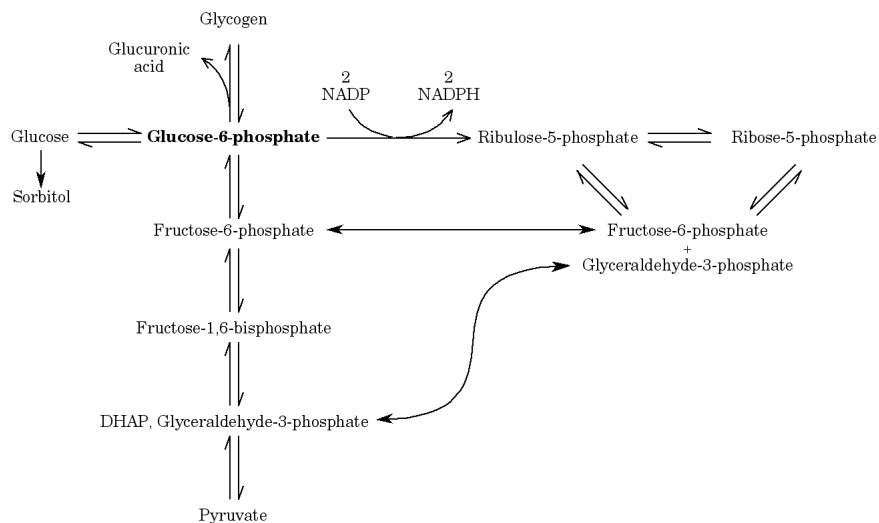


Glucose-6-phosphate in Metabolic Processes

Glucose-6-phosphate acts as a branch point for a wide variety of pathways. It can be used: 1) as a glycolytic substrate, 2) as a substrate for other synthetic reactions, 3) as a substrate for glycogen synthesis, 4) as a source of biosynthetic reducing equivalents and intermediates via the hexose monophosphate shunt, or 5) (in liver and kidney only) be converted back to glucose and released into the bloodstream.



The flow of glucose through each of these processes is tightly regulated. We have already covered the glycolytic and gluconeogenic pathways; we will now turn to other possible fates of glucose-6-phosphate.

Hexose monophosphate shunt (Pentose phosphate pathway)

Use and Importance:

The hexose monophosphate pathway is used for production of NADPH from NADP. The NADPH is required for biosynthetic reactions such as fatty acid synthesis, cholesterol synthesis, drug reduction, and as a cofactor for some non-synthetic enzymatic reactions. In addition, it is used for the production of ribose for nucleotide and nucleic acid synthesis. The hexose monophosphate pathway also allows the entry of some carbohydrates into the glycolytic pathway (especially ribose, but also some others), and therefore acts as a connection route between different pathways.

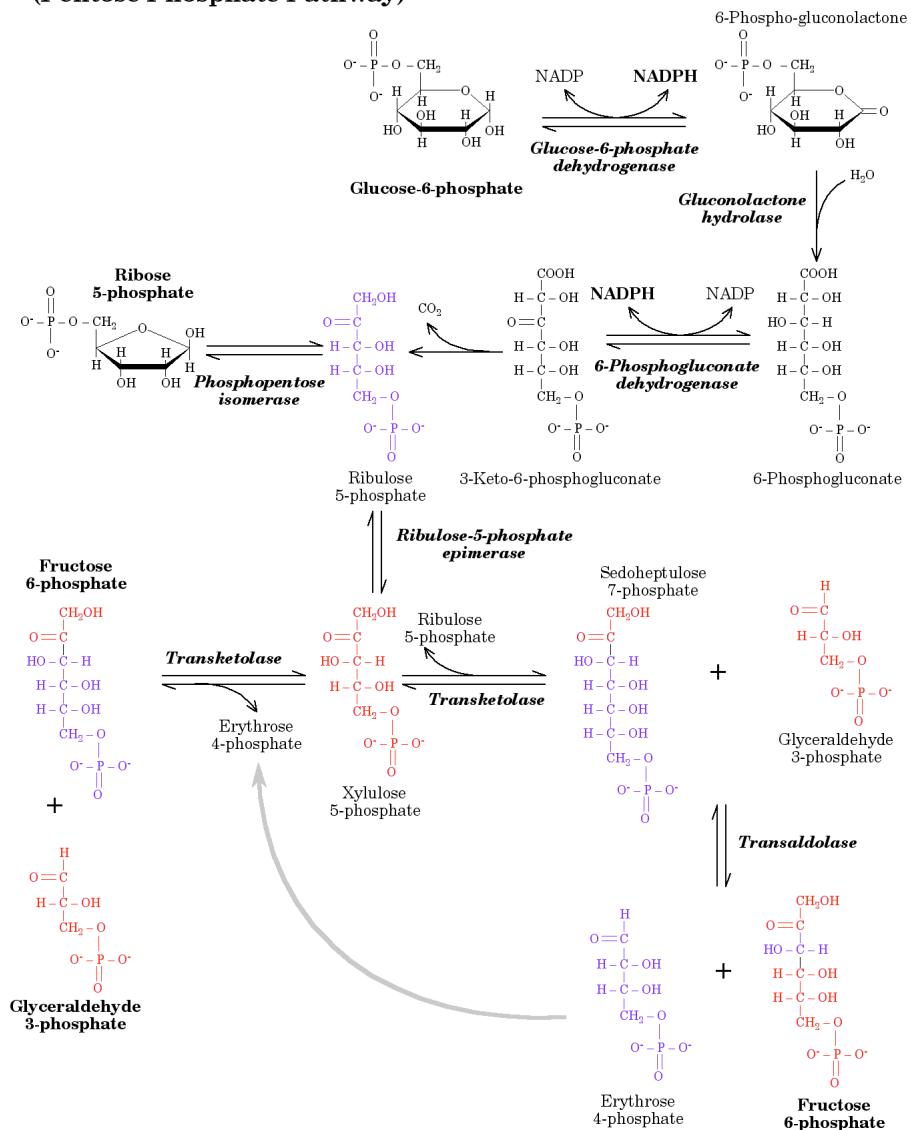
Steroidogenic tissues, red blood cells, and the liver are the major sites of hexose monophosphate pathway. Muscle has small amounts of some of the hexose monophosphate pathway enzymes, because it has little need for synthetic reactions, and therefore, little need for NADPH. The muscle, however, like **all tissues, needs to be able to synthesize ribose** in order to make nucleotides and nucleic acids.

Features of the hexose monophosphate pathway

Like the glycolytic enzymes, the enzymes of the hexose monophosphate pathway are found in the cytoplasm.

Although the hexose monophosphate pathway has fewer enzymes than glycolysis, it is somewhat more complicated. The net reaction for the pathway is somewhat variable, depending on the entry and exit points of the metabolites. The full pathway is shown below; it is important to remember that not all of these reactions occur in all tissues.

Hexose Monophosphate Shunt (Pentose Phosphate Pathway)



Hexose monophosphate pathway reactions (NADPH-generating steps)

Glucose-6-phosphate dehydrogenase

Glucose-6-phosphate dehydrogenase is the **rate-limiting step**, and **primary control point** of the NADPH-generating portion of the hexose monophosphate pathway. Glucose-6-phosphate dehydrogenase is stimulated by NADP and inhibited by NADPH. The levels of glucose-6-phosphate dehydrogenase are increased by

insulin (insulin stimulates anabolic processes, and many anabolic reactions require NADPH).

Glucose-6-phosphate dehydrogenase catalyzes the formation of the first NADPH of the pathway. The reaction is drawn as reversible, but is effectively irreversible under physiological conditions due to the instability of the product and the presence of gluconolactone hydrolase.

Gluconolactone hydrolase

The 6-phosphogluconolactone produced by glucose-6-phosphate dehydrogenase is unstable, and will undergo spontaneous hydrolysis. The hydrolase merely accelerates the process. The hydrolase reaction is effectively irreversible under physiological conditions.

6-Phosphogluconate dehydrogenase

6-Phosphogluconate dehydrogenase catalyzes the formation of the second NADPH. It has a reaction mechanism similar to that of isocitrate dehydrogenase, and like isocitrate dehydrogenase, yields an unstable product (3-keto-6-phosphogluconate) that rapidly decarboxylates to release ribulose-5-phosphate. Due to the loss of carbon dioxide in this step, the 6-phosphogluconate dehydrogenase reaction is irreversible under physiological conditions.

Note that 6-phosphogluconate contains six carbons, while ribulose-5-phosphate contains five carbons (as mentioned above, one carbon is lost as carbon dioxide).

In most cells, NADPH levels are much higher than NADP levels. NADPH has a much higher free energy than NADP, both as a result of the concentration difference, and of the fact that, like NADH, NADPH is a more energetic molecule than its oxidized counterpart. While most dehydrogenase reactions are reversible, net synthesis of NADPH is possible because the products of both dehydrogenase reactions in this pathway are rapidly and irreversibly converted to compounds that cannot act as substrates for the reverse reactions. Therefore, **the glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase reactions are irreversible because their product concentrations are never high enough to allow the reverse reactions to proceed.**

Hexose monophosphate pathway reactions (Later, reversible steps)

Although some tissues (*e.g.*, skeletal muscle) contain small amounts of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, all tissues contain the remaining enzymes in the pathway. These remaining enzymes are necessary for the synthesis of nucleotides, and for the conversion of ribose into glycolytic intermediates. All of the remaining hexose monophosphate pathway enzymes catalyze reversible reactions. Flow of carbon compounds through the remainder of the pathway is dependent on the **availability of substrates**. Other pathways regulate the availability of all of these substrates; regulation of the other pathways (especially nucleotide synthesis and breakdown, and glycolysis and gluconeogenesis) therefore indirectly regulates the flow of substrates through the non-NADPH synthesizing part of the hexose monophosphate pathway.

The ribulose-5-phosphate formed in the 6-phosphogluconate dehydrogenase reaction acts as a substrate for two different enzymes.

Ribulose-5-phosphate isomerase

The isomerase converts the ketopentose ribulose-5-phosphate to the aldopentose ribose-5-phosphate; in other words, it rearranges the molecule to move the carbonyl from the 2-position to the 1-position. The product, **ribose-5-phosphate is the precursor for all nucleotide synthesis**, and its production is an important function of the hexose monophosphate pathway.

Ribulose-5-phosphate epimerase

Epimers are carbohydrates with reversed stereochemistry at one of the carbons. Ribulose-5-phosphate epimerase converts ribulose-5-phosphate to its epimer xylulose-5-phosphate. (Note that for D-ketopentoses, only two epimers are possible; reversal of the stereochemistry at the 4-position would result in an L-monosaccharide.)

Because the isomerase and epimerase reactions are **reversible**, excess ribose-5-phosphate (resulting from the hexose monophosphate pathway or from nucleotide breakdown) can be converted to other compounds via the hexose monophosphate pathway.

Transketolase

Transketolase catalyzes **two-carbon transfer** reactions. It requires **thiamin pyrophosphate** as a cofactor.

Transketolase is used in two separate reactions in the hexose monophosphate pathway: it transfers two carbons from xylulose-5-phosphate to ribulose-5-phosphate to release the seven-carbon sedoheptulose-7-phosphate. Alternatively, it can transfer the two carbons to erythrose-4-phosphate to produce fructose-6-phosphate. In both cases, the remaining carbons from the xylulose-5-phosphate are released as glyceraldehyde-3-phosphate.

The two-carbon transfer involves covalent bond formation to the thiamin pyrophosphate. Note that the transketolase reaction is required for glyceraldehyde-3-phosphate formation from ribose-5-phosphate. Thiamin deficiency therefore prevents the entry of ribose into glycolysis, and results in accumulation of ribose, in addition to other important effects (such as inhibition of pyruvate dehydrogenase and α -ketoglutarate dehydrogenase).

Transaldolase

Transaldolase catalyzes a **three-carbon transfer** from sedoheptulose-7-phosphate to glyceraldehyde-3-phosphate, leaving erythrose-4-phosphate, and forming fructose-6-phosphate. Note that the erythrose-4-phosphate produced in this reaction is a substrate for one of the transketolase reactions. The transaldolase reaction also involves the formation of a covalent intermediate between the enzyme and substrate. In the case of transaldolase, however, the covalent intermediate is formed between the substrate and a lysine side chain of the enzyme.

Pathway variations

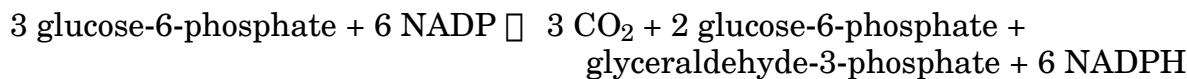
Intermediates can enter the hexose monophosphate pathway from glycolysis (which bypasses NADPH synthesis). This is especially important in muscle and other tissues that lack significant amounts of glucose-6-phosphate dehydrogenase; these tissues can therefore synthesize ribose-5-phosphate for nucleotide biosynthesis, although they produce little NADPH. Alternatively, ribose-5-phosphate can enter the pathway to be broken down for energy or converted into other compounds.

Net reactions:

The hexose monophosphate pathway can be used to synthesize both NADPH and ribose:



The hexose monophosphate pathway can also be used to synthesize NADPH, while regenerating glucose-6-phosphate (note that the fructose-6-phosphate formed in the hexose monophosphate pathway can readily be converted to glucose-6-phosphate).

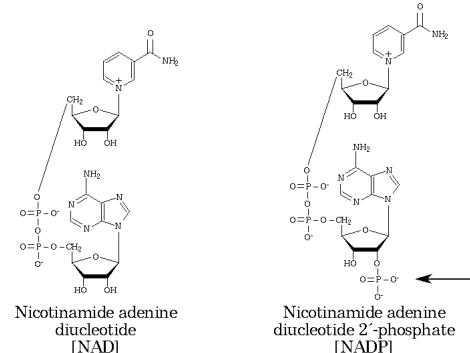


Finally, in some cases, cells need to synthesize ribose without needing NADPH, or need to convert ribose to energy. Using the reversible enzymes in the pathway, the reaction below allows these conversions to take place. Note that this last reaction is fully reversible; no NADPH are used or formed, and none of the carbons are lost as carbon dioxide.



Uses of NADPH

Chemically NAD and NADP are very similar molecules. The two molecules are structurally nearly identical (the only difference is the phosphate on the 2'-position of the adenosine ribose of NADP instead of the free hydroxyl at this position in NAD). In addition, the nicotinamide rings used for the actual chemistry of these redox cofactors have similar reduction potentials.



Physiologically, however, the two nicotinamide cofactors are quite different. NAD is used to accept electrons in catabolic processes (such as the TCA cycle), while NADPH is primarily used to donate electrons for synthetic reactions. (Note that gluconeogenesis is an exception: the synthetic process of gluconeogenesis uses NADH as an electron donor.) Most dehydrogenase enzymes are extremely specific, using either NADP(H) or NAD(H), but not both.

Under most conditions, the cellular concentration of NADPH is much higher than

the concentration of NADP. NADPH can therefore act as a strong driving force for otherwise unfavorable reactions because of both the concentration differential and the fact that NADPH contains more energy than NADP (in the same way that NADH contains more energy than NAD). In contrast, the reduction of NAD associated with a number of catabolic reactions is assisted by the fact that NAD is normally maintained at much higher concentrations than NADH.

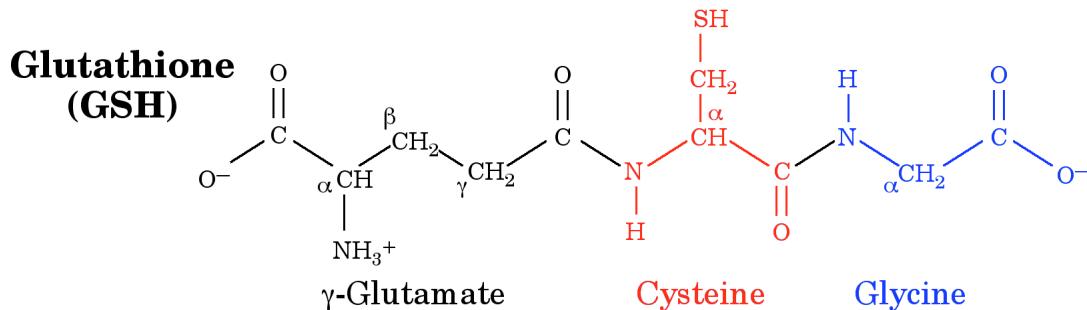
NADPH is used for a variety of electron donation reactions. In addition to its involvement in synthetic processes, in some cell types, NADPH is required to maintain normal functioning. As an example, red blood cells, which perform very few synthetic reactions, nevertheless require significant amounts of NADPH.

NADPH in red blood cells

NADPH plays two critical roles in red blood cells. Both of these roles are related to the oxygen-transport function of the red blood cell.

The first role is related to glutathione: erythrocytes require NADPH to maintain their levels of reduced glutathione. Glutathione is a tripeptide with cysteine as the middle residue. Because the cysteine sulphydryl group is important to its function, reduced glutathione is abbreviated as GSH, while oxidized glutathione, which exists as a disulfide dimer, is abbreviated as GSSG.

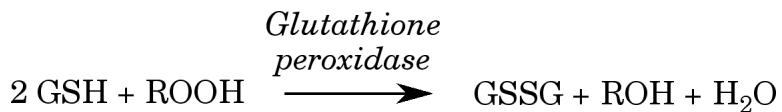
The vast majority of peptides used in humans are formed on ribosomes. In contrast, the peptide bonds in glutathione are produced enzymatically. This enzymatic synthesis results in an unusual peptide bond formed between the $\text{ }\text{\textbar}\text{-carboxyl}$ group of glutamate and the $\text{ }\text{\textbar}\text{-amino}$ group of cysteine. The structure of glutathione is shown below.



Oxygen is toxic: without reduced glutathione, peroxides spontaneously formed from molecular oxygen would oxidize the lipid components of the red blood cell membranes. Oxidized membranes are significantly less flexible than normal membranes, and result in damage to the red blood cells when the cells attempt to transit capillaries. In addition, peroxides tend to damage hemoglobin, resulting in precipitation of the protein. Insoluble aggregates of hemoglobin have severely impaired oxygen carrying capacity, and insoluble protein aggregates also tend to be inflexible enough to prevent the normal deformations of the red blood cell.

Two enzymes are required to deal with the peroxides (compounds with the general structure ROOH, where the R can be an organic compound, or, in the case of

hydrogen peroxide, hydrogen). *Glutathione peroxidase* uses glutathione to convert the peroxide to an alcohol.



Glutathione reductase then uses NADPH to regenerate the reduced glutathione.



The second role of NADPH in red blood cells is related to hemoglobin. Oxygen tends to oxidize the hemoglobin iron from +2 to the more stable +3 oxidation state (resulting in methemoglobin). This is a problem: the +3 state of heme iron binds oxygen very poorly. NADPH is used to supply reducing equivalents to **methemoglobin reductase**, the enzyme that returns the hemoglobin to the +2 oxidation state.

Glucose-6-phosphate dehydrogenase deficiency

A genetically-mediated reduction in glucose-6-phosphate dehydrogenase levels is somewhat protective against malaria. Glucose-6-phosphate dehydrogenase deficiency is an extremely common genetic abnormality, found in 5-10% of the global population. The result of the deficiency is a decreased ability to form NADPH in red blood cells.

Malaria parasites live inside red blood cells. Red blood cells normally have a life-span of about 120 days. A number of mutations (glucose-6-phosphate dehydrogenase deficiency, sickle cell anemia, and the thalassemias) shorten the life-span of the red blood cell, resulting in death of the malaria parasites that have not reached maturity. In the case of glucose-6-phosphate dehydrogenase deficiency, the most common mutations appear to decrease the half-life of the enzyme, which is effective in reducing the life-span of the cell because red blood cells lack the ability to synthesize new proteins.

The erythrocytes in individuals with glucose-6-phosphate dehydrogenase deficiency are asymptomatic unless challenged with oxidants; oxidant exposure, however, results in hemolytic anemia (a reduced number of red blood cells due to lysis of the cells). The hemolysis (*i.e.* disruption of the red blood cells) occurs because, as the membrane lipids become oxidized, the membrane becomes less flexible. Less flexible red blood cell membranes are a serious problem, because the red blood cell is larger than the typical capillary, and if insufficiently flexible, either cannot enter the capillary, or lyses as it attempts to do so.

An episode of the television series M*A*S*H had Corporal Klinger becoming very tired after being treated with an anti-malarial drug (Primaquine). This is what actually happens with glucose-6-phosphate dehydrogenase deficiency; primaquine

results in greater oxidation than the erythrocytes can tolerate, and causes hemolysis. (A minor irony; the mutation is protective against malaria, but causes intolerance to anti-malarial drugs.)

Summary

The hexose monophosphate pathway is used to produce the NADPH required for a variety of cellular functions. NADPH production is regulated by the relative levels of NADP and NADPH, which alter the activity of glucose-6-phosphate dehydrogenase, the control enzyme for the pathway.

The hexose monophosphate pathway is also used to synthesize the ribose-5-phosphate required for nucleotide and nucleic acid biosynthesis.

All of the hexose monophosphate pathway enzymes after the 6-phosphogluconate dehydrogenase step are fully reversible. These enzymes allow the synthesis of ribose-5-phosphate from the glycolytic intermediates fructose-6-phosphate and glyceraldehyde-3-phosphate, and the conversion of ribose-5-phosphate into glycolytic intermediates.

NADPH (and therefore the hexose monophosphate pathway) is part of the mechanism that red blood cells use to prevent oxygen-mediated damage to their membranes and proteins, and to maintain hemoglobin in the oxidation state required for oxygen transport.