

Triacylglycerol and Phospholipid Biosynthesis

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I. Regulation of Fatty acid Metabolism

It is energetically wasteful to have fatty acid synthesis and β -oxidation occurring at the same time. Hence these 2 metabolic pathways are reciprocally regulated. This coordinated regulation is also related to the regulation of glycolysis, and the regulation of the citric acid cycle. The end product of β -oxidation is acetyl CoA, the end product of glycolysis is pyruvate which can be converted into acetyl CoA by pyruvate dehydrogenase. Acetyl CoA is activated in the form of malonyl CoA for fatty acid biosynthesis. Acetyl CoA is oxidized into CO_2 in the citric acid cycle. In short acetyl CoA is the common metabolite of all of these metabolic pathways.

Allosteric Regulation

When fatty acid biosynthesis is turned on, the concentration of malonyl CoA rises. High concentrations of malonyl CoA prevent the degradation of fatty acids by inhibiting carnitine acyl transferase I. The inhibition of carnitine acyl transferase I stops the transport of fatty acids across the inner mitochondrial membrane. When the transport is stopped, β -oxidation stops.

Citrate activates acetyl CoA carboxylase.

Palmitoyl-CoA, stearoyl CoA and arachidyl CoA all inhibit acetyl CoA carboxylase.

Hormonal Regulation

We have already discussed the effects of phosphorylation of acetyl CoA carboxylase (ACC). ACC contains 8-12 residues that may be phosphorylated by a variety of protein kinases. These protein kinases are under hormonal control. We are already familiar with the hormone glucagon which is the signal that the blood glucose concentration is low. Glucagon binds to cell receptors activating an intracellular phosphorylation cascade which activates protein kinases that phosphorylate ACC. The phosphorylated ACC enzyme favors the inactive protameric form of the enzyme. Only a high concentration of citrate can activate the phosphorylated form of the enzyme. Conversely, a small concentration of the acyl-CoA is required to keep the equilibrium in favor of the inactive protamers.

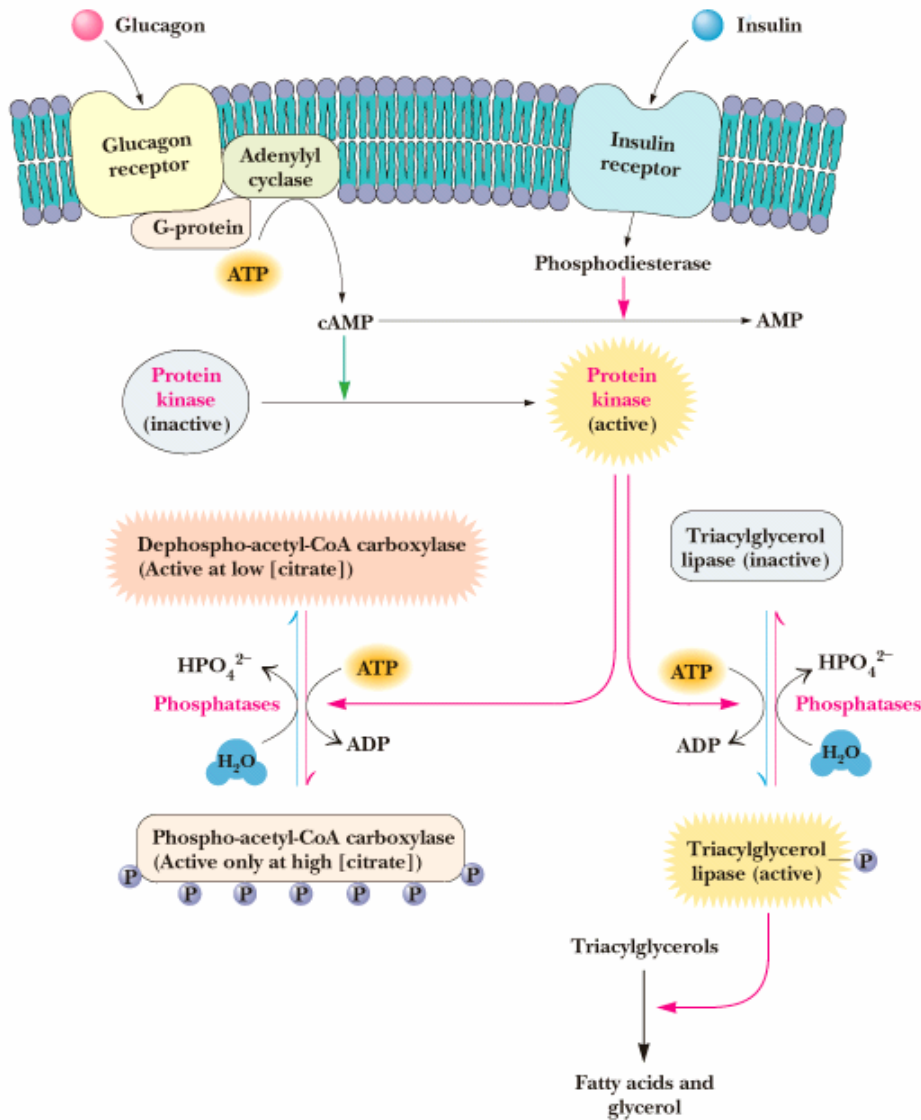
The phosphorylated ACC enzyme can be reactivated by a specific phosphatase which dephosphorylates the enzyme.

Glucagon binds to the adipose cell receptors triggering a phosphorylation cascade that activates the lipases to produce fatty acids from triacylglycerols stored in the tissue. These fatty acids are carried by serum albumin to the tissues that need them.

Thus glucagon simultaneously stops fatty acid biosynthesis and activates fatty acid transport from the adipose tissue.

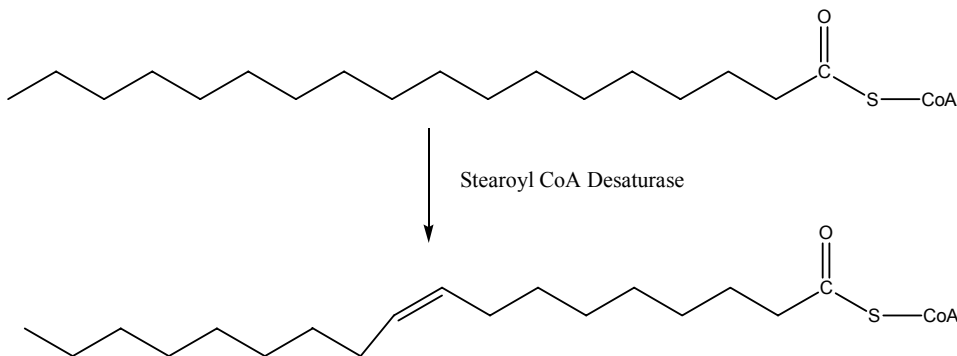
The effects of glucagon are counteracted by the hormone insulin. Insulin is the hormone that signals high blood glucose concentrations. Insulin activates the phosphodiesterase that hydrolyzes cAMP terminating the glucagon phosphorylation cascade. Insulin starts its own phosphorylation cascade that activates a variety of phosphatases. One of these phosphatases dephosphorylates the phosphorylated lipase in the adipose tissue which stops the liberation of fatty acids. Another insulin activated phosphatase

dephosphorylates ACC and thereby activating it. So insulin simultaneously turns on fatty acid biosynthesis and stops fatty acid transport from the adipose tissue.

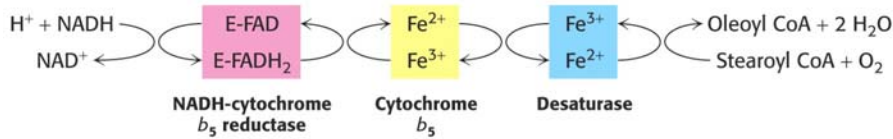


II. Desaturation of Fatty Acids.

The addition of cis double bonds in eukaryotes does not occur until the fatty acid has reached full length. The desaturation occurs in the endoplasmic reticulum. There are two common naturally occurring monosaturated fatty acids palmitoleic (16: Δ^9) acid and oleic acid (18: Δ^9). Oleic acid is produced by the dehydrogenation of stearoyl CoA. This conversion is catalyzed by stearoyl CoA desaturase which uses stearoyl CoA as the substrate.

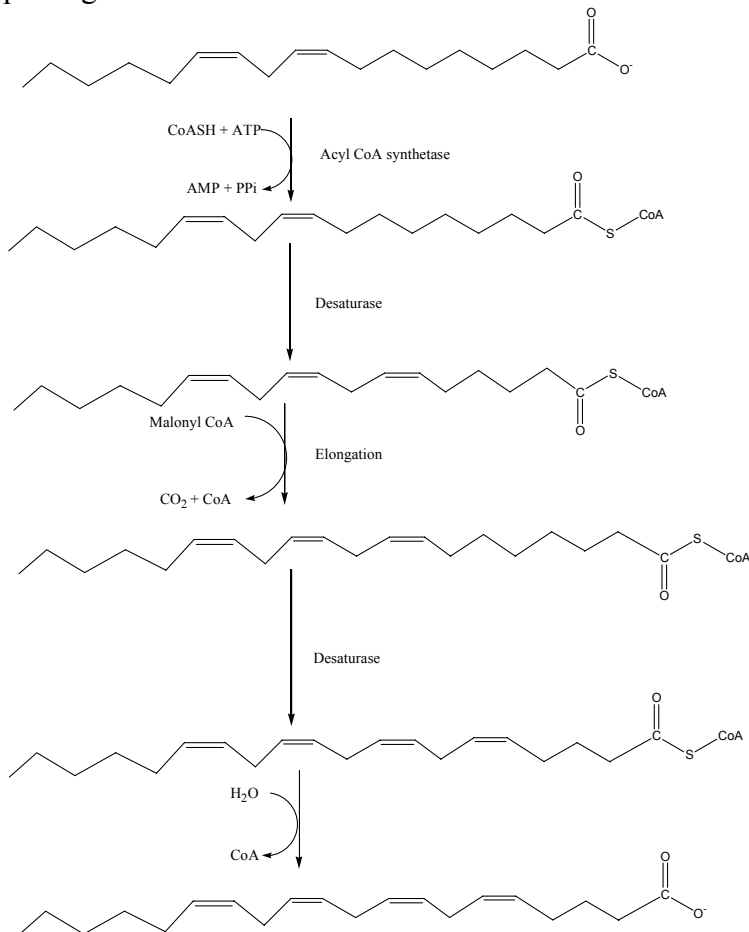


Stearoyl CoA desaturase contains a nonheme iron center, NADH and an oxygen binding site. Two other proteins are also required, cytochrome b_5 and cytochrome b_5 reductase, which is a flavoprotein. All three of these proteins are associated with the endoplasmic reticulum membrane. Cytochrome b_5 reductase transfers electrons one at a time from NADH through FAD to cytochrome b_5 . Cytochrome b_5 transfers an electron to reduce the nonheme iron from the ferric to the ferrous state. The ferrous iron coordinates to an O_2 molecule. At this iron center the cis double bond at the 9,10 position of the substrate is formed. O_2 is the terminal electron acceptor in this fatty acid desaturation cycle. 2 molecules of water are produced per oleoyl CoA which means that four electrons were transferred in the overall process. 2 electrons came from the NADH and the other 2 electrons form stearoyl CoA.



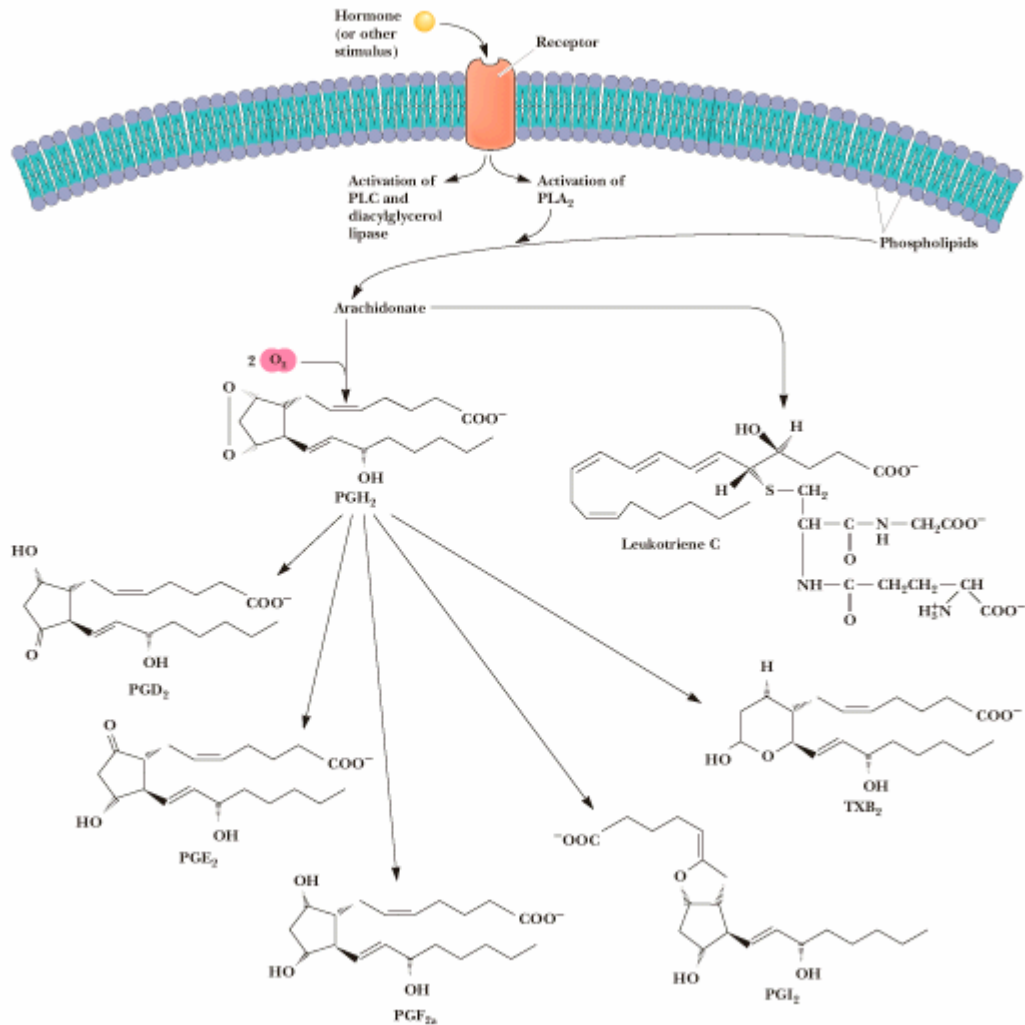
Mammals lack the enzymes to introduce cis double bonds at carbons beyond C9 in the fatty acid chain. Hence mammals cannot synthesize linoleate (18:2 $\Delta^{9,12}$) and linolenate (18:3 $\Delta^{9,12,15}$). Plants however have the ability to desaturate oleoyl CoA at the 12 position to produce linoleate or at both the 12 and 15 positions to generate linolenate. These two polyunsaturated fatty acids must come from the diet and are hence called essential fatty acids.

Mammals can synthesize arachidonic acid, plants cannot. Mammals take linoleic acid (obtained from the diet) and converted it into arachidonic acid (20:4 $\Delta^{5,8,11,14}$). The biosynthesis occurs in the endoplasmic reticulum. Arachidonic acid is an important precursor for leukotrienes, proacyclins, thromboxanes and prostaglandins.



III. Eicosanoids

Eicosanoids are 20 carbon fatty acids that are produced by the breakdown of selected phospholipids. Hormonal signals activate phospholipase A₂ which cleaves the fatty acids from the C2 position of phospholipids which is position occupied by unsaturated fatty acids. One of these unsaturated fatty acids is arachidonic acid whose biosynthesis we just discussed. The hormonal signal also activates phospholipase C which produces diacylglycerols which in turn are substrates for diacylglycerol lipase which cleaves fatty acids from the C2 position.

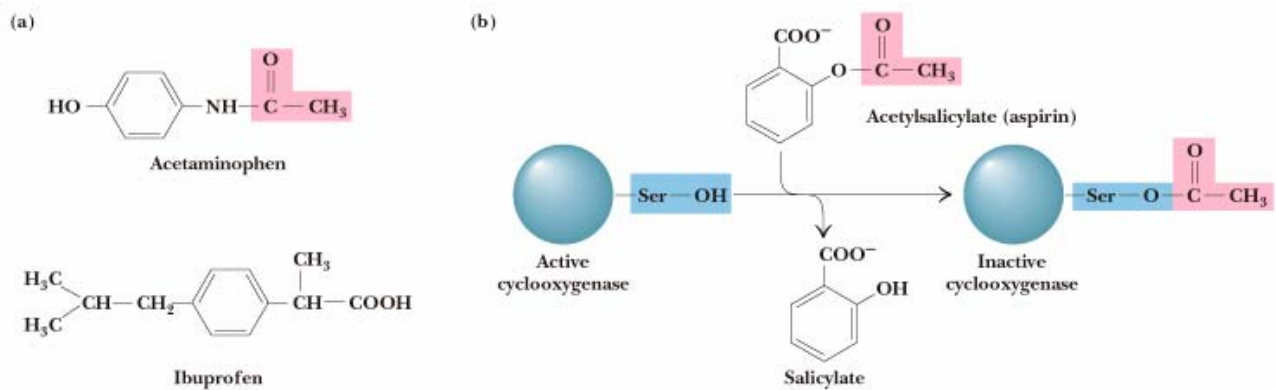


Eicosanoids are local hormones.

- Eicosanoids have short lifetimes (between 30-200 seconds).
- Eicosanoids exert their effects at very low concentrations (10^{-14} M)
- Eicosanoids act at sites near their biosynthesis.
- Eicosanoids include thromboxanes (Tx), leukotrienes, prostaglandins(PGF), ect.
- Eicosanoids are synthesized from arachidonic acid in the endoplasmic reticulum.
- The first step of eicosanoid synthesis is the simultaneous epoxidation and cyclization catalyzed by a cyclooxygenase (COX).

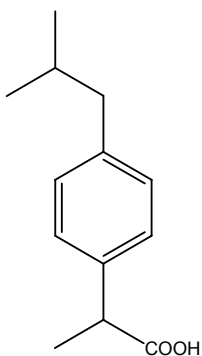
A variety of stimuli activated the release of arachidonate and the conversion into eicosanoids including histamines, hormones, proteases and proteins such as serum albumin. Thromboxane A₂ is produced by platelets to stimulate platelet aggregation. One of the most important roles of eicosanoids is involved in tissue injury, inflammation and pain sensitivity. When tissue is damaged, special inflammatory cells, monocytes and neutrophils, invade the injured tissue and interact with the smooth muscle cells and fibroblasts. This interaction stimulates arachidonate release and eicosanoid production. Examples of tissue injury which stimulates eicosanoid biosynthesis include heart attacks, rheumatoid arthritis and ulcers.

Garrett & Grisham: Biochemistry, 2/e
Figure 25.29



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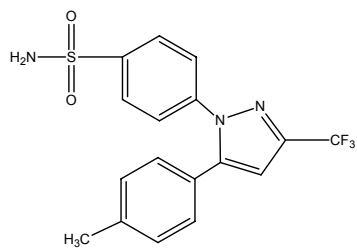
Aspirin reduces pain sensitivity by irreversibly inhibiting COX activity by transferring an acetyl group from acetylsalicylic acid to an active site serine residue which prevents the first step of eicosanoid formation. Thus aspirin prevents the formation of a great number of eicosanoids which produces a number of side effects including reducing the ability of platelets to aggregate and form blood clots and inhibiting the secretion of mucin in the stomach which protects the gastric wall.



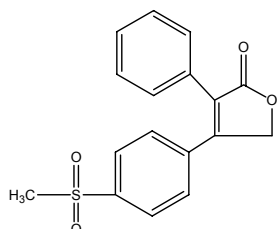
Ibuprofen (Motrin)

Ibuprofen (Motrin) and Acetaminophen (Tylenol) are nonsteroidal anti-inflammatory, anti-fever, pain relievers. Ibuprofen and acetaminophen are reversible, competitive inhibitors for the arachidonate binding site.

There are two isozymes of COX called COX-1 and COX-2. Motrin and Tylenol reversibly inhibit both of them. Aspirin irreversibly inactivates both of them. COX-1 is expressed in many tissues. COX-1 is important for the secretion of mucin, regulating gastric acid secretion, maintaining renal blood flow, and platelet aggregation. COX-2 is not expressed under normal conditions. COX-2 expression is induced by inflammatory mediators such as interleukin-1. COX-2 produces the eicosanoids that promote inflammation, fever and pain.



Celebrex

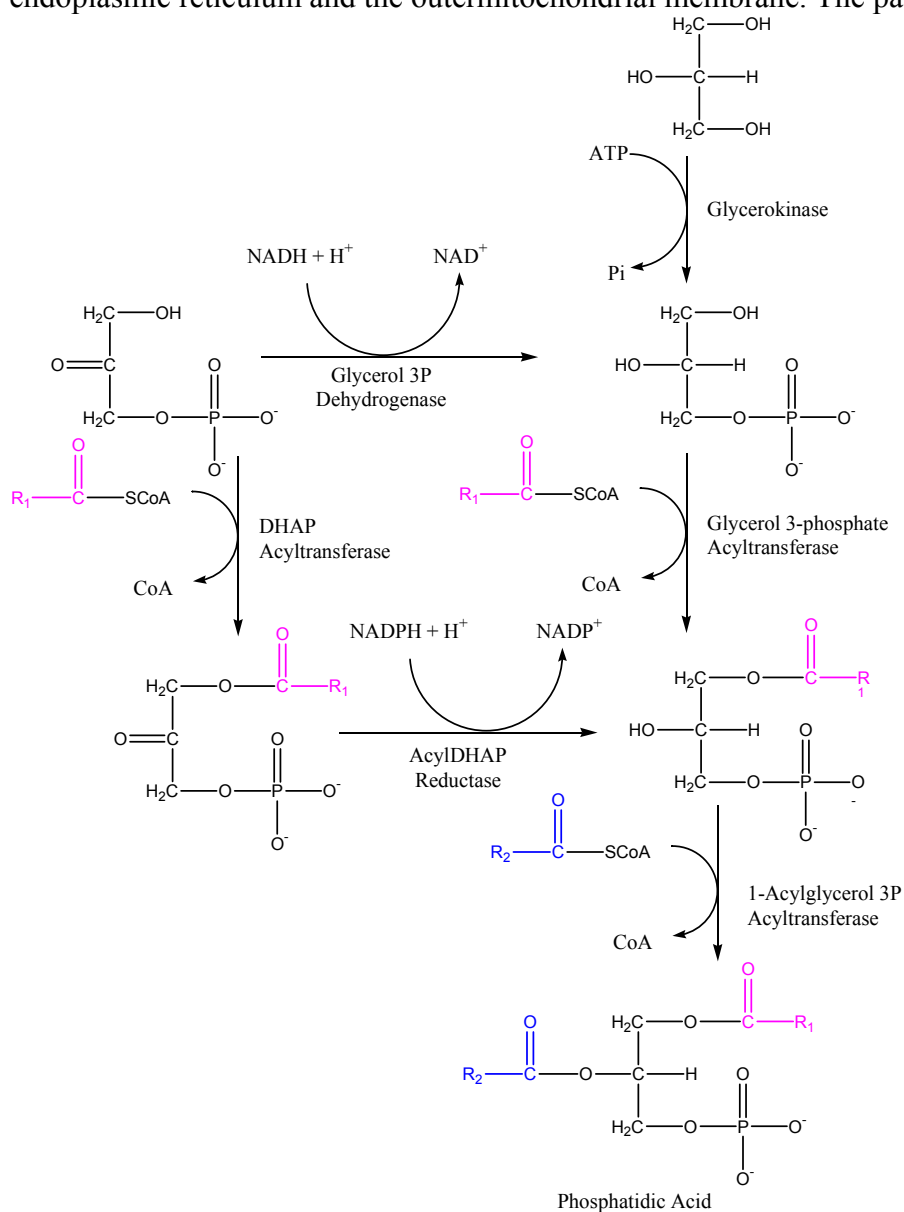


Vioxx

There is a new generation of pain killers which are COX-2 inhibitors. These inhibitors block pain, fever and swelling but do not interrupt mucin formation in the stomach or interfere with platelet aggregation by specifically targeting the COX-2 isozyme. Celebrex and Vioxx are COX-2 inhibitors Celebrex and Vioxx are weak competitive inhibitors of COX-1, but a tight binding competitive inhibitors of COX-2.

IV. Phosphatidic Acid Biosynthesis

Triacylglycerols and glycerophospholipids have glycerol as a backbone. The biosynthesis of these compounds proceeds through the formation of phosphatidic acid. Phosphatidic acid biosynthesis occurs in the endoplasmic reticulum and the outer mitochondrial membrane. The pathway is shown below.

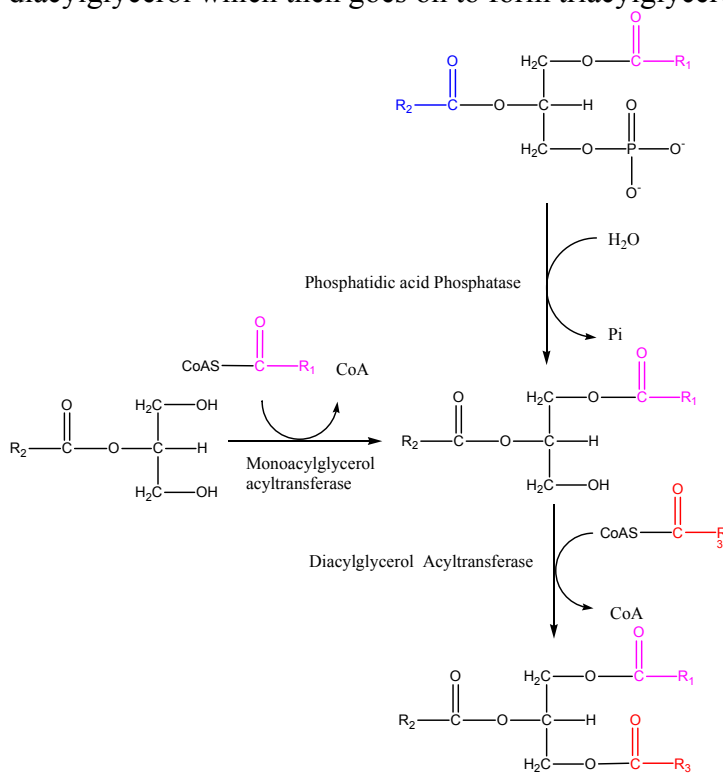


The starting point for phosphatidic acid biosynthesis begins with either glycerol 3-phosphate or DHAP. Dihydroxyacetone phosphate can be reduced by glycerol 3-phosphate dehydrogenase to form glycerol 3-P. Eukaryotes can acylate DHAP by DHAP acyltransferase to form 1-Acyldihydroxyacetone phosphate which can be reduced by acyl-DHAP reductase to form 1-Acylglycerol-3-phosphate.

Glycerol 3-phosphate acyltransferase is specific for saturated acyl-CoAs.
The eukaryotic DHAP acyltransferase is also specific for saturated acyl-CoAs.

V. Triacylglycerol Biosynthesis

Triacylglycerol biosynthesis occurs in the intestinal mucosa cells, the adipose tissue and the liver. Triacylglycerols are used primarily for energy storage. For some animals triacylglycerols also provide insulation for survival in cold climates. In the liver and adipose tissue, triacylglycerols are synthesized by diacylglycerol acyltransferase. This enzyme is found on the cytoplasmic face of the endoplasmic reticulum. A different route for triacylglycerol biosynthesis occurs in the intestinal mucosa cells. In the small intestine triacylglycerols are degraded into 2-monoacylglycerols and free fatty acids. The intestinal mucosa cells absorb the monoacylglycerols and fatty acids. A monoacylglycerol acyltransferase produces diacylglycerol which then goes on to form triacylglycerols as shown below.

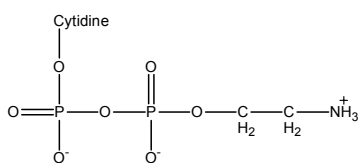
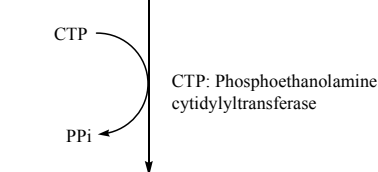
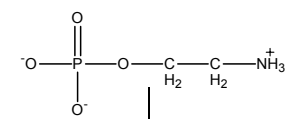
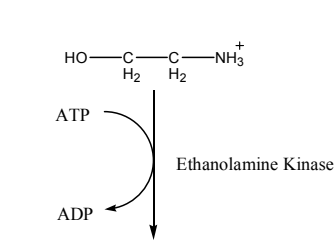


VI. Glycerophospholipid Biosynthesis

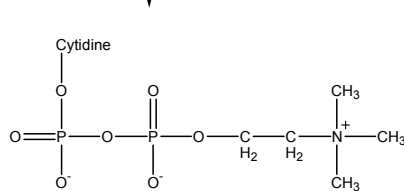
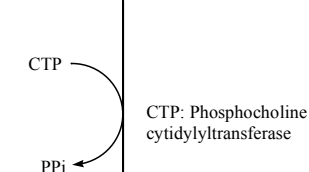
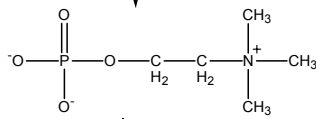
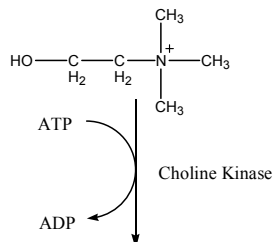
Phosphatidic acid can be converted into phospholipids. Phospholipids are the main components of biological membranes.

Phosphatidyl Choline and Phosphatidyl Ethanolamine Biosynthesis

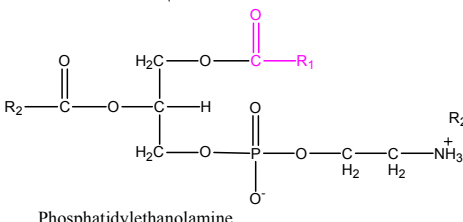
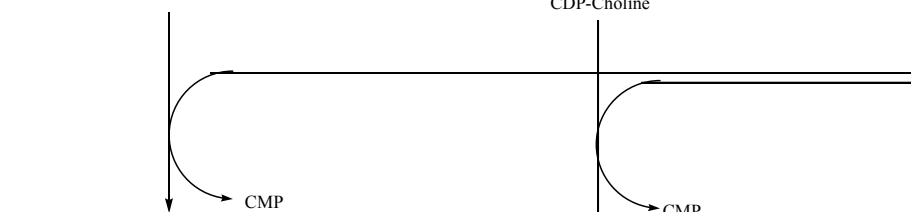
The first step in the biosynthesis of these 2 glycerophospholipids is the activation of choline or ethanolamine. The activation requires 2 steps. The first step is phosphorylation with ATP to produce phosphoethanolamine or phosphocholine.



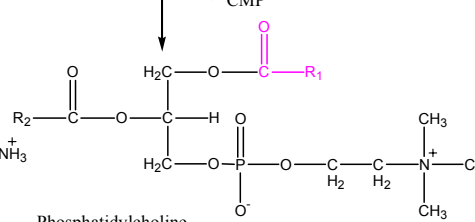
CDP-Ethanolamine



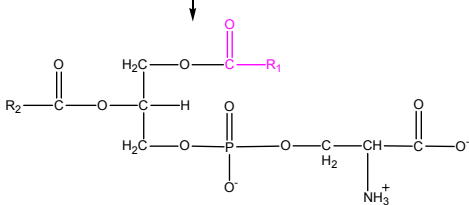
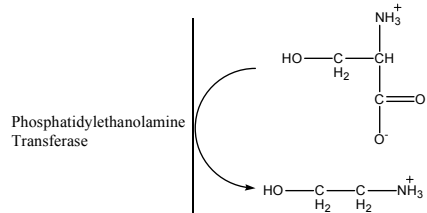
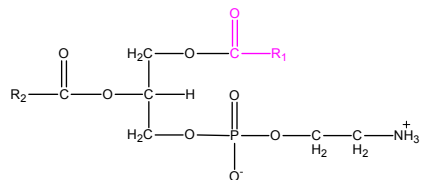
CDP-Choline



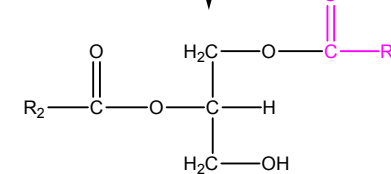
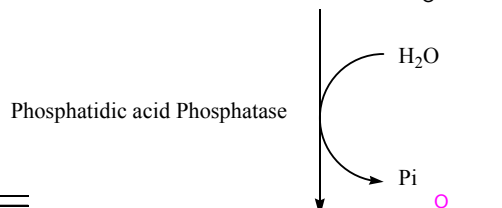
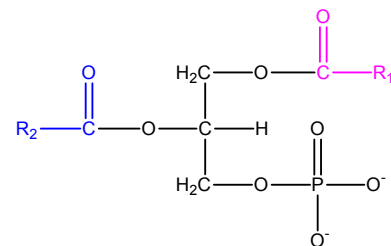
Phosphatidylethanolamine



Phosphatidylcholine



The second step involves cytidylyltransferase to generate cytosine diphosphate ethanolamine or cytosine diphosphate choline. This reaction produces pyrophosphate which of course is hydrolyzed by pyrophosphatase which makes this activation irreversible.



Phosphatidic acid is dephosphorylated by phosphatidic acid phosphatase to form diacylglycerol.

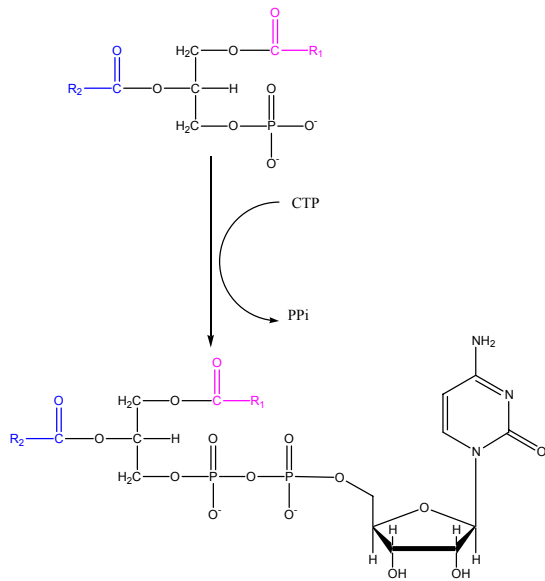
The diacylglycerol then reacts with CDP-ethanolamine or CDP-choline to form phosphatidylethanolamine or phosphatidylcholine.

The enzymes that catalyze these two reactions have incredibly long names: CDPethanolamine: 1,2-diacylglycerol phosphoethanolamine transferase and CDPcholine: 1,2-diacylglycerol phosphocholine transferase.

Phosphatidylserine

Phosphatidylserine is synthesized in mammals by phosphatidylethanolamine transferase. In this enzyme the hydroxyl group of serine attacks the phosphate group of phosphatidylethanolamine eliminating ethanolamine to form phosphatidyl serine.

Phosphatidylinositol, Phosphatidylglycerol and Cardiolipin Biosynthesis.



In order to synthesize these phospholipids, the phosphatidic acid must be activated in order to be condensed with an alcohol to produce the corresponding phospholipids. Phosphatidic acid is activated in the same manner as ethanolamine and choline were activated. CDP:phosphatidate cytidylyltransferase which reacts CTP with phosphatidate to form cytidine diphosphodiacylglycerol. This activation reaction produces pyrophosphate which of course is hydrolyzed by pyrophosphatase which makes this activation irreversible.

