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PROTECTIVE MECHANISMS AGAINST REACTIVE OXYGEN SPECIES

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This chapter focuses on mechanisms whereby the liver is protected against reactive oxygen species. Hepatic oxygen metabolism and generation of reactive oxygen species in each cell type in the liver are discussed in website chapter W-13 and in Chapter 18.

METABOLISM OF ANTIOXIDANTS IN THE LIVER

The liver is the major organ for removal of intestinally derived bacteria and toxic compounds, such as endotoxin, oxidants, and pro-oxidants. To detoxify these hazardous compounds, the liver contains high levels of low molecular weight antioxidants and enzymes that degrade reactive oxygen species. Reduced glutathione (GSH), vitamin C, vitamin E, superoxide dismutase (SOD), glutathione peroxidase, and catalase are examples (Table 19.1).

DYNAMIC ASPECTS OF GLUTATHIONE METABOLISM

Glutathione is a naturally occurring major thiol and is synthesized in cytosol in its reduced form. The thiol functions as a multipotential metabolite (1–3). GSH is translocated from cytosol to mitochondria (4) and nucleus (5), and serves as an antioxidant reducer and for detoxifying electrophilic compounds. Since enzymes that hydrolyze GSH, oxidized glutathione (GSSG), and glutathione S-conjugates are localized extracellularly, these tripeptides are translocated out of cells prior to degradation. In rodents, the liver is the major organ that excretes GSH into the circulation and bile (6,7). The liver also excretes GSSG and glutathione S-conjugates preferentially in bile (8). GSH is the major form of glutathione in bile. If fresh bile samples are exposed to air, GSH is oxidized rapidly by a mechanism that is inhibited by ethylenediaminetetraacetic acid (EDTA). GSH is excreted from hepatocytes bidirectionally in plasma and bile. In contrast to bidirectional secretion of GSH, GSSG and glutathione S-conjugates are preferentially excreted in the bile (8,9) (see Chapter 25 and website chapter W-15). However, glutathione S-conjugates formed in erythrocytes and extrahepatic tissues are also excreted into the circulation. Secretory transport of these tripeptides occurs by a carrier-mediated mechanism (10–13).

Studies in hyperbilirubinemic mutant rats [transport defect (TR^-) or Eisaihyperbilirubinemic rat (EHBR)] revealed that biliary secretion of glutathione is inhibited in these animals. They lack the ability to excrete bilirubin and non–bile acid organic anions into bile (13–16). Biliary secretion of this tripeptide occurs predominantly via an adenosine triphosphate (ATP)-dependent mechanism (see Chapters 24 and 25).

Glutathione and its S-conjugates that are excreted in the bile are degraded to constituent amino acids by γ-glutamyltransferase (GGT) and peptidases localized on the luminal surface of biliary cells (17) (see Chapter 24) and epithelial cells of the pancreas and small intestine. The extent of biliary degradation of the tripeptides differs from one species to another depending on the levels of GGT in biliary cells and pancreatic juice. In the rat, about 50% of biliary glutathione is degraded in bile. In contrast to rodents, hepatic GGT activity is extremely high in humans, rabbits, and sheep. Most of the biliary tripeptide in these species is degraded within the biliary tract. Thus,

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TABLE 19.1. ANTIOXIDANTS AND SCAVENGING ENZYMES

Antioxidants	
Glutathione	Major antioxidant in intra- and extracellular compartment (hydrophilic), cellular level: 2–10 mM, levels in arterial plasma: 5–25 μM
Other thiols	Cysteine (significantly lower than GSH)
Vitamin C	Hydrophilic antioxidant in extracellular fluid and cytosol (40–140 μM in plasma) cooperates with vitamin E
Uric acid	Final metabolite of adenosine and xanthine; strong antioxidant (for HO•); 2.6–7.5 mg/dL plasma (0.12–0.45 mM)
Bilirubin	Hydrophobic antioxidant (20 μM); circulates bound to albumin
Vitamin E	Scavenges in hydrophobic compartment; 0.5–1.6 mg/dL plasma (10–40 μM); circulates bound to LDL
β-Carotene	0.055 mg/dL serum
Coenzyme Q10	0.08 mg/dL plasma
Scavenging enzymes	
SOD	Present ubiquitously in all mammalian cells
Cu/Zn-SOD	Cytosol, erythrocytes (2,300 units/g Hb)
Mn-SOD	Mitochondria
Extracellular SOD (EC-SOD)	Plasma and endothelia cell surface, binds to heparin (low specific activity)
Catalase	Peroxisome, RBC (153,000 units/g Hb)
GSH peroxidase	Cytosol (75%), mitochondria (25%), (serenocysteine) RBC (31 units/g Hb) Se-independent (glutathione-S-transferase)
GSSG reductase	NADPH-dependent reduction of GSSG
Thioredoxin system	Redox regulation
Binding Proteins	
Albumin	Strong antioxidant (0.5 mM in plasma); mercapto- and nonmercaptalbumin
Ceruloplasmin	Protection by feroxidase activity; 15–60 mg/dL plasma (1–4 μM)
Transferin	Chelate free iron (200–400 mg/dL, 25–50 μM)
Metalothionein	Chelate heavy metals

[a]Values show the level and activity in humans.
GSH, reduced glutathione; GSSG, oxidized glutathione; HO•, hydroxyl radical; LDL, low-density lipoprotein; RBC, red blood cell; SOD, superoxide dismutase.

cyst(e)ine and other constituent amino acids are the major metabolites in the bile of these animals. In contrast, both glutathione and cyst(e)ine enter the small intestine of rodents. Since GGT is highly enriched in the brush border of intestinal epithelial cells, the remaining glutathione and related peptides are hydrolyzed completely within the lumen. The constituent amino acids are absorbed by active transport systems in the small intestine, transferred to the liver, absorbed by hepatocytes, and used again for GSH synthesis. Thus, the metabolism of biliary glutathione and related metabolites occurs via intrahepatic and enterohepatic cycles (Fig. 19.1).

GSH and its S-conjugates that appear in plasma are degraded in tissues that have GGT. In rodents, the kidney is the major organ that extracts GSH and related peptides in the circulation (18). Since renal GGT is localized on the outer surface of both apical and basolateral membranes of proximal tubule cells, glutathione and related metabolites are degraded both in luminal and contraluminal space of the renal tubules. Since 20% to 30% of low molecular weight compounds in renal arterial plasma is filtered by a single pass through the glomerulus, glutathione and its S-conjugates are degraded by brush border membranes of proximal tubules. The remaining fractions (70% to 80%) are degraded by peritubular transferase activity. Thus, GSH and its metabolites in the circulation are metabolized predominantly by a hepatorenal cycle (Fig. 19.1). Hepatic GGT activity is less than a few percent of that in the kidney of rodents. In contrast, GGT activity in human liver is as high as in the kidney. Since GGT levels in the liver and other tissues differ from one species to another, the quantitative aspects of GSH cycles may also differ depending on the enzyme activity in tissues. GSH levels in human arterial and hepatic venous plasma are significantly lower (about one-tenth) than those of rodents, which may result from higher activity of hepatic GGT in humans. Hepatic GGT is low in rodents and is localized exclusively on the luminal surface of bile canalicular membranes and biliary cells. However, high activity of rat kidney GGT is localized on the outer surface of both luminal and contraluminal membranes of proximal tubule cells. Although the presence of GGT in bile and on luminal membranes of human liver is well established, direct evidence for absence of the enzyme in sinusoidal plasma membranes is lacking. If hepatic GGT is also localized on the outer surface of contraluminal membranes of hepatocytes, GSH excreted by hepatocytes into the space of Disse may be degraded to constituent amino acids. Since the inferior vena cava in humans contains more circulating glutathione than does the artery, the intestine may possibly be the source for plasma glutathione in humans. Species-specific differences in the interorgan metabolism and transport of GSH exist.

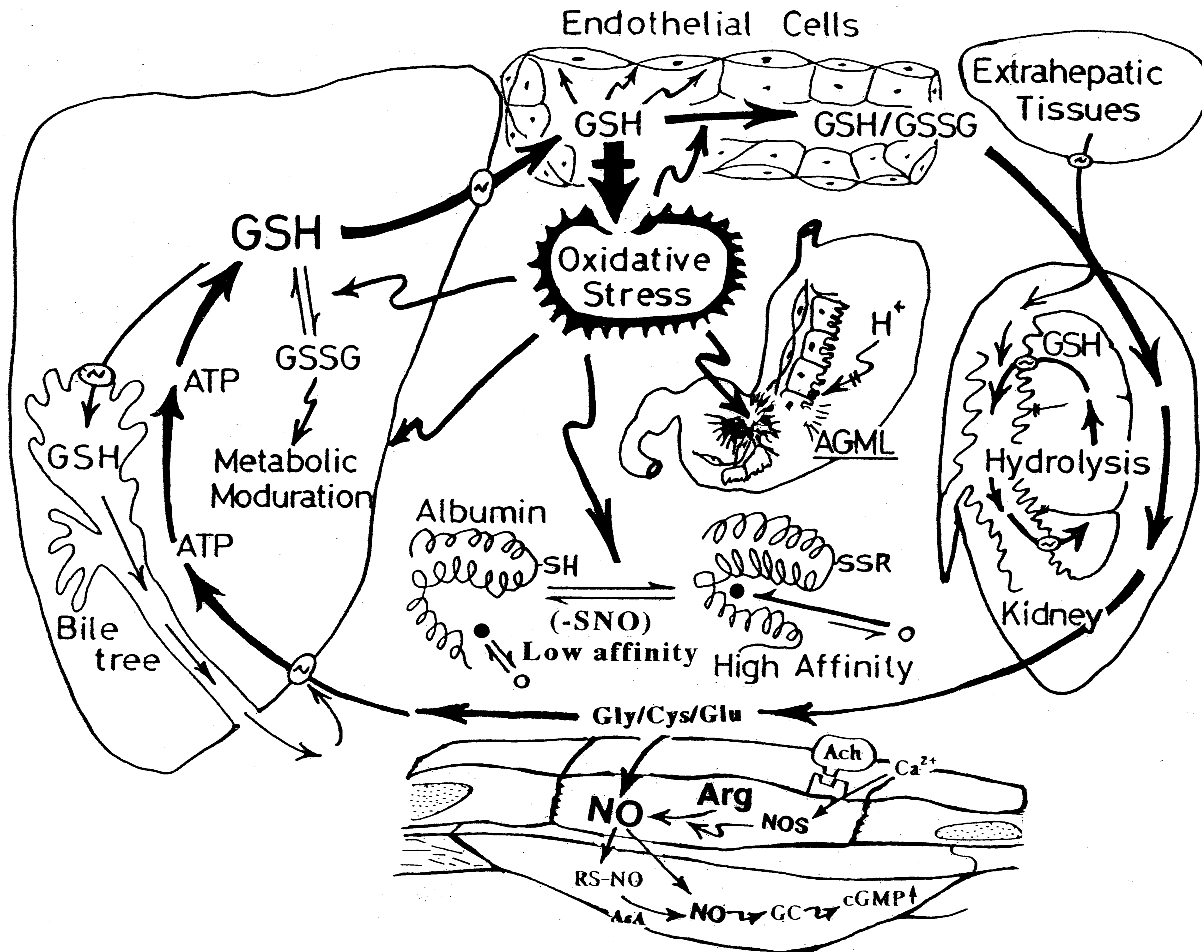

FIGURE 19.1. Interorgan metabolism of reduced glutathione (*GSH*). Glutathione is synthesized exclusively intracellularly in its reduced form (GSH). GSH is secreted by tissues, such as the liver, into the circulation and luminal compartments of epithelial tissues by some adenosine triphosphate (*ATP*)-dependent mechanism. Extracellular GSH and its S-conjugates are hydrolyzed on the outer surface of cells that have γ-glutamyltransferase (*GGT*) activity, such as the kidney and small intestine. The constituent amino acids, such as cysteine, are transported by cells and used for various metabolisms including GSH synthesis. Thus, GSH and its metabolites are handled via inter- and intraorgan cooperation in which liver, kidney, and small intestine play central roles. Both GSH and cysteine function as antioxidants, decrease oxidative stress, and regulate the redox status of various molecules. The ligand-binding activity of albumin is affected by these thiols or by nitric oxide (*NO*) through formation of mixed disulfides or a nitrosothiol at the cys35 residue; nonmercaptalbumin shows higher affinity for hydrophobic anions than does mercaptalbumin (17) while GS-NO shows lowest affinity for the ligands. *AGML*, acute gastric mucosal lesion; *EC*, vascular endothelial cells; *Ach*, acetylcholine; *NOS*, NO synthase; *Arg*, arginine; *RS-NO*, nitrosothiols; *GC*, guanylate cyclase; *AsA*, ascorbic acid.

Other Antioxidants with Low Molecular Weight

In addition to GSH, the liver also contains other antioxidants with low molecular weight such as vitamin C, vitamin E, ubiquinol, carotenoids, and bilirubin. They function in different subcellular compartments depending on their hydrophilic or hydrophobic nature. GSH and vitamin C are potent scavengers of reactive oxygen species, particularly in plasma, cytosol, and other aqueous compartments. In contrast, vitamin E and other hydrophobic antioxidants function predominantly in and around membrane/lipid bilayers.

There is a cooperative mechanism by which free radicals formed in hydrophobic domains are trapped by hydrophilic scavengers in aqueous compartments. For example, vitamin C reacts with vitamin E radicals, thereby regenerating vitamin E. Vitamin C radicals formed in soluble fractions are regenerated to vitamin C by monodehydroascorbate reductase or by spontaneous dismutation. Such synergistic action with lipophilic vitamin E is also seen with other hydrophilic antioxidants, such as cysteine. Thus, in conjunction with vitamin C, cysteine, or other hydrophilic antioxidants, vitamin E effectively scavenges free radicals occurring within lipoproteins and membrane/lipid bilayers of the liver and

other tissues (Fig. 19.2). Current topics about vitamin E are described elsewhere (19).

Although the α-form of tocopherol is about ten times more abundant in plants than is its γ-form, only the former is effectively accumulated in the liver. Studies by Arai's group (20) revealed the presence of hepatic-binding protein specific for α-tocopherol. They isolated α-tocopherol transfer protein (α-TTP) from rat liver cytosol, which specifically binds vitamin E and enhances its transfer between membranes. α-TTP also localizes in human liver. The complementary DNA (cDNA) for human α-TTP isolated from a human liver cDNA library predicts 278 amino acids with a molecular mass of 31,749, and the sequence exhibits 94% similarity with rat α-TTP at the amino acid level. The recombinant human α-TTP expressed in *Escherichia coli* exhibits both α-tocopherol transfer activity and cross-reactivity to the anti-(rat α-TTP) monoclonal antibody. Northern blot analysis revealed that human α-TTP is also expressed in the liver. The human and rat α-TTPs show structural similarity with other apparently unrelated lipid-binding/transfer proteins, i.e., retinaldehyde-binding protein present in retina, and yeast SEC14 protein, which possesses phosphatidylinositol-phosphatidylcholine transfer activity. *In situ* hybridization analysis revealed a single α-TTP gene corresponding to the 8q13.1-13.3 region of chromosome 8, which is identical to the locus of a recently described clinical disorder, ataxia with selective vitamin E deficiency.

Patients with isolated vitamin E deficiency have an impaired ability to incorporate α-tocopherol into lipoproteins in the liver and usually have symptoms and signs of spinocerebellar dysfunction before adolescence. The three frame-shift mutations in α-TTP gene were identified. A 744delA mutation accounts for 68% of the mutant alleles

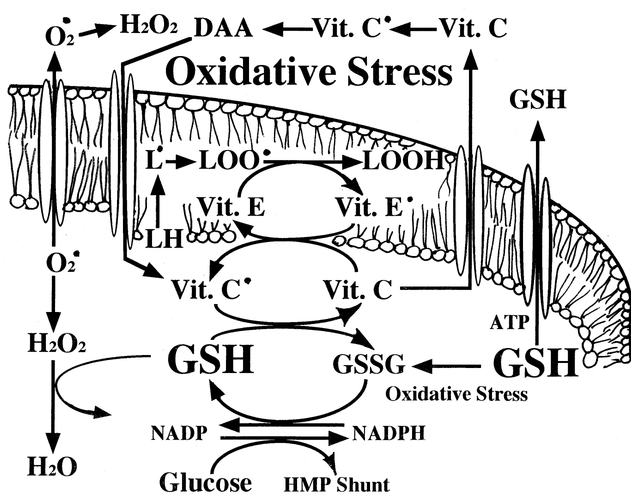

FIGURE 19.2. Radical chain reactions and antioxidant network in membranes and aqueous compartments. *$O_2^\bullet$*, superoxide radical; *LH*, lipids; *L•*, lipid radicals; *LOO•*, peroxy radical; *Vit.E*, vitamin E; *Vit.E•*, vitamin E radical; *Vit.C*, vitamin C; *Vit.C•*, vitamin C radical; *DAA*, dehydroascorbic acid.

in the 17 families analyzed and appears to have spread in North Africa and Italy. This mutation correlates with a severe phenotype but alters only the C-terminal tenth of the protein. Two other mutations were found in single families. Accumulated evidence suggests that α-TTP is abnormal in these patients. Arai's group (21) studied a patient from an isolated Japanese island who began to have ataxia, dysarthria, and sensory disturbances in the sixth decade of life. The vitamin E concentration in the serum of the patient was low (1.2 mg/mL). Exons of his gene for the α-TTP were analyzed by DNA sequencing. The patient was homozygous for a point mutation that replaces histidine (CAT) with glutamine (CAG) at position 101 of the gene for α-TTP. When expressed in COS-7 cells, the missense mutation produced a functionally defective α-TTP with approximately 11% of the transfer activity of the wild-type protein. Of the 801 island inhabitants examined, 21 were heterozygous for the His101Gln mutation. In all affected subjects, including the patient, this mutation co-segregated with an intron-sequence polymorphism. Heterozygotes were phenotypically normal and had serum vitamin E concentrations that were on average 25% lower than those of normal subjects. Thus, α-TTP is a determinant of serum vitamin E concentrations and an abnormality in this protein causes spinocerebellar dysfunction. The finding of α-TTP gene mutations in the patients substantiates the therapeutic role of vitamin E as a protective agent against neurologic damage in this disease.

Since vitamin C is highly water soluble, this antioxidant functions primarily in cytosol and extracellular fluid. In fact, when plasma was incubated in the presence of radical generating agents, plasma levels of ascorbic acid decreased rapidly (22). Studies using a cytochrome *c* derivative that circulates bound to albumin and, thus, has a prolonged half-life *in vivo*, revealed that significant amounts of ascorbyl radicals are generated in the circulation of normal animals (23,24). These antioxidants function in different subcellular compartments depending on their hydrophilic or hydrophobic nature. There is a cooperative mechanism by which free radicals formed in hydrophobic domains are trapped by hydrophilic scavengers in aqueous compartments (25,26) (Fig. 19.2) (see Chapter 18). The antioxidant activity of bilirubin was initially reported by Bernhard et al. (27). This pigment is as potent as vitamin E in inhibiting the ultraviolet (UV)-induced peroxidation of linoleic acid (28). Bilirubin also inhibits autooxidation of polyunsaturated fatty acids (29). Since hepatocytes have transport systems for bilirubin (see Chapters 20 and 25 and website chapter 💻 W-16), the circulating bilirubin rapidly undergoes transhepatic transport from plasma to bile. Hence, bilirubin may function as a potent scavenger for reactive oxygen species in plasma, hepatocytes, bile, and the small intestinal lumen. Bernhard et al. suggested that bilirubin may enhance the intestinal absorption of vitamin A and linoleic acid by inhibiting the oxidation of these molecules.

When oxidized, bilirubin is converted to stable and water-soluble biliverdin, and excreted in bile without conjugation by glucuronic acid. In the presence of singlet oxygen, bilirubin is degraded to dioxethane and endoperoxide (29). The former is degraded to methylvinylmaleimide and imide dipyrole dialdehyde, whereas the latter is converted to hematinic acid and propentdyopent (30). The rate of bilirubin degradation is enhanced in patients with sepsis or asphyxia. Owing to their relative hydrophobic properties, both conjugated and unconjugated bilirubin circulate, but predominantly the latter bind to albumin. Small amounts of bilirubin also circulate bound to lipoproteins particularly in patients with hypo- and analbuminemia. Thus, plasma bilirubin may function as an antioxidant in the binding sites of albumin and lipoproteins and regions of membrane/lipid bilayers.

Stellate cells (Ito cells or fat-storing cells) contain significantly large amounts of fat and carotenoids; more than 90% of total vitamin A is localized in these cells (see website chapter 💻 W-20). Although the antioxidant properties of carotenoids have been well documented, the role for stellate cells and retinoids in the metabolism of reactive oxygen species in and around hepatic sinusoids remains to be elucidated.

ANTIOXIDANT ENZYMES

After scavenging reactive oxygen species, some metabolites of low molecular weight antioxidants, such as GSSG and vitamin C and E radicals, are regenerated enzymatically or nonenzymatically, whereas other metabolites of sacrificial scavengers, including bilirubin and uric acid, undergo irreversible degradation. Enzymatic regeneration of the oxidized scavengers may occur principally intracellularly at the expense of GSH, reduced nicotinamide adenine dinucleotide (NADH), or reduced nicotinamide adenine dinucleotide phosphate(NADPH). Under physiologic conditions, the regenerating systems for antioxidants may function sufficiently to maintain their steady-state levels. Since the liver is the main filtering organ for hazardous xenobiotics, high levels of enzymes that metabolize reactive oxygen species (e.g., SOD, catalase, and glutathione peroxidase) are present in the liver (Table 19.1).

Mammalian tissues and cells have three types of SOD isozymes: the first (Cu/Zn-SOD), the homodimer, localizes in cytosol and contains 1 mol of Cu and Zn per mole of monomeric subunit; the second (Mn-SOD), homotetramer, localizes in mitochondrial matrix and contains 1 mol of Mn per subunit; the third (extracellular SOD, EC-SOD), homotetramer, contains 1 mol of Cu and Zn per mole of monomeric subunit and localizes bound to cell surface matrix via its heparin-binding domain. Both Cu/Zn-SOD and Mn-SOD effectively dismutate superoxide radicals and generate hydrogen peroxides by a diffusion-limited mechanism, whereas the specific activity of EC-SOD is significantly lower than that of other isozymes. The physicochemical properties and catalytic mechanism of Cu/Zn-SOD are well understood (31). Crystallographic analysis of Cu/Zn-SOD suggests that high specific activity of the enzyme (3,000 units/mg) is due, at least in part, to a positively charged lysyl cluster near the active site that drives substrate superoxide anion electrostatically to the Cu-containing catalytic site (32). The specific activity of the enzyme purified from erythrocytes of diabetic patients was significantly lower than that from normal human subjects. Protein chemical analysis suggested that low specific activity of the enzyme from diabetic patients is due to nonenzymatic glycation of lysine residues (Lys^{3}, Lys^{9}, Lys^{122}, and Lys^{128}). This observation supports the hypothesis that the positively charged lysyl cluster plays an important role in maintenance of its high catalytic activity. Since mitochondria are highly enriched in Mn-SOD, this enzyme may be the principal one in the dismutation of superoxide radicals within the mitochondrial matrix. Cu/Zn-SOD has been postulated to localize predominantly in cytosol; however, the enzyme also localizes bound to the cytoplasmic surface of mitochondria and peroxisomes. Thus, this isozyme can dismutate superoxide radicals very effectively at the outer surface of these organelles. Such subcellular localization of Cu/Zn-SOD may be important to minimize free radical injury caused by superoxide-dependent reactions in cytosol and nucleus. In this context, mutation of Cu/Zn-SOD has recently been found in patients with familiar amyotrophic lateral sclerosis (32). Interestingly, the affinity of mutant r-SOD to mitochondria and peroxisomes decreased significantly. Thus, changes in subcellular localization of this isozyme may play an important role in the pathogenesis of familiar amyotrophic lateral sclerosis.

Under physiologic conditions, the half-life of the superoxide radical is about 5 seconds. Hence, in compartments with low SOD activity, the superoxide radical is also dismutated nonenzymatically to hydrogen peroxide. Although EC-SOD is localized in plasma and on the vascular endothelial cell surface, total activity of the enzyme in extracellular compartments is low. Thus, nonenzymatic dismutation of superoxide radicals may be important in extracellular compartments with low SOD activity. In fact, some animals, such as rodents, lack this isozyme.

Hydrogen peroxide is relatively stable when compared with other reactive oxygen species, such as superoxide and hydroxyl radicals. Thus, hydrogen peroxide may be more toxic to cultured cells than is superoxide radical (33). However, this need not be the case *in vivo*. Hydrogen peroxide derived from superoxide radicals is further metabolized to water by glutathione peroxidase and catalase. There are two isozymes of glutathione peroxidase inside cells: one is selenium-dependent and the other selenium-independent (34). Both isozymes are highly enriched in human liver. The relative ratio of Se-dependent to -independent enzyme in rat

liver is about 2. The Se-independent type of the enzyme is identical with one of the isozyme of glutathione S-transferase (GST 1-1 or YaYa), which catalyzes the conjugation of epoxides and other electrophils with GSH (35). Glutathione S-transferases are a group of isozymes with overlapping specificity whose characteristics have been detailed elsewhere (see website chapters W-14 and W-15). The Se-dependent enzyme of the liver is a homotetramer that is synthesized in the cytosol. The amino acid residue at position 45 is catalytically active selenocysteine. Although a major fraction of the enzyme (75%) is localized in the cytoplasmic compartment of hepatocytes, the remainder enters into mitochondria. Hence, conversion of hydrogen peroxide to water occurs in cytosolic and mitochondrial compartments. The substrate is not only hydrogen peroxide but organic peroxides (ROOH) of a lipophylic nature also serve. Selenium deficiency is often associated with cardiomyopathy, liver necrosis, and renal injury. It has been postulated that Se deficiency is the cause of Keshan disease (36). The content of Se in the soil of the Keshan area in China is extremely low, resulting in low Se content in the diet of people living in this area. Patients with Se deficiency show lethal cardiomyopathy. Administration of Se markedly improves the clinical signs of Keshan disease, which suggests that oxidative stress caused by Se-deficiency may underlie the pathogenesis of this disease.

GSH and its redox cycle also play critical roles in catabolizing hydrogen peroxide and other peroxides. In fact, cytotoxicity of hydrogen peroxide increases with low cellular levels of GSH (37). Furthermore, treatment of hepatocytes with L-buthionine sulfoximine, a specific inhibitor of GSH synthase, or 1,3-bis(chloroethyl)-1-nitrosourea, an inhibitor of glutathione reductase, potentiates the cytotoxic effect of hydrogen peroxide.

Since catalase in the liver is localized predominantly in peroxisomes, this enzyme might be the most significant one for degradation of hydrogen peroxide generated in this organelle (see Chapter 18). Hydrogen peroxide is amphipathic and easily penetrates across membrane/lipid bilayers (38). Injury of endothelial cells and hepatocytes caused by hydrogen peroxide was inhibited by erythrocytes, which have high activity of catalase (153,000 units/g Hb). Rao et al. (39) reported that erythrocytes play an important role in preventing cold-storage–induced endothelial cell injury of the liver. Since the protective effect of erythrocytes against hydrogen peroxide-induced cell injury disappeared after treating cells with aminotriazole, an inhibitor of catalase, extracellular hydrogen peroxide can be degraded by intracellular enzyme (40). Hepatic catalase may also function in degrading hydrogen peroxide generated in extraperoxisomal compartments of the liver. The Michaelis' constant (K_m) value of catalase for hydrogen peroxide is much higher than that of glutathione peroxidase. Hence, glutathione peroxidase has been postulated to degrade low levels of hydrogen peroxide physiologically, while catalase might function when cellular levels of hydrogen peroxide are increased. Consistent with this view are the findings in acatalasemic patients who do not show lethal signs, except for oral gangrene. In Japanese patients with acatalasemia, the defect is inherited as an autosomal-recessive trait (41). Although catalase activity in the heart is only 2% of that in the liver, the enzyme is localized predominantly in peroxisomes and degrades hydrogen peroxide four times more efficiently than does glutathione peroxidase. Cellular levels of catalase and glutathione peroxidase differ from one species to another and show negative correlation; erythrocytes with high glutathione peroxidase activity show low catalase activity, whereas those with low peroxidase activity have high catalase activity. The two enzymes, therefore, appear to compensate for each other in scavenging hydrogen peroxide in intra- and extracellular compartments.

Although reactive oxygen species occur both intracellularly and extracellularly, levels of antioxidants and related enzymes in the former compartments are much higher than those in the latter. Why the metabolism of reactive oxygen species and their scavengers is organized differently in the two compartments is unknown. Oxidative stress, the hepatic circulation, ischemia, and reperfusion-induced liver injury are discussed in website chapter W-13 and Chapter 18.

The blood volume circulating through digestive organs comprises about 25% of the cardiac output. Exchange of fluid and solutes between sinusoidal plasma and hepatocytes may be affected by the circulatory status of the liver and metabolic conditions of sinusoidal lining cells. Contraction of sinusoidal endothelial cells and fat-storing Ito cells may decrease the vascular bed in the liver, which could decrease the time and space for interaction of hepatocytes with components in sinusoidal plasma. The hydrostatic pressure of the portal blood is relatively low and, hence, decreases the shear stress for sinusoidal endothelial cells. Presumably because of the circulatory status in the liver, hepatic endothelial cells are able to maintain fenestrae (see Chapter 30 and website chapter W-26). Since the size of endothelial fenestrae (about 0.1 um in diameter) is significantly larger than the Stokes' radius of albumin (about 0.014 um) and other plasma proteins, metabolites in sinusoidal plasma interact freely with plasma membranes of hepatocytes. However, sinusoidal endothelial cells and the space of Disse are highly enriched with proteoglycans, such as heparan sulfates. Hence, the functional size of endothelial fenestrae may be considerably smaller than the size observed morphologically. In fact, the concentration of albumin and globulin in hepatic lymph is significantly lower than in sinusoidal plasma, and the ratio of the former to the latter protein in venous plasma (0.7) is lower than in hepatic lymph (0.82). These observations suggest that the transfer of circulating albumin and other plasma proteins to the space of Disse (and to hepatic lymph) may be limited by the sieve plates, particularly when endothelial cells or fenestrae are contracted. Although serotonin induces nitric

oxide (NO)-dependent vascular relaxation, it also promotes contraction of endothelial fenestrae (42,43). Serotonin increased portal pressure of an isolated perfused liver of the rat and decreased uptake for albumin-bound cholephilic ligands, such as sulfobromophthalein (44). Thus, endothelial cells and their sieve plates may regulate the extent of interaction of constituents in portal plasma and hepatocytes. In this context, xanthine oxidase induced contraction of fenestrae of cultured hepatic endothelial cells by an SOD-inhibitable mechanism (44). Thus, superoxide or its metabolites could induce contraction of sinusoidal endothelial cells and their fenestrae.

The superoxide radical interacts with NO [endothelium-derived relaxing factor (EDRF)], thereby causing vascular contraction. EDRF is inactivated by superoxide, suggesting that reactive oxygen species may play an important role in regulating the circulatory status of tissues (see website chapter 🖳 W-32). Although the activities of sinusoidal endothelial cells and Kupffer cells of normal rats to generate reactive oxygen species and NO are relatively low, they increase significantly after treating animals with bacteria (*Propionibacterium acnes*) and endotoxin (45). Thus, such reactive oxygen species as superoxide and NO could also play important roles in the regulation of sinusoidal circulation and hepatic transport across sinusoidal lining cells.

Because NO has high affinity for hemoproteins, it reacts with not only guanylate cyclase but also with other heme proteins, such as mitochondrial electron transfer complexes (see Chapter 39). Thus, the state-3 (ATP-synthesizing state) respiration of mitochondria is inhibited reversibly by NO; cytochrome *c* oxidase (complex IV) is the major site for inhibition (46). The inhibitory effect and EDRF function of NO are enhanced by physiologically low oxygen tensions (47). Thus, cellular production of ATP is regulated pivotally by the coordination of vascular and mitochondrial actions of NO depending on the local concentration of molecular oxygen (48). NO also inhibits ATP synthesis and growth of *E. coli* in an oxygen concentration-dependent manner (49). Thus, a cross-talk of molecular oxygen, superoxide, and NO constitutes a supersystem for the regulation of the circulatory status and energy metabolism and plays a critical role in the defense mechanism against bacterial infection (Fig. 19.3).

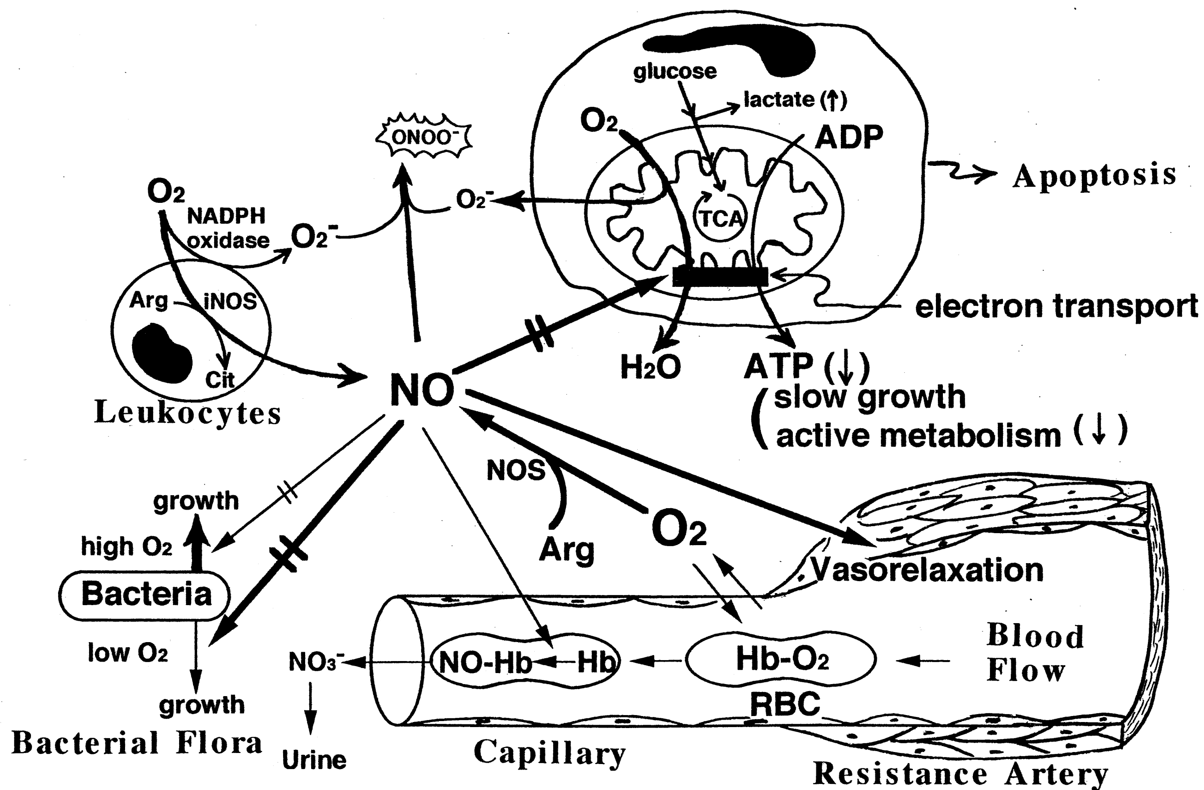

FIGURE 19.3. Supersystem driven by a cross-talk of nitric oxide (*NO*) and oxyradicals regulates circulation, adenosine triphosphate (*ATP*) synthesis, and bacterial growth. NO increases oxygen supply to peripheral tissues by decreasing arterial resistance while it inhibits ATP generation by mitochondria. Biologic activities of NO are enhanced by decreasing local oxygen tensions. Cross-talk of NO, superoxide, and molecular oxygen regulates the circulatory status, mitochondrial ATP generation, and the growth of bacteria. *ADP*, adenosine diphosphate.

ISCHEMIA AND REPERFUSION-INDUCED LIVER INJURY (SEE ALSO CHAPTER 18)

Although liver transplantation is a potential therapy for patients with severe liver injury, a second transplantation of the liver is often required predominantly because of primary nonfunction of a graft. Transient ischemia followed by reperfusion of a tissue also occurs during radical surgical resection. Pathologic metabolites generated during ischemia or reperfusion perturb the circulatory status leading to endothelial cell injury. These changes may underlie the pathogenesis of hepatocellular injury by cold-preservation or postischemic reperfusion of the liver. Kawamoto et al. (50,51) reported that transient occlusion followed by reperfusion of hepatic vessels markedly increased xanthine oxidase activity in plasma of systemic and portal blood. Under such conditions, hepatic circulatory status and transport function for albumin-associated cholephilic ligands were significantly impaired by a mechanism that was inhibited by a long-acting and site-directed SOD derivative that circulates bound to albumin (52,53). Thus, vascular endothelial cells can be impaired by superoxide radicals or related metabolites that are formed during reperfusion (54). Because the mitochondrial electron transport system in ischemic tissues is fully saturated with electrons, molecular oxygen in reperfused blood may undergo one electron reduction by reacting with the released electron to generate superoxide radicals. Since endothelial cells are enriched in xanthine oxidase, the enzyme is also a candidate for generation of superoxide radicals in reperfused liver. Kupffer cells also generate reactive oxygen species particularly when they are primed by endotoxin and various cytokines (see Chapter 30 and 31 and website chapter W-26). Thus, cold storage followed by reperfusion of the liver may potentiate the activity of Kupffer cells to generate reactive oxygen species.

Vascular endothelial cells may be injured by reactive oxygen species generated by xanthine oxidase enriched in these cells and/or NADPH-oxidase of leukocytes. Superoxide radicals enhance the adhesion of leukocytes to vascular endothelial cells. Ischemia and reperfusion enhance adhesion of circulating neutrophils to sinusoidal endothelial cells, thereby perturbing the microcirculation in the liver. Hence, activation of leukocytes may also underlie the pathogenesis of reperfusion injury of the liver. Reperfusion injury of the liver and other organs was significantly inhibited by a long-acting and site-directed SOD (50–58). Administration of this SOD derivative markedly inhibited liver injury caused by warm ischemia that followed reperfusion of the graft and improved the survival (59).

Inoue et al. (59–61) developed a fusion gene encoding human Cu/Zn-SOD and a C-terminal heparin-binding domain that has a high affinity for heparan sulfates. When injected intravenously, the fusion SOD binds to heparan sulfates on vascular endothelial cells and hepatic sinusoidal lining cells. The heparin-binding fusion SOD markedly inhibited liver injury caused by ischemia and reperfusion, suggesting that superoxide radicals in and around endothelial cells of hepatic sinusoid play critical roles in its pathogenesis. Thus, site-directed SOD and related enzymes that specifically regulate superoxide metabolism in the region of vascular endothelial cells may have therapeutic potential for decreasing reperfusion injury of the liver and other tissues, and for inhibiting primary nonfunction of a graft. Since reactive oxygen species also underlie the pathogenesis of liver injury, regulation of these hazardous species by site-directed enzymes and antioxidants may also be important.

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