TABLE 14–4 Glucogenic Amino Acids, Grouped by Site of Entry

Pyruvate	**Succinyl-CoA**
Alanine	Isoleucine*
Cysteine	Methionine
Glycine	Threonine
Serine	Valine
Threonine	**Fumarate**
Tryptophan*	Phenylalanine*
α-Ketoglutarate	Tyrosine*
Arginine	**Oxaloacetate**
Glutamate	Asparagine
Glutamine	Aspartate
Histidine	
Proline	

Note: All these amino acids are precursors of blood glucose or liver glycogen, because they can be converted to pyruvate or citric acid cycle intermediates. Of the 20 common amino acids, only leucine and lysine are unable to furnish carbon for net glucose synthesis.

*These amino acids are also ketogenic (see Fig. 18–21).

the result would be the consumption of ATP and the production of heat. For example, PFK-1 and FBPase-1 catalyze opposing reactions:

$$\text{ATP} + \text{fructose 6-phosphate} \xrightarrow[\text{PFK–1}]{} \text{ADP} + \text{fructose 1,6-bisphosphate}$$

$$\text{Fructose 1,6-bisphosphate} + \text{H}_2\text{O} \xrightarrow[\text{FBPase–1}]{} \text{fructose 6-phosphate} + \text{P}_i$$

The sum of these two reactions is

$$\text{ATP} + \text{H}_2\text{O} \longrightarrow \text{ADP} + \text{P}_i + \text{heat}$$

These two enzymatic reactions, and a number of others in the two pathways, are regulated allosterically and by covalent modification (phosphorylation). In Chapter 15 we take up the mechanisms of this regulation in detail. For now, suffice it to say that the pathways are regulated so that when the flux of glucose through glycolysis goes up, the flux of pyruvate toward glucose goes down, and vice versa.

SUMMARY 14.4 Gluconeogenesis

- Gluconeogenesis is a ubiquitous multistep process in which pyruvate or a related three-carbon compound (lactate, alanine) is converted to glucose. Seven of the steps in gluconeogenesis are catalyzed by the same enzymes used in glycolysis; these are the reversible reactions.
- Three irreversible steps in the glycolytic pathway are bypassed by reactions catalyzed by gluconeogenic enzymes: (1) conversion of pyruvate to PEP via oxaloacetate, catalyzed by pyruvate carboxylase and PEP carboxykinase; (2) dephosphorylation of fructose 1,6-bisphosphate by FBPase-1; and (3) dephosphorylation of glucose 6-phosphate by glucose 6-phosphatase.
- Formation of one molecule of glucose from pyruvate requires 4 ATP, 2 GTP, and 2 NADH; it is expensive.
- In mammals, gluconeogenesis in the liver and kidney provides glucose for use by the brain, muscles, and erythrocytes.
- Pyruvate carboxylase is stimulated by acetyl-CoA, increasing the rate of gluconeogenesis when the cell already has adequate supplies of other substrates (fatty acids) for energy production.
- Animals cannot convert acetyl-CoA derived from fatty acids into glucose; plants and microorganisms can.
- Glycolysis and gluconeogenesis are reciprocally regulated to prevent wasteful operation of both pathways at the same time.

14.5 Pentose Phosphate Pathway of Glucose Oxidation

In most animal tissues, the major catabolic fate of glucose 6-phosphate is glycolytic breakdown to pyruvate, much of which is then oxidized via the citric acid cycle, ultimately leading to the formation of ATP. Glucose 6-phosphate does have other catabolic fates, however, which lead to specialized products needed by the cell. Of particular importance in some tissues is the oxidation of glucose 6-phosphate to pentose phosphates by the **pentose phosphate pathway** (also called the **phosphogluconate pathway** or the **hexose monophosphate pathway;** Fig. 14–20). In this oxidative pathway, $NADP^+$ is the electron acceptor, yielding NADPH. Rapidly dividing cells, such as those of bone marrow, skin, and intestinal mucosa, use the pentoses to make RNA, DNA, and such coenzymes as ATP, NADH, $FADH_2$, and coenzyme A.

In other tissues, the essential product of the pentose phosphate pathway is not the pentoses but the electron donor NADPH, needed for reductive biosynthesis or to counter the damaging effects of oxygen radicals. Tissues that carry out extensive fatty acid synthesis (liver, adipose, lactating mammary gland) or very active synthesis of cholesterol and steroid hormones (liver, adrenal gland, gonads) require the NADPH provided by the pathway. Erythrocytes and the cells of the lens and cornea are directly exposed to oxygen and thus to the damaging free radicals generated by oxygen.

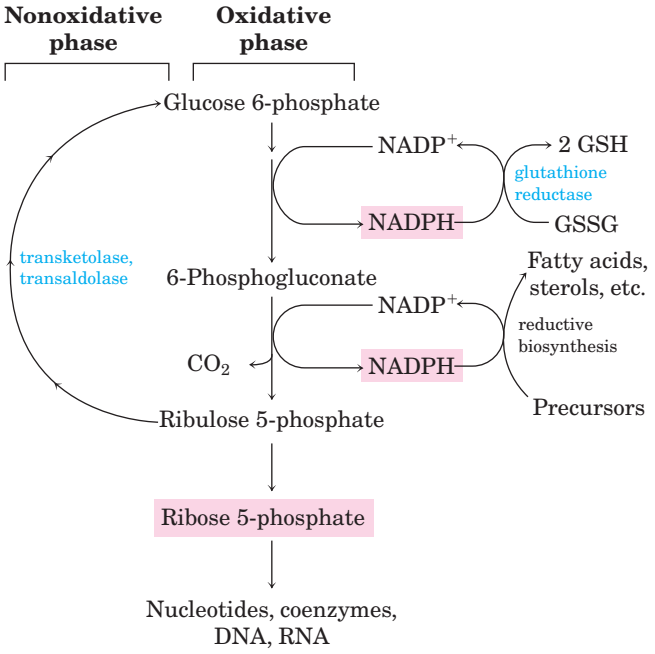


FIGURE 14–20 General scheme of the pentose phosphate pathway. NADPH formed in the oxidative phase is used to reduce glutathione, GSSG (see Box 14–3) and to support reductive biosynthesis. The other product of the oxidative phase is ribose 5-phosphate, which serves as precursor for nucleotides, coenzymes, and nucleic acids. In cells that are not using ribose 5-phosphate for biosynthesis, the nonoxidative phase recycles six molecules of the pentose into five molecules of the hexose glucose 6-phosphate, allowing continued production of NADPH and converting glucose 6-phosphate (in six cycles) to CO_2.

By maintaining a reducing atmosphere (a high ratio of NADPH to $NADP^+$ and a high ratio of reduced to oxidized glutathione), they can prevent or undo oxidative damage to proteins, lipids, and other sensitive molecules. In erythrocytes, the NADPH produced by the pentose phosphate pathway is so important in preventing oxidative damage that a genetic defect in glucose 6-phosphate dehydrogenase, the first enzyme of the pathway, can have serious medical consequences (Box 14–3). ■

The Oxidative Phase Produces Pentose Phosphates and NADPH

The first reaction of the pentose phosphate pathway (Fig. 14–21) is the oxidation of glucose 6-phosphate by **glucose 6-phosphate dehydrogenase (G6PD)** to form 6-phosphoglucono-δ-lactone, an intramolecular ester. $NADP^+$ is the electron acceptor, and the overall equilibrium lies far in the direction of NADPH formation. The lactone is hydrolyzed to the free acid 6-phosphogluconate by a specific **lactonase,** then 6-phosphogluconate undergoes oxidation and decarboxylation by **6-phosphogluconate dehydrogenase** to form the ketopentose ribulose 5-phosphate. This reaction generates

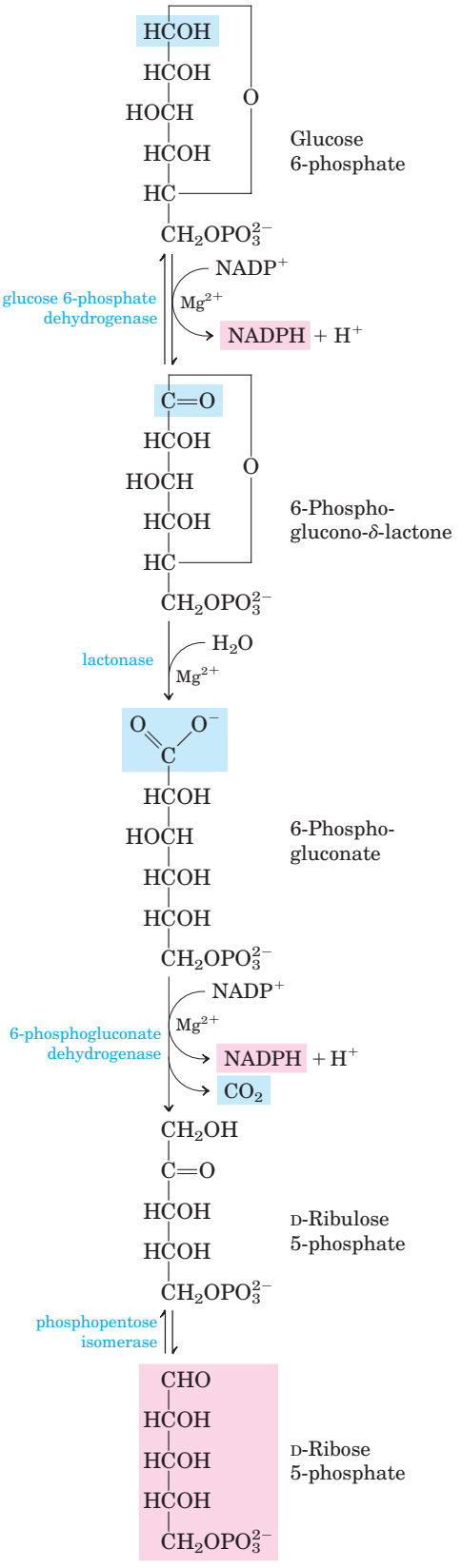


FIGURE 14–21 Oxidative reactions of the pentose phosphate pathway. The end products are ribose 5-phosphate, CO_2, and NADPH.

BOX 14–3 BIOCHEMISTRY IN MEDICINE

Why Pythagoras Wouldn't Eat Falafel: Glucose 6-Phosphate Dehydrogenase Deficiency

Fava beans, an ingredient of falafel, have been an important food source in the Mediterranean and Middle East since antiquity. The Greek philosopher and mathematician Pythagoras prohibited his followers from dining on fava beans, perhaps because they make many people sick with a condition called favism, which can be fatal. In favism, erythrocytes begin to lyse 24 to 48 hours after ingestion of the beans, releasing free hemoglobin into the blood. Jaundice and sometimes kidney failure can result. Similar symptoms can occur with ingestion of the antimalarial drug primaquine or of sulfa antibiotics or following exposure to certain herbicides. These symptoms have a genetic basis: glucose 6-phosphate dehydrogenase (G6PD) deficiency, which affects about 400 million people. Most G6PD-deficient individuals are asymptomatic; only the combination of G6PD deficiency and certain environmental factors produces the clinical manifestations.

G6PD catalyzes the first step in the pentose phosphate pathway (see Fig. 14–21), which produces NADPH. This reductant, essential in many biosynthetic pathways, also protects cells from oxidative damage by hydrogen peroxide (H_2O_2) and superoxide free radicals, highly reactive oxidants generated as metabolic byproducts and through the actions of drugs such as primaquine and natural products such as divicine—the toxic ingredient of fava beans. During normal detoxification, H_2O_2 is converted to H_2O by reduced glutathione and glutathione peroxidase, and the oxidized glutathione is converted back to the reduced form by glutathione reductase and NADPH (Fig. 1). H_2O_2 is also broken down to H_2O and O_2 by catalase, which also requires NADPH. In G6PD-deficient individuals, the NADPH production is diminished and detoxification of H_2O_2 is inhibited. Cellular damage results: lipid peroxidation leading to breakdown of erythrocyte membranes and oxidation of proteins and DNA.

The geographic distribution of G6PD deficiency is instructive. Frequencies as high as 25% occur in tropical Africa, parts of the Middle East, and Southeast Asia, areas where malaria is most prevalent. In addition to such epidemiological observations, in vitro studies show that growth of one malaria parasite, *Plasmodium falciparum*, is inhibited in G6PD-deficient erythrocytes. The parasite is very sensitive to oxidative damage and is killed by a level of oxidative stress that is tolerable to a G6PD-deficient human host. Because the advantage of resistance to malaria balances the disadvantage of lowered resistance to oxidative damage, natural selection sustains the G6PD-deficient genotype in human populations where malaria is prevalent. Only under overwhelming oxidative stress, caused by drugs, herbicides, or divicine, does G6PD deficiency cause serious medical problems.

An antimalarial drug such as primaquine is believed to act by causing oxidative stress to the parasite. It is ironic that antimalarial drugs can cause illness through the same biochemical mechanism that provides resistance to malaria. Divicine also acts as an antimalarial drug, and ingestion of fava beans may protect against malaria. By refusing to eat falafel, many Pythagoreans with normal G6PD activity may have unwittingly increased their risk of malaria!

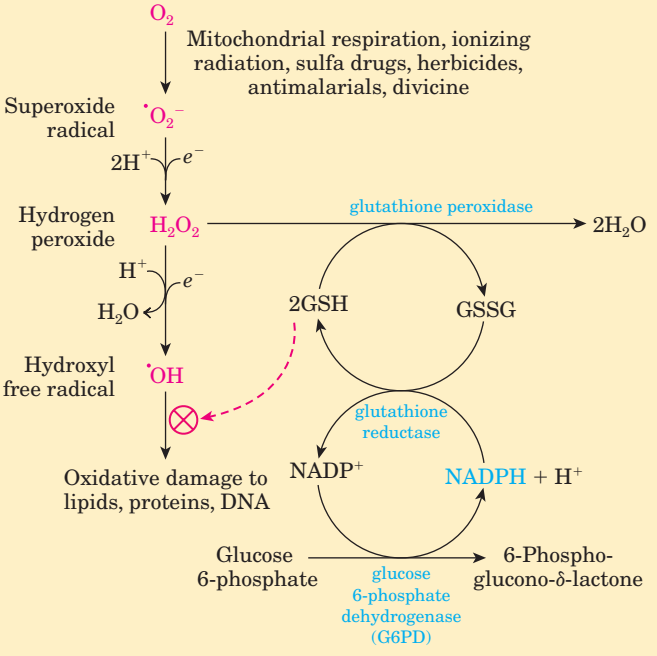


FIGURE 1 Role of NADPH and glutathione in protecting cells against highly reactive oxygen derivatives. Reduced glutathione (GSH) protects the cell by destroying hydrogen peroxide and hydroxyl free radicals. Regeneration of GSH from its oxidized form (GSSG) requires the NADPH produced in the glucose 6-phosphate dehydrogenase reaction.

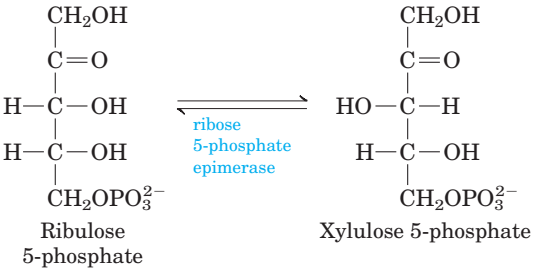
a second molecule of NADPH. **Phosphopentose isomerase** converts ribulose 5-phosphate to its aldose isomer, ribose 5-phosphate. In some tissues, the pentose phosphate pathway ends at this point, and its overall equation is

$$\text{Glucose 6-phosphate} + 2\text{NADP}^+ + \text{H}_2\text{O} \longrightarrow \text{ribose 5-phosphate} + \text{CO}_2 + 2\text{NADPH} + 2\text{H}^+$$

The net result is the production of NADPH, a reductant for biosynthetic reactions, and ribose 5-phosphate, a precursor for nucleotide synthesis.

The Nonoxidative Phase Recycles Pentose Phosphates to Glucose 6-Phosphate

In tissues that require primarily NADPH, the pentose phosphates produced in the oxidative phase of the pathway are recycled into glucose 6-phosphate. In this nonoxidative phase, ribulose 5-phosphate is first epimerized to xylulose 5-phosphate:



Ribulose 5-phosphate — Xylulose 5-phosphate

Then, in a series of rearrangements of the carbon skeletons (Fig. 14–22), six five-carbon sugar phosphates are converted to five six-carbon sugar phosphates, completing the cycle and allowing continued oxidation of glucose 6-phosphate with production of NADPH. Continued recycling leads ultimately to the conversion of glucose 6-phosphate to six CO_2. Two enzymes unique to the pentose phosphate pathway act in these interconversions of sugars: transketolase and transaldolase. **Transketolase** catalyzes the transfer of a two-carbon fragment from a ketose donor to an aldose acceptor (Fig. 14–23a). In its first appearance in the pentose phosphate pathway, transketolase transfers C-1 and C-2 of xylulose 5-phosphate to ribose 5-phosphate, forming the seven-carbon product sedoheptulose 7-phosphate (Fig. 14–23b). The remaining three-carbon fragment from xylulose is glyceraldehyde 3-phosphate.

Next, **transaldolase** catalyzes a reaction similar to the aldolase reaction of glycolysis: a three-carbon fragment is removed from sedoheptulose 7-phosphate and condensed with glyceraldehyde 3-phosphate, forming fructose 6-phosphate and the tetrose erythrose 4-phosphate (Fig. 14–24). Now transketolase acts again, forming fructose 6-phosphate and glyceraldehyde 3-phosphate from erythrose 4-phosphate and xylulose 5-phosphate (Fig. 14–25). Two molecules of glyceraldehyde 3-phosphate formed by two iterations of these reactions can be converted to a molecule of fructose 1,6-bisphosphate as in gluconeogenesis (Fig. 14–16), and finally FBPase-1 and phosphohexose isomerase convert fructose 1,6-bisphosphate to glucose 6-phosphate. The cycle is complete: six pentose phosphates have been converted to five hexose phosphates (Fig. 14–22b).

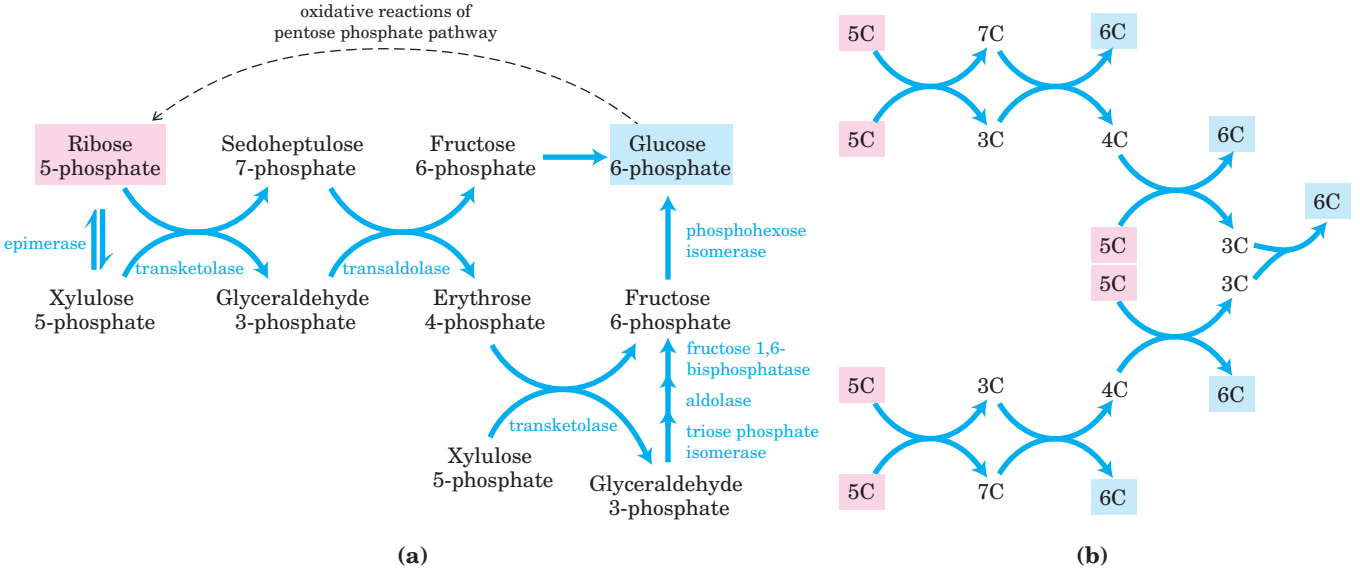
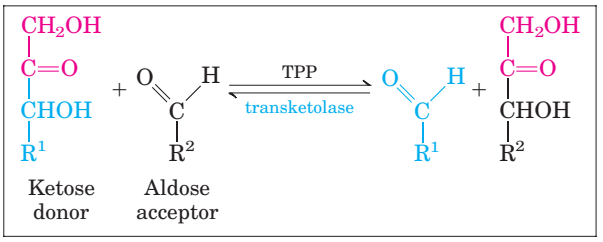
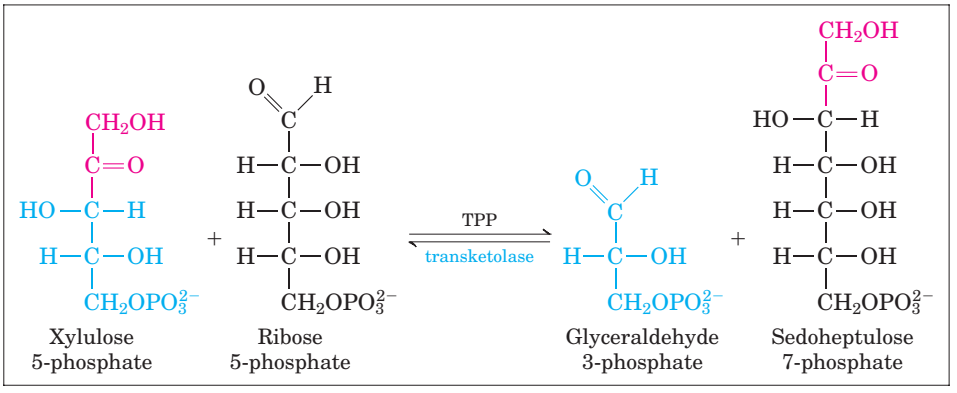


FIGURE 14–22 Nonoxidative reactions of the pentose phosphate pathway. (a) These reactions convert pentose phosphates to hexose phosphates, allowing the oxidative reactions (see Fig. 14–21) to continue. The enzymes transketolase and transaldolase are specific to this pathway; the other enzymes also serve in the glycolytic or gluconeogenic pathways. **(b)** A schematic diagram showing the pathway from six pentoses (5C) to five hexoses (6C). Note that this involves two sets of the interconversions shown in **(a).** Every reaction shown here is reversible; unidirectional arrows are used only to make clear the direction of the reactions during continuous oxidation of glucose 6-phosphate. In the light-independent reactions of photosynthesis, the direction of these reactions is reversed (see Fig. 20–10).



(a)



(b)

FIGURE 14–23 **The first reaction catalyzed by transketolase.** **(a)** The general reaction catalyzed by transketolase is the transfer of a two-carbon group, carried temporarily on enzyme-bound TPP, from a ketose donor to an aldose acceptor. **(b)** Conversion of two pentose phosphates to a triose phosphate and a seven-carbon sugar phosphate, sedoheptulose 7-phosphate.

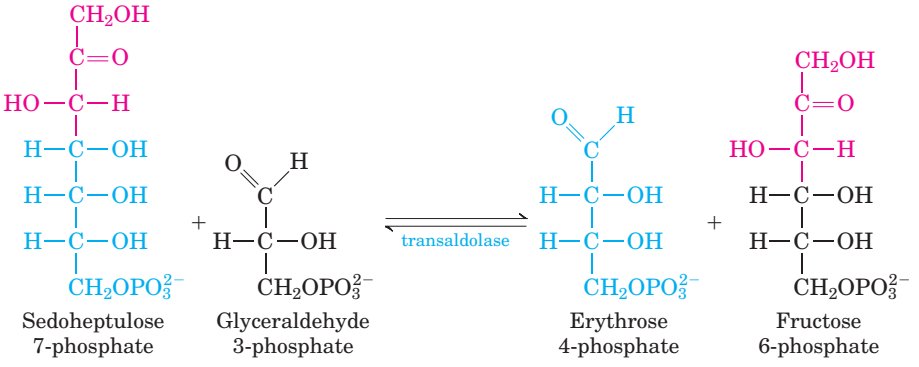


FIGURE 14–24 **The reaction catalyzed by transaldolase.**

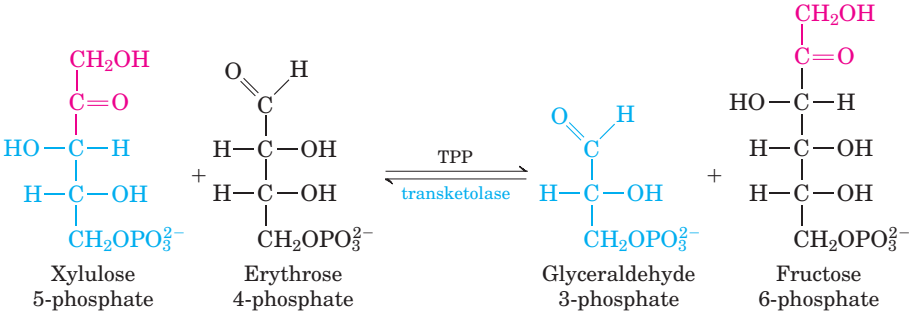
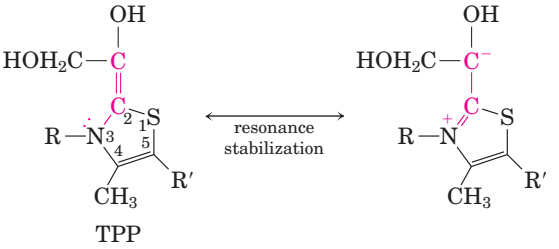


FIGURE 14–25 **The second reaction catalyzed by transketolase.**

Transketolase requires the cofactor thiamine pyrophosphate (TPP), which stabilizes a two-carbon carbanion in this reaction (Fig. 14–26a), just as it does in the pyruvate decarboxylase reaction (Fig. 14–13). Transaldolase uses a Lys side chain to form a Schiff base with the carbonyl group of its substrate, a ketose, thereby stabilizing a carbanion (Fig. 14–26b) that is central to the reaction mechanism.

The process described in Figure 14–21 is known as the **oxidative pentose phosphate pathway.** The first two steps are oxidations with large, negative standard free-energy changes and are essentially irreversible in

(a) Transketolase



(b) Transaldolase

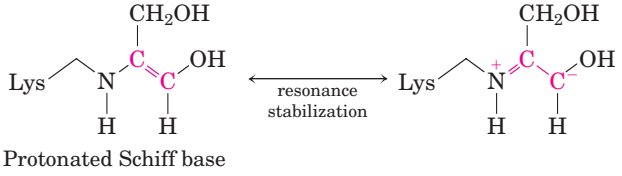


FIGURE 14–26 Carbanion intermediates stabilized by covalent interactions with transketolase and transaldolase. (a) The ring of TPP stabilizes the two-carbon carbanion carried by transketolase; see Fig. 14–13 for the chemistry of TPP action. **(b)** In the transaldolase reaction, the protonated Schiff base formed between the ε-amino group of a Lys side chain and the substrate stabilizes a three-carbon carbanion.

the cell. The reactions of the nonoxidative part of the pentose phosphate pathway (Fig. 14–22) are readily reversible and thus also provide a means of converting hexose phosphates to pentose phosphates. As we shall see in Chapter 20, a process that converts hexose phosphates to pentose phosphates is crucial to the photosynthetic assimilation of CO_2 by plants. That pathway, the **reductive pentose phosphate pathway,** is essentially the reversal of the reactions shown in Figure 14–22 and employs many of the same enzymes.

All the enzymes in the pentose phosphate pathway are located in the cytosol, like those of glycolysis and most of those of gluconeogenesis. In fact, these three pathways are connected through several shared intermediates and enzymes. The glyceraldehyde 3-phosphate formed by the action of transketolase is readily converted to dihydroxyacetone phosphate by the glycolytic enzyme triose phosphate isomerase, and these two trioses can be joined by the aldolase as in gluconeogenesis, forming fructose 1,6-bisphosphate. Alternatively, the triose phosphates can be oxidized to pyruvate by the glycolytic reactions. The fate of the trioses is determined by the cell's relative needs for pentose phosphates, NADPH, and ATP.

Wernicke-Korsakoff Syndrome Is Exacerbated by a Defect in Transketolase

In humans with Wernicke-Korsakoff syndrome, a mutation in the gene for transketolase results in an enzyme having an affinity for its coenzyme TPP that is one-tenth that of the normal enzyme. Although moderate deficiencies in the vitamin thiamine have little effect on individuals with an unmutated transketolase gene, in those with the altered gene, thiamine deficiency drops the level of TPP below that needed to saturate the enzyme. The lowering of transketolase activity slows the whole pentose phosphate pathway, and the result is the Wernicke-Korsakoff syndrome: severe memory loss, mental confusion, and partial paralysis. The syndrome is more common among alcoholics than in the general population; chronic alcohol consumption interferes with the intestinal absorption of some vitamins, including thiamine. ■

Glucose 6-Phosphate Is Partitioned between Glycolysis and the Pentose Phosphate Pathway

Whether glucose 6-phosphate enters glycolysis or the pentose phosphate pathway depends on the current needs of the cell and on the concentration of $NADP^+$ in the cytosol. Without this electron acceptor, the first reaction of the pentose phosphate pathway (catalyzed by G6PD) cannot proceed. When a cell is rapidly converting NADPH to $NADP^+$ in biosynthetic reductions, the level of $NADP^+$ rises, allosterically stimulating G6PD and thereby increasing the flux of glucose 6-phosphate through the pentose phosphate pathway (Fig. 14–27). When the demand for NADPH slows, the level of $NADP^+$ drops, the pentose phosphate pathway slows, and glucose 6-phosphate is instead used to fuel glycolysis.

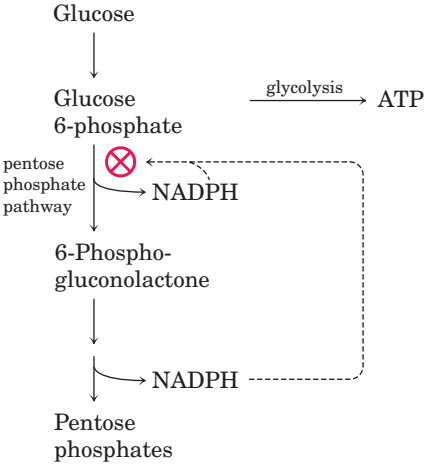


FIGURE 14–27 Role of NADPH in regulating the partitioning of glucose 6-phosphate between glycolysis and the pentose phosphate pathway. When NADPH is forming faster than it is being used for biosynthesis and glutathione reduction (see Fig. 14–20), [NADPH] rises and inhibits the first enzyme in the pentose phosphate pathway. As a result, more glucose 6-phosphate is available for glycolysis.

SUMMARY 14.5 Pentose Phosphate Pathway of Glucose Oxidation

- The *oxidative* pentose phosphate pathway (phosphogluconate pathway, or hexose monophosphate pathway) brings about oxidation and decarboxylation at C-1 of glucose 6-phosphate, reducing $NADP^+$ to NADPH and producing pentose phosphates.
- NADPH provides reducing power for biosynthetic reactions, and ribose 5-phosphate is a precursor for nucleotide and nucleic acid synthesis. Rapidly growing tissues and tissues carrying out active biosynthesis of fatty acids, cholesterol, or steroid hormones send more glucose 6-phosphate through the pentose phosphate pathway than do tissues with less demand for pentose phosphates and reducing power.
- The first phase of the pentose phosphate pathway consists of two oxidations that convert glucose 6-phosphate to ribulose 5-phosphate and reduce $NADP^+$ to NADPH. The second phase comprises nonoxidative steps that convert pentose phosphates to glucose 6-phosphate, which begins the cycle again.
- In the second phase, transaldolase (with TPP as cofactor) and transketolase catalyze the interconversion of three-, four-, five-, six-, and seven-carbon sugars, with the reversible conversion of six pentose phosphates to five hexose phosphates. In the carbon-assimilating reactions of photosynthesis, the same enzymes catalyze the reverse process, called the *reductive* pentose phosphate pathway: conversion of five hexose phosphates to six pentose phosphates.
- A genetic defect in transketolase that lowers its affinity for TPP exacerbates the Wernicke-Korsakoff syndrome.
- Entry of glucose 6-phosphate either into glycolysis or into the pentose phosphate pathway is largely determined by the relative concentrations of $NADP^+$ and NADPH.

Key Terms

Terms in bold are defined in the glossary.

glycolysis 522
fermentation 522
lactic acid fermentation
hypoxia 523
ethanol (alcohol) fermentation 523
isozymes 526
acyl phosphate 530
substrate-level phosphorylation 531
respiration-linked phosphorylation 531
phosphoenolpyruvate (PEP) 532
mutases 534
isomerases 534
lactose intolerance
galactosemia 537
thiamine pyrophosphate (TPP) 540
gluconeogenesis 543
biotin 544
pentose phosphate pathway 549
phosphogluconate pathway 549
hexose monophosphate pathway 549

Further Reading

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Problems

1. Equation for the Preparatory Phase of Glycolysis Write balanced biochemical equations for all the reactions in the catabolism of glucose to two molecules of glyceraldehyde 3-phosphate (the preparatory phase of glycolysis), including the standard free-energy change for each reaction. Then write the overall or net equation for the preparatory phase of glycolysis, with the net standard free-energy change.

2. The Payoff Phase of Glycolysis in Skeletal Muscle In working skeletal muscle under anaerobic conditions, glyceraldehyde 3-phosphate is converted to pyruvate (the payoff phase of glycolysis), and the pyruvate is reduced to lactate. Write balanced biochemical equations for all the reactions in this process, with the standard free-energy change for each reaction. Then write the overall or net equation for the payoff phase of glycolysis (with lactate as the end product), including the net standard free-energy change.

3. Pathway of Atoms in Fermentation A "pulse-chase" experiment using ^{14}C-labeled carbon sources is carried out on a yeast extract maintained under strictly anaerobic conditions to produce ethanol. The experiment consists of incubating a small amount of ^{14}C-labeled substrate (the pulse) with the yeast extract just long enough for each intermediate in the fermentation pathway to become labeled. The label is then "chased" through the pathway by the addition of excess unlabeled glucose. The chase effectively prevents any further entry of labeled glucose into the pathway.

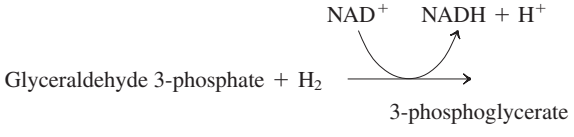
(a) If [1-^{14}C]glucose (glucose labeled at C-1 with ^{14}C) is used as a substrate, what is the location of ^{14}C in the product ethanol? Explain.

(b) Where would ^{14}C have to be located in the starting glucose to ensure that all the ^{14}C activity is liberated as $^{14}CO_2$ during fermentation to ethanol? Explain.

4. Fermentation to Produce Soy Sauce Soy sauce is prepared by fermenting a salted mixture of soybeans and wheat with several microorganisms, including yeast, over a period of 8 to 12 months. The resulting sauce (after solids are removed) is rich in lactate and ethanol. How are these two compounds produced? To prevent the soy sauce from having a strong vinegar taste (vinegar is dilute acetic acid), oxygen must be kept out of the fermentation tank. Why?

5. Equivalence of Triose Phosphates ^{14}C-Labeled glyceraldehyde 3-phosphate was added to a yeast extract. After a short time, fructose 1,6-bisphosphate labeled with ^{14}C at C-3 and C-4 was isolated. What was the location of the ^{14}C label in the starting glyceraldehyde 3-phosphate? Where did the second ^{14}C label in fructose 1,6-bisphosphate come from? Explain.

6. Glycolysis Shortcut Suppose you discovered a mutant yeast whose glycolytic pathway was shorter because of the presence of a new enzyme catalyzing the reaction:



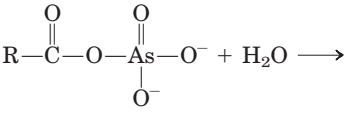
Would shortening the glycolytic pathway in this way benefit the cell? Explain.

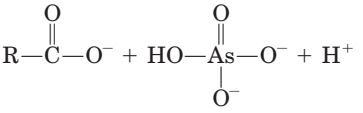
7. Role of Lactate Dehydrogenase During strenuous activity, the demand for ATP in muscle tissue is vastly increased. In rabbit leg muscle or turkey flight muscle, the ATP is produced almost exclusively by lactic acid fermentation. ATP is formed in the payoff phase of glycolysis by two reactions, promoted by phosphoglycerate kinase and pyruvate kinase. Suppose skeletal muscle were devoid of lactate dehydrogenase. Could it carry out strenuous physical activity; that is, could it generate ATP at a high rate by glycolysis? Explain.

8. Efficiency of ATP Production in Muscle The transformation of glucose to lactate in myocytes releases only about 7% of the free energy released when glucose is completely oxidized to CO_2 and H_2O. Does this mean that anaerobic glycolysis in muscle is a wasteful use of glucose? Explain.

9. Free-Energy Change for Triose Phosphate Oxidation The oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate, catalyzed by glyceraldehyde 3-phosphate dehydrogenase, proceeds with an unfavorable equilibrium constant ($K'_{eq} = 0.08$; $\Delta G'° = 6.3$ kJ/mol), yet the flow through this point in the glycolytic pathway proceeds smoothly. How does the cell overcome the unfavorable equilibrium?

10. Arsenate Poisoning Arsenate is structurally and chemically similar to inorganic phosphate (P_i), and many enzymes that require phosphate will also use arsenate. Organic compounds of arsenate are less stable than analogous phosphate compounds, however. For example, acyl *arsenates* decompose rapidly by hydrolysis:





On the other hand, acyl *phosphates,* such as 1,3-bisphosphoglycerate, are more stable and undergo further enzyme-catalyzed transformation in cells.

(a) Predict the effect on the net reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase if phosphate were replaced by arsenate.

(b) What would be the consequence to an organism if arsenate were substituted for phosphate? Arsenate is very toxic to most organisms. Explain why.

11. Requirement for Phosphate in Ethanol Fermentation In 1906 Harden and Young, in a series of classic studies on the fermentation of glucose to ethanol and CO_2 by extracts of brewer's yeast, made the following observations. (1) Inorganic phosphate was essential to fermentation; when the supply of phosphate was exhausted, fermentation ceased before all the glucose was used. (2) During fermentation under these conditions, ethanol, CO_2, and a hexose bisphosphate

accumulated. (3) When arsenate was substituted for phosphate, no hexose bisphosphate accumulated, but the fermentation proceeded until all the glucose was converted to ethanol and CO_2.

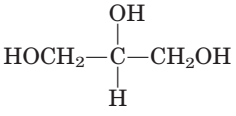
(a) Why did fermentation cease when the supply of phosphate was exhausted?

(b) Why did ethanol and CO_2 accumulate? Was the conversion of pyruvate to ethanol and CO_2 essential? Why? Identify the hexose bisphosphate that accumulated. Why did it accumulate?

(c) Why did the substitution of arsenate for phosphate prevent the accumulation of the hexose bisphosphate yet allow fermentation to ethanol and CO_2 to go to completion? (See Problem 10.)

12. Role of the Vitamin Niacin Adults engaged in strenuous physical activity require an intake of about 160 g of carbohydrate daily but only about 20 mg of niacin for optimal nutrition. Given the role of niacin in glycolysis, how do you explain the observation?

13. Metabolism of Glycerol Glycerol obtained from the breakdown of fat is metabolized by conversion to dihydroxyacetone phosphate, a glycolytic intermediate, in two enzyme-catalyzed reactions. Propose a reaction sequence for glycerol metabolism. On which known enzyme-catalyzed reactions is your proposal based? Write the net equation for the conversion of glycerol to pyruvate according to your scheme.



Glycerol

14. Severity of Clinical Symptoms Due to Enzyme Deficiency The clinical symptoms of two forms of galactosemia—deficiency of galactokinase or of UDP-glucose:galactose 1-phosphate uridylyltransferase—show radically different severity. Although both types produce gastric discomfort after milk ingestion, deficiency of the transferase also leads to liver, kidney, spleen, and brain dysfunction and eventual death. What products accumulate in the blood and tissues with each type of enzyme deficiency? Estimate the relative toxicities of these products from the above information.

15. Muscle Wasting in Starvation One consequence of starvation is a reduction in muscle mass. What happens to the muscle proteins?

16. Pathway of Atoms in Gluconeogenesis A liver extract capable of carrying out all the normal metabolic reactions of the liver is briefly incubated in separate experiments with the following ^{14}C-labeled precursors:

(a) [^{14}C]Bicarbonate, $HO-{}^{14}C(=O)O^-$

(b) [1-^{14}C]Pyruvate, $CH_3-C(=O)-{}^{14}COO^-$

Trace the pathway of each precursor through gluconeogenesis. Indicate the location of ^{14}C in all intermediates and in the product, glucose.

17. Pathway of CO_2 in Gluconeogenesis In the first bypass step of gluconeogenesis, the conversion of pyruvate to phosphoenolpyruvate, pyruvate is carboxylated by pyruvate carboxylase to oxaloacetate, which is subsequently decarboxylated by PEP carboxykinase to yield phosphoenolpyruvate. The observation that the addition of CO_2 is directly followed by the loss of CO_2 suggests that ^{14}C of $^{14}CO_2$ would not be incorporated into PEP, glucose, or any intermediates in gluconeogenesis. However, when a rat liver preparation synthesizes glucose in the presence of $^{14}CO_2$, ^{14}C slowly appears in PEP and eventually at C-3 and C-4 of glucose. How does the ^{14}C label get into PEP and glucose? (Hint: During gluconeogenesis in the presence of $^{14}CO_2$, several of the four-carbon citric acid cycle intermediates also become labeled.)

18. Energy Cost of a Cycle of Glycolysis and Gluconeogenesis What is the cost (in ATP equivalents) of transforming glucose to pyruvate via glycolysis and back again to glucose via gluconeogenesis?

19. Glucogenic Substrates A common procedure for determining the effectiveness of compounds as precursors of glucose in mammals is to starve the animal until the liver glycogen stores are depleted and then administer the compound in question. A substrate that leads to a *net* increase in liver glycogen is termed glucogenic, because it must first be converted to glucose 6-phosphate. Show by means of known enzymatic reactions which of the following substances are glucogenic:

(a) Succinate, $^-OOC-CH_2-CH_2-COO^-$

(b) Glycerol, $CH_2(OH)-C(H)(OH)-CH_2(OH)$

(c) Acetyl-CoA, $CH_3-C(=O)-S\text{-}CoA$

(d) Pyruvate, $CH_3-C(=O)-COO^-$

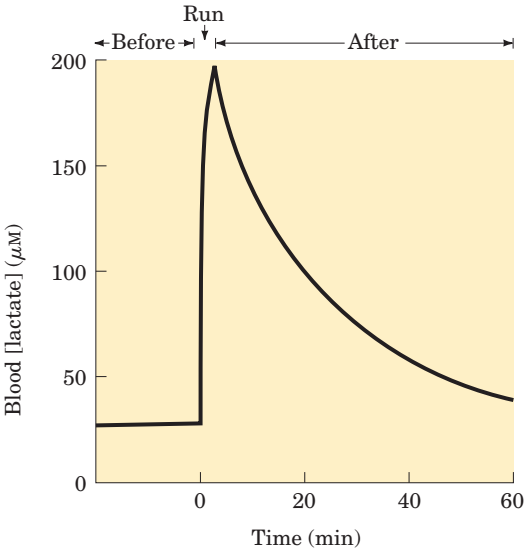
(e) Butyrate, $CH_3-CH_2-CH_2-COO^-$

20. Ethanol Affects Blood Glucose Levels The consumption of alcohol (ethanol), especially after periods of strenuous activity or after not eating for several hours, results in a deficiency of glucose in the blood, a condition known as hypoglycemia. The first step in the metabolism of ethanol by the liver is oxidation to acetaldehyde, catalyzed by liver alcohol dehydrogenase:

$$CH_3CH_2OH + NAD^+ \longrightarrow CH_3CHO + NADH + H^+$$

Explain how this reaction inhibits the transformation of lactate to pyruvate. Why does this lead to hypoglycemia?

21. Blood Lactate Levels during Vigorous Exercise The concentrations of lactate in blood plasma before, during, and after a 400 m sprint are shown in the graph.



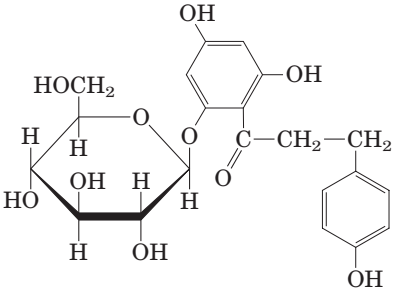
(a) What causes the rapid rise in lactate concentration?

(b) What causes the decline in lactate concentration after completion of the sprint? Why does the decline occur more slowly than the increase?

(c) Why is the concentration of lactate not zero during the resting state?

22. Relationship between Fructose 1,6-Bisphosphatase and Blood Lactate Levels A congenital defect in the liver enzyme fructose 1,6-bisphosphatase results in abnormally high levels of lactate in the blood plasma. Explain.

23. Effect of Phloridzin on Carbohydrate Metabolism Phloridzin, a toxic glycoside from the bark of the pear tree, blocks the normal reabsorption of glucose from the kidney tubule, thus causing blood glucose to be almost completely excreted in the urine. In an experiment, rats fed phloridzin and sodium succinate excreted about 0.5 mol of glucose (made by gluconeogenesis) for every 1 mol of sodium succinate ingested. How is the succinate transformed to glucose? Explain the stoichiometry.



Phloridzin

24. Excess O_2 Uptake during Gluconeogenesis Lactate absorbed by the liver is converted to glucose, with the input of 6 mol of ATP for every mole of glucose produced. The extent of this process in a rat liver preparation can be monitored by administering [^{14}C]lactate and measuring the amount of [^{14}C]glucose produced. Because the stoichiometry of O_2 consumption and ATP production is known (about 5 ATP per O_2), we can predict the extra O_2 consumption above the normal rate when a given amount of lactate is administered. However, when the extra O_2 used in the synthesis of glucose from lactate is actually measured, it is always higher than predicted by known stoichiometric relationships. Suggest a possible explanation for this observation.