BB 422

OUTLINE:

Introduction and review
Transport
Glycogenolysis
Glycolysis
Other sugars
Pasteur: Anaerobic vs Aerobic
Fermentations — Exam-1 material

Pyruvate
Krebs' Cycle
Oxidative Phosphorylation — Exam-2 material
Electron transport
Chemiosmotic theory: Phosphorylation

Fat Catabolism
Fatty acid Catabolism — Exam-3 material
Mobilization from tissues (mostly adipose)
Activation of fatty acids
Transport; carnitine
Oxidation: β-oxidation, 4 steps:
Protein Catabolism
Amino-Acid Degradation
Dealing with the nitrogen; Urea Cycle
Dealing with the carbon; Seven Families
Nucleic Acid & Nucleotide Degradation

PHOTOSYNTHESIS:

Overview of Photosynthesis
Key experiments:
Light Reactions
energy in a photon
pigments
HOW
Light absorbing complexes–"red-drop experiment"
Reaction center
Photosystems (PS)
PSII – oxygen from water splitting
PSI – NADPH
Proton Motive Force – ATP
Overview of light reactions

ANABOLISM I: Carbohydrates

Carbon Assimilation – Calvin Cycle
Stage One – **Rubisco** } Know mechanism
Carboxylase
Oxygenase
Glycolate cycle
Stage Two – making sugar
Stage Three – remaking Ru 1,5P$_2$
Overview and regulation
Calvin cycle connections to biosynthesis } Know pathway
C4 versus C3 plants
Kornberg cycle – glyoxylate

Carbohydrate Biosynthesis in Animals
precursors
Cori cycle
Gluconeogenesis — Know pathway
reversible steps
irreversible steps – four
energetics
2-steps to PEP in mitochondria: Pyr carboxylase-biotin & PEPCK
FBPase
G6Pase

Glycogen Synthesis
UDP-Glc
Glycogen synthase
branching

Pentose-Phosphate Pathway
oxidative-NADPH
non-oxidative-Ribose 5-P

Regulation of Carbohydrate Metabolism
Acetyl-CoA/Pyruvate
Pyruvate/PEP
F6P/FBP: Fru 2,6P$_2$
Glc/Glc6P: sequestration
Glycogen: PKA/PP1
Gene control

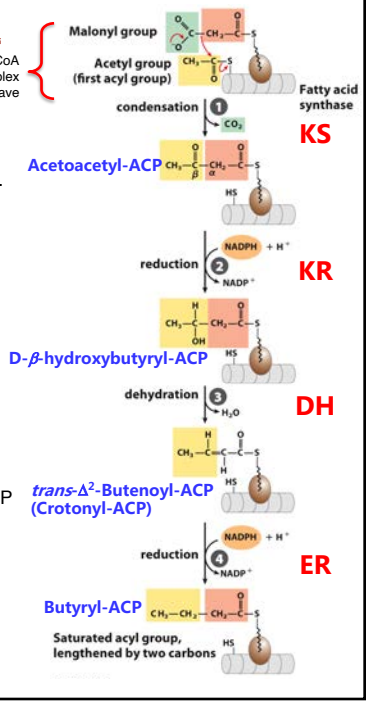
Anaplerotic reactions

Biosynthesis of Lipids
Fatty Acids
contrasts
location & transport
Synthesis
Regulation
Diversification
elongation
desaturation
Eicosanoids

End of Exam-4 material

Fatty Acid Biosynthesis

Note that malonyl-CoA and acetyl-CoA have already been attached to complex via thioester linkages to enzyme and have shed their CoA attachments.



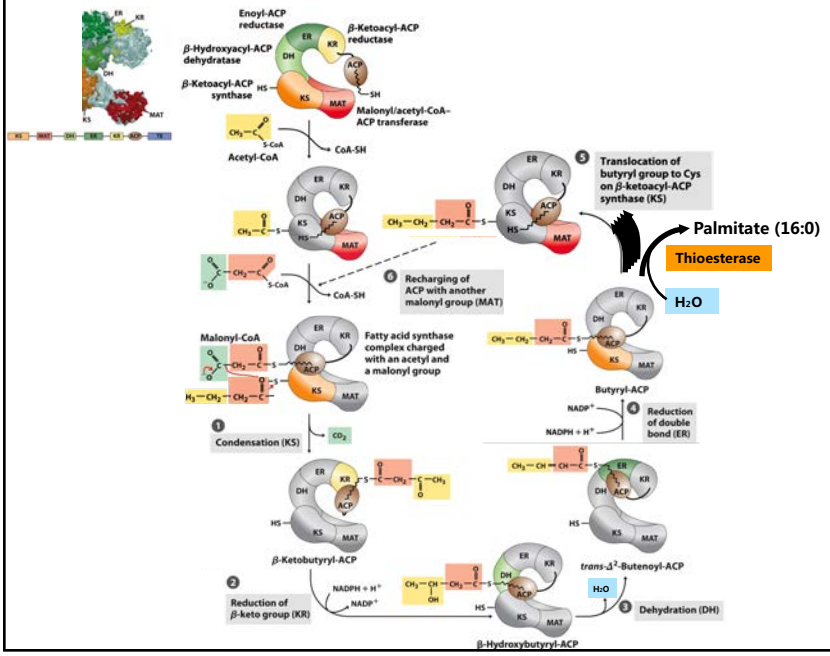
Step 1: Condensation reaction attaches two C from acetyl group (or longer fatty acyl chain) to two C from malonyl group.
- release of CO_2 activates malonyl group for attachment
- creates β-keto intermediate (acetoacetyl-ACP)
- Catalyzed by β-ketoacyl-ACP synthase (KS)

Step 2: First Reduction: NADPH reduces the β-keto intermediate to an alcohol.
- carbonyl at C-3 reduced to form D-β-hydroxybutyryl-ACP
- NADPH is 2e^- donor
- catalyzed by β-ketoacyl-ACP reductase (KR)

Step 3: Dehydration: OH group from C-2 and H from neighboring CH_2 are eliminated, creating double bond (trans-alkene).
- OH and H removed from C-2 and C-3 of β-hydroxybutyryl-ACP to form *trans*-Δ^2-butenoyl-ACP
- catalyzed by β-hydroxyacyl-ACP dehydratase (DH)

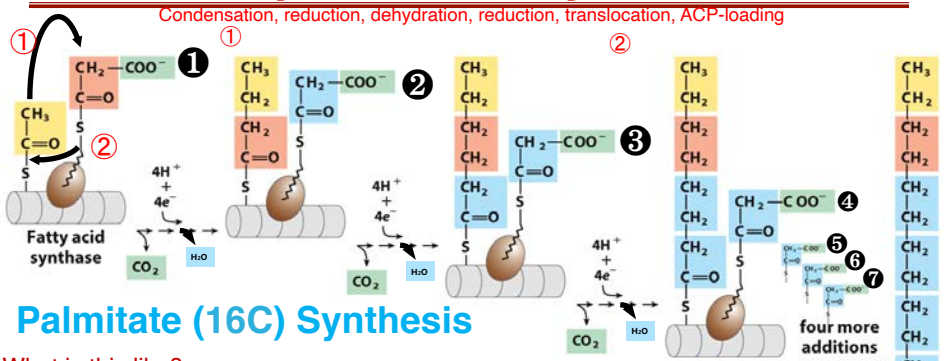
Step 4: Second Reduction: NADPH reduces double bond to yield saturated alkane.
- NADPH is the electron donor to reduce double bond of *trans*-Δ^2-butenoyl-ACP to form butyryl-ACP.
- catalyzed by enoyl-ACP reductase (ER)

Fatty Acid Biosynthesis



Fatty Acid Biosynthesis

Condensation, reduction, dehydration, reduction, translocation, ACP-loading



Palmitate (16C) Synthesis

What is this like?

Protein synthesis; where the peptidyl-tRNA (acyl-ACP) puts the growing peptide onto the incoming AA-tRNA (malonyl-ACP)

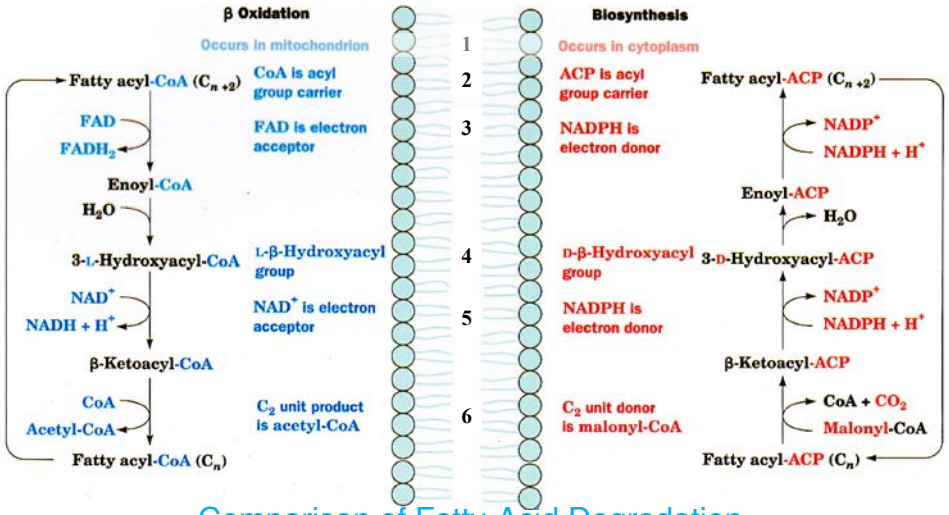
7 acetyl-CoA + 7 CO_2 + 7 ATP → 7 malonyl-CoA + 7 ADP + 7 P_i

acetyl-CoA + 7 malonyl-CoA + 14 NADPH + 14 H^+ → palmitate (16:0) + 7 CO_2 + 8 CoA + 14 $NADP^+$ + 6 H_2O

8 acetyl-CoA + 14 NADPH + 14 H^+ + 7 ATP → palmitate (16:0) + 8 CoA + 14 $NADP^+$ + 7 ADP + P_i + 6 H_2O

If you add in the transport (8x2=16): 23 ATP

Fatty Acid Biosynthesis



Comparison of Fatty-Acid Degradation
versus
Fatty-Acid Synthesis

ANABOLISM II: Biosynthesis of Fatty Acids & Lipids

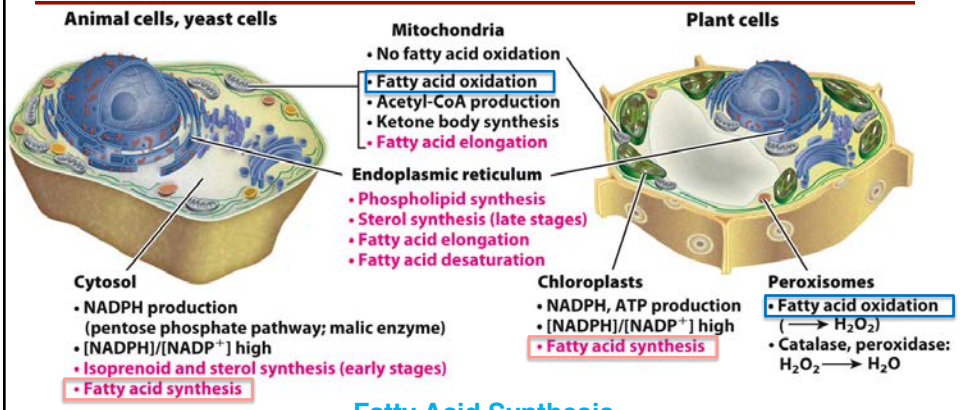
ANABOLISM II: Biosynthesis of Fatty Acids & Lipids

1. Biosynthesis of fatty acids
2. Regulation of fatty acid degradation and synthesis
3. Diversification of fatty acids
   a. Elongation/desaturation
   b. Eicosanoids (Prostaglandins and Thromboxane)

End of Exam-4 material

4. Assembly of fatty acids into triacylglycerol and phospholipids
5. Metabolism of isoprenes
   a. Ketone bodies and Isoprene biosynthesis
   b. Isoprene polymerization
      i. Cholesterol
      ii. Steroids & other molecules
      iii. Regulation
      iv. Role of cholesterol in human disease

Fatty Acid Biosynthesis



Fatty Acid Synthesis

Cytosol for animals

Chloroplast for plants

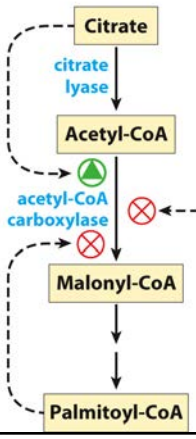
Both of these compartments are where there are ample Sources of NADPH:
pentose phosphate pathway, malic enzyme, and photosynthesis

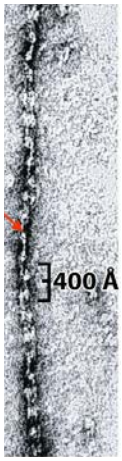
How does cell regulate which pathway is working?

Fatty Acid Biosynthesis

Control of Fatty-Acid Synthesis

- **Acetyl CoA carboxylase (ACC) catalyzes the committal step.**
- **Allosteric Control**
  - Inhibited when energy is needed, fatty acids are acylated for degradation, and inhibit ACC.
    - ACC is feedback-inhibited by **palmitoyl-CoA**.
  - ACC is *activated* by **citrate**.
    - Citrate is made from acetyl-CoA in mitochondria (acetyl-CoA^{mt}).
    - High [Citrate] signals excess energy to be converted to fat.
  - When [acetyl-CoA]^{mt} ↑, [citrate]^{mt} ↑ ….. citrate is exported to cytosol
- **Hormonal Control**
  - Glucagon and epinephrine: leads to activation of AMP-dependent Protein Kinase (AMPK)
    - Phosphorylation inactivates ACC
    - Phosphorylation reverses the polymerization → monomers (inactive)
  - Insulin: leads to activation of Protein Phosphatase 2A
    - Dephosphorylation activates ACC
    - When dephosphorylated, ACC polymerizes into long filaments (active)
- **Changes in gene expression**
  - example: excess of certain polyunsaturated fatty acids (eicosanoids) bind to transcription factors called peroxisome proliferator-activated receptors (PPARS)



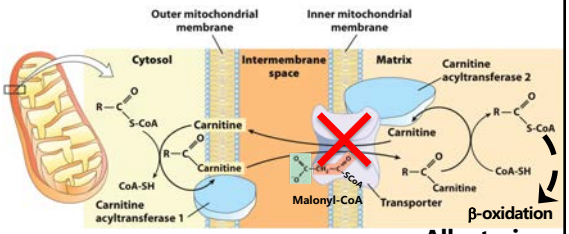


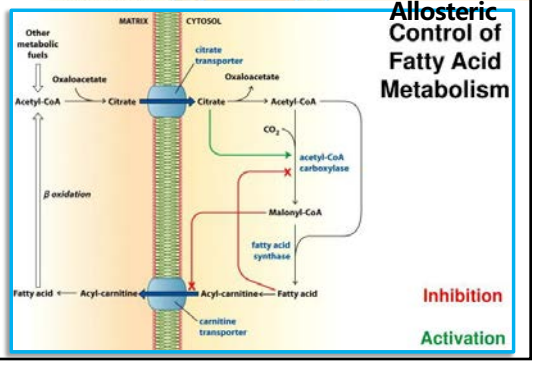
glucagon, epinephrine trigger phos inactivation

Fatty Acid Biosynthesis

Recall how β-oxidation starts: Acyl-Carnitine/Carnitine Transport:

- β oxidation of fatty acids occurs in mitochondria.
- If fatty acyl-CoAs are not transported in, they cannot be degraded
- Transport is via carnitine transporter.
- Blocking β oxidation with initial committed product of fatty-acid synthesis: malonyl-CoA





Allosteric Control of Fatty Acid Metabolism

Reciprocal Control of Fatty-Acid Degradation *versus* Fatty-Acid Synthesis

ensures that fat synthesis and oxidation don't occur simultaneously

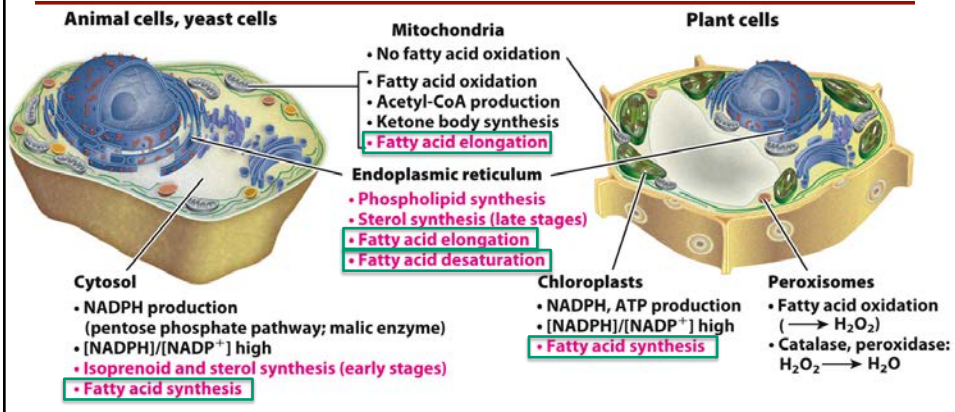
ANABOLISM II: Biosynthesis of Fatty Acids & Lipids

1. Biosynthesis of fatty acids
2. Regulation of fatty acid degradation and synthesis
3. Diversification of fatty acids
   a. Elongation/desaturation
   b. Eicosanoids (Prostaglandins and Thromboxane)

End of Exam-4 material

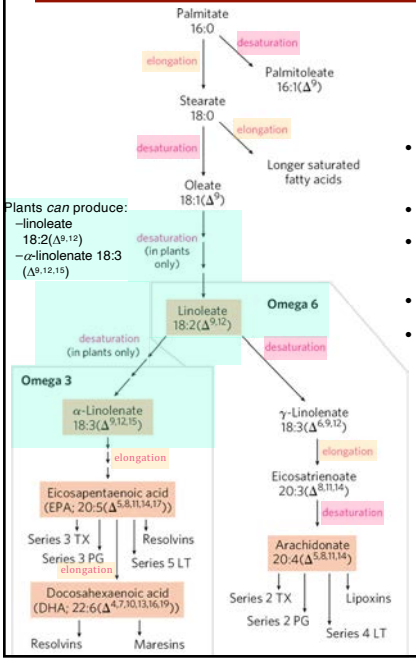
4. Assembly of fatty acids into triacylglycerol and phospholipids
5. Metabolism of isoprenes
   a. Ketone bodies and Isoprene biosynthesis
   b. Isoprene polymerization
      i. Cholesterol
      ii. Steroids & other molecules
      iii. Regulation
      iv. Role of cholesterol in human disease

Fatty Acid Biosynthesis



Elongation and Desaturation

Fatty Acid Biosynthesis



Diversification of Palmitate: Elongation

Fatty Acid Elongation Systems

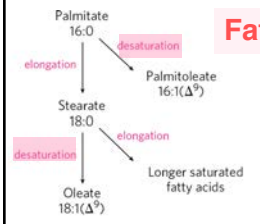
- Elongation systems in the endoplasmic reticulum and mitochondria create longer fatty acids.
- Use CoA derivatives
- As in palmitate synthesis, each step adds units of 2 C. Use malonyl-CoA.
- Stearate (18:0) is the most common product.
- Plants make linoleate (Ω6) and α-linolenate (Ω3), animals can't; But they can elongate these ingested essential FA
  - These fatty acids are "essential" to humans.
    - –Polyunsaturated fatty acids (PUFAs) help control membrane fluidity.
    - –PUFAs are precursors to eicosanoids
  - The ratio of omega-6 to omega-3 fatty acids in the diet, if too high, can lead to cardiovascular disease

Eicosanoids

PG = Prostaglandins
TX = Thromboxanes
LT = Leukotrienes

Fatty Acid Biosynthesis

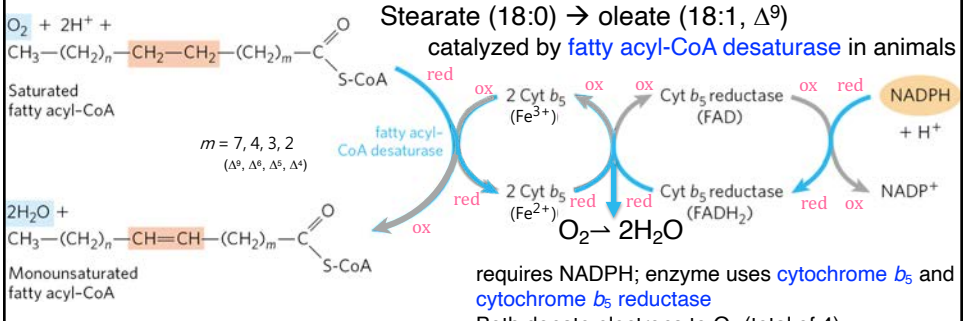
Diversification of Palmitate: Desaturation



Fatty Acyl-CoA Desaturase

- Looks like a mixed-function oxidase: OXIDASE
- Humans have Δ^4, Δ^5, Δ^6, and Δ^9 desaturases but *cannot* desaturate beyond Δ^9.

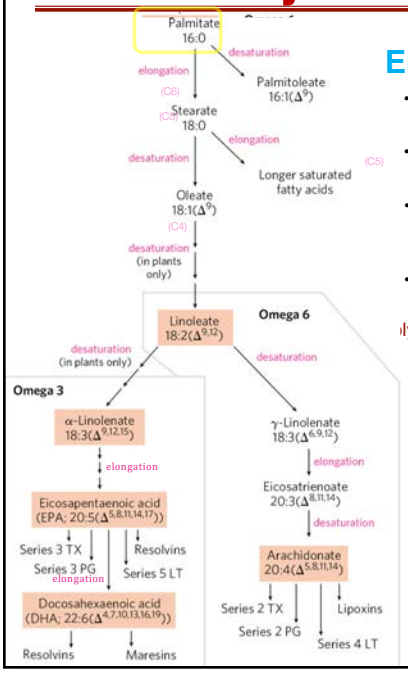
FOR EXAMPLE: for a *Δ^9-desaturation*
Palmitate(16:0) → palmitoleate(16:1, Δ^9)
Stearate (18:0) → oleate (18:1, Δ^9)
catalyzed by fatty acyl-CoA desaturase in animals



requires NADPH; enzyme uses cytochrome b_5 and cytochrome b_5 reductase
Both donate electrons to O_2 (total of 4)

In plants & bacteria the desaturases work on PL, not fatty-acyl CoA; needed for rapid changes in fluidity

Fatty Acid Biosynthesis



Animals can do, plants can do

Eicosanoids: Synthesis and Classes

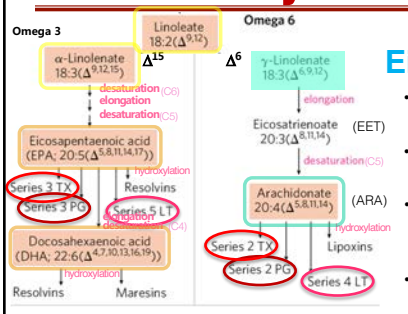
- Linoleate (18:2($\Delta^{9,12}$) gets desaturated on both sides = γ-linolenate (18:3($\Delta^{6,9,12}$) & α-linolenate (18:3($\Delta^{9,12,15}$).
- Elongation to C20, and further desaturation of γ-linolenate (18:3($\Delta^{6,9,12}$) gives Arachidonate, 20:4($\Delta^{5,8,11,14}$)
- Elongation to C20, and TWO further desaturation of α-linolenate (18:3($\Delta^{9,12,15}$) gives EPA, 20:5($\Delta^{5,8,11,14,17}$). More of the same gets DHA, 22:6($\Delta^{4,7,10,13,16,19}$)
- These desaturations are all going closer to the carboxylate and can be accomplished by animals.

lyunsaturated fatty acids (PUFAs)) bioactive paracrine

PG = Prostaglandins
TX = Thromboxanes
LT = Leukotrienes

- Created from the arachidonate or EPA, which are incorporated into membranes phospholipids.
- In response to stimuli (hormone, etc.), phospholipase A_2 is activated
- The unsaturated C-2 fatty acid is released

Fatty Acid Biosynthesis

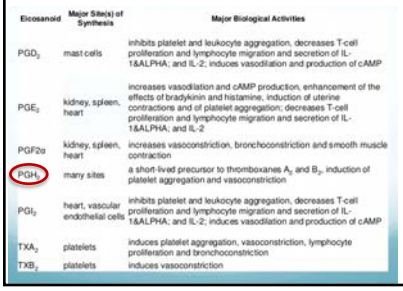


Animals can do, plants can do

Eicosanoids: Synthesis and Classes

- Linoleate (18:2($\Delta^{9,12}$) gets desaturated on both sides = γ-linolenate (18:3($\Delta^{6,9,12}$) & α-linolenate (18:3($\Delta^{9,12,15}$).
- Elongation to C20, and further desaturation of γ-linolenate (18:3($\Delta^{6,9,12}$) gives Arachidonate, 20:4($\Delta^{5,8,11,14}$)
- Elongation to C20, and TWO further desaturation of α-linolenate (18:3($\Delta^{9,12,15}$) gives EPA, 20:5($\Delta^{5,8,11,14,17}$). More of the same gets DHA, 22:6($\Delta^{4,7,10,13,16,19}$)
- These desaturations are all going closer to the carboxylate and can be accomplished by animals.

From these Eicosanoids (20C Polyunsaturated fatty acids (PUFAs)) bioactive paracrine signaling molecules are made:

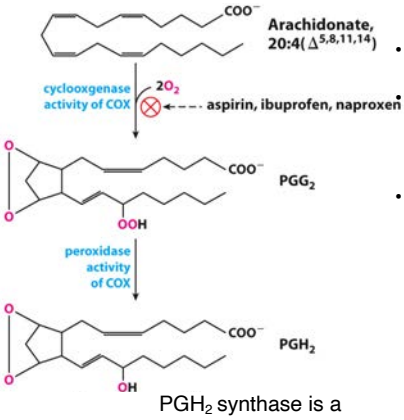


Eicosanoid	Major Site(s) of Synthesis	Major Biological Activities
PGD_2	mast cells	inhibits platelet and leukocyte aggregation, decreases T-cell proliferation and lymphocyte migration and secretion of IL-1&ALPHA; and IL-2; induces vasodilation and production of cAMP
PGE_2	kidney, spleen, heart	increases vasodilation and cAMP production, enhancement of the effects of bradykinin and histamine, induction of uterine contractions and of platelet aggregation; decreases T-cell proliferation and lymphocyte migration and secretion of IL-1&ALPHA; and IL-2
$PGF2\alpha$	kidney, spleen, heart	increases vasoconstriction, bronchoconstriction and smooth muscle contraction
PGH_2	many sites	a short-lived precursor to thromboxanes A_2 and B_2; induction of platelet aggregation and vasoconstriction
PGI_2	heart, vascular endothelial cells	inhibits platelet and leukocyte aggregation, decreases T-cell proliferation and lymphocyte migration and secretion of IL-1&ALPHA; and IL-2; induces vasodilation and production of cAMP
TXA_2	platelets	induces platelet aggregation, vasoconstriction, lymphocyte proliferation and bronchoconstriction
TXB_2	platelets	induces vasoconstriction

PG = Prostaglandins
TX = Thromboxanes
LT = Leukotrienes

- Created from the arachidonate or EPA, which are incorporated into membranes phospholipids.
- In response to stimuli (hormone, etc.), phospholipase A_2 is activated
- The unsaturated C-2 fatty acid is released

Fatty Acid Biosynthesis



Bengt Samuelsson
Nobel Prize 1982

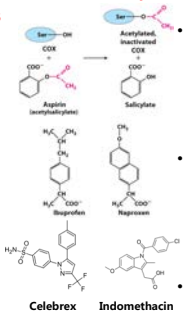
PGH_2 synthase is a cyclooxygenase/peroxidase enzyme that functions in the smooth ER.

- Step 1: PGH_2's cyclooxygenase activity adds 2 O_2 to form PGG_2.
- Step 2: PGH_2's peroxidase activity converts peroxide to alcohol, creates PGH_2.

Eicosanoids: Cyclic Pathway

- PGH_2 synthase (cyclooxygenase, or COX) has isozymes
- COX-1 is ubiquitous and catalyzes synthesis of prostaglandins that regulate *gastric mucin secretion and other smooth muscle functions*
- COX-2 is inducible (except in brain) by immune response and acts at the sites of inflammation. It catalyzes synthesis of prostaglandins that mediate *pain, inflammation, and fever.*

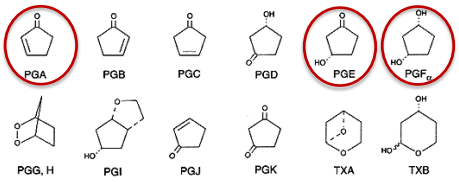
Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit COX isozymes



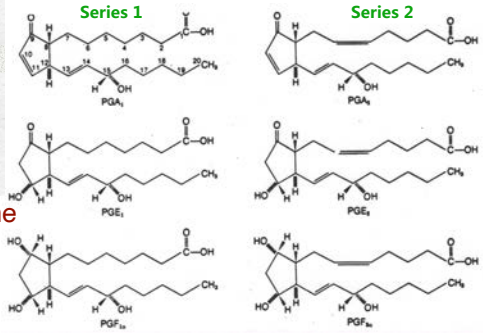
- Aspirin (acetylsalicylate) is an irreversible inhibitor.
  - acetylates a Ser in active site
  - blocks active site in both COX isozymes
- Ibuprofen, naproxen, and indomethacin are competitive inhibitors.
  - resemble substrate; also block active site in both isozymes
- Celebrex and Vioxin are specific for COX-2

Fatty Acid Biosynthesis

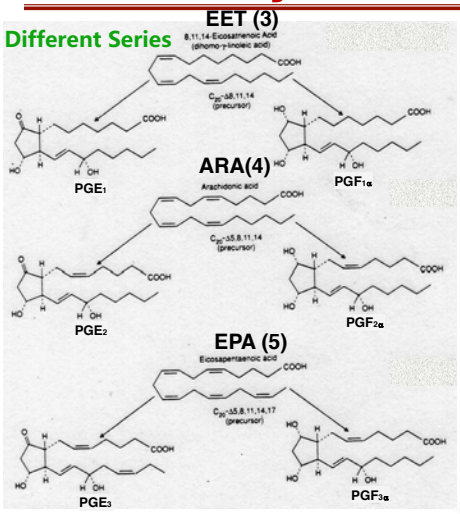
Prostaglandin & Thromboxane: Synthesis and Classes



Different Classes



Different Series

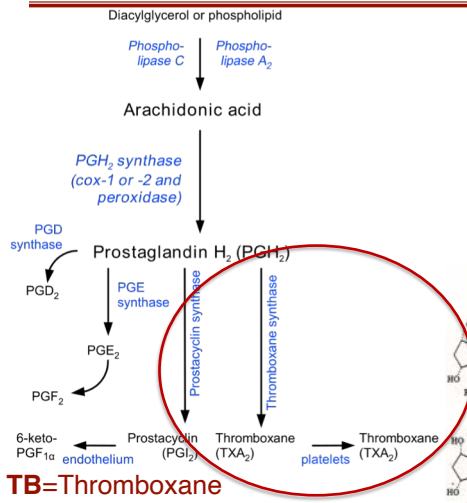


PG = Prostaglandins/**TB**=Thromboxane

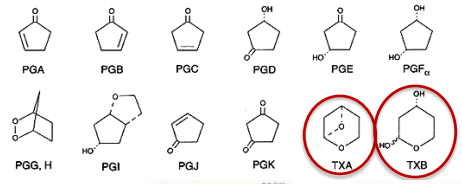
–letters-after are for number and oxidation state of oxygens and ring (**Classes**)

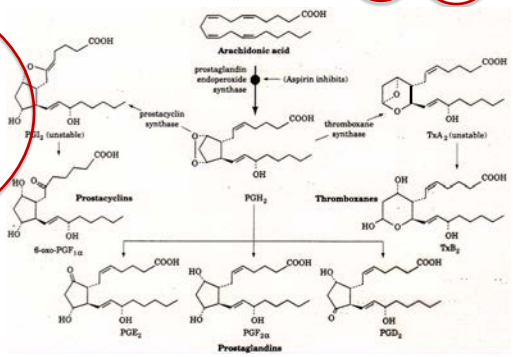
–subscript is the number of double bonds (**Series**)

Fatty Acid Biosynthesis



Thromboxane: Synthesis and Classes





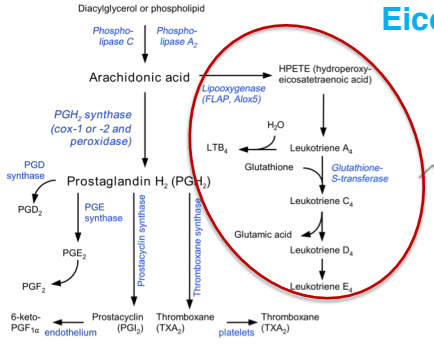
TB=Thromboxane

–letters-after are for number and oxidation state of oxygens and ring (**Classes**)

–subscript is the number of double bonds (**Series**)

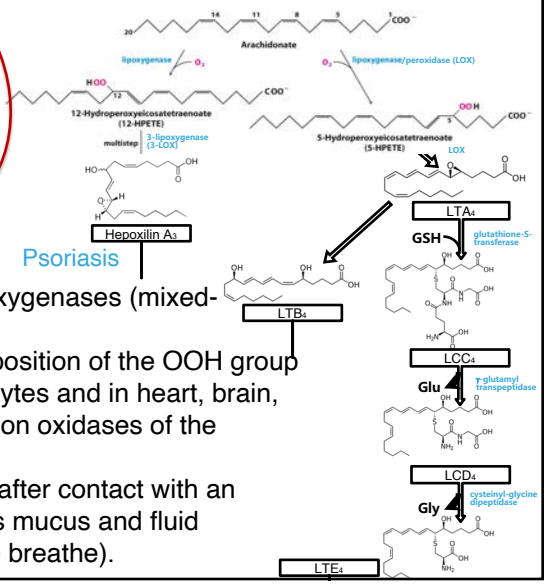
Platelet aggregation and wound healing

Fatty Acid Biosynthesis



Eicosanoids: Linear Pathway

Synthesis of Leukotrienes

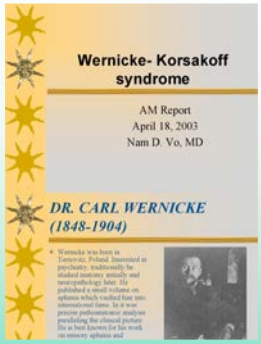


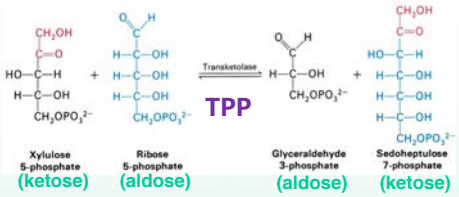
Psoriasis

- O_2 added to arachidonate via lipoxygenases (mixed-function oxidases) (LOX)
- Creates species that differ in the position of the OOH group
- LOX enzymes are found in leukocytes and in heart, brain, lung, and spleen, are mixed-function oxidases of the cytochrome P-450 family.
- Inflammatory chemicals released after contact with an allergen; airways constrict, excess mucus and fluid produced (e.g., asthma; difficult to breathe).

Clinical Correlations

Transketolase Deficiency





- This is called **Wernicke-Korsakoff Syndrome**
- It presents as a mental disorder: memory loss and partial paralysis
- Often seen in alcoholics with poor nutrition

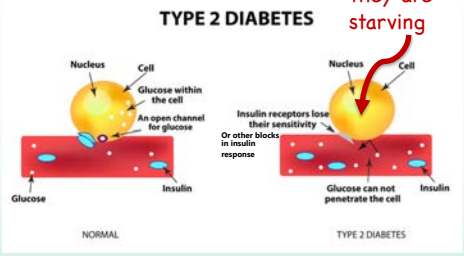
- There are no mutations in the transketolase gene, which are likely embryonically lethal. So, it appears that it arises from a deficiency in thiamine used for TPP

Clinical Correlations

Cells think they are starving

Diabetes Mellitus

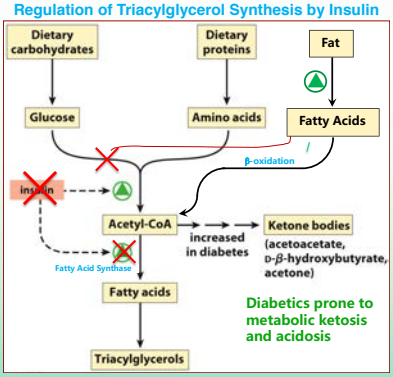
- Chronic disease of metabolism
- Diagnosed with *Glucose Tolerance Test*.
- Two types:
  - Lacking Insulin-Type I
  - Lacking insulin response-Type 2 (T2D)
- Over 220 million people have T2D
- Discovery by Oskar Minkowsky and Josef von Mering in 1889 that the pancreas fails to produce a substance that regulates blood glucose. Sweet urine
- Also smells of acetone, but got labeled a carbohydrate disorder. It's more a disorder of fatty-acid metabolism



Clinical Correlations

Diabetes Mellitus

- In muscle and many other tissues, Fatty Acids will block glucose metabolism. Presumable to spare glucose for the brain and replenishing glycogen stores.
- Within hours after a meal, there is a switch to ketone bodies
- FA inhibit PDHC and PFK-2
- Advantages for survival, but is a disadvantage during obesity.
- The effect of high [FA] in the blood, which is a symptom of obesity, is to accentuate this effect and the glucose uptake is severely compromised and the pancreas thinks you have just ate and produces more insulin, to which you don't respond.



End of Material for Exam 4