disaccharidase	Substrate and product
○ Sucrase - isomaltase	– Sucrose → glucose + fructose – Maltose → glucose – Terminal α – 1, 4 linkage of maltose and – α- limit dextrins → glucose
○ Lactase	– Lactose → glucose + galactose
○ Maltase	– Maltose, maltotriose → glucose – Terminal α – 1, 4 linkage of maltose, – α- limit dextrins → glucose

Disaccharides ⟶ Monosaccharides

- The combined actions of maltase, isomaltase and sucrose yield glucose molecules from a-limit dextrins.
- Isomaltase is necessary to split the α-1, 6 link

- Digestion continues at the brush border (apical) membrane (BBM aka AM) of the enterocytes.
- Disaccharides are present in the diet, and some of these are derived from the hydrolysis of oligosaccharides.
- The disaccharides also in the small intestinal BBM hydrolyse the disaccharides to monosaccharides (membrane digestion).
- The milk disaccharide “lactose” is formed by the monosaccharides glucose and galatose.
- Lactase hydrolyzes lactose into galactose and glucose.
- The rate of hydrolysis (digestive activity) of lactase is low, so that the rate of hydrolysis of lactose limits the rate of absorption of its monosaccharides (galactase and glucose).
- In about 80% of humans, their BBM lactase activity falls after weaning, and they cannot tolerate large amount of milk or milk products.
- These persons are said to be lactose-intolerant or “milk tolerant” or to have “primary lactase deficiency”.
- Most persons of Northern Western European ancestery (“Caucasians”) have persistently high levels of BBM lactase, so they can enjoy drinking milk and dairy products into their adult years.
- When a Caucasian develops milk intolerance, it is usually due to a disease-related decline in BBM lactase activity, such as from celiac disease.
- Fasting has little effect on the activity of lactase, but does reduce the activity of sucrase.
- Sucrose (“sugar”) is formed by the monosaccharides glucose and fructose.
- Sucrose is also formed from the action of maltase (aka glucoamylase) on the maltose and maltotriose derived from starch digestion.
- The activity of sucrase is increased when the dietary load of sucrase is increased.

➢ Enterocyte Monosaccharide Transporters

- There are 3 enterocyte monosaccharide transporters.
- $SGLT_1$, Na^+ - dependent glucose/ galactose co-transporter in brush border (apical) membrane (BBM)
- $GLUT_5$, Na^+ - independent fructose transporter in BBM
- $GLUT_2$, Na^+ - independent monosacharide transported in basolateral membrane (BLM), which traffics from cytosol to AM in response to CHO-containing meal.

$SGLT_1$

- Glucose and galactose are transported with Na^+ by the Na^+ - dependent transporter $SGLT_1$ in the enterocyte BBM.
- This is called secondary Na^+ -dependent active transport, in which the hexose transport is energized by the Na^+ electrochemical gradient caused by the BBM Na^+-K^+ pump (Na^+/K^+ ATPase).
- Water is absorbed in large amounts with the $SGLT_1$-transported monosaccharides glucose and galactose, as well as with the Na^+ which is cotransported by $SGLT_1$ across the BBM.

GLUT5

- Fructose uptake across the BBM is by Na^+ - independent facilitated diffusion, using the transporter $GLUT_5$.
- Shortly after the oral intake of carbohydrates, $GLUT_2$ in the enterocyte cytosol trafficks from a cytosolic pool to and is inserted into the BBM to facilitate further the uptake of glucose, galactose and fructose.
- Nutrients taken up across the AM may be further metabolized in the enterocytes.

GLUT2

- $GLUT_2$ is Na^+- independent, and also facilitates the exit of those three monosaccharides across the BLM (basolateral membrane) and into the portal circulation.

- **Colonic Digestion and Absorption of Carbohydrates**
  - The colon plays a role in the partial recovery of unabsorped dietary carbohydrate (CHO) (intermediate and end products of anaerobic bacterial fermentation of carbohydrates).
  - Bacterial fermentation of carbohydrates lead to increased bacterial production of hydrogen (H_2) gas, and this underlies the principle of the breath hydrogen test for small intestinal bacterial overgrowth (SIBO) or carbohydrate malabsorption.
  - Undigested / unabsorbed colonic CHO is metabolized by bacteria to short chain fatty acids (SCFA).
  - SCFA are absorbed into colonocytes, where they are used as the main fuel for these cells.
  - An abnormal increase of CHO carbohydrate in the colon (such as from CHO malabsorption in the small intestine) leads to an excess of luminal SCFA, acidic stools, flatulence and bloating, and osmotic diarrhea.

- A test dose of carbohydrate may be labelled with ^{13}C or ^{14}C; malabsorption or SIBO leads to less absorption of labelled carbohydrate, less production of CO_2, and less ^{13}C / ^{14}C being detected in the breath.

"Go as far as you can see; when you get there,
you'll be able to see farther"

J. P. Morgan

SO YOU WANT TO BE A GASTROENTEROLOGIST!

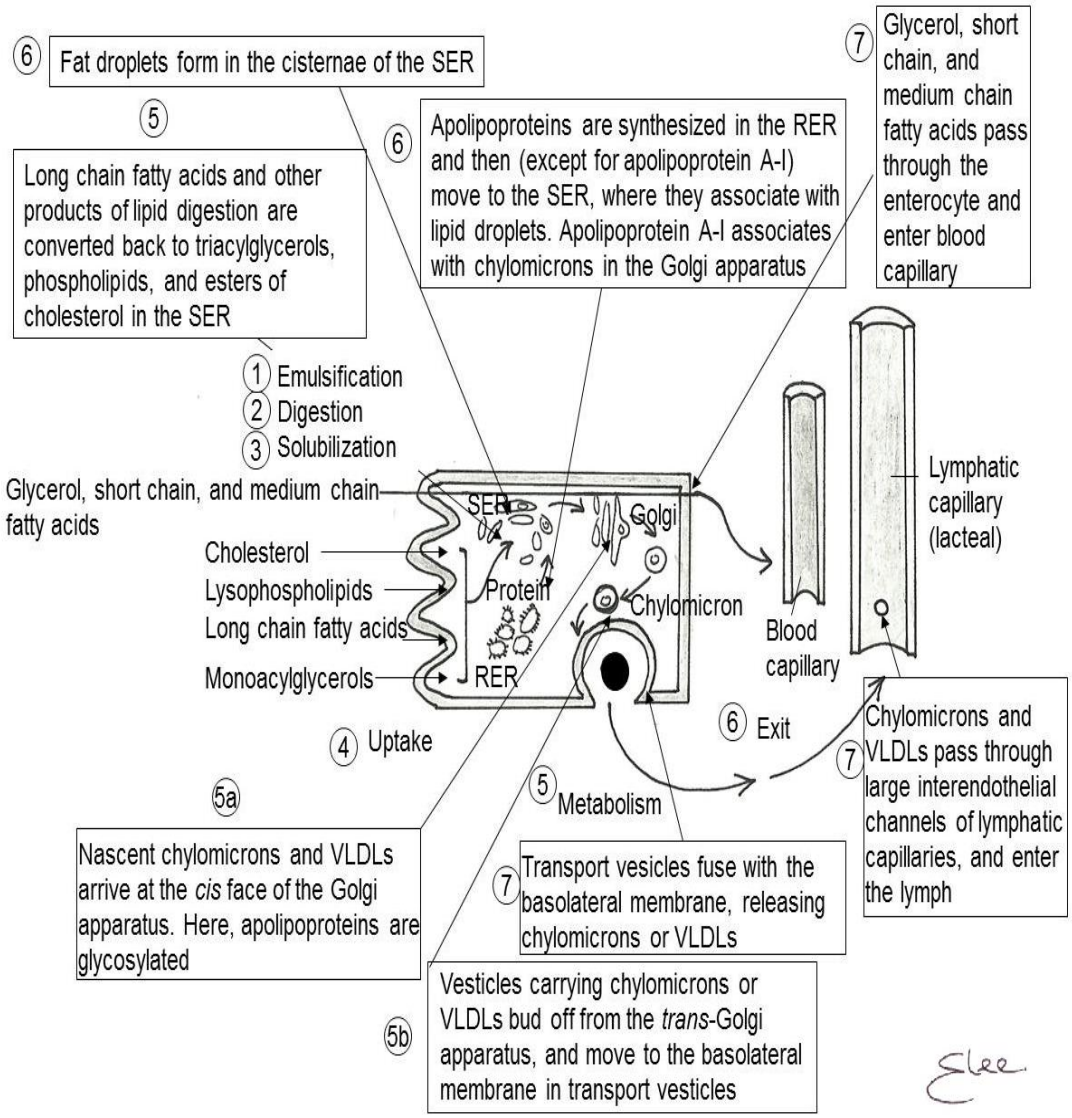
The intestinal brush border membrane (BBM) LPH (lactase-phlorizin hydrolase) gene expression is regulated single-nucleotide polymorphism (SNO).

- Give the method to distinguish in Caucasians between primary and acquired lactase deficiency, on the basis of the LPH SNPs
  - Primary lactase (Lactose Intolerance) deficiency – Genotype CC
  - Secondary deficiency – Genotypes TC and TT

Digestion and Absorption of Triglycerides

- The process of lipid absorption is complex, and includes a role for the pancreatic emulsification, bile salt micellar solubilization, the intestinal unstirred water layer, uptake across the enterocyte BBM, lipid binding proteins in the cytosol of the enterocyte, metabolism by way of the enterocyte endoplasmic reticulum and golgi apparatus, synthesis of and binding to apolipoproteins, and transport across the enterocyte BLM into the lymphatics and portal blood.

- Enterocyte BBM uptake, metabolism and BLM exit of lipids



Adapted from: *Medical Physiology*, Figure 45-14, page 967, Second Edition, 2009

- Origin
  - More than 90% of dietary fat is composed of TAGs (triacylglycerols, aka – triglycerides [TG]), which contain 3 fatty acyl esters (medium-or long-chain saturated or polyunsaturated fatty acids) of glycerol.
  - Animal TAGs contain a low P/S ratio (polyunsaturated-to-saturated) of fatty acids, whereas there is high P/S ratio in vegetable TAGs.
  - This bile content of lipid compares with 4-6 g/day of dietary phospholipids, 0.5 g/day of dietary cholesterol from animal cell membranes, and 70-100 g/day of dietary TAGs.

- Give the processes of emulsification, micellar formation, digestion and absorption of dietary triglycerides.

➢ Emulsification

- Food preparation, chewing of lipids in the mouth and churning, of lipids in the stomach causes their of emulsification.
- Bile contains a rich source of endogenous lipids: 10-15 gm/day of lecithin (phosphatidylcholine), and 1-2 g/day of cholesterol (free, or unsaturated), plus 2-6 g/day of lipid from desquamated intestinal cells.
- The surface of the emulsion droplet is made up of multiple layers of monoacylyglycerol, lysolecithins and other phospholipids as well as FAs.
- The surface monolayer of phospholipids, denatured protein, polysaccharides, and FAs prevents the small emulsion droplets from coalescing into large droplets.
- With emulsification of the lipid (mostly TAGs, DAGs [diglycerides], cholesterol esters [CEs], and non-polar lipids), small droplets are formed with a high ratio of droplet surface area to volume.
- This high surface area optimizes the potential for lipid digestive enzymes to perform their function.

➢ Digestion

- Digestive lipases (gastric, milk, pancreatic) work on the oil-water interfaces of lipids.
- Gastric Lipase
  - Accounts for ~ 25% of TG lipolysis
  - Present in gastric fundus
  - Hydrolyzes TG, but not PL (phospholipids) or cholesterol esters (CE)
  - Chewing and mixing of food in stomach produces an instable emulsion
  - In healthy infants, the activity of pancreatic lipase is low, and the infant relies on gastric lipase and human milk lipase to digest lipid in the diet.
  - In healthy adults, gastric lipases break down about 15% of dietary TAGs.
  - Gastric lipases are serine hydrolases with unique properties.
    - Are optimally active at a pH of 4
    - Are stable in the acid milieu of the stomach

- Are not digested by gastric pepsin
- Are not inhibited by the monolayer which covers the emulsified lipids

- Gastric lipase removes the sm3-fatty acid from TAGs, leaving DAGs and FAs. The long-chain FAs hydrolyzed from TAG by the gastric lipase remain in the lipid droplets.
- Gastric lipase releases some Fas from dietary TAGs, and these FAs release cholecystokinin (CCK) and gastric inhibitory peptide (GIP) from the duodenal mucosa.

- Human milk lipase
  - The lipase in human milk is similar to the pancreatic carboxyl ester hydrolase.
  - Human milk lipase is not affected by acid or pepsin in the stomach.
  - Milk lipase is protected from proteolysis by bile salts.
  - When milk lipase enters the duodenum, it hydrolyzes TAGs, DAGs, MAGs, cholesterol esters, and fat-soluble vitamins.
  - Bile salts enhance the activity of milk lipase. For this reason, milk lipase is also known as bile-salt stimulated milk lipase.
  - Cholesterol ester hydrolase (CEH) (aka pancreatic esterase, cholesterol esterase, and lysophospholipase), acts on esterified cholesterol (CE) and MAG, to form free cholesterol and glycerol.
  - PL, BA (bile acids) and FA (from TG through action of gastric lipase), and CE coat TG droplets, increasing the surface area of the droplet which is available for lipolysis by pancreatic enzymes
  - The surface coat of the TG droplet makes the emulsion stable and gives it a large surface area, but prevents the lipolytic zone of the pancreatic lipase from gaining access to the TG.
  - PLA2 (phospholipase A2) is activated by bile salts and Ca^{2+}
  - Some of the PL on the coat of the TG emulsion is removed by PLA2 (phospholipase A2)
  - Part of the emulsion remains as unilamellar and multilamellar lipid vesicles.
  - 2 FA and IMG (monoglyceride) are released to be solubilized in the bile salt micelles

SO YOU WANT TO BE A GASTROENTEROLOGIST!

- Give the names of the three pancreatic enzymes involved in the lipolysis of triglyceride (TG).
  - Of course TG lipase and colipase, as well as PLRP2 (pancreatic lipase-related protein).

- Give the differences between pancreatic PLRP2 and TG lipase.
  - PLRP2
    - Has greater substrate specificity
    - Breaks down phospholipids and galactolipids
    - No need for colipase
    - No sensitivity to bile salts
    - Appears before birth; persists into adulthood

- Give the effects of pancreatic enzymes on phospholipids and cholesterol.
  - Phospholipids
    - PLA2 (pancreatic phospholipase A2) is secreted as a proenzymes, and is cleaned by trypsin in its active form
    - PLA2 hydrolyzes the 2-position FA from PC (phosphatidyl choline).
    - PLA2 is mostly in the lumen of the upper small intestine; a small amount is attached to the enterocyte BBM
  - Cholesterol
    - CEL (cholesterol ester lipase, aka pancreatic cholesterol esterase) hydrolyzes dietary cholesterol ester to free cholesterol
    - CEL is fully active in the pressure of the bile salts cholic and chenodeoxycholic acid

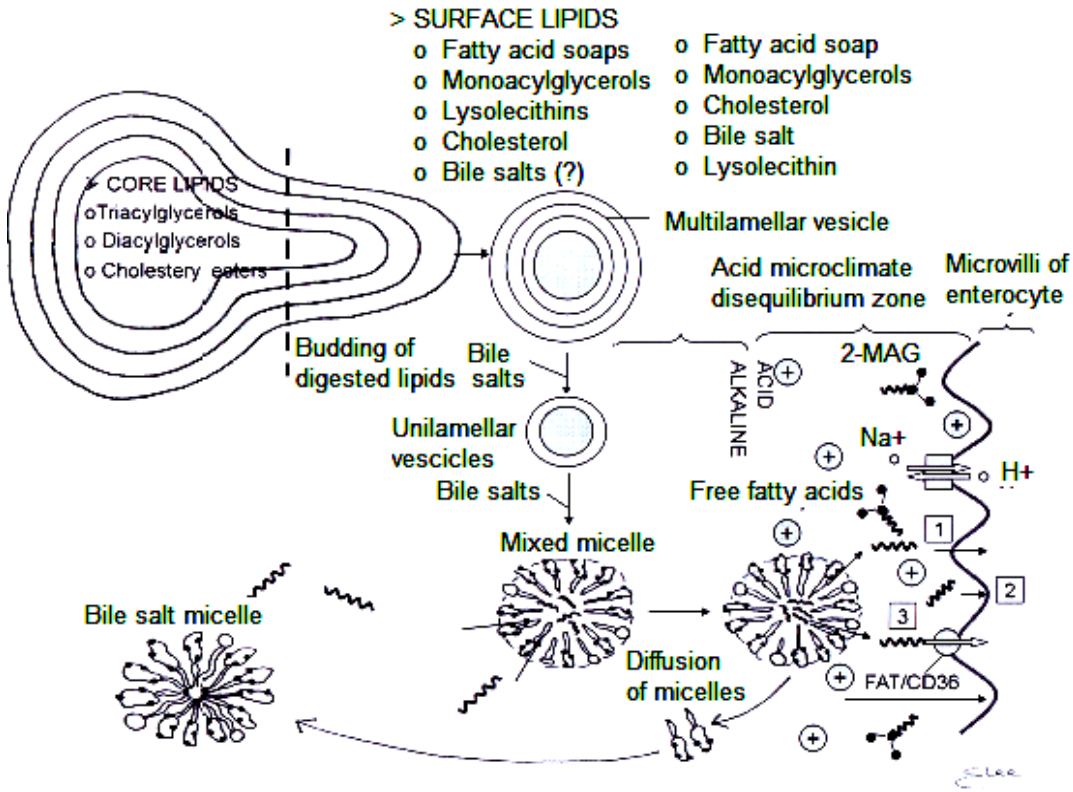
  - Pancreatic lipase
    - The constituents of the monolayer arising from lipid digestion inhibit the activity of lipase.
    - The surface active protein and phospholipids displace lipase from the oil-water interface.
    - This displacement of lipase from the oil-water interface prevents the lipase access to the lipid droplet.
    - In turn this prevents the lipase to begin the next step in the lipid digestion process.

- Pancreatic trypsin cleaves procolipase into lipase and enterostatin (a satiety factor).
- CCK contracts the gallbladder and relaxes the sphincter of Oddi.
- As a result, bile flows into the duodenal lumen.
- CCK also stimulates the secretion of pancreatic enzymes including lipase, pro- colipase, cholesterol ester hydrolase and esterase.
- Colipase and the pancreatic lipase form a complex.
- The pancreatic lipase is adsorbed to the surface lipids, and it's the active hydrolytic sites cannot access the lipid (TAG) and begin digestion of lipid .
- When co-lipase is present, it displaces bile acids from the active hydrolytic sites of pancreatic lipase.

- Maximizing TG-lipase: colipse
- For the TG lipase to achieve its maximum lipolytic activity, there must be
  - Colipase to bind to bile salts and to reduce their inhibitory effect on lipase activity
  - Pancreatic ductular HCO_3- secreted into the duodenal lumen to achieve an intraluminal pH of 6 to 8

- The colipase causes a conformational change in the lipase, opening access to the hydrolytic site to the TAG, and hydrolysis of TAGs begin.
  - The pancreatic lipase-mediated hydrolysis of TAGs removes FAs from the sm1 and sm3 positions.
  - This leaves sm2- FAs and glycerol.
  - PLA2 (phospholipase A2) from the pancreas or Paneth cells acts on glycersphospholipids to form lysophospholipids plus sm2-FAs.
  - Bile salts activate PLA2, just as they activate milk lipase.
  - The TAGs at or near the surface of the lipid emulsion droplet are broken down to MAGs and FAs.
  - TAGs from the core of the emulsion droplet move towards the surface and continue the supply of substrate for hydrolysis by the pancreatic lipase.
  - With digestion of the TAGs, more MAGs and FAs accumulate in the surface lipids.
  - Note that there are completing and balancing roles for bile salts and lipase activity
    - Bile salts inhibit lipase (that's one reason that colipase is needed)
    - Bile salts reduce the optimum pH for lipase activity from 8 to 6.
  - Also note
    - pH < 6, FA poorly soluble bile salt micelles, but readily diffuse across the enterocyte AM
    - pH < 5, glycine-conjugated bile salts precipitate

- Pancreatic TG lipase acts in duodenal lumen, but also attaches to enterocyte AM (brush border membrane), where it is involved in lipolysis TG → FA plus MG (monoglyceride) cholesterol esters → free cholesterol
- A small portion of the surface lipids of the emulsion droplet form a multilamellar liquid-crystalline-micelle.



➢ Liver and gallbladder – micellar solubilization

- When the concentration of bile salts (BS) in the duodenum is above the CMC (critical micellar concentration negatively-charged spheres, simple micelles are formed.
- Simple BS micelles solubilize FA, MG (monoglyceride), and Chol (free cholesterol), forming mixed micelles (50-80 nm).
- The surface area-to-volume ratio of the vesicles becomes larger as the particles become smaller (emulsion droplet to multilamellar vesicle, multilamellar to unilamellar vesicle, unilamellar vesicles to mixed micelle).
- As the area-to-volume ratio of these vesicles increase, the rate of TAG hydrolysis becomes faster.
- If the CMC is not present in the intestinal lumen, lipid malabsorption results.

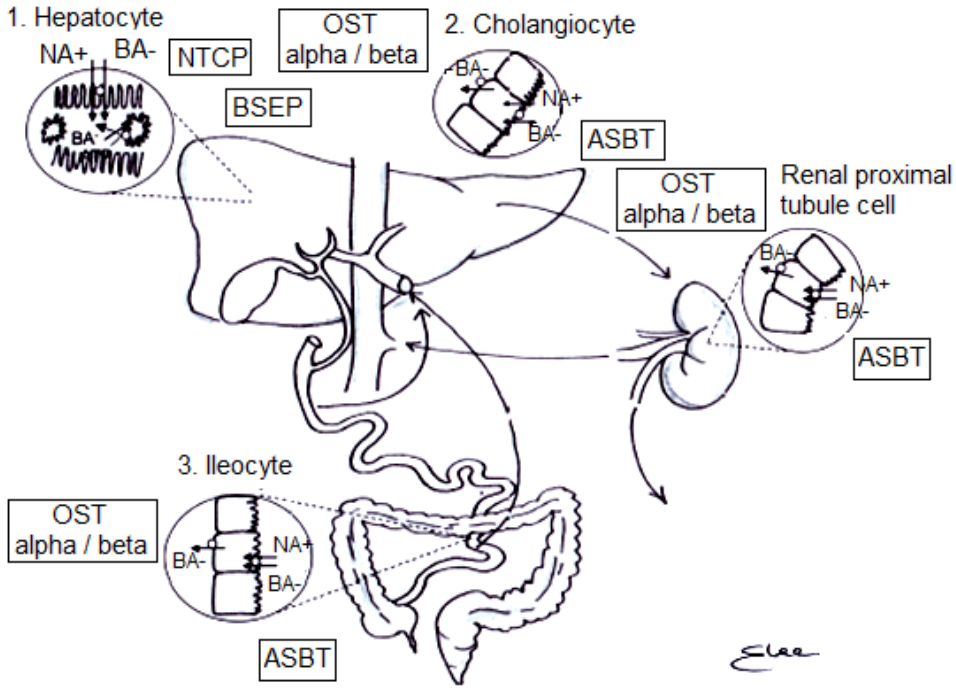
- In the absence of bile salts and therefore bile salt micelles, some lipid may still be transferred to the AM (brush border membrane) of the enterocyte in the unilamellar and the multilamellar lipid vesicles.
- The hydrophobic portions of the lipids in the mixed micelle face inwards towards the core.
- The BSM contains several forms of lipids, and is known as a mixed micelle (MM).
- When the CMC is present in the lumen, lipids are solubilized and can be absorped.
- Mixed micelle carry lipids through the acidic unstirred layer to the surface of the enterocyte.

➢ Enterohepatic circulation of bile acids

- The topic of the enterohepatic circulation of bile acids has been considered in detail in the chapter on the liver. As a helpful reminder of this complex process, a figure is shown here.

."You cannot solve a problem with the same mind that created it"

Albert Einstein



Abbreviations: 1) Hepatocyte: BA^-, bile acid; NTCP, Na^+ taurochoate transporting polypeptide (SLC10A1); BSEP, bile salt export pump (ABCB11); 2) Cholangiocyte: OST, organic solute transporter; 3) Ileocyte: OST, organic solute transporter; ASBT, apical sodium bile acid transporter (SLC10A2); 4) Proximal renal tubular cell: OST, organic solute transporter; ASBT, apica bile acid transporter (SLC10A2)

Adapted from: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Ninth Edition, 2010, Figure 64-3, page 1082.

- Cytosol of the enterocyte
  - Lipid-binding proteins
    - L-FABP (liver-type fatty acid binding protein) for MG and lysophosphatidylcholine
    - I- FABP (intestinal FABP), for FAs
    - ILBP (ileal lipid-binding protein)
  - Characteristics
    - Jejunum > ileum
    - Villus, crypt cells
    - Unsaturated > saturated fatty acids
    - Long-chain fatty acids >>> medium or short-chain fatty acids

- Functions
  - Intracellular transport of lipids to ER for resynthesis of TG from FA plus MG
  - Intracellular control of lipid metabolism
  - Gene expression

- Intestinal unstirred water layer
  - Adjacent to the brush border membrane (BBM aka apical membrane [AM]) of the enterocytes in the upper small intestine, there is a layer of poorly stirred water, the unstirred water layer (UWL).
  - The UWL has dimensions of thickness, surface area, acidity and viscosity (from secreted mucus).
  - The diffusion coefficient of solutes through the UWC diffusion barrier is slower than in the bulk phase of the liquid in the intestinal lumen.
  - In the small intestine, the UWL creates an acid microenvironment between the alkaline contents in the lumen, and the AM (in the stomach, the UWL creates an alkaline microenvironment).
  - The mixed micelles diffuse across the UWL (unstirred water layer) present adjacent and external to the enterocyte BBM.
  - The BBM NHE1 transporters H^+ into the UWL, creating an acid microclimate in the UWL.
  - In the acid microclimate of the UWL, the FAs in the bile salt micelle lose their solubility in the micelles and partition into the acidic UWL.
  - The FA in the acidic UWL become protonated, and can then diffuse across the BBM, or be moved by a transporter (CD36 / FAT [fatty acid transporter])
  - Once the FA, MG and Chol are released and absorbed from the bile salt micelles, the simple micelles comprise of BA shuttle back into the intestinal lumen, where more lipolytic products (FA, MG, Chol) are solubilized, and are now ready to diffuse across the UWL and through the BBM enterocyte.
  - Thus, the bile salts in the micelle are not absorbed intact in the jejunum, but their contents are absorbed.

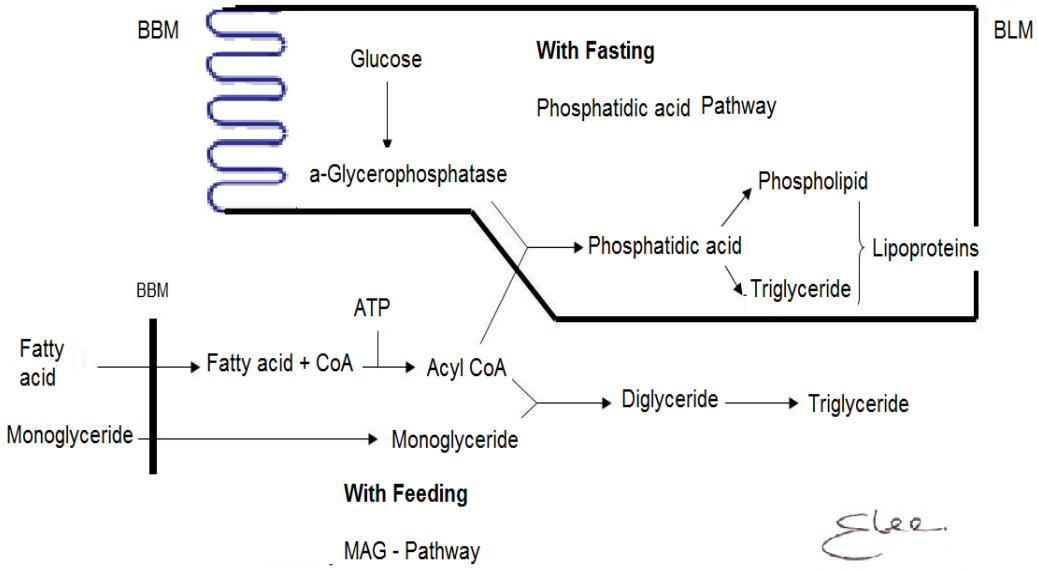
- Enterocyte
  - 2-MAG, fatty acids, lysophospholipids, and cholesterol leave the mixed micelle and enter an acidic microenvironment created by the apical Na^+-H^+ exchanger.
  - The lipids enter the enterocyte by (1) non-ionic diffusion; (2) incorporated into the enterocyte membrane (collision), or (3) carrier mediated transport.

- Intestinal unstirred water layer
  - Brush border membrane (BBM)
    - Passive diffusion of protonated fatty acids (FA)
    - BBM transport proteins for FA
      - CD36 / FAT (fatty acid transporter)
      - FATP4 (fatty acid transport proteins) / SCF (solute carrier family) 27 – expression may be regulated by PPA, RXR, TNF-α
      - LACS (long-chain fatty acyl-CoA synthetase) in cytosol of enterocyte
    - BBM transport protein for cholesterol
      - nPC 1L1 (Niemann-Pick-C1-like protein) also known as mixed micelle [MM])
  - The bile acid micelle (BAM) slowly diffuses across the UWL, and acts as a reservoir for the high concentration of lipids.
  - Cholesterol, lysophospholipids, long chain fatty acids (LCFA), monoacylglycerol are presented to the AM of the enterocyte.
  - Once the BAM reaches the BBM, these lipophilic lipids in the BAM may move either directly into the highly lipophilic BBM, or may partition from the BAM into a liquid phase and then be transported across the BBM.
  - Extracellular LCFAs may bind directly to a fatty acid transport protein (FATP) dimer complex in the AM, and be transported into the enterocyte.
  - Alternatively, LCFAs may bind first to CD36, which then transfers the LCFA to FATP dimer.

Adapted from: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Figure 45.12 and 45.13, page 965-966, Ninth Edition, 2010.

- Endoplasmic reticulum (ER) of the enterocyte
  - Once the lipophilic lipids such as long-chain fatty acids [LCFAs], monoacylglycerols [MAGs], lysophospholipids and cholesterol are absorbed, there is a complex series of intracellular metabolic steps.
  - In the enterocyte cytosol, there are numerous fatty acid binding proteins (FABPs) which lower the cytosolic concentration of FAs, and carry lipids to the SER (smooth endoplasmic reticulum).
  - Intracellular LCFAs are coupled to coenzyme A (CoA) by long-chain fatty acyl-CoA synthetase (LACS), preventing their efflux, cytoplasmic fatty acid binding protein (FABP) acts as a buffer for LCFA.
  - In the SER, the products of lipid digestion which have crossed the AM are metabolized back to the more lipophilic TAGs, CEs (cholesterol esters) and phospholipids.

- Metabolic fate of absorbed fatty acids and monoglyceride in enterocytes



Abbreviations: BBM, apical brush border membrane (BBM); BLM, basolateral membrane (BLM)

Adapted from: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Figure 100-6, page 1704, Ninth Edition, 2010.

- These lipophilic products of digestion form lipid droplets in the cisternae of the SER.
- After a meal, the large amounts of MAGs and FAs, which entered the enterocyte through the BBM, are re-esterified by the MAG-pathway.
- Phosphatidic acid pathway (active during fasting)
  - Alpha glycerophosphate is acylated to phosphatidic acid, then to either phospholipid or TG (SER)
- Acetyl CoA synthetase activates especially the LCFAs to be used in both the MAG and the phosphatidic acid pathways.
- The fatty acids in the liver undergo metabolism, by β-oxidation, to acetyl CoA, or reform TAG.
- Acetyl CoA may be used for
  - Entry into the citric acid cycle to produce energy
  - Production of acetoacetate, to be used for energy not by the liver but instead by the brain, kidney, and muscle.
  - Production of ketone bodies (acetoacetate, acetone and β-hydroxybutyrate) which may accumulate in excess during fasting or diabetic ketoacidosis.

- Monoglyceride pathway (feeding)
  - Absorbed FA is activated to acyl coenzyme A, and TG is synthesized in SER (smooth endoplasmic reticulum)
- During fasting, FAs entering the enterocyte across the BM are re-esterified by the phosphatidic acid pathway.
- This phosphatidic acid in the enterocyte is derived from G-3-P (glycerol-3-phosphate), produced from glucose and amino acids, or from the breakdown of biliary lecithin).

➢ Apolipoprotein

- The proteins which are secreted by the enterocyte RER are called "apolipoproteins" (or apoproteins).
- The apolipoproteins bind to the TAGs and CEs to form of chylomicrons and very-low-density lipoproteins (VLDL) in the tubular endoplasmic reticulum and Golgi apparatus.
- The chylomicrons and the VLDLs contain the same apolipoproteins.
- The chylomicrons are larger (~250 nm) than the VLDLs (30-80 nm).
- Chylomicrons contain mostly exogenous (dietary lipids), whereas the VLDLs contain mostly endogenous lipids.
- Apo A-I / -II
- Apo B-48 (apoprotein B 48)
  - Intestinal post-transcriptional editing of apo B mRNA by way of deamination of cytidine produces a stop codon and Apo B-48, rather than apo B-100 as is produced in hepatocytes.
  - Absorbed FFA plus monoglyceride is reformed to TG (triglyceride)
  - Absorbed cholesterol is re-esterified to cholesterol ester (CE)
  - TG + CE is packaged into nascent chylomicrons
  - Nascent chylomicrons (NCM) contain (approximately)
    - TG 90
    - PL 8
    - CE, FSV 2
    - FSV
    - Apo A-I, -II, -IV, apo B-48
  - NCM cross the BLM (basolateral membrane) of the enterocytes and then pass into the interstitial space.
  - NCM in lacteals pass through the thoracic duct, and into the systemic circulation
  - In the interstitial space
    - Apo c- I is added to the NCM.

- Apo c- II activates LPL (lipoprotein lipase), and apo c – III inhibits LPL activity.
- LPL releases TG from the CMR (chylomicron remnant), depending on the balance between apo C – II and apo C – III.
- The CMR gains apo E, which targets the CMR to the CMR-R (CMR receptor) on the hepatocyte.
- The CMR is taken up into the hepatocyte by way of the CMR-R

➢ Golgi Apparatus

- Apolipoproteins A-1 from the RER associated with chylomicrons do not traffic to the SER, but instead go directly to the Golgi.
- At the cis face of the golgi, VLDL apolipoproteins arrive at the *cis* face of the Golgi apparatus, are glycosylated, containing the nascent chylomicron (NCM) and VLDL.
- The vesicles which carry chylomicrons or VLDLs bud off from the *trans*-Golgi apparatus, forming transport vesicles.
- These vesicles carrying chylomicrons and VLDL diffuse to the BLM.
- The chylomicrons and VLDL cross the BLM into the lymphatics by the process of exocytosis.

➢ Basolateral membrane of enterocyte

- Chylomicrons and VLDL exit the enterocyte across the basolateral membrane (BLM) by a process of exocytosis.
- Glycerol, as well as short- and medium- chain length fatty acids avoid the apolipoproteins, transport vesicles, and lymphatics. And pass directly from the enterocyte cytosol, across BLM, and into the portal circulation.
- The wall of the lymphatics is highly permeable due to large fenestrations in its endothelial wall.
- The chylomicrons and the VLDLs readily cross through the fenestrations and into the lymphatic circulation.
- Chylomicrons and VLDL enter the intestinal lymphatics, from which they pass to the thoracic duct and from there to the systemic circulation.

➢ Hepatocytes

- The hepatic Fas re-esterified with glycerol to TAG are bound to lipoprotein, forming VLDL (very low density lipoprotein).
- VLDL may be stored in the liver, or exported from the liver for use by the peripheral tissue, where the VLDL are broken down by LDL.
- In the fasting state, VLDL shuttle endogenous TAG to muscle and adipose tissue, whereas chylomicrons are important in the fed state.
- Apo B-100

- Binds to LDL (low-density lipoprotein) R (receptor) [LDLR]

- Apo C – I reduces uptake of chylomicron remnants by liver
- Apo E
  - Present in VLDL, LDL, HDL, IDL
  - Binds to LDL
  - Removes serum chylomicron remnants
  - Acts as a cofactor to activate LCA (lecithin-cholesterol acyltransferase) which esterifies cholesterol to cholesteryl ester
- Apo A– I / - II
  - Apo A – I is a major component of HDL
  - Accepts cholesterol from cell membrane when lipid state of the body is low.

SO YOU WANT TO BE A HEPATOLOGIST!

- Give the mechanism by which cholesterol is transferred to the liver from non-hepatic tissues.
  - Hepatic LCAT, in the presence of apo A-I, esterifies cholesterol (free cholesterol

(free cholesterol ---------- LCAT / apo A – I → Cholesterol ester [CE]

  - CCTP (cholesterol ester transfer protein) is made in the liver and associates with HDL in the plasma

(HDL-CE ---------- CETP → CE)

  - PLTP (phospholipid transfer protein) also aids in the transfer of cholesterol from non-hepatic tissue to the liver.

TRICK QUESTION

Abetalipoproteinemia results in the small intestine and liver not making lipoprotein with TG.

- Give the correct mechanism of the lipoprotein – poor lipoproteins in abetalipoproteinemia, as well as the often quoted incorrect / incomplete mechanism.
  - Yes, in abetalipoproteinemia the synthesis of Apo B is reduced, but the real cause of the lipoprotein-poor lipoproteins is the mutation of the MTP (microsomal triglyceride transfer protein).

- Adipocyte
  - TG (triglyceride / and Ch (Cholesterol) in the liver are bound to VLDL
  - TG bound in VLDL is started in adipocytes
  - During fasting, activation of H-SL (hormone-sensitive lipase) in the adipocytes releases FFA from adipocytes into the serum.
  - FFA in the serum are bound to albumin.
  - Albumin-bound FFA are transported to tissues, including the liver. For the resynthesis of TG an PL (phospholipids).
  - In the hepatocyte cytosol, FFA are bound to FABP (fatty acid binding protein)
  - FABP directs FFA towards
    - sER for VLDL synthesis
    - peroxisomes for beta-oxidation
  - Peroxisome proliferating agent regulate transcription of FABPs.
  - Source of fatty acids
    - Fasting, VLDL ---HDL---→ FFA
    - Feeding, chylomicrons
  - Lipolysis of VLDL in the liver ----→ nascent HDL
  - Lipolysis of chylomicrons in the intestine ------→ nascent HDL
  - Apo a –I and apo A – II are the major apolipoproteins of HDL
  - Apo a- I / - II can recycle between HDL and chylomicrons.
- Nuclear receptor transcription factors
  - LXR-α and –β are nuclear receptor transcription factors which are activated by cholesterol and its derivatives.
  - LXR-α and –β form complexes with RXR (retinoid X receptors).
  - The LXR-RXR complexes bind to LXRE (LXR response element)
  - LXR-α and β form complexes bound to LXRE (LXR response element).

SO YOU WANT TO BE A HEPATOLOGIST!

When the serum triglyceride (TG) concentration is markedly elevated, the serum is lipemic (i.e. cloudy). If persons with cholestatic liver disease have marked hyperglyceridemia, the serum is not lipemic.

- Give the reason for the unexpected lack of lipemic serum in cholestatic liver disease when the serum TG levels are increased.
    - In the presence of cholestasis, there are 3 LDL-like lipoproteins which bind serum TG.
        - Normal LDL
        - LP-Y (lipoprotein Y)
        - LP-X (lipoprotein X)
    - In cholestasis, most of the TGs are bound to LP-Y as well as LDL, and the serum remains clear (non lipemic).

- Homeostasis
    - Lipids activate the LXR-RXR complexes bound to LXRE to regulate the expression of genes involved in bile acid synthesis, cholesterol metabolism and fatty acid synthesis.
    - In the presence of bile acids (BAs), several of the layers of lipid in the surface lipid coating are displaced, leaving a unilamelar vesicle.
    - When a critical concentration of BSs is present in the intestinal lumen (known as the critical micellar concentration [CMC] about 5 mm), a negatively charged sphere of BAs forms.
    - This sphere of BA is called a bile acid micelle (BAM).
    - The surface lipids are still comprised of FAs, MAGs, PLs, cholesterol and bile salts.
    - As more and more BSs are added, the unilamellar vesicles are transformed into negatively charged spheres, the bile acid micelles (BAM).
    - The hydrophilic portion of the unilamellar bile acid vesicles face outwards, where they interface with the fluid contents of the intestinal lumen.

Clinical Tip

- The performance characteristics of qualitative fat analysis by the staining of spot a stool sample with Sudan dye reflects why this screening test is not used in adult gastroenterology:
    - Sensitivity, 78%
    - Specificity, 70%

Intestinal Cholesterol Digestion and Absorption

Small Intestine

- Digestion and absorption
  - Cholesterol in the diet is in the form of esters.
  - Cholesterol esters are hydrolysed by pancreatic cholesterol esterase, forming free cholesterol
  - Dietary and secretory cholesterol are in the ester from, and the free cholesterol must just be de-esterified to free cholesterol by pancreatic cholesterol ester hydrolase (CEH).
  - The free cholesterol is solubilized in BAMs with TAGs and PLs (phospholipids); forming mixed micelles.
  - The mixed micelles diffuse through the intestinal water layer (WWL), and up close to the BBM (brush border membrane) of the enterocyte.
  - The lipid constituents partition from the mixed micelle and cross the BBM of the enterocytes, by passive diffusion through the lipid bilayer of the BBM, as well as by transport proteins in the AM transporter for cholesterol is NPC1L1 (the Niemann – Pick C1 like transporter protein).

- Endoplasmic reticulum of the enterocyte
  - Enterocytes absorb, metabolize and export dietary (exogenous) as well as secreted (endogenous) lipids.
  - In the enterocyte, and more so in the hepatocyte, cholesterol is synthesized by way of HMG-CoA coreductase.
  - Once the absorbed and synthesized cholesterol is in the enterocyte, it is esterified by ACAT (acyl CoA ester cholesterol transferase), in the endoplasmic reticulum.
  - TG, CE (cholesterol esters) and phospholipids (PL) are packaged for export
    - TG and PL are synthesized in SER
    - Apolipoproteins are synthesized in RER
    - VLDL and chylomicrons are formed in the ER and Golgi apparatus
    - During fasting
      - VLDL (very-low density lipoproteins) (FA from PL) are formed.

- During feeding
  - Chylomicrons (FA from food TG) are formed
  - 750-6000 nm
  - Coat of chylomicrons is PL, CE, and apolipoproteins
  - Core of chylomicrons is TG
  - Apo A coats VLDL, Apo A and B coat chylomicrons

- Apoproteins
  - The enterocytes synthesize apoprotein I and II (A-I and A-II).
  - Esterified cholesterol is solubilized by binding to apoprotein.
  - A-I and A-II are part of the apoproteins forming chylomicrons in the enterocyte.
  - The apolipoproteins pass to the Golgi apparatus, where they are glycosylated.

- Basolateral membrane of the enterocyte
  - The chylomicrons remnants in the fed state and VLDLs pass through large interendothelial channels of lymphatic capillaries, and enter the lymph fluid in the lymphatic system.
  - The ATP-binding cassette (ABC) transporter, ABCA1 translocates cholesterol, and phospholipids (PLs) to the BLM.
  - Dietary triacylglycerol (TAG, aka triglycerides) and cholesterol are exported across the enterocyte BLM as chylomicrons.
  - Chylomicrons are 80% to 90% TAG, plus cholesterol, phospholipids, and apoproteins are secreted by the enterocyte, passing through the BM.
  - Chylomcrons pass from the BLM to lymphatics, to the thoracic duct, and into the systemic circulation.
  - The glycerol and fatty acids are digested and leave the chylomicrons. which now contain just cholesterol.
  - The fatty acids in the liver undergo metabolism, by β-oxidation, to acetyl CoA, or they acerol to reform TAG.
  - Acetyl CoA may be used for
    - Entry into the citric acid cycle to produce energy
    - Production of acetoacetate, to be used for energy not by the liver but instead by the brain, kidney, and muscle.
    - Production of ketone bodies (acetoacetate, acetone and β-hydroxybutyrate) which may accumulate in excess during fasting or diabetic ketoacidosis.

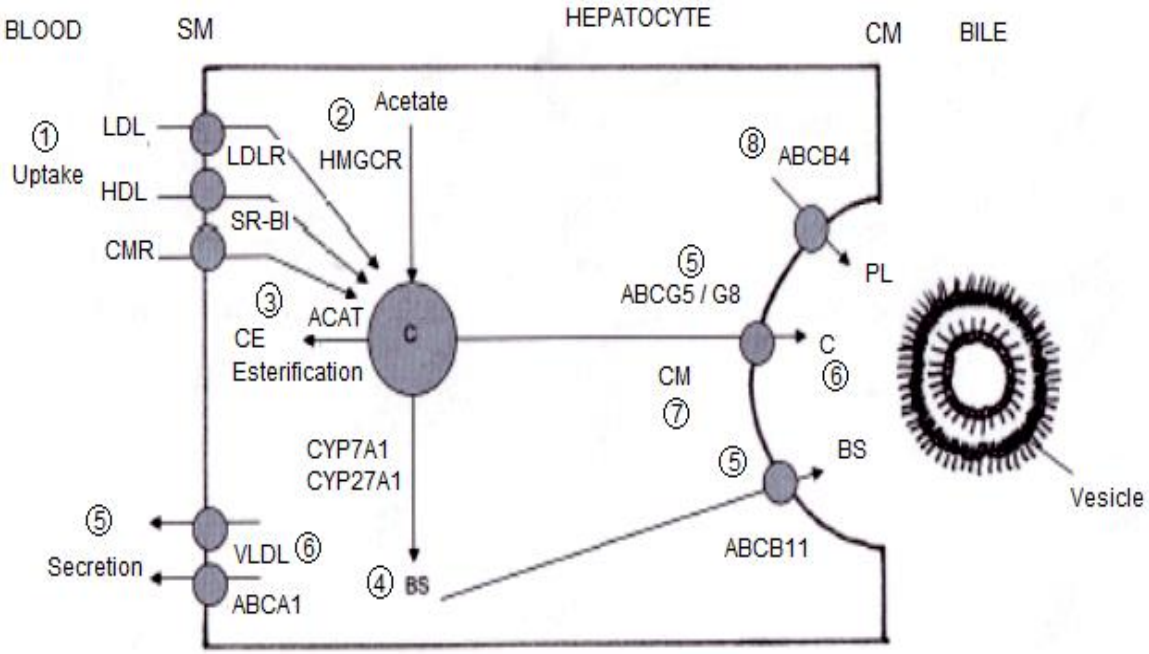
- The hepatic Fas re-esterified with glycerol to TAG are bound to lipoprotein, forming VLDL (very low density lipoprotein).
- VLDL may be stored in the liver, or exported from the liver for use by the peripheral tissue, where the VLDL are broken down by LDL.
- In the fasting state, VLDL shuttle endogenous TAG to muscle and adipose tissue, whereas chylomicrons (CM) are important in the fed state.

➢ Systemic circulation-remnant chylomicron

- LPL is present in the walls of the capillaries in muscle and adipose tissue.
- The TAG in chylomicrons are partially broken down by lipoprotein lipase (LPL).
- The remnant chylomicrons bound to the LDL- and LDL-related receptors enter the hepatocyte by receptor-mediated endocytosis.

Hepatic Cholesterol Synthesis

The topic of hepatic cholesterol synthesis and lipoprotein metabolism are not usually texted in examinations of basic / non-subspecialist gastroenterology or hepatology.



Abbreviations: CH, cholesterol; CM, canalicular membrane; SM, sinusoidal membrane

- Hepatocyte

  - 1) **Uptake**: Cholesterol in portal blood is bound mostly to LDL, with lesser amounts bound to HDL and to cholesterol-enriched chylomicrons are called remnant (CMRs).

  - The hepatic **uptake** of cholesterol is mediated by three proteins:
    - The low-density lipoprotein (LDL) receptor (LDLR) for LDL
    - Scavenger receptor class B type I (SR-BI) for high-density lipoprotein (HDL), and
    - The chylomicron remnant receptor (CMRR) for chylomicron remnants (CMR).

  - Some CMR bind to SM LDLR delivered to the liver as well as to LDL-related receptor.

  - The chylomicron and VLDL cross the sinusoidal membrane (SM) of the hepatocyte and mix with, the absorbed cholesterol synthesized de novo through the action of HMG CoA reductase.

  - CM-Rs contain a small amount of TAGs

  - Hepatocyte SR-B1 (for HDL)
    - HDL-CE binds to SR-B1 (scavenger receptor class B type 1) on the hepatocyte.
    - SR-B1 leads to the uptake of HDL-CE (this is not a precess of endocytosis).
    - HDL-CE may also transfer its cholesterol ester (CE) to VLDL, IDL and LDL.
    - This transfer of HDL-CE occurs through the action of CETP (cholesterol ester transfer protein).

  - This removal of cholesterol from peripheral tissues by SR-B1 and CETP, and the eventual excretion of cholesterol in bile is called reverse cholesterol transport.

  - Also, the TAG broken down by LPL contains certain long-chain fatty acids (LCFA) which are taken up only by the liver.

  - These LCFA cross the SM of the hepatocyte by facilitated uptake as well as “flip-flow” across the membrane.

  - At the Golgi apparatus the apolipoproteins are glycosylated.

  - This VLDL-derived cholesterol leaves the non-hepatic tissue by the cholesterol efflux transporter (CET), ABCA1.

- This cholesterol binds to HDL (high density lipoproteins).
- The ATP binding cassette (ABC) transporter ABCA1 may translocate, either directly or indirectly, cholesterol and phospholipids to the cell surface, where they appear to form lipid domains that interact with amphipathic a-helices in apolipoproteins.
- This interaction solubilizes these lipids, and nascent HDL particles dissociate from the cell.
- The same steps are followed for the association with apoproteins, glycosylation of apolipoproteins, the budding off of chylomicrons and VLDL from the Golgi apparatus, and the transport of the chylomicrons across the canalicular membrane (CM) of the hepatocyte and into the bile.
- 2) **Biosynthesis**: The liver also synthesizes cholesterol de novo from acetyl CoA and mevalonate.
- Biosynthesis of hepatic cholesterol (CH) from acetate is regulated by the rate limiting enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR).
- The enzyme HMG-CoA reductase is in the membrane of the ER (endoplasmic reticulum) of the hepatocyte.
- The activity of HMG-CoA reductase is increased by
  - ↓ cell cholesterol
  - ↓ cell mevalonate
  - ↑ cell demand for metabolites derived from mevalonate
  - ↑ gene translation
  - Depletion of the bile acid pool
- This upregulation occurs by the process of
  - ↑ stability HMG-CoA reductase.
- The activity of HMG-CoA reductase is reduced by fasting, high levels of cholesterol in cells, and sterol acting on sterol regulatory elements (SRE) and sterol regulatory element binding proteins (SREBPs).
- 3) Part of the cholesterol is esterified by acyl-coenzyme A: cholesterol acyltransferase (ACAT) for storage in the liver.
- Free cholesterol plus the phospholipid lecithin, are acted upon by the enzyme LCAT lecithin cholesterol acyltransferase.
- The LCAT forms cholesterol esters plus lysolecithin from the free cholesterol and lecithin.

- The HDL is now enriched with cholesterol esters (CE), forming HDL-CE.
- 4) A propotion of cholesterol is used for the synthesis of bile salts via the 'neutral' and the 'acidic' pathways, as regulated by two rate limiting enzymes.
- These rate limiting enzymes are
  - Cholesterol 7α-dehydroxylase (CYP7A1), the "neutral" pathway, and
  - Sterol 27-hydroxylase (CYP27A1), the "acidic" pathway.
- 5) The bile salts cross the CM by ABCB11
- The small amount of TAG delivered to the hepatocytes in the CMRs undergoes breakdown into glycerol and fatty acids (FAs).
- In the hepatocyte, lysosomes break down to CMRs.
- 6) Some of the cholesterol is used for the formation of very low density lipoprotein (VLDL), which is secreted into the blood by ABCA1 (ATP-binding cassette transporter).
- The digestion of VLDL by LDL yields cholesterol (and phospholipid) from the surface of the VLDLs.
- This VLDL-derived cholesterol leaves the non-hepatic tissue by the cholesterol efflux transporter (CET), ABCA1.
- This cholesterol binds to HDL (high density lipoproteins).
- 7) Chylomicrons are 80% to 90% TAG, plus cholesterol, phospholipids, and apoproteins are secreted by the enterocyte, passing through the BLM.
- CM pass across the BLM to lymphatics, to the thoracic duct, and into the systemic circulation by ABC65/68.
- The glycerol and fatty acids are digested and leave the chylomicrons. which now contain just cholesterol.
- These cholesterol-enriched chylomicrons (CM) are called remnant chylomicrons (RCM).
- The RCM contain small amounts of DAG, and large amounts of cholesterol.
- The RCMs pass in the systemic circulation to the liver.

➢ Hepatic excretion of cholesterol across the canalicular membrane (CM)

- The apoproteins (except for apolipoprotein [A-I] traffic to the SER, where they associate with lipid droplets.
- Apolipoprotein A-I is associated with chylomicrons in the Golgi apparatus.
- Apolipoproteins solubilize cholesterol and phospholipids, and generate nascent HDL particles.

- This interaction solubilizes the lipids and generates nascent HDL.
- The nascent HDL particles dissociate from the cell.
- Nascent chylomicrons and VLDLs (very low density lipoproteins) arrive at the cis face of the Golgi apparatus.
- Some cholesterol is used for the formation of very-low-density lipoprotein (VLDL), which is secreted into the portal blood.
- The vesicles which carry chylomicrons or VLDLs bud off from the trans-Golgi apparatus, forming transparent vescicles.
- The transport vesicles traffic to the BM, releasing their chylomicron or VLDLs by exocytosis.
- Glycerol, short chain, and medium chain fatty acids pass through the enterocyte, across the BM, and enter blood capillary.
- These products avoid the apolipoproteins, transport vesicles, and lymphatics.
- The synthesis is regulated by two rate-limiting enzymes cholesterol 7α-hydroxylase (CYP7A1), the “neutral pathway”, and sterol 27-hydrolase (CYP27A1), the “acidic” pathways.
- The HDL particles leave the hepatocyte.
- Cholesterol (Chol), bile salts (BS), and phospholipids (PL) are secreted across the canalicular membrane by the three lipid transporters ABCG5/G8, ABC B11, and ABCB4, respectively.
- 8) PL (phospholipid) cross CM by ABCB4.
- CM secretion: ABCG5/ G8, ABCB11, ABC B4; ABC transporters in AM; PL, phospholipids

Adapted from: *Sleisenger and Fordtran’s Gastrointestinal and Liver Disease,* Ninth Edition, 2010, Figure 65-4.

Absorption of Fat Soluble Vitamins A, D, E, and K

- Gastric acid and proteolysis releases the esterified fat soluble (FS) vitamins from their associated proteins.
- The free form of the FS vitamins is formed from dietary esters by the action of the carboxyl ester hydrolases in pancreatic juice and in the AM of the enterocytes.

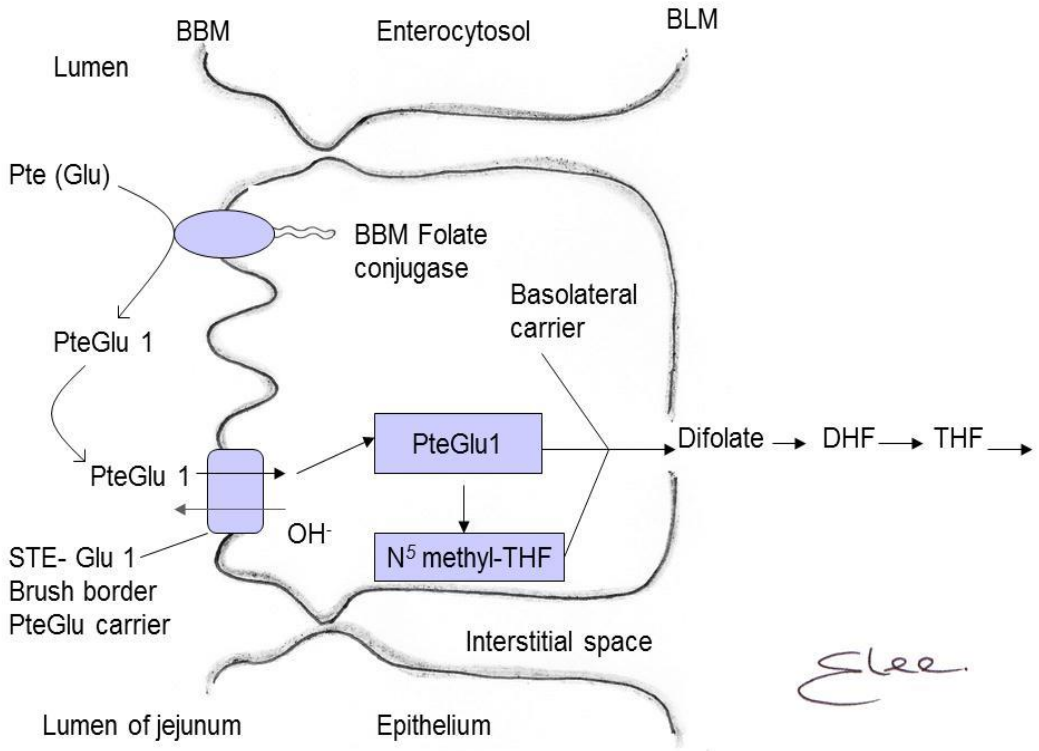
- The assimilation of the fat soluble (FS) vitamins A, D, E, and K, generally follows that of the other lipids. Gastric HCl, and proteolysis releases the esterified FS vitamins from their associated proteins.

Absorption of Water Soluble Vitamins

Folic Acid

- Tetrahydrofolae (THF) is essential for the synthesis of DNA.

FOLATE ABSORPTION



Abbreviations: BBM, brush border (apical) membrane; BLM, basolateral membrane; DHF, dihydrofolate; THF, tetrahydrofolate

Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Figure 45-15, page 970.

- Diet
  - Dietary folate is comprised of pteridine, p-amino benzoates and seven glutamate molecules (PteGlu7; aka folate polyglutamate or pteroylpolyglutamate).
  - Dietary folate is similar to medicinal folate, other that having several glutamate residues.

- Give the physiology of the absorption of dietary folic acid.
  - BBM folate conjugase (aka peptidase) removes (i.e., deconjugates) all but one glutamate from the dietary folate molecule, leaving PteGlu1 (monoglutamate).
  - PteGlu1 is transported into the enterocyte by the BBM carrier Ste-Glu1.
  - Ste-Glu1 is a folate-OH^- exchanger
  - The pH of 5 is optimum for BBM folate conjugase and the folate-OH- exchanger.

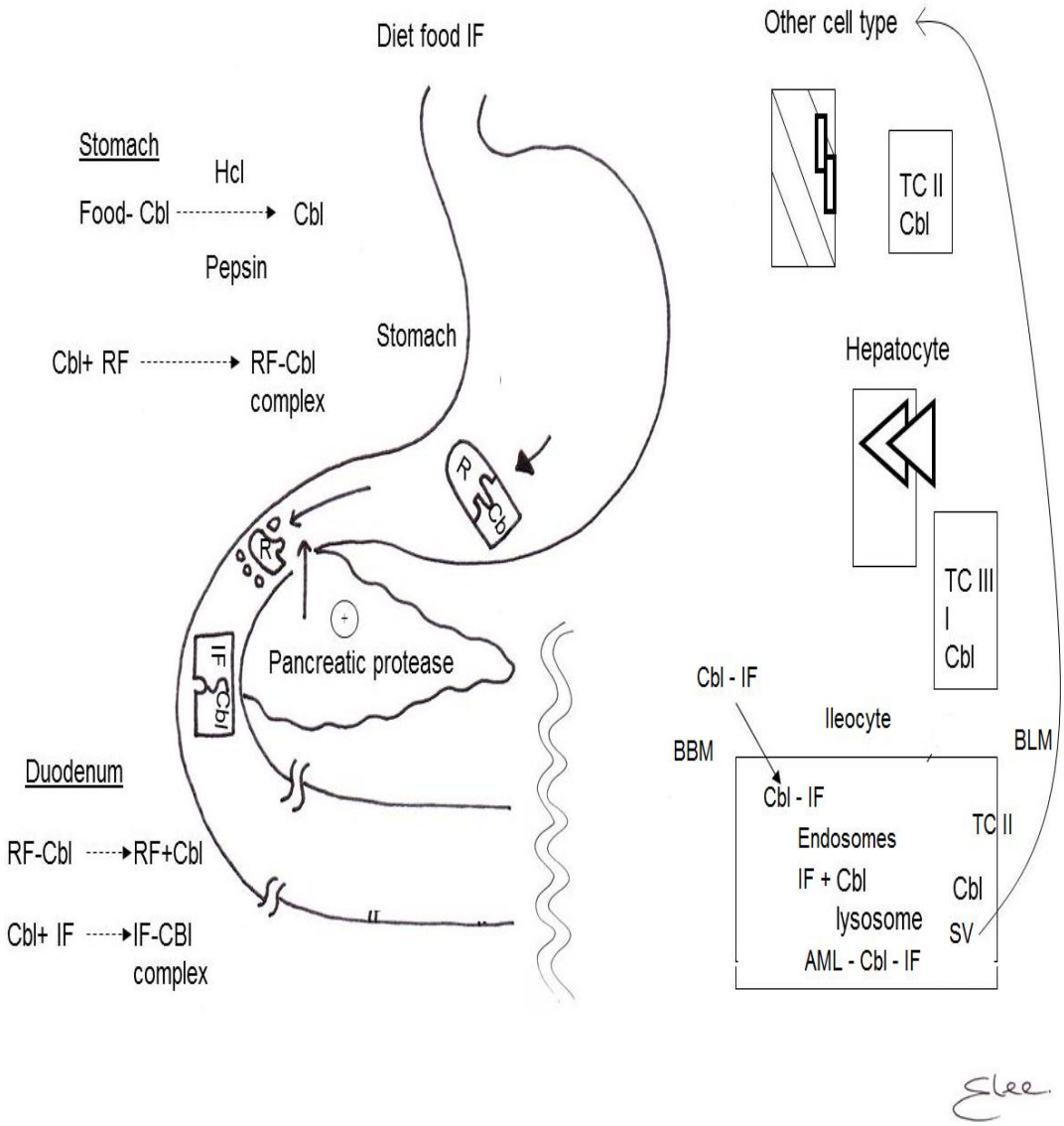
- Cytosol
  - The PteGlu1 is metabolized in the enterocyte cytosol by difolate reductase to dihydrofolate (DHF).
  - DHF is further metabolized to the biologically active tetrahydrofolate.
  - After the cell has reduced PteGlu 1 to THF by adding four H^+s, it then converts THF to 5, 10 - methylene - THF, thus breaking down serine to glycine in the process.
  - This 5, 10 - methylene THF is the methyl donor in the conversion of the nucleotide deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) in the synthesis of DNA.
  - A second reaction converts this 5, 10- methylene-THF to N^5 - methyl-THF, which can then act as a methyl donor in the synthesis of methionine.
  - THF has three parts: the biologically active pterridine moiety, a p-aminobenzoate, and a glutamate.
  - THF traffics across the cytosol to the basolateral membrane (BM).

- Jejunum – BLM
  - At the BLM, an unknown carrier transports PteGlu1 and N5-TNF into the portal circulation.

- Give the physiology of the absorption of cobalamin (vitamin B12).

Vitamin B_{12} (cobalamin, CBl) Absorption

ABSORPTION OF VITAMIN B_{12} (Cbl)



Abbreviation: Cbl, cobalamin (vitamin B12); PP, pancreatic proteases; SV, secretory vesicle; TC II, transcobalamine II; TC III, transcobalamine III

- Diet
  - Cobalamin (Cbl) is synthesized by microorganisms, and dietary Cbl is ingested only in animal products.
  - Some Cbl may also be present in bile.
- Stomach
  - In the stomach, Cbl is released from animal proteins as a result of the actions of HCl and pepsin.
  - The glycoprotein R factor (RF, aka, haptocorrin) is secreted by salivary glands and gastric glands.
  - The free Cbl binds to haptocorrin.
  - Haptocorrin preferentially binds Cbl at the acidic pH in the stomach
- Duodenum
  - Intrinsic factor is secreted by the gastric parietal cells.
  - If mixes with gastric juice, which is mixed with food, and is emptied into the duodenum.
  - Intrinsic factor secretion from the gastric parietal cell is stimulated by Ach, histamine and gastrin (just as is the case for the HCl secretion from the parietal cell).
  - Cbl is released from the RF-Cbl complex in the alkaline pH in the duodenum.
  - Intrinsic factor (IP) is strongly bound to cobalamin at pH > 3
  - IF resists proteolytic breakdown (conformational changes and glycosylation)
  - The Cbl now binds to IF in the presence of Ca^{2+}, rather than to R-factor.
  - Both the biliary and dietary Cbl bind to IF.
- Pancreas
  - Pancreatic proteases continue to digestion of protein to release Cbl
  - IF is resistant to pancreatic proteases, but the pancreatic proteases degrade the R factor.

- Ileum – enterocyte BBM
  - The Cbl-IF complex passes along the small intestine to the ileum.
  - In the Ileum, a Cbl-IF carrier transporters the complex across the ileocyte BBM.
  - In the cytoplasm of the ileal enterocyte, endosomes containing Cbl-IF are formed.
  - The endosomal Cbl-IF is acted upon the cytoplasmic lysosomes degrade the IF, and releasing Cbl back into the cytosol.
  - The released Cbl in the ileal enterocyte cytosol is incorporated into secretory vesicles.
  - The secretory vesicles are bound to transcobalamin II (TCII).
  - The Cbl/TCII complex may be stored in the liver or secreted into bile.
  - The receptor for Cbl in the ileal enterocyte BBM is AML (cubilin-amnionless)
  - AML-cobalamin IF translocates Cbl to the BLM.
  - Cobalamin (vitamin B12) exits the ileal enterocyte across the basolateral membrane (BLM), binding to transcobalamin II.
  - The secretory vesicle releases the Cbl-TCII complex across the basolateral membrane (BLM) of the enterocyte and into the portal circulation.
  - Transcobalamin II – cobalamin is taken up by body cells in which the vitamin B12 is released.
  - In the liver, Cbl acts as a coenzyme for H:MMT (homocysteine methionine methytransferase). The methionine is a methyl donor for DNA synthesis, and is invloved in the synthesis of coenzymes.

"Happiness is like jam

You can't spread it even a little without getting some on yourself"

Anonymous

Minerals

Calcium

Calcium (Ca^{2+}) absorption is by energy-dependent transcellular transport at low Ca^{2+} concentrations, and paracellular diffusion at high concentrations. Both of these processes are stimulated by vitamin D.

- Give the Ca^{2+} transporters which are vitamin D (1, 25 – $[OH]_2$) responsive, and name which one is rate-limiting to the absorption of Ca^{2+}.

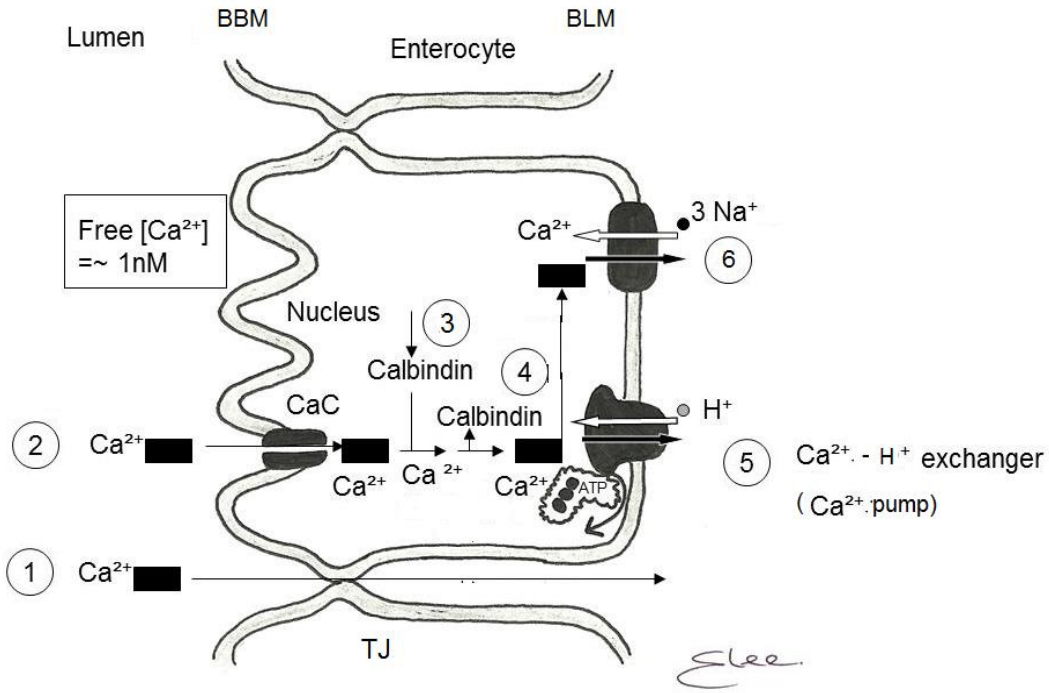
    - The bioavailability of Ca^{2+} depends upon the balance between free and bound Ca^{2+}
    - Most Ca^{+2} is absorbed in the upper small intestine.
    - Vitamin D increases steps 2, 4, and especially step 3 (↑ synthesis of calbindin)

    Abbreviations: AM, apical brush border (apical) membrane; BM, basolateral membrane; CaC, calcium channel in AM; Ca^{2+} - H^+ exchanger, calcium pump in BM

Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009; Figure 45-17, page 974; and from *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Figure 100-20, page 1723, Ninth Edition, 2010.

- 1) Along the length of the small intestine, some Ca^{2+} is passively absorbed across the TJs (tight junctions) into the paracellulaar spaces.
- 2) In the duodenum, Ca^{2+} crosses the BBM through a Ca^{2+} channel (CaC).
- In the cytosol, absorbed Ca^{2+} is bound to the protein "calbindin" (aka calbindin D9K).
- Calbindin 39 is the rate-limiting step for the intestinal absorption of Ca^{2+}.
- 3) The binding of Ca^{2+} calbindin → ↓ Ca_i^{2+} the intracellular concentration of Ca^{2+}.
- As a result of this ↓ Ca^{2+}_i, more Ca^{2+} crosses through the BBM CaC.
- The Ca^{2+} - calbindin complex traffics to the BLM.
- 4) At the BLM, Ca^{2+}- is released from the Ca^{2+}-calbindin complex

  (Ca^{2+}- calbindin → Ca^{2+} + calbindin)
- 5) The Ca^{2+} crosses the BLM by the energy (ATP) – dependent Ca^{2+} pump (aka calcium-dependent ATPase [Ca^{2+} - H^+ exchanger])
- 6) Ca^{2+} also crosses the BLM by the Na+ - Ca^{2+} exchanger.



- Vitamin D (1,25-dihydroxyvitamin D) enhances Ca^{2+} absorption by four mechanisms:
  - BBM: ↑Ca^{2+} uptake across the BBM by the CaC (calcium channel)
  - Cytosol: ↑the synthesis of calbindin, the rate-limiting step
  - BLM: ↑ transport of Ca^{2+} by the Ca^{2+} - H^+ exchanger (Ca^{2+} pump) and the Na^+-Ca^{2+} exchanger.

There are three vitamin D responsive transporters for Ca^{2+}

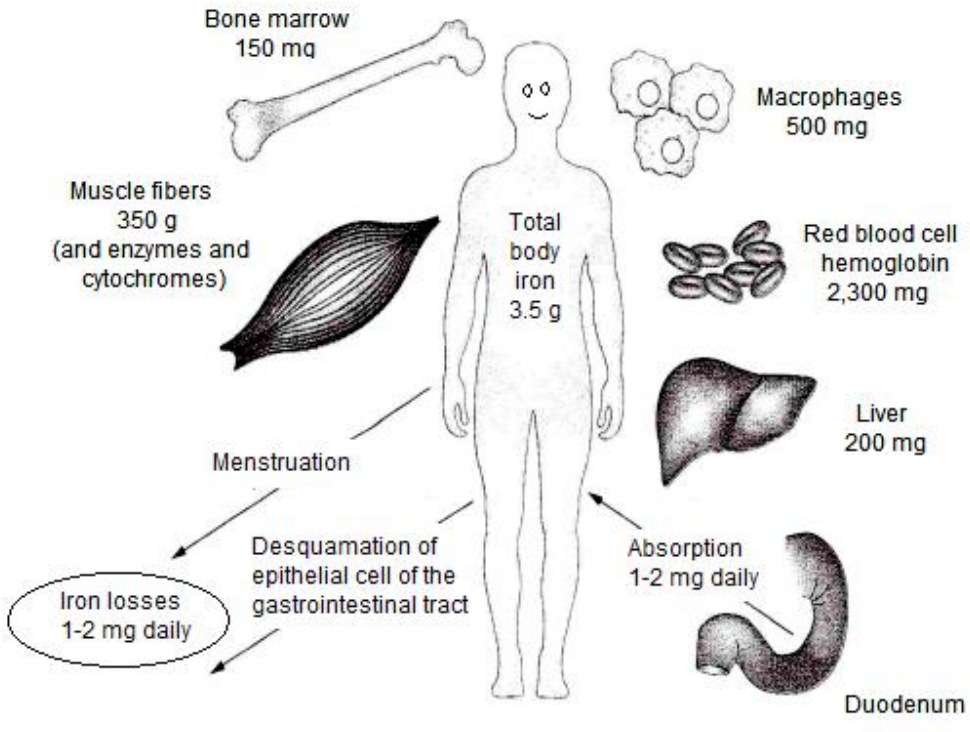
- BBM
  - Non-voltage-gated calcium channels (CaC)
- Cytosol
  - Calbindin 3 9 (aka calbindin D9K), which is calcium-binding protein, and the rate-limiting st in the intestinal absorption of Ca^{2+}
  - CALB1 (aka calbindin D28K)
- BLM
  - Calcium-dependent ATPase (Ca^{2+} pump, Ca^{2+} - exchanger

Magnesium (Mg^{2+})

- Most Mg^{+2} uptake is carrier-mediated in the ileum.
- Lesser amounts of Mg^{+2} are absorbed along the length of the small intestine, presumably by passive diffusion.
- Mg^{+2} absorption is not influenced by vitamin D.

Iron

IRON HOMEOSTASIS IN HEALTH



Adapted from: Stein J, et al. *Nat. Rev. Gastro. Hep*. Figure 1, page 599-610, 2010

- Body iron storage (BIS)
  - Approximately 3500 mg of iron is stored by the human body.
  - Most of the iron in the body is present in red blood cells as hemoglobin (~2,300 mg).

- Approximately 10% of body iron is present in myoglobin in muscle fibers, as well as in other tissues in enzymes and cytochromes (350 mg).

- The remaining body iron is stored in the liver (200 mg), macrophages (500 mg), as well as bone marrow (150 mg).

"The difference between how a person treats the powerless versus the powerful is as good a measure of human character as I know."

Robert Sutton

Iron absorption

- The process of the absorption of luminal iron is complex.

- The daily intake of dietary iron far exceeds body needs (25 mg versus 1 mg).

- About 1-2 mg of iron is lost daily from the body by way of the desquamation of epithelial cells of the skin, gastrointestinal tract, bile ducts and urinary tract, and via blood loss during menstruation.

- Blood and therefore iron may be lost from the body from bleeding caused by pathological processes, or through the generous donation of blood.

- The loss of iron from the body is not controlled

- Body iron balance is controlled by the modification of iron absorption by the intestine.

- Give the physiology of the absorption of non-heme iron.

➢ Luminal Factors and Iron Absorption (IA)

- Iron absorption from the digestive tract is the sole means by which iron homeostasis is regulated: (i.e., there is no regulation of iron secretion or loss from the body).

- Duodenal enterocytes are the major site of Fe^{2+} absorption.

- There are numerous luminal factors which increase or decrease iron absorption (IA).

Increase (IA)	Decrease (IA)
○ Reducing agents	○ Intraluminal binders
– HCl	– Phosphate
– Ascorbic acids	– Phylates
○ Chelating agents	– Oxalates
– Organic acids	– Dietary fiber
– Amino acids	

➢ Duodenocyte BBM digestion and absorption

- 1) Dietary non-heme Fe^{3+} (ferric) is reduced to Fe^{2+} (ferrous) by HCl within the gastric lumen.
- 2) Non-heme Fe^{3+} is also reduced to Fe^{2+} by the duodenal enterocyte AM, ferric reductase [Dcytb].
- Fe^{2+}, arising from dietary Fe^{3+} in the gastric lumen, is solubilized by chelating agents such as ascorbic acid, amino acids and organic acids.
- 3) Fe^{2+} from non heme Fe^{3+} crosses the duodenal BBM by the Fe^{2+} -H+ transporter DMT1 (divalent metal transporter, aka SLCIIA2), which cotransports Fe^{2+} with H^{+}.

➢ Duodenocyte cytoplasm

- 4) In the cytosol, Fe^{2+} binds to mobilferrin.
- Fe – mobilferrin diffuses to the BLM.
- At the BM, Fe^{2+} is removed from mobilferrin by FP1.
- Mobilferrin traffics back to the BBM to bind to more non-heme Fe^{2+}.
- 5) At the BLM, Fe^{2+} is oxidized to Fe^{3+} by hephestin (a forroxidase).
- 6) The Fe^{3+} binds to BLM FPNI (ferroportin transporter, aka IREE1) and is transported into the portal blood.
- 7) Fe^{3+}in portal blood is bound to transferrin

- Plasma Iron

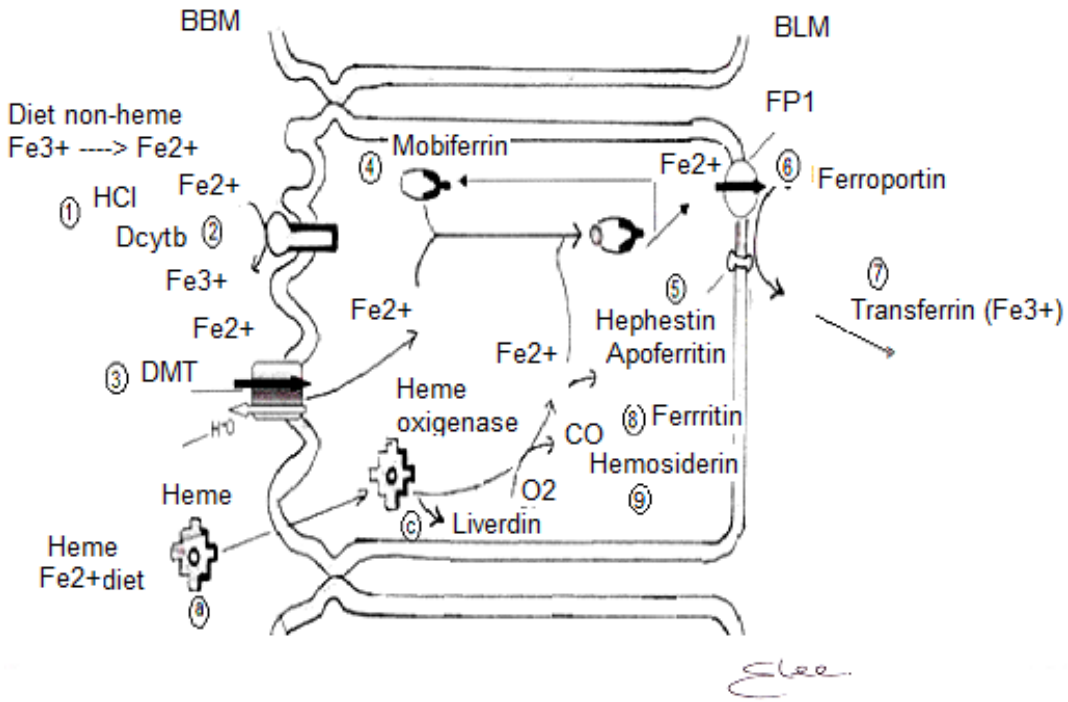
  - 8) Some Fe^{3+} which is bound to plasma transferrin moves back across the BM into enterocytes, where the Fe^{3+} is bound to apoferritin.

  - Apoferrin plus Fe^{3+} form soluble protein “ferritin”.

  - Ferritin is the major storage form of iron, and ferritin may be detected in the blood as well as in tissues.

  - Some iron in the cell is bound to hemosiderin. Hemosiderin is insoluble and remains in the cytoplasm.

VILLOUS DUODENOCYTE



Abbreviations: AM, apical brush border membrane; BM, basolateral membrane; Dcytb, duodenal cytochrome B; DMT1, divalent cation transport 1; HCP1, heme carrier protein 1; Hox1, heme oxygenase

Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009, Figure 45-18, page 975; and from *Sleisenger and Fordtran’s Gastrointestinal and Liver Disease,* Figure 100-21, page 1724, Ninth Edition, 2010

Note: Crypt duodenocyte: The TR (tranferrin receptor in the BLM of the duodenal crypt cells can take up Fe^{3+} from venous portal blood.

- Give the names of 10 proteins involved in the absorption of iron, and in the balance of body iron stores (BIS).

There are numerous proteins involved in the absorption of iron and in the balance of body iron stores (BIS).

- Villus duodenocytes
  - BBM
    - Ferroreductase ($Fe^{3+} \rightarrow Fe^{2+}$)
    - DMT1
      - DMT1 (IRE [iron-responsive element]) mRNA
      - DMT1 (non-IRE) mRNA
    - Mobiferrin (an integrin, as well as a ferric reductase)
    - HCP1 (heme-carrier protein 1)
  - Cytosol
    - Heme oxygenase
    - Ferritin (iron storage protein)
    - B2 microglobulin, associated with TR (transferrin receptor) and with HFE
  - BLM
    - Hephestin (a ferroxidase): $Fe^{3+} \rightarrow Fe^{2+}$
    - FPN1 (ferroportin)
    - Transferrin receptor (TR)
  - PVB (portal venous blood)
    - Apo TF (apotransferrin)
  - Liver
    - Hepcidin

- Crypt duodenocytes
  - HFE protein
    - ↑ TR (transferrin receptor)-mediated uptake of iron from transferrin-bound Fe in PVB (portal venous blood) across the BM (basolateral membrane) of the duodenocyte crypt cells.

Abbreviations: BBM, brush border membrane, BLM, basolateral membrane

- Heme-Iron
  - a) Heme-Fe^{2+} crosses the AM by an unknown mechanism.
  - b) In the cytosol of the enterocyte, heme oxygenase (Hox1) oxidizes the heme-Fe^{2+} to Fe^{3+}.
  - In the cytoplasm of the duodenal enterocyte, Fe^{3+} either binds to ferritin or is transported across the BM by means of ferroportin.
  - c) The remaining biliverdin in heme is reduced to bilirubin, which is eventually excreted in bile (please see the Liver Chapter, excretion of bilirubin)

➢ Control of body iron stores (BIS) by the modification of iron absorption in the duodenocyte

- Give the role of hepatic hepcidin and IL-6 in the control of body iron absorption and balance.

  - Hepcidin (HFE protein), hemojuvelin (HJV) and transferrin receptor 2 (TFR2) participate in the hepatic iron-sensing mechanism that regulates hepcidin expression.

  - HFE is secreted in hepatic Kupffer cells in an amount which is related to body iron stores (BIS).

  - Hepcidin is a negative regulator of duodenal iron absorption: when ↓ hepcidin → ↑ iron absorption (IA) by DMT and FP1.
    - ↑BIS → ↑ HFE → ↓ BIS - ↑ HFE
    - ↓BIS → ↓ HFE → ↑ IA

  - Iron absorption in enterocytes activates BMP6 expression, and the delivery of BMP6 to the liver.

  - In the liver, BMP6 binds to type I and II receptors (BMPR1 and BMPR2) as well as to the co-receptor HJV.

  - BMP6 bound to BMPR1 and HJV phosphorylates SMAD1, SMAD5 and SMAD8, and formation of complex with SMAD4.

  - When the body iron stores (BIS) are low, BMP (bone morphogenetic protein) senses the ↓ enteric iron stores.

  - ↓ enteric iron stores → ↓ BMP6 expression

  - Hepatic Kupffer cells secrete less hepcidin into the blood.

- Hepcidin reduces iron release by macrophages (and thereby increases macrophage iron stores) and also reduces iron absorption by duodenal enterocytes by moving FP1 away from the BM, to reduce the amount of dietary iron in the circulation.

COMPLEX CONTROL SYSTEM

↑ BIS → ↑ Enteric factors-↑BMP / BMPR1 → ↑ HFE → ↓1A → ↓BIS

↓ BIS → ↓ HFE → ↑ 1A → ↑ BIS enteric stores → ↓ BMP / BMPR1 → ↓ BMP → ↓ BMP6

- Less hepcidin upregulates DMT1 and FP1, thereby increasing AM Fe^{+2} uptake into and BM Fe^{3+} transfer out of the duodenocyte.
- Increased uptake and transfer of iron continues until the low BIS have been restored to normal.
- Once the BIS are normal, hepcidin falls, and the DMT1 – FP1-mediated increased iron absorption returns to the normal lower level of iron uptake across AM and exit across BM.

- IL-6
  - Iron absorption is inversely related to body iron stores (BIS), and to the level of the pro-inflammatory cytokine IL (interleukin)-6.
  - Higher levels of IL-6 stimulate the hepatic secretion of hepcidin.
  - With ↑IL-6 and ↑ hepcidin, less iron is released from duodenocytes and from macrophages
  - Hepcidin gene expression is up-regulated during infection and inflammation by proinflammatory cytokines – mainly IL-6, involving JAK dependent activation of STAT3.

HFE – related hereditary hemachromatosis (HH)

- In HH, loss of functional HFE protein leads to aberrant hepatocellular sensing of plasma iron, inappropriately low levels of hepcidin, diminished macrophage iron stores, and greater duodenal iron absorption.

- Give the role of HFE, B2-microglobulin and TR (transferrin receptor) in the duodenal crypt cells in the control of body iron stores, and the impact of defective HFE, as in HH (hereditary hemochromatosis).

    - TR in the BM of the duodenal crypt cells has the ability to retake from the PVB in response to BIS (body iron stores)
    - When BIS↑, transferrin saturation is increased, and TR takes more iron into the duodenocyte
    - This iron binds to HFE, B2-microglbulin and TR
    - The duodenocyte is now storing more iron
    - The duodenocyte containing more iron and reflecting ↑ BIS migrat up the villus, and becomes available to take up and transfer ir from the lumen across the BBM and from the cytosol into P\ across the BLM, using FPN1.
    - The increased iron in the villus cell signals DMT1 and FPN1 reduce their activity, thereby decreasing iron absorption when B are increased.
    - The complete process may be summarized as follows:

➢ ↑ BIS → ↑ TS (transferrin saturation) → ↑ TR uptake of transferrin-bound iron → ↑ crypt duodenocyte iron bound to TR, HFE, B2-microglobulin →↓ activity of DMT1 and FPN1 → ↓ iron absorption (this is known as the "mucosal block" of iron absorption, i.e. when there is ↑ TS, TR-HFE-B2-microglobulin in the duodenocyte block further iron absorption by blocking the activity of FPN1 and DMT1)

- When HFE is defective, as in HH, there is ↑ TS (transferrin saturation), but TR-HFE-B2 microglobulin does not respond to the ↑ BIS, the mucosal block of iron absorption fails, and DMT1 and FPN1 continue to transport iron, thereby further increasing BIS.

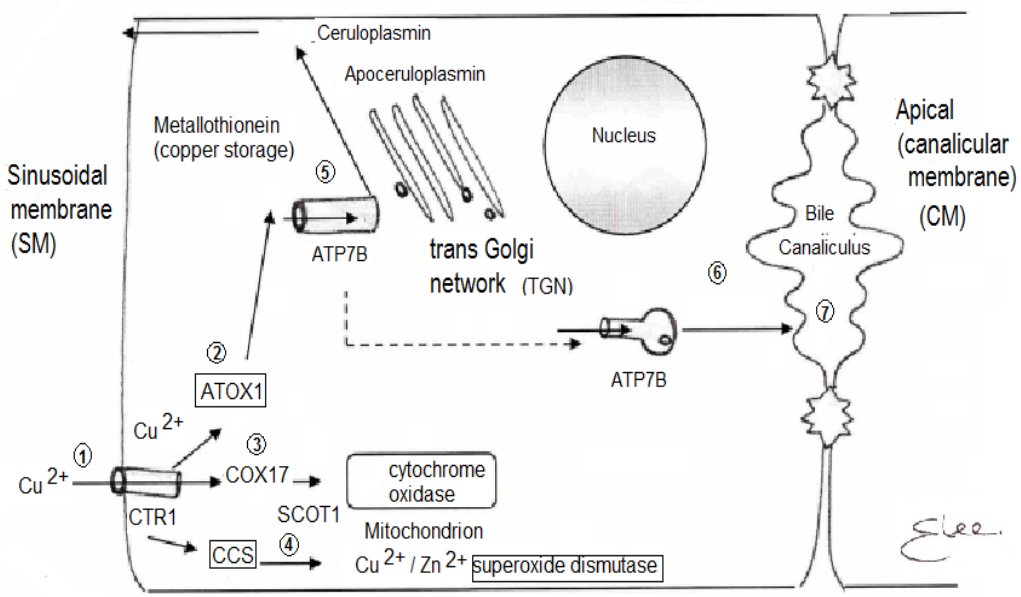
CLINICAL PHYSIOLOGICAL CORRELATION – Iron Metabolism

Background: In *HFE*-related hereditary hemochromatosis, loss of functional HFE protein (hepcidin) leads to aberrant hepatocellular sensing of plasma iron, with inappropriately low levels of hepcidin, diminished macrophage iron stores, and inappropriately greater duodenal iron absorption.

- Draw a picture of the absorption of dietary iron by the duodenal enterocyte, and the role of both the intestine and the liver in
  - Causing hereditary hemochromatosis
  - Responding to either decreased or increased body iron stores
  - Responding to a chronic inflammatory process

Copper Metabolism

- Small Intestine
  - In the enterocytes Cu^{2+} is absorbed by active and passive transport
- Hepatocytes



Adapted from: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Figures 75-1 and 75-2, page 1250, Ninth Edition, 2010; and Cox DN. *Am J Hum Genet* 1995;56:828-834.

- 1) Copper (Cu) is taken up into the hepatocyte from albumin and other carriers in blood by the copper transporter CTR1.
- Cu^{2+} across SM (sinusoidal membrane) in CTR1 and is bound to low-molecular-weight copper chaperones ATOX1, COX17 or CCS.
- 2) ATOX1 chaperone targets ATP7B (Wilson ATPase)
- 3) COX17 cytochrome oxidase targets cytochrome oxidase.
- 4) CCS targets Cu^{2+}/ Zn^{2+} superoxide dismutase
- 5) ATP7B traffics to the trans-Gogi network (TGN) in the hepatocyte cytoplasm.
- 6) ATP7B binds to COMMD.
- The Cu+ - ATP7B which traffics to COMMD1 in the canalicular membrane is transported into the bile canaliculus across the CM.

Mucosal Immunity

- Give 10 components of GALT (gut-associated lymphoid tissue).
  - PP (Peyer's patches) in the lamina propria
  - FAE (follicle – associated epithelium)
  - M cells (microfold cells)
    - A conduit to PPs by way of macrophages and DCs
  - Macrophages
  - Antigen-presenting cells (APC; such as DCs, B lymphocytes, macrophages presenting antigen to T lymphocytes)
  - APCs (macrophages, monocytes, dendritic cells)
    - Process extracellular proteins from class II MHC molecules to CD4+ T cells
    - Process intracellular proteins from class I MHC molecules to CD8+ T cells
    - Non – MHC class I molecules interact with CD 8+ T cells
    - Lamina propria dendritic cells, Peyer's patches express IL-4, IL-10
    - Control tolerance and interaction with T cells
  - DCs (dendritic cells)
    - Directly sample self- and non-self-antigens in the intestinal lumen
    - Together with macrophages internalize these non-self-antigens and contribute to the induction of immune tolerance
  - T and B lymphocytes (activated in PPs to express integrin $\alpha 4\beta 7$)
    - The lamina propria (LP) is the main effector site of GALT
    - Function of B cells
      - Antigen presentation
      - T cell activation
      - Autoantibody production
      - Cytokine production (especially TNF-α and IL-6)
  - MLNs (mesenteric lymph nodes; $\alpha 4\beta 7$ – containing lymphocytes home to Mad CAM-1 ligands)
  - IELs (intestinal epithelial lymphocytes

- Contribute to innate and to adaptive immunity)
- Transepithelial antigen transport
  - Most food proteins are hydrolyzed to non-immunogenic peptides and amino acids
  - 2% of food antigens (mostly glycoproteins) are absorbed
  - Transport ↓ - food, acid
    ↓ - alcohol, alkalinity
- Phase I
  - Antigen – specific IgE antibodies bind to Fc R on surface of IECs
  - Fc R accelerates transfer of antigen
  - Mast cell – independent
  - IgE antibody band to IEC
- Phase II
  - Mast cell – dependent
  - Mast cells release cytokines
  - IELs express cytokine receptors (IL-1, -2, -6, -10, -12, -15, GM-CS [granulocyte – monocyte colony – stimulating factor], and IFN [interferon - γ])
  - TJs open

- PAMPs (pathogen – associated molecular patterns; receptors for PAMPs on cell surface [e.g., TLRs] or in cells [e.g., NOD2])
- MHC (major histocompatibility complex [in rodents], known as HLA in humans)
- Costimulatory molecules for activation of CD^{8+} regulatory T cells (examples: ICAM-1, LFA-1, B7[CD80])
- LPLs (lamina propria lymphocytes), including LPMNCs (lamina propria mononuclear cells), e. g., IgA^{+}-plasma cells)
- Microbiota – important in the induction of oral tolerance
- In healthy persons, the intraepithelial cells (IECs) stimulate regulatory $CD8^{+}$ T cells.
- In IBD, the IECs stimulate $CD4^{+}$ T cells.
  - Intraepithelial lymphocytes (IELs) – Th1 and Th2
- >98% of IELs are T cells, mostly γδ TCR (rather than αβ TCR in the systemic immune system)
- STAT – 4 → ↑ IFN - δ → activates STAT-1 and T-bet
- T-bet
  - ↑ Th1 cytokine production (↑ IL-12 receptor B2, ↑ IL18)

- ↓ Th2 cytokine production
- Thus
  - STAT-4 ↑ IL-12R and IL-18
  - STAT-6 and GATA-3 ↑ IL-4, IL-5 and IL-13 (Th2 cytokines dendritic cells (DCs])

The gastrointestinal tract has physiological as well as immunologic barriers.

- In the context of immune – mediated non-toxic reactions to food, give the physiologic and immunologic barriers of the GI tract.

- Lumen
  - pH
  - pepsin, trypsin
  - bile acids
- Motility
- Membrane
  - Columnar intestinal epithelial cells (IECs; non professional antigen – presenting cells [APCs])
  - Tight junctions (TJs)
  - Mucus
  - Trefoil factors from stomach (TFF1, TFF2) and intesti (TFF3)
  - Defensins
  - Lipid composition of brush border (apical) membra (AM)
  - Normal epithelial cell turnover
  - AM peptidases
- GALT/ MALT
  - Innate immunity
    - NK (natural killer) cells
    - PMNs (polymorphonuclear leucocytes)
    - Macrophages
    - Epithelial cells
    - TLRs (protease – resistant proteins secreted by stomach [TLR1 and intestine [TLR3])
  - Adaptive immunity
    - IEL intraepithelial lymphocytes
    - LP-L (lamina propria lymphocytes)

- Peyer's patches
- S-IgA (secretary IgA)
- Cytokines

- Immune suppression (tolerance)
  - Main players: dendritic cells, IECs, Tregs (regulatory T cells)
- Main function of GALT
  - High dose tolerance: binding of antigen to receptor of T cells, wi no costimulatory signals → energy of lymphocytes
- Low – dose tolerance
  - Types of Tregs
    - CD4+ Th3 → TGF-β
    - CD4+ Th_1 → TL-10
    - γδ T cells → CD4+ 25+ Treg
    - CD 8+ Ts (suppressor T cells)

Crohn Disease (CD)

Useful background

- Incidence
  - $10/10^5$ per year in USA / Canada and Northe Europe
- Prevalence
  - $200 / 10^5$
  - Female-to-male ratio of 1.3:1
- Genetics
  - Concordance rate among monozygotic twins, 67 (for UC, only ~15%)
    -

Useful statistics

- Perianal disease occurring before intestinal inflammation, 24%
- Proximal intestinal Crohn disease and distal disease
  - Early 1/3, no
  - Late 3/3, yes
- Intra-abdominal abscess in ~25% of Crohn patient during the lifetime of their disease
- Angular cheilitis, 8%

- PSC (primary sclerosing cholangitis) does occur in Crohn disease (4%), especially Crohn colitis

- Give the defective innate immunity, abnormal autophagia, and excessive response in adaptive immunity which occur in Crohn disease, and contribute to the pathogenesis of this chronic inflammatory condition.
  - MHC lass II cells presents antigen with abnormal epitope to CD3 receptor of T cells

Clinical Curiosities

- The sensitivity of plain films of the abdomen for AMI is only 30%, until infarction occurs.
- Abdominal pain out of proportion to the physical findings is usually common in AMI, except for AMI due to NOMI, in which case there may be little or no pain in ~25% for sufferers.

- Give 10 genetic, microbiome and mucosal epithelial defense mechanisms which may have a pathophysiological implication in the development of CD.

➢ Genetic changes

- "It is estimated that known genetic associations account for only about 20% of the genetic variance underlying, susceptibility to inflammatory bowel disease" (Abraham & Cho, NEJM 2009;361: 2066-78)
- Familial clustering of cases of IBD
- Twin studies
- NOD2 (Nucleotide Oligomerization Domain 2) (host- microbiome interactions)
  - "carriage of disease-associated allelic variants on both chromosomes confers an odds ratio for Crohn disease of 17.1…., and heterozygous persons have an odds ratio of 2.5 …… for the disease" (Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, Table 105.3, page 1943)
  - Associated with
    - Younger onset of CD
    - Ileal disease
    - Stricturing disease
  - About 25% of CD patients have abnormal NOD2 / CARD5 but penetrance is < 1%

- NODs acts as an intracellular sensor, binding to muramyl dipeptide of bacterial dipeptide
- NOD2 is expressed in Paneth cells, which are in the crypts and produce defensins
- Defects in NODs impair the normal antibacterial responses to luminal antigens
  - Autophagy
  - Innate immune response
- Components of the IL-23 type 17 helper T cell (Th 17) pathway
- ATG 16 G1, the autophagy gene, as well as the immunity- related GTPase M protein (IRGM) intracellular components such as organelles, apoptotic bodies, and microbes
- IRGM (immunity related GTPase family member M)

➢ Mucosal repair and barrier function

- Polymorphisms in proximity to the gene encoding EP_4 ($PTGE_4$) in Crohn disease (CD).

➢ Microbiome (microorganisms which inhabit the GI tract)

- Persons with CD and UC have a reduced number and diversity, as compared with controls, of the mucosa-associated phyla Firmicutes and Bacteroidetes

➢ Defense of the epithelium

- Lumen
  - Acid
  - Blie
  - Pancreatic enzymes
  - Mucus
- Motility changes
- Enterocytes
  - Tight junctions
  - α-defensins
  - Toll like receptors
  - Cell matrix adhesion
  - Epithelial cell development or proliferation
  - Restitution of epithelial cells after injury

- Stress of the endoplasmic reticulum

- MALT/GALT (immunopathogenesis)
  - ↑ type or amount of luminal antigens which stimulate an inflammation and sustain an abnormally increased immune response
  - ↑ intestinal permeability ("leakiness") to antigens
  - In NOD2 homozygotes there are
    - ↓ defensin production by crypt Paneth cells
    - ↓ sensing of intracellular bacteria or antigens
  - Abnormal antigens are taken up by APC (antigen presenting cells), such as dendritic cells, macrophages
  - B cells secretory immunoglobulins
  - Dendritic cells
  - T cells (Peyer's patches, mesenteric lymph nodes, lymphoid follicles) when activated produce integrin a4B7 and CCR9 (a chemokine receptor)
  - Pattern recognition receptors (innate immune cells) Intestinal vasculature-adhesion molecules (selections, integrins) and chemokines (secreted cell attractants)
  - Leukocyte migration
- Autophagia
  - Autophagy of antigen in proteasomes is abnormal (e.g. autophagy-related 16-like 1 gene)

- Interactions become active once exposed to binding of costimulatory signals:
  - TNF to TNF-R
  - CD40 to CD40 ligand
  - B7 to CD28

- The p40 subunit on IL-20 and IL-23, together IL-6 and TGF-β lead to differentiation of T cells into Th-17

- ↑ NFkB in mononuclear cells lead to ↑ IL-1, -6 and TNF, as well as adhesion molecules and chemokines.

- TNF causes
  - ↑synthesis of proteins noted above
  - With IFN-α, causes ↑ MHC class II expression on epithelial cells
  - ↑ neutrophil activation

- The Th1 and Th17 immune response is increased further through leucocyte trafficking
  - Mononuclear expression of adhesion molecules
  - Rolling of leucocytes (WBC) along endothelium through expression of selectins and integrins
  - Integrins bind to intestinal receptors

Integrins	Ligands	Site of binding
α4β7	Mucosal adhesin	Endothelium of venules in LP (M CAM-1)
αEβ7	Cellular E-cadherin	IELs

- Monocytes and granular cells in LP release proinflammatory substances which initiate and maintain inflammation:
  - Prostaglandins
  - Leukotrienes
  - Nitric oxide (NO)
  - Reactive oxygen species (ROS)
  - Proteases
    - Collagenase
    - Matrix metalloproteinases

Abbreviations: IEL, intraepithelial cells; LP, lamina propria

"The meaning of life is to fill your three score and ten years with love, respect and compassion for others."

Grandad

Immunosuppression Treatment

- Give 5 immunosuppressive agents commonly used in gastroenterology or hepatology (for example for Crohn disease, ulcerative colitis, autoimmune hepatitis, liver), and for each give their mode of action, common toxic effects, and recommended monitoring.

Agent	Mode of action	Monitoring	Toxic effects
○ Cyclosporine (CyA), tacrolimus	Calcineurin inhibitor: which suppresses IL-2-dependent T cell proliferation	Blood level of CyA, cholesterol, magnesium, Creatinine, BP, BS	Renal, neurologic, hyperlipidemic, hypertension, hirsutism
○ Sirolimus (Rapamycin)	Inhibition of MTOR, which disrupts IL-2 induced intracellular signaling in lymphocytes	Blood level	Neutropenia, thrombocytopenia, hyperlipidemia
○ Prednisone	Alter gene transcription of steroid response elements (SRE); cytokine inhibitor (IL-1, IL-2, IL-6, TNF, and IFN gamma)	BP, BS, annual eye exam, DEXA scan	(see previous question)
○ Azathioprine	Inhibition of T and B cell proliferation by interfering with purine synthesis (↓DNA/RNA)	White blood cell count, liver enzymes	Bone marrow suppression, hepatotoxicity
○ Mycopheno-late mofetil (Cellsept)	Inhibition of T and B cell proliferation by interfering with purine synthesis	White blood cell count	Diarrhea, bone marrow suppression
○ Methotrexate	Folate antimetabolite (↓DNA)	Liver biopsy after 1,500 mg (only 2 years maintenance therapy)	Hepatic fibrosis Bone marrow suppression

○ OKT3	Blocking of T cell CD3 receptor, depletion of effector T cells and T regs, preventing stimulation by antigen	$CD3^+$ count	Cytokine release syndrome, pulmonary edema, increased risk of infections
○ IL-2 receptor blocker	Competitive inhibition of IL-2 receptor on activated lymphocytes	None	Hypersensitivity reactions with basiliximab

Abbreviations: BP, blood pressure; BS, blood sugar; DEXA, DEXA scan for bone mineral density; IFN, interferon; IL, interleukin; LEs, liver enzymes; MTOR, mammalian target of rapamyacin; TNF, tumour necrosis factor.

Adapted from: Martin P, and Rosen HR. *Sleisenger & Fordtran's gastrointestinal and liver disease: Pathophysiology/Diagnosis/Management* 2006: pg. 2049.

Pathophysiology of UC

- Humoral Immunity
  - ↑ plasma cells producing IgG1 and IgG3 antibodies food, bacteria and self (autoantibodies, including a 40 kDa autoantigen to normal colonic epithelium)
  - pANCA in ~ 75% of UC patients (IgG1 anti-50kDa nuclear envelop protein in myeloid cells), and may be a marker for more aggressive disease.
  - Antibodies to bacterial antigens
    - Anti-CBir 1 9antibody to flagellin from Clostridium species), in 6% of UC patients, and is a marker for development of pouchitis
    - Anti-PMOC (outer membrane porin C of E. Coli)

- Cellular Immunity
  - Innate immunity
    - Bacteria produce immune response in monocytes, macrophage and dendritic cells
    - Involves PPRs (pattern-recognition receptors, e.g. TLRs [toll-like receptors], NLRs [NOD-like receptors])
    - Activation of TLRs and NLRs activates NF-kB (nuclear factor kB)

- NF-kB increases transcription of genes coding for proinflammatory cytokines (e.g. IL-1, -6, -8, TNF), chemokines, adhesion molecules and costimulatory molecules
- NF-kB increases maturation of dendritic cells, which enhances further antigen presentation

- Adaptive Immunity
  - LP (lamina propria) lymphocytes express α4β7, a surface adhesion molecule which attracts CD4+ and CD8+ T cells
  - IELs (intraepithelial lymphocytes) is normal or ↓ MUC
  - ↑ production of
    - Monocytes (in circulation)
    - Macrophage (in mucosa)
    - Granulocytes
  - Both Th1 and Th2 response, as well as Treg, NKT and Th17
  - Th1
    - Differentiation of T cells into Th1 pathway is stimulated by IL-12
    - Production of IL-2 and IFN (interferon)-γ
  - Th2
    - ↑ IL-4, -5, -10, -13 → enhance the humoral immune response
  - Treg
    - Produce IL-10 and TGF-β
    - Inflammation
  - NK T cells (natural killer) T cells produce IL-5 and -13, which are cytotoxic to colonic epithelium
  - Th17 produce IL-6 and -17
  - Functions of IL-17
    - Proinflammatory
    - Stimulates
      - T cell activation
      - Macrophages
      - Fibroblasts
      - Epithelial cells
    - T17 inhibited by Th1, Th2 cells stimulated by IL-6, -12, -23R, and TGF-β
  - ↑ mucosal cytokines → ↑ release of metalloproteinase from fibroblasts

- ↑ mucosal content of active macrophage and neutrophils release
  - Leukotrienes
  - NO (nitric oxide)
  - ROS (reactive oxygen species)
  - PAF (platelet-activating factor)

"The key to a good speech: a brilliant opening and a brilliant ending, and keep them as close together as possible"

Peter Ustinov

Celiac Disease

- Give the immunopathogenesis of celiac disease.

➢ Mucosa
  - The toxicity of gluten is due to the gliadins contained in gluten.
  - The main toxic gliadins is prolamin
  - The toxic portion of prolamin is QQQPF (glutamine-glutamine-glutamine-proline-phenylalanine)

➢ Lamina propria dendritic cells
  - Gluten is absorbed and passed to lamina propria.
  - Dendritic cells which express HLA-DQ2 or –DQ8 bind gluten.
  - The gluten bound to the dendritic cells is presented to T cells which have the TCR (α / β T cell receptor).
  - The neutral glutamines bind to 4, 6 and 7 of the antigen binding grove of the HLA-DQ2 molecule.

➢ Lymphocytes
  - The TCR containing T cells (including α / γ TCR in IELs) activate B and other T lymphocytes.
  - The activated B and T lymphocytes produce proinflammatory cytokines.

➢ Cytokines
  - The proinflammtory cytokines (IL-4, -5, -6, -10; IFN (interferon)-γ, TGF (transforming growth factor)-β cause:
    - Enterocyte damage
    - ↑ HLA-DQ2 gene expression

- tTG
  - The increased and activated B cells produced type 2 tTG (tissue transglutaminase).
  - tTG deaminates neutralizes neutral glutamine residues in gluten to negatively charged glutamic acid residues in DGPs (deamidated gliadin peptides).
  - The DGP increase the injurious T cell responses.
- IL-15
  - ↑ IL-15 ("bridges the innate and adaptive immune responses in celiac disease") causes
    - IEL ↑ IFNα
    - LP CD4+ T cells ↑ response
- HLA class II molecules
  - The HLA class II molecules DQ2 and DQ8 are required for but are not sufficient by themselves for the development of CD: 50% of Americans are positive for one of these molecules, but only 1% develop CD. However, negative HLA DQ2 or DQ8 rule out CD as a cause of the enteropathy (high negative predictive value)
  - There develops ↑ apoptosis of the villous tip enterocytes, proliferation of enterocyte production in the crypts, population of the villus with immature enterocytes which lack the normal digestive and absorptive function.
  - Malabsorption begins and worsens with shortening of the villi which occurs if the ↑ crypt production cannot keep up with the ↑ apoptosis.

"Resources may influence the community standard of care"

Jacque Guilbert, MD,

SO YOU WANT TO BE A GASTROENTEROLOGIST!

- Give the suggested immunopathogenesis of refractory celiac disease (RCD).
    - ↑ IL-15 in mucosa of small intestine → ↓ SMAD3 dependent TGF-β signaling in T lymphocytes
    - Initial normal expression of T cell surface markers (CD3+, CD8+, CD4-) and normal TC (T-cell receptors, polyclonal plasma cell immunophenotype) (still normal cytologically)
    - ↓ TGF-β signaling
        - Continued intestinal inflammation in celiac small intestine
    - T cells become aberrant, becoming phenotypically abnormal (on flow cytometry determination), with immune dysregulation including monoclonal expansion, CD3 in the cytoplasm, CD108 on the surface of the T cells, loss of TCR, CD3, CD8, CD4 (on flow cytometry determination)
    - Once > 29% of T cells are abnormal, the RCD I is said to have progressed to RCD II
    - EATL (enteropathy-associated T-cell lymphoma) is on a continuum with RCD II, both of which are associated with HLA-DQ2 homozygosity
    - EATL is said to have occurred when
        - The T cells are cytologically abnormal
        - Tumor masses develop
    - In RCD II, steroids, azathioprine, infliximab are ineffective against the aberrant monoclonal T cells, and 2-CDA (cladribine) or ASCT (autologous stem cell transplantation) may be necessary

Eosinophilic Disorders of the GI Tract

- Eosinophils
    - Eosinophils are bilobed nucleated granulocyte(s) which differentiate from myeloid progenitor cells into mature eosinophils which contain bright birefringent cationic granules which have a high affinity for the acidic dye eosin
- Bone marrow
    - The eosinophils in the bone marrow mature under the influence of the hematopoiesis-specific transcription factors
        - GATA-1
        - GATA-2
        - PU1

- C/EBP (enhancing binding protein family)

The bone marrow is also stimulated to produce and release eosinophils to the peripheral blood by

- Eotaxin
- GM-CSF
- GATA-192
- C/ERP

- GI tract
  - Ingestion of allergen
  - $\alpha_4\beta_7$ – integrin and β_2-integrin pathway control the movement of eosinophils into the small and large intestines.
  - Eosinophils are directed to traffic to the gut site of allergen exposure
    - IL-5
    - Eotaxin
  - Eosinophils release
    - EDEPs (eosinophil-derived granule proteins)
    - ECP (eosinophil cationic protein)
    - EDP (eosinophil derived neurotoxin)
    - EPO (eosinophil peroxidase)
    - MBP (major basic protein)
  - These EDGP cationic proteins have
    - Antiviral activity
    - Ribonucleus activity
    - Degranulate mast cells

Adapted from: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* page 425, Ninth Edition, 2010

Food Allergies

Useful Background: Definitions

- The failure to develop normal oral tolerance, or the loss of oral tolerance, results in the development of food allergy.
- Adverse food reaction
  - "any untoward reaction occurring after the ingestion of a food or a food addition....."
  - ".....and may be the result of toxic or non-toxic reactions"
- Toxic reactions
  - "will occur in any exposed individual upon ingestion of a sufficient dose."

- Non – toxic reactions
  - "…..depend on individual susceptibilities...."
  - "…..may be immune – mediated....or non – immune – mediated"
- Immune – mediated non – toxic reactions – food allergy or hypersensitivity
- Non – immune – mediated non – toxic reactions – food intolerance

Source: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* page 139, Ninth Edition, 2010.

SO YOU WANT TO BE A GASTROENTEROLOGIST! – Food allergies

- Give 5 clinical or laboratory features associated with IgE-mediated food – induced symptoms.
  - Atopy
    - Atopic dermatitis
    - Allergic rhinitis
    - Allergy
  - ↑ serum
    - IgE
    - Eosinophils
  - ↓ serum
    - Hemoglobin/ iron
    - Albumin
  - Positive skin prick tests

- Give the steps in IgE – and non-IgE-mediated food allergy.

➢ IgE-mediated food "allergy"

- APCs, T and B cells act with MHC class II molecules to present T cell epitopes (small peptide fragments) to the T cells as peptide – MHC complex.
- T cells with the appropriate TCR (T cell receptor) bind the peptide – MHC complex
- Binding of the peptide – MHC complex to TCR results in
  - Proliferation of T cells
  - Production of cytokines
  - IL-4 IgE response
- IL-4 IgE activates Th2 cells.
- Th2 cells act on B cells with the appropriate antigen-specific receptors.

- The B cells produce antigen-specific IgE antibodies.
- Antigen-specific IgE antibodies binds to Fc I & II receptors on APCs (macrophages, mast cells, basophils).
- Histamine, prostaglandins, leukotrienes are released.
- The APCs are prepared for their next exposure to the antigen in question.
- Normally there is tolerance:
  - ↑ secretion of IgA & IgG
  - ↓ systemic T-cell responses
- In genetically predisposed persons, the release of these mediators leads to immediate hypersensitivity
  - Vasodilation
  - Smooth muscle contraction (bronchospasm)
  - Mucus secretion
- The activated mast cells may also release cytokines leading to late-phase inflammation.
- With subsequent exposure to the same dietary antigen, soluble proteins are presented to Th1, Th2 and Th3 (regulatory) cells.

➢ Non – IgE – mediated food "allergy"
- APC ± T cells become active and secrete.
  - TNF – α, and result in dietary protein – induced enterocolitis syndrome (cow's milk, soy protein)
  - IL-4 ± IL-5, and result in allergic eosinophilic gastroenteritis

Intestinal Ischemia

Terminology: AMI (acute mesenteric ischemia)

- Arterial
  - SMAE (SMA embolus)
    - Thrombus in SMA distal to the takeoff of the ileocolic artery is minor, proximal is major
    - If no peritonitis, consider TTT (transcatheter thrombolytic therapy)
  - NOMI (non-occlusive mesenteric ischemia)
    - Splanchnic vasoconstriction
    - Seen in hypoperfusion (shock, MI, CCF, arrhythmia, digitalis); CABG; chronic renal failure, hemodialysis, peritoneal dialysis
    - Treat with infusion of papaverine
  - SMAT (SMA thrombosis)
    - Occurs in area of narrowing from atherosclerosis
    - May occur on top of CMI (chronic mesenteric ischemia)

        - Important to distinguish between acute versus chronic thrombosis: “the absence of collateral vessels or the presence of collaterals with inadequate filling of the SMA indicates an acute occlusion and demands prompt intervention” (Feldman M., et al. *Sleisenger and Fordtran’s Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, page 2035), i.e. SMA infusion of papaverine followed by surgery for peritoneal signs
    - FSI (focal segmental ischemia)
  - The approximate frequency of these causes of AMI are:
    - SMAE, 50%
    - NOMI, 25%
    - SMAT, 10%
    - MVT (mesenteric venous thrombosis), 10%
    - FSI, 5%

- Give the anastomotic circulation between the celiac axis (CA) and the superior mesenteric artery (SMA), and between the SMA and the inferior mesenteric artery (IMA).

CA ↔ SMA	SMA ↔ IMA
○ Superior pancreaticoduodenal arte (comes off CA)	- Marginal artery of Drummond
○ Inferior pancreaticoduodenal arte (comes off SMA)	- Central anastomotic artery - Arc of Riolan

  - The central anastomotic artery or the arc of Riolan may be called the “meandering artery” when SMA or IMA are obstructed.

"In the end, it's not going to matter how many breaths you took, but how many moments took your breath away"

Shing Xiong

SO YOU WANT TO BE A GASTROENTEROLOGIST!

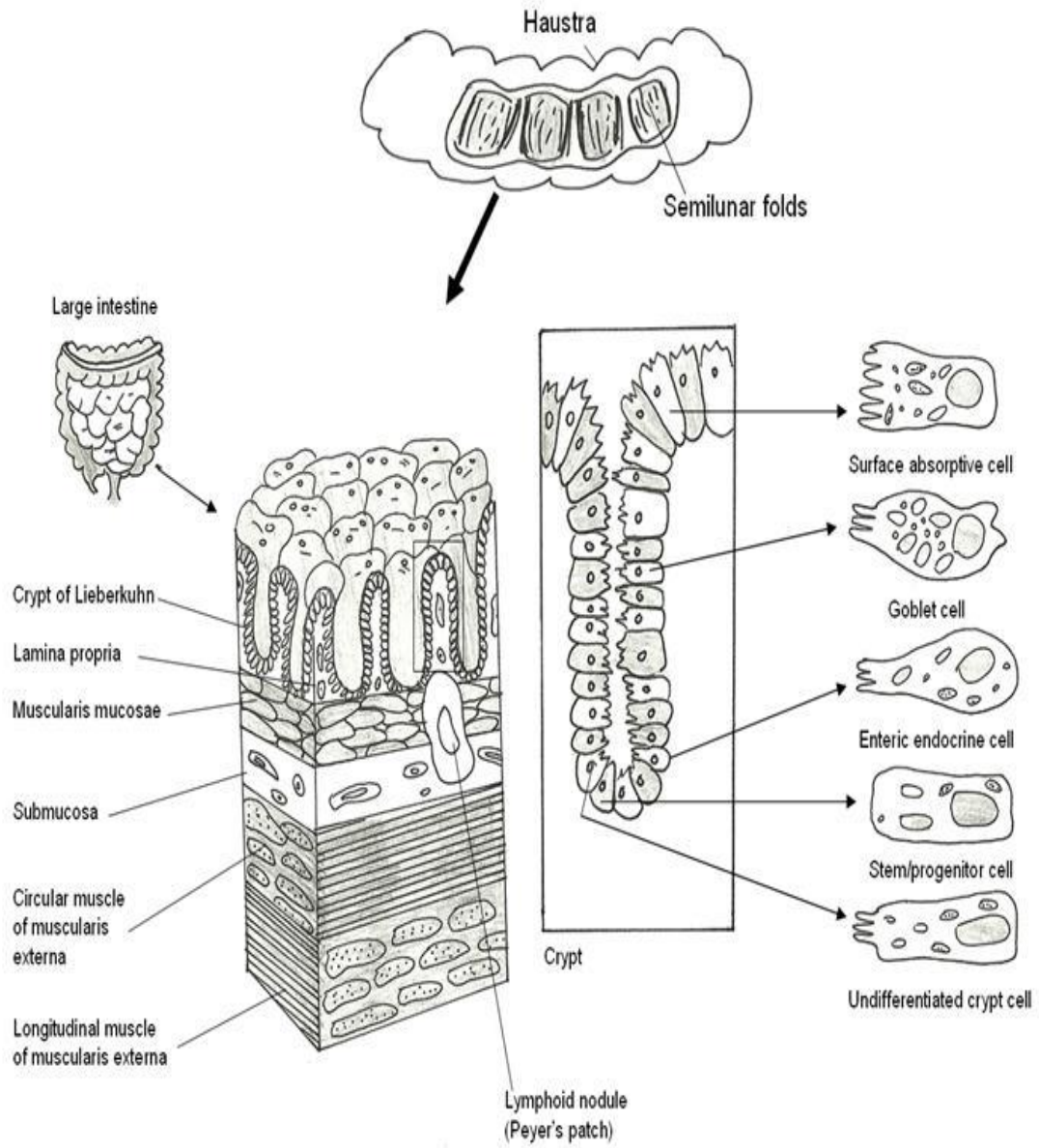
- Give the mechanism of tissue injury which occurs with reperfusion following a period of ischemia.
  - During ischemia, the enzyme XDH (xanthine dehydrogenase) becomes XO (xanthine oxidase)
  - During reperfusion, XO produces reactive oxygen radicles
  - Reactive oxygen radicals cause
    - ↑ permeability of microvessels
    - ↑ necrosis of epithelial cells
    - ↑ LT (leukotriene) B4 release
    - ↑ PAF (platelet-activating factor)
  - LTB4 and PAF → neutrophil adherence / migration → ↑ proteases → ↑ damage to endothelium

- Give 3 factors which are associated with ↑ risk of AMI (acute myenteric ischemia) in young persons.
  - Vasoactive drugs (e.g. amphetamine, phenylephrine)
  - Cocaine use
  - Thrombophilia

- Give the limitation of ultrasound studies, including Duplex and Doppler flowmetry, in diagnosing AMI
  - The peripheral portion of vessels is not seen
  - Reliable diagnosis of AMI due to NOMI not possible
  - Just because blood flow is reduced does not prove ischemia, since even total occlusions may not be causing symptoms when collaterals have developed.

COLON

Structure



Adapted from: *Medical Physiology*, Second Edition, Boron Walter F. and Boulpaepemile L. 2009, Figure 41-3, page 885.

- The wall of the colon is comprised of an inner circular and an outer longitudinal muscle layer.
- The longitudinal muscle layer is discontinuous, running in three 6-to-10 mm wide bands (teniae coli) from the cecum to the rectum.
- There is no muscle between the teniae coli, leading to three bulges in the wall of the colon, called the “haustra”.
- The haustra are between the teniae coli
- Because these muscles are tonically contracted and then suddenly relax, their size changes with contraction and relaxation of the muscle bands.
- When the muscle coats contract, the flat mucosal surface of the colon appears as folds, known as the semilunar folds or the plicae semilunaris.
- The cecum, ascending and descending colon are less mobile than the transverse colon and sigmoid colon, each of which has a mesentery.
- The ileocecal valve (ICV) appears as two transverse folds.
- The ICV is anatomically not a sphincter, but has a resting tone of 20 mm Hg and so is capable of functioning in this manner.
- When the terminal ileum is distended, the ICV pressure falls so that small intestinal contents pass into the cecum.
- The (vermiform) appendix is attached to the medial wall of the cecum of adults. Its function in humans is unknown.
- The S-shaped sigmoid colon is angulated, particularly at the rectosigmoid junction, where the colon begins to have a peritoneal surface.
- The colonic mucosa becomes smoother in the rectum, which passes between the sacrum and the uterus in females, or between the sacrum and the bladder in males.
- The inner circular muscle of the colon becomes thicker and ends as the internal anal sphincter (IAS).
- A layer of elastic fibers and the outer longitudinal layer of the rectum separate the IAS from the outer external anal sphincter (EAS).

- The EAS is skeletal muscle, and is under voluntary control.

Main diseases

- Increase or decrease in stool output (diarrhea/constipation)
- Fecal incontinence
- Inflammatory bowel disease (IBD; Crohn disease or ulcerative colitis)
- Irritable bowel syndrome (IBS)
- Colorectal cancer (CRC)

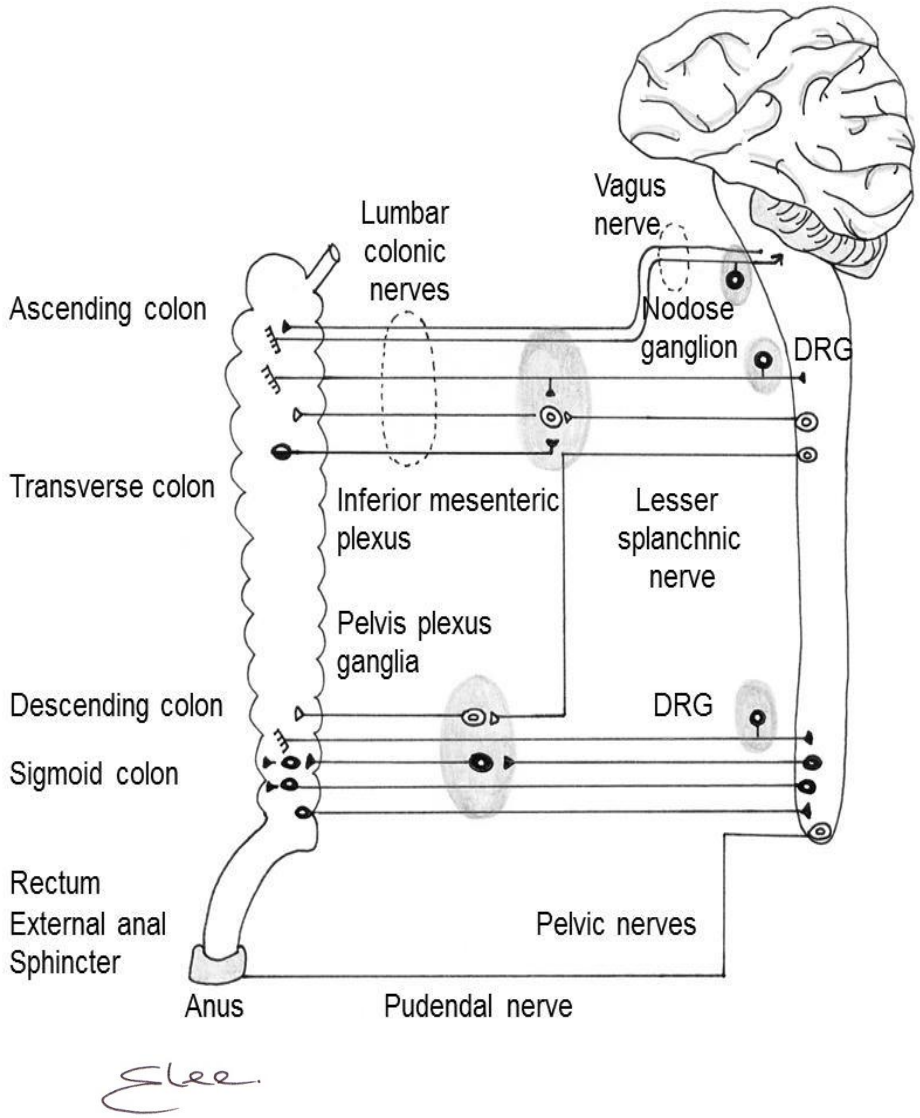
Movement/Transit

- Give the physiology of colonic motility.
  - The functions of the colon include mixing, absorption/secretion of water, metabolism, transit, storage, and control of fecal evacuation.
  - Unlike small intestine where slow waves propagate predominantly aborad (down the colon, way from the mouth and towards the anus), in the colon slow waves propagate over short distances both up and down the colon.

➢ Parasympathetic and Sympathetic Nervous System

- Enteric nerve cell bodies in the colon receive input from both parasympathetic and sympathetic pathways.
- Extrinsic nerves provide a greater control over the colon than over the small intestine.
- Parasympathetic efferent pathways from the dorsal motor nucleus of the vagus in the brainstem pass through the vagus nerve to the colon and through prevertebral sympathetic ganglia to the colon, through the lumbar colonic nerves.
- Parasympathetic pathways from nuclei in the sacral spinal cord also run through the pelvic nerves and either synapse in the pelvic plexus ganglia, or run directly into the colonic wall.

INNERVATION OF THE COLON



Abbreviations: DRG, dorsal root ganglia

Adapted from: *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Figure 98-3, page 1662, Ninth Edition, 2010

- Parasympathetic innervation
  - ↓ muscle activity
  - ↓ blood flow
  - ↓ secretion
  - ↑ contraction of IC (ileocecal) function and IAS (internal anal sphincter)

IAS inhibitory innervation	EAS stimulatory innervation
Lumbar sympathetic	Pudendal nerve

- Sacral parasympathetic
  - Sympathetic pathways consist of preganglionic neurons which project out of the prevertebral ganglia.
  - Viscerofugal enteric neurons project out of the bowel to the prevertebral ganglia.
  - There are also spinal afferents with cell bodies in dorsal root ganglia (DRG) which run
    - through both the lesser splanchnic and lumbar colonic nerve pathway, and
    - via the pelvic nerves and ganglia to the rectum.
  - These include sensory neurons that transmit non-nociceptive information about the distention of the rectum.
  - Afferent pathways consist of vagal neurons from the proximal colon with cell bodies in the nodose ganglion (NG).
  - These synapse with sympathetic postganglionic neurons, either in the inferior mesenteric plexus or in the pelvic plexus.

"Is there an upper age limit as to who should be offered a liver transplantation? "Remember that the patient's physiology is more important than chronology when making the selection decision."

Grandad

- Enteric Neuron System (ENS)

- Give the location of the nerve cell bodies which occur in plexuses in the various histological layers of the colon.

Location of nerve cell bodies	Histological layer
Meissner plexus	Mucosa / Submucosa
Schabadasch plexus	Circular muscle
Auerbach plexus	Longitudinal muscle

- Motor neuron
  - Excitatory
    - Acetylcholine
    - Tachykinins
      - Substance P
      - Neurokinin A
  - Inhibitory
    - NO (nitric oxide)
    - ATP (adenosine triphosphate)
    - VIP (vasoactive intestinal polypeptide)
    - PACAP (pituitary adenyl cyclase activating peptide)

- Motor activity
  - The pudendal nerve (S2) supplies the pelvic diagram (PD) and external anal sphincter (EAS); S3 and 4 nerves supply the puborectalis.
  - Extrinsic sympathetic and parasympathetic /afferent and efferent nerves from the lumbosacral cord run through the pelvic plexus and innervate the internal anal sphincter (IAS) and the rectum.
  - Motor activity in the proximal colon (cecum, ascending and transverse colon) are controlled by myogenic, neurogenic (vagus and pelvic nerves) and hormonal factors.

- This motor activity causes
  - mixing (non-propulsive segmentation caused by slow wave activity, creating haustra), and
  - peristalsis (mass peristalsis, propulsion distally of fecal material)
- Motor activity in the distal colon is mostly segmentation, until a mass peristalsis fills the rectum and the process of defecation is begun.
- A lump of feces will move quickly over several centimetres, then stop for awhile, then propel forward again; these are called “mass movements”.
- The mass movements occlude the colonic lumen, push in the caudal direction and gradually fill the rectosigmoid bowel.
- The intermittent rhythmic contractions that cause the mass movement of feces are neurogenic, initiated by extrinsic innervation, and are not linked to electrical slow waves.
- During the intermittent ring contractions, the haustra temporarily disappear.

- Colonic stimulus → activation of IPANS (intrinsic primary afferent neurons) → excitation of
  - Ascending excitatory nerves, proximal contraction
  - Descending inhibitory nerves, distal relaxation

- Colonic transit is controleed by the parasympathetic and sympathetic nervous systems

- Rhythmic myoelectric activity from layer of middle of circular smooth muscle
  - Ingestion, digestion and absorption of food
  - MMCs move food to ileocecal valve
  - Monophasic, ileal propagating pressure plus inhibition of phase contractions of the ileocecal junction, pumps chime into the cecum within 90 mm of a meal
  - Cecal filling triggers proximal colonic propagating contractions
  - MPOs (myenteric potential oscillations)
    - ↓ 10-12 per minute
    - ↓ arise from myenteric plexus
    - MPO-related contractions summate to result in longer and stronger contractions
  - Slow waves
    - Arise from submucosal border of inner circular muscle

- 2-4 per minute

- Colonic motility
  - Feeding pattern
    - Segmental (mixing) activity
  - Fasting (propulsive) pattern
  - HAPC (high-amplitude propagated contractions, aka mass movements)
  - LACA (low-amplitude contractile activity)

- CCK and Motor Activity
  - The tonic ring contractions are myotonically produced.
  - The pacing of the regular rhythmic contractions is by way of electrical slow waves, slower in the colon (every 10 sec.) versus faster in the small intestine (every 6 sec).
  - After eating and the entry of food into the duodenum, there is release of CCK.
  - CCK increases the neurally mediated motor function of the ileum and the colon (especially the sigmoid colon), known as the “gastrocolic reflex”.
  - The movement (transit, aka craniocaudic translocation) of feces is more marked in the colon than is the mixing flow.
  - Flow from the stomach to the cecum takes 1-2 hours, but flow from the cecum to the rectum takes 5-7 days.
  - Transit through the transverse and descending colon is relatively faster than through the rest of the colon.
    - MPOs and slow waves in colon
      - Non propagating waves mix stool
      - Excitatory motor neurons cause MPOs to summate and lead to HAPCS (high-amplitude propagating contractions)
    - The HAPC overcomes the RMC (rectal motor complex)
    - The rectum fills, and dilates to accommodate the stool bolus
    - Stretching of the rectal wall
      - Simultaneously activates an enteric descending inhibitory reflex to relax the IAS
      - Extrinsic reflex pathway to contract EAS

- Colonic and Rectal Motor Patterns

- Non-propagating
  - Random
  - Segmenting and mixing
  - 2-4 cpm (cycles per minute)
- Propagating
  - Summation of MPOs (myenteric potent oscillations)
  - Aka high-amplitude propagating sequence or HAPCs (high-amplitude propagati contractions)
- Rectal motor complex (RMC, aka PRMA [periodic rectal motor activity])

➢ ICC (interstitial cells of Cajal) in the colon

- ICC-MP
  - Myenteric plexus
    - Pacemakers for rapid oscillations in MPOs of circular and longitudinal muscle
- ICC-SM
  - Submucosal plexus
    - Pacemakers for high amplitude, slow waves
- ICC-IM
  - Intramuscular, between circular and longitudinal muscles
    - Integrate pacemaker activity and neuronal inputs to smooth muscle

CLINICAL PHYSIOLOGICAL CHALLENGE – Constipation

Case: A 30 year-old mother of three pre-schoolers presents with a 3 month history of increasing constipation, by which she means three BM's per week, passing hard stools. There is no abdominal pain and no rectal bleeding. She is otherwise well.

- Give the normal physiology of colonic transit. Classify the medications used to treat constipation. Give the safety of these medications during pregnancy and lactation.

Irritable Bowel Syndrome (IBS) and Functional Abdominal Pain

The irritable bowel syndrome (IBS), and functional abnormal (pain (FAP) are two pain syndromes which affect the GI tract. The focus in recent years has switched from the possibility of their pathogenesis resting on abnormal motility, to the concept of abnormal pain perception (visceral hypersensitivity).

- Components of IBS / FAP may include
  - Dysregulation of CNS – ENS (central [C] and enteral [E] nervous systems)
  - ↑ pain perception
  - ↓ coping strategies
  - ↓ social net works

➢ Physiology: Transmission of ascending visceral pain

- First-order neurons
  - Visceral C-fibers → thoracolumbar sympathetic nervous system → dorsal horn of spinal cord
- Second-order neurons
  - Cross the spinal cord
  - Ascend in
    - Spinothalamic pathway tracts
    - Spinoreticular pathway tracts
    - Reticulothalamic tracts
- Synapse of second – order neurons in the thalamus with third – order neurons which synapse in the limbic system.
  - Sensory – discriminative component:
    - Insula – input from sensory thalamus and nucleus tractus solitaries
    - Integrates sensory and emotional information
    - ACC (anterior cingulated cortex) – modulates affective component of pain
    - Primary somatosensory cortex

- Visceral afferent fibers
  - Parasympathetic
    - Vagus (→ medulla)
    - Pelvic nerves (→ S2 to S4)
  - Sympathetic
    - Superior cervical ganglion (T1 to T4)
    - Celiac ganglion (T5 to T12)
    - Superior mesenteric ganglion (to celiac ganglion)
    - Inferior mesenteric ganglion (L1 to L3)

- Descending modulation of pain
  - Endorphin -/ enkephalin – mediated inhibitory system
  - Cortex/ limbic system → midbrain/ medulla → dorsal horn of spinal cord
    - Directly inhibits nociceptive projection on second – order neurons
    - Indirectly inhibits spinal cord interneurons
  - Dorsal horn of cortex modulates (↑ or ↓ transmission; gate control theory) the amount of input from the gut to the peripheral afferents.
  - The descending inhibition of afferent nociceptive impulses from peripheral sites is achieved through the serotonergic and epinephrine pathways, modifying endorphin activity.
  - These descending inhibitory systems are diffuse (DNIC [diffuse noxious inhibitory control], and are not activated normally in functional abdominal pain [FAP]).
  - In FAP, there may be ↑ afferent signals, and ↓ descending pain control, producing hypersensitivity (↑ pain response) to a painful signal, possibly due to cleavage in the serotonin (5-hydroxytryptomine [5-Ht]) receptors, 5-HT_1, 5-HT_3, 5-HT_4 or 5-HT reuptake.
  - Also, the ACC (anterior cingulated cortex, the cortical motivational – affective component) is abnormal in FAP/ IBS (irritable bowel syndrome).

➢ Pathophysiology

While the cause of IBS (**irritable bowel syndrome**) is unknown, there are many suggestions for the etiology.

- Give 4 proposed pathophysiological causes of IBS.

  - Genetic
    - ↑ concordance in monozygotic is dizygotic twins
    - SCN5A (sodium channel) mutation
  - Nervous system
    - Psychological factors
      - Depression, anxiety, somatization
      - Abuse sexual, physical, emotional
      - Childhood stress
    - hypervigilance (↑ attention to pain)Cent dysregulation
      - PET scanning changes in ACC (anter cingulate cortex) activity with rectal distention
  - Diet
    - Wheat, lactose, fructose, sorbitol
    - Food-exclusion diets may be successful, especia when IBS-sufferer has a positive IgG antibo response to the food
  - GI tract
    - Motility
    - HAPCs (high-amplitude propagated contractions):
      - ↓ in IBS-C
      - ↑ in IBS-D
    - ↑ gastrocolic reflex
    - Transit
      - ↑ in IBS-D
      - ↓ in IBS-C
    - ↑ frequency and duration of jejunal contractions
    - Visceral hypersensitivity
      - Dysfunction
        - IBS-D ↑ sympathetic adrenergic dysfunction
        - IBS-C ↑ vagal dysfunction
      - Abnormal sensation
        - Spinal cord (dorsal horn)
        - CNS
      - Abnormal receptors
        - TRPV1 (transient receptor potential vanilloid -1)
        - NMDA (N-methyl-D-aspartate)
        - PARs (proteinase-activated and serine proteas receptors)
        - Inflammation trigger

- Give 8 observations which suggest that inflammation may play a role ("trigger") in the pathophysiology of post-infectious IBS.
  - ↑ mast cells in muscularis externa
  - ↑ TNF-α
  - ↑ SIBO (mall intestinal bacterial overgrowth)
  - ↑ lymphocytes around myenteric plexus
  - ↑ neuron degeneration
  - Post-infection IBS
    - ↑ permeability
    - ↑ enterochromaffin cells
    - ↑ CD3, CD4, and CD8 T cells
    - ↑ macrophages

Please see Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, Table 118.4, page 2101, "Management Recommendations for Irritable Bowel Syndrome".

Functional abdominal pain syndrome (FAPS)

- The explanation of FAPS arises from the biopsychosocial model of illness, with the experiencing of pain arising from physiological, psychological (emotional) and cognitive (CNS – gut neuraxis) components

- There is
  - Upregulation of nociceptors in the mucosa
  - Sensitization of the visceral afferent nerves
  - Increased visceral efferent nerve signals in the brain (brain – gut dysregulation)

Avoid Common Clinical Mistakes!

It is often suggested that it is important clinically to avoid the use of narcotics and benzodiazepine (BZ) in IBS and FAP

- The basis for this concept:
  - Narcotics and "benzo's" (benzodiazepines) must be avoided in FAP/ IBS
    - ↑ pain sensitivity
    - ↓ pain threshold
    - ↑ dependency
    - Narcotic bowel syndrome
    - Do not withhold analgesia in person with an "acute abdomen", since several (6) studies have shown that giving such patients sufficient pain-relieving analgesia does not delay the making of the correct diagnosis of the cause of the acute pain.

- Another common mistake
  - Surgical lysis of adhesions for chronic post-operative pain: "avoid laparoscopic adhesiolysis". The problem is, this common clinical recommendation is not evidence – based

In the context of the patient with intra-abdominal pathology, what is the Kehr sign, and give its mechanism?

- Any cause of irritation of the diaphragm, for example from subdiaphragmatic abscess, hematoma, or splenic rupture, the pain m be referred to the left shoulder.

- "Referred pain is ordinarily located in the cutaneous dermatomes th share the same spinal cord level as the affected visceral inputs" (Yar JC & Friedman LS. *Sleisenger and Fordtran's Gastrointestinal and Liv Disease.* Figure 31-1, page 163, Ninth Edition, 2010).

"Simplify complex issues"
Grandad

Classification of Laxatives

- Give a classification of laxatives, their mechanism(s) of action, and their potential limitations.

Laxative class	Laxative agents	Mechanism of action	Potential limitations
➢ Bulk laxatives	○ Natural fibre (e.g. psylium seed husk) ○ Semisynthetic fibre (e.g. methylcellulose) ○ Synthetic fibre (e.g. polycarbophil, polyethylene glycol macogol)	– Luminal water binding increases stool bulk and reduces consistency	• Abdominal distention
➢ Osmotic salts	○ Magnesium hydroxide, Magnesium citrate, Magnesium sulphate, Sodium phosphate	– Osmotic water binding	• May cause electrolyte abnormalities; to be used with caution in patients with renal or cardiac failure
➢ Disaccharides and sugar alcohols	○ Lactulose ○ Sorbitol	– Osmotic water binding	• Bacterial fermantation with bloating, flatulence • Less effective in slow transit due to bacterial degradation
➢ Stool softener	○ Paraffin oil ○ Docusate	– Luminal water binding increases stool bulk an reduces consistency	• Abdominal discomfort and cramps
➢ Stimulant laxatives	○ Diphenylmethane derivatives (bisacodyl, sodium picosulphate) ○ Anthraquinone derivatives (senna, aloe, cascara)	– Induce colonic contractions by acting on enteric nerves – Decrease colonic absorption of water and electrolytes	• Abdominal discomfort and cramps

Printed with permission: Tack J. Current and Future Therapies for Chronic constipation. *Best Practice and Research Clinical Gastroenterology* 2011; 25: 151-158.

Intestinal Gas

SO YOU WANT TO BE A GASTROENTEROLOGIST!

- Name 6 major intestinal gases.
    - From inspired air – N_2, O_2, CO_2
    - from metabolism – H_2, CH_4, CO_2, H_2S

- Give why it is suggested to persons with "intestinal gas" to eat slowly and to drink at the end of meals.

For every 10 mL of fluid consumed, we swallow about 18 mL of air!

- Give the mechanism to explain why breath Hydrogen levels may be falsely low and thereby misrepresent the presence of SIBO (small intestinal bacterial overgrowth).

H2 is produced by intestinal bacteria, some of which consume H_2 to reduce sulfate to sulfide, or use H_2 to reduce O_2 to CH_4 (methanogenic bacteria).

- After eating garlic – containing foods, why does flatus not smell of garlic?

The odoriferous ("smelly") sulfur-containing gas which is derived from garlic is absorbed and excreted in the breath, not in the stools.

- Give the normal 24-hour volume of passage of flatus which is passed per rectum, and the number of individual passages.

The average person passes per rectum about 2200 mL of flatus per day, falling to about 400 mL per day and a low-fiber diet. The frequency of passages ("farts") is about 10 per day.

- Give the relative rate of passage of substances along the GI tract.

Gas faster than liquids or solids.

- Distinguish between the terms "bloating" and "distention".
    - Bloating – "subjective sensation of a swollen abdomen, full belly, abdominal pressure, or excess gas" (no correlation between measured/ intestinal content of gas and symptom of bloating – symptom of bloating is due to a disturbance in the gas.
    - Distention ".... an objective increase in [abdominal] girth".

(*Sleisenger and Fordtran's Gastrointestinal and Liver Disease*. Figure 31-1, page 238, Ninth Edition, 2010.

Defecation

- Give the muscles involved in defecation.

Condition	Type of Muscle	
	Skeletal Muscle	Smooth Muscle
○ At rest	– Relax ▪ Puborectalis ▪ EAS	– Relax ▪ IAS
○ Contract – Puborectalis – EAS, IAS	– Contract ▪ Diaphragm (mov down) ▪ Levator ani (mov up) ▪ Rectus mus (moves inwards)	– Contract ▪ Rectum ▪ Urge to purge

Abbreviations: EAS, external anal sphincter; IAS, internal anal sphincter

- Give the nerves involved in defecation.
  - ○ Anorectum
    - – Sensory and motor function of pudendal nerve (S2 to S4)
  - ○ Rectum (distention)
    - – S2 to S4 parasympathetic nerves, which travel with the splanchnic (and not the pudendal) nerves
    - – Parasympathetic stimulation contracts and sympathetic stimulation relaxes.
  - ○ Rectal mucosa and myenteric plexus – myelinated and unmyelinated nerve fibers which mediate:
    - – Reflexes (viscerovisceral, rectoanal contractile and sympathetic reflex
    - – Stretch (distentions) causes
      - ▪ Rectoanal inhibitory reflex, with ↓ resting pressure
      - ▪ Rectoanal contractive reflex response
  - ○ Pelvic floor
    - – The striated muscles of the pelvic floor (including the external anal sphincter) are supplied by motor neurons with cell bodies in the spinal cord and with axons that run in the pudendal nerves (S2).

- Give the anatomical components of fecal continence.
  - Anus
    - 2 cm to 4 cm muscular tube, closed at rest
    - The spongy vascular tissue underneath the squamous epithelium in the anal canal are also important to maintain fecal continence.
  - Anorectal angle
    - At rest, 90^{o}
    - Squeeze, 70^{o} (acute)
    - Defecation, 110 – 130^{o} (obtuse)
  - Internal anal sphincter (IAS) – 0.3 cm to 0.5 cm expansion of rectal smooth muscle.
  - External anal sphincter (EAS) – 0.6 cm to 1.0 cm expansion of levator ani muscles.
  - Contributions of pressure of anal sphincter (IAS plus EAS), %

		IAS	EAS
- Resting		75	25
- Response to distentio	▪ Sudden	40	60
	▪ Constant	65	35

  - Seal of the anus
    - Tonic activity of IAS plus EAS
    - Anal mucosal folds
    - Anal vascular cushions
    - Flap – like valve of puborectalis muscle
    - Normally the puborectalis muscles (PR) is contracted, so that there is a sharp 90° angle between the axis of the rectum and the anal canal. When the PR muscle relaxes, the rectoanal angle becomes <90%.
    - The levator ani is comprised of three muscles (puborectalis, pubococcygeus, and ileococcygeus) which form the pelvic diaphragm and a puborectal sling.
    - When the levator ani contracts, there is a rise of the pelvic diaphragm (PD), the rectum and anus.
    - This rise in the PD causes a decrease of the anal-rectal angle, and straightening of the rectum.
    - When the pelvic floor relaxes, the anal-rectal angle increases.
    - Receptors lead to reflex relaxation of internal anal sphincter (IAS).
    - The myotonically-produced tonic ring contraction involves the IAS.

- The tone in the IAS is reduced or increased by neurogenic mechanisms (NANC inhibitory and cholinergic stimulatory motor nerves).
- This process of ↑ storage of stool continues until the person elects to have a BM (bowel movement), or until the mechanism to provide continence fail, and there is incontinence.
- As the rectum distends further, the signal of discomfort and the need to defecate is transmitted centrally.

CLINICAL PHYSIOLOGICAL CHALLENGE – Fecal incontinence

Case: A 60 year old mother of three children presents with fecal incontinence. There is no overflow diarrhea or urinary incontinence.

- Give
  - The physiology of defecation
  - The approximate normal values of anorectal sphincter pressure and volumes of balloon distention to achieve rectal sensation.
  - The tests used to investigate fecal incontinence, including sensory, motor and biomechanical function, as well as rectal capacity and compositive sensory motor function.
  - The medical, endoscopic and surgical treatments of fecal incontinence.

- The striated and voluntarily-innervated skeletal muscle of the external anal sphincter (EAS) surrounds the IAS.
- Signals also arise in the rectus muscles of the abdominal wall and thorax, stimulating central sensory pathways.
- The defecation reflex is initiated from these local and central afferents; a ring contraction beginning at the rectosigmoid junction, relaxation of the levator ani muscles, leading to relaxation of the IAS through NANC nerves.
- The pelvic diaphragm is composed of the levator ani, puborectalis, pubococygeus and ileococcygeus skeletal muscles. The pelvic diaphragm has holes through which pass the urethra, rectum, and vagina in females.
- When the IAS relaxes and intraabdominal pressure is voluntarily increased, defecation occurs.
- The function of the IAS may be assessed clinically by rectal manometry, a test which may be performed as part of the investigation of the incontinence:

➢ Nerve supply

- Give the process of normal defecation
  - Maintenance of fecal continence and the process of defecation are complex and integrated processes.
  - Continence of stool is controlled by the internal and external anal sphincters, the rectum, the pelvic diaphragm, and the coordinated innervation of these of these structures.

➢ Muscle sequences with voluntary defecation

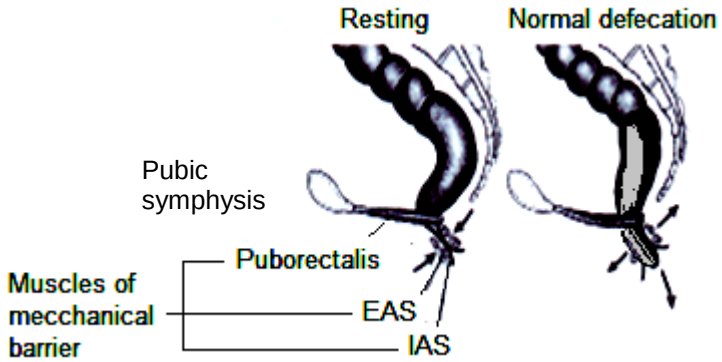
- Sit on toilet
  - Perineum (anorectal junction) descends
  - ↑ ARA (anorectal angle)
- Conscious decision to follow the "urge to purge"
  - Relax EAS
  - Relax puborectalis ↑ ARA even more
  - Levator ani contracts
    - Perineum descends more
  - Stool is "funneled" into anal canal
  - Beginning one hour before defecation, there is
    - ↑ colonic phasic and tonic activity (gastrocolonic response)
    - ↑ frequency of propagating pressure waves (which represer the preparatory phase to defecation)
    - This gastrocolonic response occurs with 300 kcal, especia from fat, and may result from the food-induced effects on HT3 receptors on vagal afferent
  - These propagating waves push stool into the rectum, and
    - Stimulate sacral spinal efferent mechanoreceptors
    - Activate an enteric descending inhibitory reflex (DIR)
  - The DIR causes transient
    - ↓ IAS pressure
    - ↑ EAS pressure
  - The mechanoreceptors cause the defecatory urge, a defecation can be postponed with
    - Receptive rectal accommodation (↑ rectal volume, with no intrarectal pressure, mediated by inhibitory nerves
    - Voluntary suppression of the defecatory urge
    - ↓ gastric emptying
    - ↓ small bowel transit

- ↓ colonic transit (by ↓ proximal colonic propagating pressu waves)

- Straining
  - ↑ perineal descent
  - ↑ ARA (anorectal angle)
  - Contraction of
    - Diaphragm
    - Abdominal muscles
  - If defecation is not to be postponed, then there is
    - ↓ IAS pressure
    - Colonic motility pushes stool into the rectum.
    - Stool moves into upper anal canal
    - The rectum distends to accommodate the stool.
    - Rectal compliance increases, the intraluminal pressure does not increase, and the rectum continues to receive stool.
    - Anorectal junction descends with sitting
    - Rectum descends further with straining (contraction of diaphragm and abdominal wall [valsalva maneuver]
    - As feces enters the rectum, the rectum expands and the sensory mechano- and chemoreceptors are stimulated, giving the sensation of “the urge to purge”.
    - When the colon pushes stool into the rectum, the rectum adapts, stretches and stores the stool
    - When the rectal distention reaches a point where the person senses the distention, vagal effects are stimulated
    - If the person wished to wait to have a “bowel movement” (BM), s/he voluntarily causes the stimulation of the external anal sphincter (EAS), and the stool stays in the rectum
    - If the person wishes to respond to the sensation of rectal distention and to have a BM, they push down with their abdominal muscles, tighten the pelvic floor, and relax the EUS
    - The vagal efforts cause the internal anal sphincter (IAS) to relax, the puborectalis relaxes, the rectum straightens, and stool is expelled
    - Sitting and straining increase cause ↑ anorectal angle
    - Puborectalis relaxed with ↑ anorectal angle
    - Levator ani muscles contract
    - Perineum descends

- Stool moves further into anal canal
- Voluntary ↓ EAS pressure
- Successful expulsion of stool

- Rectal distension by large volume of stool stimulates reflex relaxation of internal anal sphincter → stool moves into the upper anal canal → urge
- Sitting or squatting causes descent of anorectal junction
- Straining produces further rectal descent
  - Both activities serve to increase anorectal angle → less resistance to outflow
- External anal sphincter (EAS) relaxes voluntarily
- Puborectalis relaxes (↑ anorectal angle)
- Levator ani contracts → perineum descend (↑ anorectal angle); stool funnelled into anal canal and expelled by increasing strain-induced, intrarectal pressure
- Colonic contractions propagating toward anus may allow evacuation without further straining.



- Puborectalis muscle – may contain sensory receptors to distinguish between flatus and feces (sampling reflex)
- Once defecation occurs, the rectosigmoid colon becomes empty, and is ready to be filled again with the mass movement of feces.
- The EAS contracts.

Adapted from: Schey R, et al. Am J Gastroenterol. 2012;107(11):1624-33.

Dissynergic defecation

If the process of normal defecation is abnormal and poorly co-ordinated, dyssynergic defecation may occur, resulting in constipation or a form of constipation predominant (c), irritable bowel syndrome (IBS; C-IBS):

- Colonic transit
  - ↓ (slow)
- Rectal sensitivity
  - ↓ (hyposensitive)
- Rectal muscle action
  - ↓ (↓ compliance, ↓ propulsion) IAS / EAS
  - Relaxation
- Straining
  - ↓ coordination of straining (abdominal muscles and diaphragm) and change in angle of puborectalis)

<u>Rectal Manometry</u>

- A useful clinical test in the patient with incontinence.

➢ Anorectal Sphincter Pressure*

○ Anorectal sphincter resting pressure	58 mm Hg
○ Pressure squeeze during voluntary contraction	130 mm Hg
○ Rectal pressure during straining	34 mm Hg
○ Duration of the voluntary contraction	12 sec

➢ Rectal Sensation

○ Balloon volume at first rectal sensation	100 cc ml
○ Balloon volume at defecation urge	180 cc ml

➢ Balloon Expulsion Test	12 sec
➢ Inhibitory Anorectal Reflex	Present

- Rectal Compliance 1.6-5 cc

*Approximate Normal Values used in Clinical Practice

Fecal Incontinence

- When any of the normal components which contribute to fetal continence are dysfunctional, fecal incontinence (FI) may develop
- Fecal incontinence (FI) is a frequent cause of major embarassment to sufferers
- FI is common in the elderly, infirm, after obstetrical injury
- FI may occur with diarrhea, and the patient with diarrhea must be carefully questioned, since they may really be suffering from incontinence

There are numerous components of the normal processes for the maintenance of fecal continence which may become disordered and cause incontinence:

- Muscle weak puborectalis
  - ↓ resting and squeeze pressures of IAs and EAS
  - ↓ sensation of rectum and anus
  - ↓ rectal capacity
  - ↑ fluidity of stools

- Give the approximate % contribution of various mechanisms to the development of fecal incontinence.

Mechanism	%
Dysfunction of anal sphincter	75
↓ pudendal nerve function	50
↓ rectal sensation	50
↓ rectal compliance	40

For complete details of mechanisms, causes, and pathophysiology of fecal incontinence, see *Sleisenger and Fordtran's Gastrointestinal and Liver Disease,* Table 171, page 243, Ninth Edition, 2010.

SO YOU WANT TO BE A GASROENTEROLOGIST!

- In the context of fecal incontinence, what is “rectal agnosia”?
    - Rectal agnosia is loss of the normal sampling reflex, leading to loss of the ability to distinguish between flatus and feces.

- What is the “anal wink”, and what is the significance of its loss?
    - Loss of the normal contraction of the EAS on touching the perianal skin to elicit the anocutaneous reflex (“anal wink”) suggests impaired afferent or efferent nerve damage.

- What is the use of neurophysiologic testing to measure PNTML (pudendal nerve terminal motor latency time)?
    - PNTML accesses the function of the pudendal nerve, a cause of fecal incontinence. Unfortunately, pudendal neuropathy may be present even in the pressure of a normal PNTML time (false-negative). Also note that symptom assessment does not always correlate with the severity of manometric findings.

- What is “biofeedback therapy”, and in what type of incontinence is biofeedback training most effective?

Biofeedback therapy is a form of neuromuscular training which is based on techniques of operant conditioning. The intention of feedback training is to improve.

- The strength of the muscles of the anal sphincter
- Anorectal sensation
- Coordination of the muscles involved with voluntary contraction after rectal distention (abdominal, gluteal, EAS).
- Biofeedback is as effective as electrical stimulation of the pudendal or sacral nerve.

- Give 4 techniques used to investigate fecal incontinence.

Function	Test	Parameters measured
○ Sensory function	– Latex ballon distension – Barostat bag distension – Electrical stimulation – Brain 'image'	• Threshold volumes • Threshold volumes and pressures • Mucosal electrosensitivity thresholds • Mucosal thermosensitivity thresholds • Rectal evoked potentials • Function magnetic resonance imaging
○ Motor function	– Prolonged manometry – Prolonged barostat	• Phasic contractile activity • Tonic contractile activity, phasic contractile events
○ Biomechanical function	– Barostat pressure-volume curve – Impedance planimetry	• Compliance • Compliace, rectal wall stree and strain
○ Capacity / size	– Barostat study under fluoroscopy – MRI	• Diameter • Rectal dimensions
○ 'Composite' sensorimotor function	– Ballon expulsion – Evacuation proctography – MR defaecography	• Evacuatory function • Evacuatory function • Evacuation function

Printed with permission: S. M. Scott et al. *Best Practice and Research Clinical Gastroenterology* 2011; 25: 103-118

Colorectal Cancer (CRC)

This section on oncogenesis provides the background for considering CRC.

➢ Cellular process

- Give the cellular processes through and by which a balance of cell growth is achieved, describe the programs which trigger apoptosis and control proliferation, and use colorectal cancer as an example of the disruption of normal cell proliferation leading to oncogenesis.

➢ Cell growth

- Proliferation
- Differentiation
- Senescence
- Apoptosis (programmed cell death)

➢ Apoptosis

Definition: "Apoptosis is an energy [ATP]-dependent, genetically programmed form of death [controlled cell suicide] that typically results in controlled deletion of individual cells (Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, page 1420).

- Membrane – bound death receptors (THF-R, Fas and DR5)
  - Activate caspase 8
  - Caspase 8 activates BID (bd-2 interacting domain)
- Activation of expression of the tumour suppression gene TP53, ↑ bax/bcl-2 ratio
  - Caspases are intracellular cysteine proteases

- Give the pathophysiology of apoptosis.

➢ Cellular process of programmed cell death

- Caspase-mediated
  - Proteolytic enzymes which contain cysteine at their active site
  - Cleave polypeptides at aspartate residues
- Non-caspase-mediated apoptosis
  - JNK (c-jun N-terminal kinase) inactivation by phosphorylation of Bc1-XL (protective protein is lost, leading to apoptosis)

- Toxic biochemical process
  - External pathway
    - Acting on cell surface receptors (death domain [ligand])
      - Fas-ligand
      - TNF-R1 receptor
      - TRIAL (TNF-related apoptosis-inducing ligand) receptors
  - Internal pathway
    - Within the cell
    - Recruitment of adaptor molecules
      - FADD (Fas-associated death domain)
      - TRADD (TNF receptor-associated death domain)
    - FADD and TRADD activate procaspase 8
    - Procaspase 8 forms DISC (death-inducing signaling complex)
    - Procaspase 8 forms caspase 8
    - Caspase 8 acts on Bcl-2 (B cell lymphoma / leukemia family), cleaving Bid into t Bid
    - T Bid causes Bax to translocate to the mitochondria
    - In the mitochondria, Bax aggregates with Bak
    - Bax-Bak increases permeability (“permeabilizing” proteins)
    - Other permeabilizing proteins besides Bax-Bak including
      - Bmf, from the cytoskeleton
      - Bim, from ER
    - There are proteins which protect the mitochondrial membranes from ↑ permeability, such as Bcl-2 and Bcl-XL
    - The net effect on permeability depends on the balance between pro- and anti-permeabilizing protein effects
    - Permeable mitochondria release cytochrome C and SAME (binds inhibitors of the caspase proteins; thereby increasing the effect of the caspase proteins and acting like a pro-death molecule)
    - Inhibitors of the caspase proteins which are bound by SMAC include
      - IAPs (inhibitors of apoptosis proteins)
      - AIF (apoptosis-inducing factor; aka A paf)
    - Formation of the aptosome activates caspase 9
    - Caspase 9 activates caspase 3
    - Active caspase 3 causes apoptsis by A-1 ATP-dependent process
    - These are mitochondrial related processes
    - These are non-apoptosome process, which by-pass the mitochondria

- For example, stress to ER
  - Activates caspase 9, and then caspase 3
  - Impairs protein folding

- "in the presence of ATP, ell death can proceed by apoptosis, but when mitochondria are deenergized, the mechanism of call death is necrosis" (Feldman M., et al. *Sleisenger and Fordtran's Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, page 1421).
- These proteases act out the aspartate residues of their substrates
  - The pro apoptotic bax and bak and the antiapoptotic Bcl-2 and bcl-1 converge on the mitochondria
  - The mitochondria release cytochrome c
  - The apoptosome
    - Formed from cytochrome c, caspase 9 and Apaf 1
    - Activates several caspases, which lead to cell death (apoptosis)
- Histological features of apoptosis
  - Cytoplasm
    - Condensed, convoluted
  - Nucleus
    - Chromatin condensed
    - Convoluted
    - Fragmentation
  - Cell surface
    - Convoluted
  - Membrane – bound, cell residue – filled apoptotic bodies

SO YOU WANT TO BE A HEPATOLOGIST!

You are asked to look at a liver biopsy which shows a necroinflammatory reaction, and to comment if there is apoptosis or necrosis.

- Give the histopathological signs of apoptosis.
  - Apoptosis is "bland", i.e., there is not the attraction of lymphocytes, neutrophils, eosinophils and macrophage, which is seen with necrosis.
  - Cell
    - Small
  - Nucleus
    - Shrunken
  - Nuclear chromatin
    - Condensed at margin of nucleus
  - Plasma membrane
    - Blebs (membrane-bound bodies from fragmented cells)

- Proliferation
  - Proliferation occurs when cells transition from G0 arrest into the active cell cycle.
  - Proliferation is achieved by external stimuli acting through regulatory peptide growth factors, extracellular matrix molecules, and cell – cell adhesion molecules.
  - The adhesion molecules (e.g., integrins, adherins, selectins, proteoglycans) modulate external growth stimuli, and thereby the cytoskeleton of the cell.
  - There are 3 main types of receptors on the surface of cells which initiate cell signaling and alter the transcription of genes.
    - Tyrosine kinases
    - Serine and threonine kinases
    - G-protein coupled receptors
  - Two examples of signaling pathways which regulate the cell cycle and proliferation of intestinal epithelial cells include
    - Wnt-β-catenin - ↓ p21 - ↑ proliferation extracellular EGF, CSF-1, PDGF, and
    - IGF-↑ cyclin D1 gene expression
  - Proliferation may be balanced by growth – inhibiting signals, e.g.
    - TGF-β mediated arrest of the cell cycle at the G1 phase by the induction of the transcription of p^{15}, p^{21} and p27K1P1

CLINICAL PHYSIOLOGICAL CHALLENGE – CRC

Case: A 65 year old man presents with colorectal cancer (CRC). He wishes to know whether his family is at risk.

- Give the genes associated with CRC.
  - Three types of genes are induced in the transformation of normal to malignant cells:
    - Oncogenes (↑ cell growth)
    - Tumour suppressor genes (↓ growth)
    - DNA repair genes (↑ genome instability)

    - Mutations in tumour suppressor genes alter the normal function of these genes, such as
      - The APC gene which inhibits Wnt signaling; the MEN 1 gene which regulates histone methyltransferase; E-cadherin, which interrupts cell - cell interactions

        - SMAD+, which is included in the transduction of TGF-β,
        - TP53 which regulates DNA repair and apoptosis
    - Tumour suppressor genes may undergo gene deletions; these gene deletions lead to polymorphisms, which on a molecular level distinguish between paternal and maternal alleles.

About 6% of us will develop CRC. CRC develops from benign growths in the bowel, polyps. Since benign polyps can be found and removed before they become malignant, their CRC is a potentially reventable cancer.

- Give the abnormal molecule pathways involved in the pathogenesis of sporadic colorectal cancer (CRC).
    - Activation of oncogenes, e.g. K-Ras

SO YOU WANT TO BE A GASTROENTEROLOGIST!

- Give the name of the gene in the APC gene family which increases the risk of colon cancer (CRC) in Ashkenazi Jewish persons, and the gene which may be altered and to thereby increase the risk of CRC from the metabolism of nutrients and environmental agents.
    - In Ashkenazi Jewish persons
        - I 1307 K
    - Environmental risk
        - Methylene tetrahydrofolate reductase
        - N-acetyltransferase-1 and -2

- Give 10 examples of molecular genetic changes associated with sporatic or familial / hereditary colorectal cancer (CRC).

    - There are multiple genes which are altered in at least some patients with CRC, and these gene mutations may become important at different stages in the development of CRC.

➢ Stability genes

- MMR (DNA mismatch repair) gene
    - Germline mutations
        - hMLH1
        - hMSH2
        - hMSH6

- BER (base-excision repairs) gene
  - MUTYII (mutated BER gene; aka MYN)
  - MMR gene mutations load to MSI (microsatellite instability)
  - MSI in 15% sporatic and 55% of HNPCC CRC (colorectal cancers)
- There are three important classes of these sporatic CRC-associated gene
  - Proto-oncogenes
  - Tumor suppressor genes
  - MMR (mismatch repair) genes
- Other important gene mutations include
  - RAS / RAF / MAPK pathway, with mutation in BRAF gene
  - Expression pattern of micro RNAs
  - Non-MMR genes
- The gene mutations in CRC occur against a background of CIN (chromosomal instability)
- CIN occurs in . 80% of CRC
- Candidate genes for CIN include genes responsible for
  - Mitotic spindle checkpoint
  - DNA damage checkpoint
  - Control of number of centrosomes

➢ MMR (mismatch repair) genes

- MSI (microsatellite instability)
- Alterations in hMLH, hPMS1, hPMS2, hMSH2, hMSH3, hMSH6
- ↓ repair of mismatches in base pairs → MSI → DNA replication errors → tandem repeat DNA sequences
- Seen in only 15% of sporatic CRC

➢ Non-MMR gene changes

- CIMP (CPG island methylator phenotype)
- MSI may be caused by mutations in MMR genes, or by epigenetic silencing mechanism
- Definition: “Epigenetics is the study of clonal changes in gene expression without accompanying changes in DNA coding sequences” (Feldman M., et al. *Sleisenger and Fordtran’s Gastrointestinal and Liver Disease.* 9th Edition. Saunders/Elsevier, Philadelphia, 2010, page 2203).
- Hypermethylation of the hMLA, promotor, with inactivation of hMLH1.

- Seen in 70% of sporatic MSI tumors.
- Common pathway for the development of serrated adenomas.

➢ ↑ proto-oncogenes and oncogenes (activation)

- Oncogenes
  - Genes which encode
    - A normal cellular protein to be produced in ↑ amounts, or
    - An abnormal, structurally altered protein with ↑ activity
  - Activation of oncogenes (proto-oncogenes → oncogenes):
    - Gene transduction/ insertion
    - Gene rearrangement
    - Gene amplification
    - Point mutation
  - RAS genes (related to G proteins), H-RAS, K-RAS and N-RAS, encode 21-kd proteins
  - Half of CRCs have point mutations in K-RAS
  - RAS activation leads to phosphorylation of severe and threonine kinases
  - C-Myc is a member of the Myc family of nuclear oncogenes which may be overexpressed in GI cancers
- ↑ activation of proto-oncogenes
  - K-RAS
  - N-RAS
  - H-RAS
- Role in sporatic CRC
  - Activating point mutations (especially in K-RAS gene):
    - Adenomas < 1 cm 10%
      > 1 cm 58%
    - Carcinoma 47%

- Loss (inactivation) of tumor suppressor genes
  - APC gene
    - Allelic losses at chromosome locations
      - 5q
      - 17p
      - 18q
  - Truncation of the APC protein
  - Occur in 60% to 80% of sporatic adenomas / carcinomas
  - Loss of DCC (deleted in colon cancer)
  - Low of DPC4 / SAMD4 (loss of activation of TGF-β receptors)
  - Loss of p53 tumor suppressor activity from deletion of chromosome 17p leads to loss of cells, thereby preventing damaged DNA from going from G1 to S phase of the cell cycle.
  - Loss of TP53 gene
  - These polymorphisms include
    - SNPs (single nucleotide polymorphisms)
    - RFLPs (restriction fragment length polymorphisms)
    - Microsatellite polymorphisms
  - On allelic deletion (aka loss of heterozygosity [LOH]) is the loss of an allele from one parent.
  - This allelic deletion or LOH causes inactivation of some tumour suppressor genes.
  - P53 is a nuclear phosphoprotein, end point mutations in the TP53 gene is seen in over half of sporadic CRC.
  - The DNA mismatch repair system connects the spontaneous mismatching of nucleotides arising from slippage of microsatellite DNA.
  - This slippage of microsatellite DNA occurs during the normal replication of DNA, leads to strand.
  - The APC gene (adenomatous polyposis coli gene) is part of the chromosomal instability pathway leading to CRC.
  - Germline mutations of APC are seen in FAP (familial adenomatous polyposis), and somatic APC mutations are seen in most sporadic CRCs.
  - A premature stop code is produced, and the resulting protein product is truncated, and is associated with functional changes in important protein-protein interactions

SO YOU WANT TO BE A GASTROENTEROLOGIST

- Give the role of APC gene function, nuclear β-catenin, the Wat signal pathway, the LEF (lymphoid enhancer factor) / TCF (T-cell factor) family in the development of CRC.
  - Normal
    - APC (tumor suppressor) gene
      - Association with E-cadherin → cell-cell adhesion
      - Binds β-catenin → ↑ phosphorylation
      - ↓ β-catenin - ↓ stimulation of Wnt-Tcf signal pathway
        - ↓ proliferation
        - ↑ apoptosis
  - APC gene mutation
    - ↓ cell-cell adhesion
    - ↓ β-catenin phosphorylation
      - ↑ unphosphorylated β catenin complex with LEF / TCF
      - ↑ Wnt-Tcf
    - Target genes
      - ↑ proliferation
      - ↓ apoptosis
- Give 4 biochemical changes that occur during the development of CRC.
  - ↑ CEA (carcinoembryonic antigen)
  - ↑ matrix metalloproteinases
    - MMP-1
    - MT1 – MMP
  - ↑ EGF (vascular endothelial growth factors) increase angiogenesis and vasculization
  - ↑ selectins (on endothelium)
  - ↑ sialoglycoproteins (on tumor)

"Home is the place they know us best and still loves us"

William Barclay

Probiotics

Probiotics are an area of active research interest into examining their role in a variety of intestinal disorders such as Traveler's diarrhea and in inflammatory bowel disease.

- Give the proposed mechanisms of beneficial action of probiotics in the GI tract.

- Pathogenic luminal bacteria
  - ↓ pathogen binding
  - ↓ decrease luminal pH
  - Produce antibacterial bacteriocine
  - Alter ratio of bneficial probiotic bacteria is pathogenic bacteria

- GI epithelial cells
  - ↑ barrier function
  - ↑ promote cytoprotective responses
  - ↑ cell survival
  - ↑ mucin production

- Intestinal immune function
  - ↓ pro-inflammatory cytokines
  - ↑ tolerogenic cytokine profiles and regulatory pathways
  - ↑ Secretory IgA

- Neuromodulation
  - ↑ mu-opiod and cannabinoid receptors on epithelial cells
  - ↓ visceral hypersensitivity and stress response

- Nutritional benefit
  - Assist in the breakdown of undigestable foods, to produce usable nutrients

Adapted from: Cioba MA. Clin Gastroenterol Hepatol. 2012;10(9):960-968.

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