CHAPTER II

REVIEW OF THE LITERATURE

The Liver

The liver is the largest gland in the body. It is positioned immediately beneath the diaphragm in the right side of the peritoneal cavity. In the human body it has two main lobes, the right lobe is much larger than the left lobe but in rats it has four lobes (Wells, 1964). The lobes of the liver are made up of many functional units called lobules (Figure 1). A lobule consists of specialized epithelial cells, called hepatocytes or parenchymal cells arranged in irregular, branching, and interconnected plates around a central vein. Rather than capillaries, the liver has larger spaces lined by endothelium called sinusoids, through which blood passed. The sinusoids are also party lined with stellate retculoendothelial (Kupffer's) cells. These phagocytes destroy worn-out white and red blood cells, bacteria, and other foreign matter in the blood draining from the gastrointestinal tract (Tortora and Grabowski, 2000).

The liver receives blood from two sources, the hepatic artery with oxygenated blood, and the hepatic portal vein with deoxygenated blood containing newly absorbed nutrients, drugs, and possibly microbes and toxin from the gastrointestinal tract. Branches of both hepatic artery and hepatic portal vein carry blood into liver sinusoids, where oxygen, most of the nutrients, and certain poisons are extracted by the hepatocytes. Products manufactured by the hepatocytes and nutrients needed by other cells are secreted back into the blood. The blood drains into the central vein. Central veins drain into larger veins often called sublobular veins and these in turn drain into the hepatic veins and empty their blood into the inferior vena cava (Figure 2). Branches of the hepatic portal vein, hepatic artery, and bile duct typically accompany each other in their distribution through the liver. Collectively, these three structures are called a portal triad or portal tract (Tortora and Grabowski, 2000).

The liver is subdividing into functional lobule to regard as a unit of hepatocyte that region which is irrigated by a terminal branch of the distributing veins. This unit is called the hepatic acinus (Reppaport, 1956) (Figure 3). Cells in the hepatic acinus can be subdivided into zones (Reppaport, 1956). Zone I called periportal area would be closest to the vessel and consequently the first to be affected by or to alter the incoming blood. Cell in zone II called midzone would be second to respond to the blood and zone III called centrilobular or periacinal area would be portal vein blood that has been previously altered by cells in both zone I and II. The zonation is important in the description and interpretation of patterns of degeneration, regeneration, and specific toxic effects in the liver parenchyma relative to the degree or quality of vascular perfusion of the hepatic cells. As a result of the sinusoidal blood flow, the oxygen gradient, the metabolic activity of the hepatocytes, and the distribution of hepatic enzymes varies across the three zones. The distribution of liver damage due to ischemia and exposure to toxic substances can be explained using this zonal interpretation (Ross et al., 2003).

Cells in zone I are the first to receive oxygen, nutrients, and toxins from the sinusoidal blood and are the first to show morphologic changes following bile duct occlusion (bile stasis). These cells are also the last to die if circulation is impaired and the first to regenerate. On the other hand, cells in zone III are the first to show ischemic necrosis (centrilobular necrosis) in situations of reduced perfusion and the first to show fat accumulation. They are the last to respond to toxic substances and bile stasis. Normal variations in enzyme activity, the number and size of cytoplasmic organelles, and the size of cytoplasmic glycogen deposits are also seen between zone I and III. Cells in zone II have functional and morphologic characteristics and responses intermediate to those of zones I and III (Ross et al., 2003).

The liver performs many vital functions (Romanes, 1986) including; first, metabolizing the products of digestion through the portal vein (principally degradation

products of protein and carbohydrates); second, the storage and release of substances (principally glucose) so as to maintain a constant level in the blood; and third, the synthesis, conjugation and transformation of substances e.g. formation of protein, detoxification of poisonous substances, production of carbohydrates from protein. All of these are endocrine function which alter the composition of blood traversing the liver.

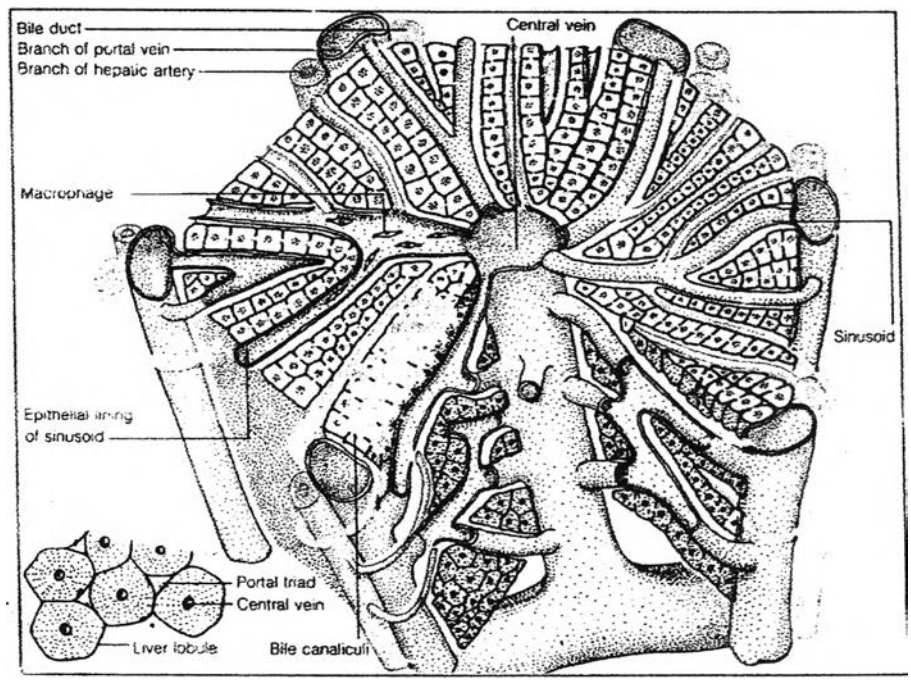


Figure 1. A liver lobule (Landau, 1980)

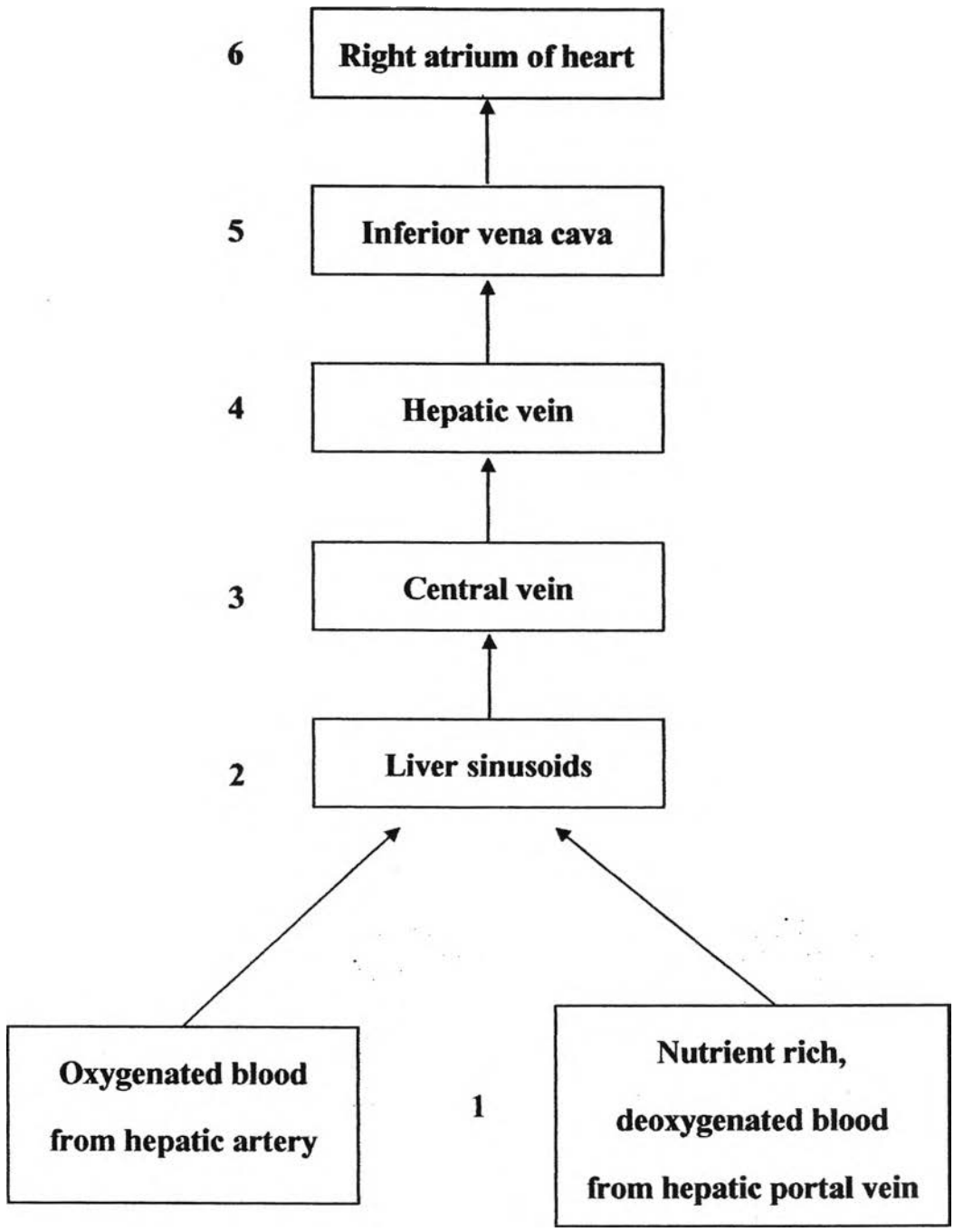


Figure 2. Blood flow through the liver and return to the heart. (Tortora and Grabowski, 2000)

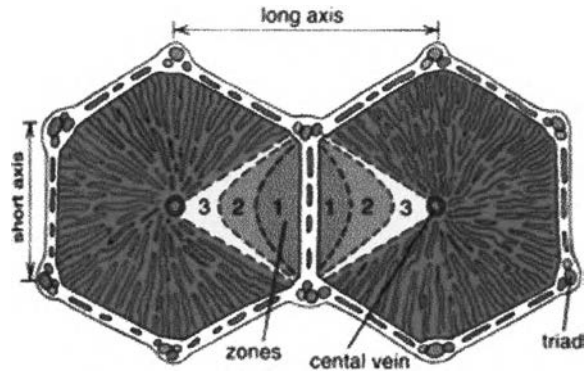


Figure 3. The hepatic acinus (Ross et al., 2003)

The Liver and Lipid Metabolism

Free fatty acids normally cycle between the liver and peripheral adipocytes without any appreciable accumulation of lipids within hepatocytes. The flux of free fatty acids to the liver can come from 3 general sources. Triglycerides in peripheral adipocytes can be enzymatically hydrolyzed by hormone-sensitive lipase to yield glycerol-3-phosphate and free fatty acids. A second source of free fatty acids comes from dietary triglycerides in the form of chylomicrons which are hydrolyzed by the enzyme lipoprotein lipase. Endogenous fatty acid synthesis represents a third source of fatty acids to the liver. When hepatic glycogen stores are saturated, acetyl CoA from the metabolism of excess dietary glucose or amino acids can be diverted to lipogenic pathways forming free fatty acids.

In the context of fatty acid storage, mobilization, and metabolism, 2 major metabolic pathways exist in the liver. Fatty acids may be incorporated with glycerol-3-phosphate into a storage form. This is accomplished through the sequential esterification of acetyl CoA with glycerol-3-phosphate to form triglycerides. After being packaged with specific apoproteins to form the lipoprotein, very low-density lipoproteins (VLDL), the triglycerides can be secreted by the liver and exported back

to the peripheral adipocytes. Fatty acids may also proceed down the catabolic pathway of mitochondrial β-oxidation in which 2 carbon fragments are successively removed, liberating acetyl CoA for further oxidation by the tricarboxylic acid (TCA) cycle. When the oxidative capacity of the TCA cycle is exceeded, excess acetyl CoA can also be diverted to form ketone bodies (Fong et al., 2000).

Within this schema, numerous control points for the integration of lipid metabolism are under the influence of hormonal and autocrine mechanism (Fong et al., 2000). Insulin, for example, exerts an anti-lipolytic effect by inhibiting hormone sensitive lipase, thereby reducing the flux of free fatty acids to the liver. Within the liver, insulin acts to inhibit mitochondrial β-oxidation and stimulate the synthesis of fatty acids from glucose. In contrast, hormones such as epinephrine and norepinephrine, save as the major counteregulatory hormones that stimulate the mobilization of free fatty acids from adipocytes by up-regulating the action of hormone-sensitive lipase (Carey, 1998). At the level of the liver, glucagon stimulates β-oxidation and inhibits endogenous fatty acid synthase and endogenous fatty acid synthesis (Foster and McGarry, 1982). At the same time, cortisol has also been shown to increase lipolysis by stimulating the activity of lipoprotein lipase (Saleh, 1999).

Nonalcoholic Steatohepatitis (NASH)

Nonalcoholic steatohepatitis (NASH) is a common liver injury in which the histopathological abnormalities mimic those of alcoholic steatohepatitis (Ludwig et al., 1980). Histologically, NASH is similar to alcoholic-induced hepatitis with the presence of macrovesicular steatosis, mixed inflammatory cell infiltration of the lobules, hepatocyte ballooning and necrosis, Mallory bodies, and perisinusoidal fibrosis or cirrhosis (Figure 4) (Ludwig et al., 1980). NASH is considered as the part of the spectrum of nonalcoholic fatty liver disease (NAFLD), which includes fat alone

and fat with nonspecific inflammation, in addition to NASH (Matteoni et al., 1999 and Younossi, 1998).

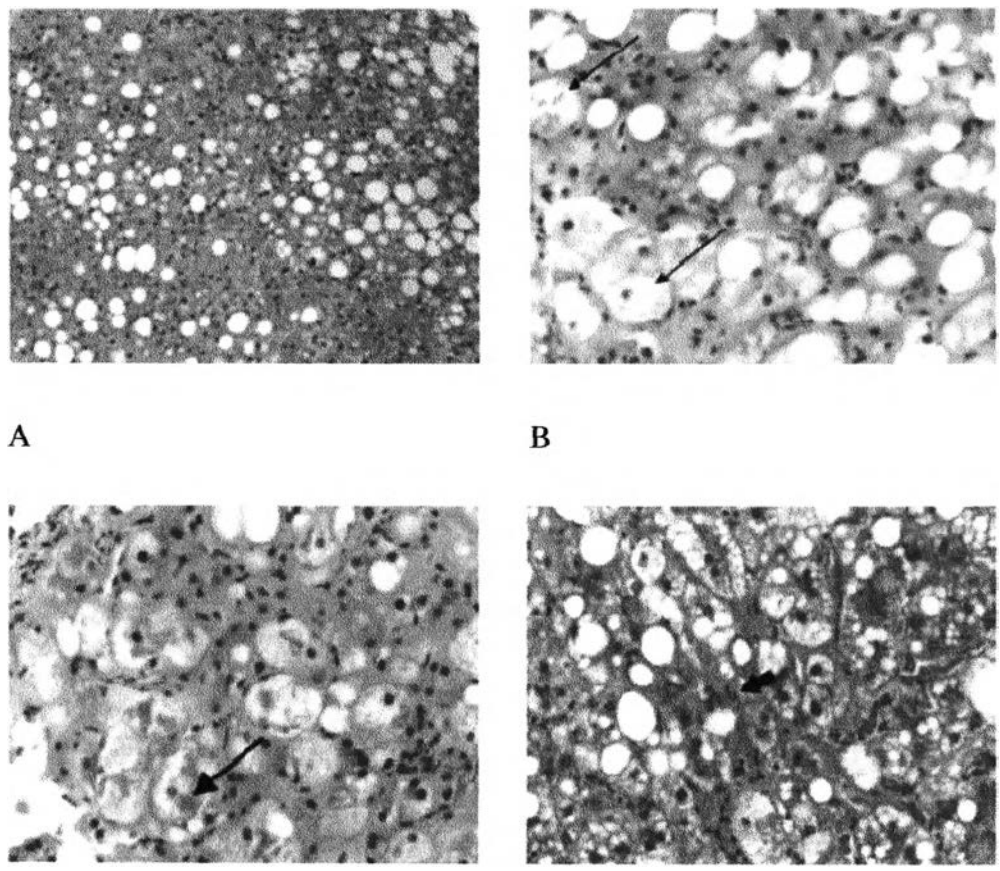


Figure 4. Hematoxylin and eosin stain of human liver histopathology in NASH; macrovesicular steatosis (A), an enlarged view showing steatosis, ballooning changes (arrows), and lobular inflammation (B), Hematoxylin and eosin stain highlighting Mallory bodies (C) (arrows), Masson trichrome stain highlighting extensive sinusoidal fibrosis (D) (arrowhead). (Grant and Lisker-Melman, 2004)

1. Pathogenesis of NASH

The pathogenesis of NASH remains unclear. The "two-hit" hypothesis was proposed by Day and James in 1998. The "first hit" is thought to be an accumulation of fat, specifically fatty acids and triglycerides, within the liver. Once steatosis is present, cellular adaptations occur through altered signaling pathways. This is a result of chronic exposure to increased levels of oxidant stress, which allow the cell to survive in the new environment. However, the cell is now vulnerable to the "second hit", possibly environmental and/or genetic in origin, which leads to either apoptosis or more likely cellular necrosis, accompanied by inflammation.

1.1 First Hit

Steatosis occurs when fat homeostasis becomes unbalanced. Normally, triglycerides, the main components of fat in the liver, are derived from the esterification of free fatty acids that accumulate within the liver. These free fatty acids accumulate through two major pathways. They are packaged with albumin via gut absorption or lipolysis of adipose tissue and transported to the liver, or they are synthesized within the liver through lipogenesis. Fatty acids that accumulate in the liver can then either undergo oxidation within hepatocyte mitochondria, peroxisomes or microsomes, or be esterified into triglycerides. The triglycerides are then secreted as very low-density lipoprotein (VLDL), through exocytosis (Koteish and Diehl, 2001).

Homeostasis of fat metabolism can be deranged by increased delivery of free fatty acids to the liver, increased synthesis of fatty acid in the liver, decreased β-oxidation of fatty acids in the liver, and/or decreased synthesis or secretion of VLDL. Alterations in insulin signaling (the ability of the adipocyte to respond to changes in glucose levels) and fat metabolism appear to promote steatosis. As previously mentioned, insulin resistance has been shown to be present in the majority of patients

with NASH (Marchesini et al., 1999; Pagano et al., 2002 and Chitturi et al., 2002) and is also found in association with diabetes mellitus, obesity, and hypertriglyceridemia. Elevated insulin level affects both the adipocyte and hepatocyte, but in different ways. In the adipocyte, increased circulating insulin promotes lipolysis with subsequent increased delivery of free fatty acids to the liver. In the hepatocyte, fatty acid synthesis is stimulated and oxidation of fatty acids is inhibited (Kaplan, 1998). Additionally, elevated insulin levels may increase the degradation of apolipoprotein B 100, a component of VLDL, thus leading to the inability of triglycerides to be transported out of the liver (Neuschwander-Tetri, 2001). Ultimately, triglycerides accumulate in the liver because of increased synthesis from greater flow of free fatty acids to the liver and possibly decreased production or secretion of VLDL (Harrison, 2002).

Leptin, expressed primarily by adipocytes, is an important regulatory hormone of energy homeostasis, and may play a role in the development of hepatic steatosis. This hormone is thought to promote insulin resistance and alter insulin signaling in hepatocytes resulting in increasing hepatocellular fatty acid production (Kaplan, 1998). Elevated levels of leptin are found in a majority of patients with NASH (Uygun et al., 2000), irrespective of body mass index, and may be related to hyperlipidemia.

Peroxisome proliferator-activated receptors (PPAR) are a family of nuclear receptors and have been recognized as major signaling-transducing molecules in post-receptor insulin signaling. Peroxisome proliferator-activated receptor-α (PPARα) is expressed in tissues with high rates of oxidative phosphorylation and is a regulator of lipid use via microsomal, peroxisomal, and mitochondrial oxidative systems (Angulo, 2002). Additionally, it may promote mitochondrial synthesis of uncoupling protein-2 (UCP-2), an oxidative phosphorylation chain uncoupler (Chavin et al., 1999). Alterations in the function of this system may lead to alter lipid homeostasis.

1.2 Second Hit

The factors leading to the development of hepatocyte inflammation, ballooning degeneration, necrosis, and fibrosis are still being investigated. However, data now suggest that a cascade of events occurs at the hepatocyte level may cause a select number of patients with steatosis and progress to NASH. The precise role and interaction of each of these factors is under intense study.

Oxidative stress is thought to be one driving catalyst behind the progression of steatosis to NASH. Oxidative stress is generated through multiple sources. Free fatty acids that have accumulated within the hepatocytes undergo oxidation via mitochondria, microsomes, and peroxisome generating reactive oxygen species (ROS). The increased free fatty acids also induce upregulation of CYP2E1 (Zanger and Novak, 1997), and the enzyme acts as a catalyst for further lipid peroxidation. Other cytochrome p450 pathways, such as CYP3A4, have been implicated as a source of further lipid peroxidation as well (Leclercq et al., 2000). Chronic oxidative stress leads to the depletion of the natural antioxidant pool (e. g., glutathione), and results in excess reactive oxidative species with the hepatocyte over time. The generation of excess ROS, in addition to triggering lipid peroxidation of cellular membrane, leads to release of tumor necrosis factor (TNF)-α, a proinflammatory cytokine, via hepatocytes, Kupffer cells, and adipose tissue (Kern et al., 1995). This upregulation of TNF-α activates specific redox-sensitive kinases, such as IKK-β, which upregulate proinflammatory pathways as well as enhance insulin resistance (Yuan et al., 2001 and Kim et al., 2001).

1.3 Development of Fibrosis

Stellate cells are primarily responsible for fibrogenesis, and are a principal cytokine target in liver fibrosis. These resting cells must be activated by

initiation and then perpetuation (Friedman, 1993). Initiation appears to involve noncytokine stimuli such as MDA and 4-hydroxynonenal, products of lipid peroxidation. Cytokines are also critical to stellate cell perpetuation. Kupffer cell-derived proinflammatory cytokines, IL-1, IL-6, and TNF-α , are involved in the stimulation of stellate cells (Melgert et al., 2001). TNF-α secretion leads to upregulation of NF-κβ, which stimulates other proinflammatory cytokines such as IL-6, tissue growth factor-β (TGF-β), and platelet-derived growth factor, which may induce fibrogenesis.

Oxidative Stress

Oxidative stress has been defined as a disturbance of the balance between antioxidants and prooxidants (free radicals and other reactive oxygen and nitrogen species) with increased levels of pro-oxidants leading to potential damage (Halliwell, 1997; Sies, 1997 and Betteridge, 2000). This imbalance can be an effect of depletion of endogenous antioxidants, low dietary intake of antioxidants and/or increased formation of free radicals and other reactive species.

1. Free Radicals and Other Reactive Species

A free radical in simple terms may be defined as an atom or molecule that contains one or more unpaired electrons and is capable of independent existence (Sodergren, 2000). An unpaired electron is an electron that occupies an orbital alone, but electrons usually associate in pairs in orbitals of atoms and molecules. Free radicals are generally more reactive than non-radicals due to their unpaired electron, but different types of free radicals vary widely in their reactivity (Slater, 1984 and Halliwell and Chirico, 1993). The oxygen molecule (O_2) qualifies as a free radical because it contains two unpaired electrons, but it is not particularly reactive due to a special electron arrangement that makes the reactions with oxygen spin restricted (Halliwell and Gutteridge, 1990). However, when oxygen is partly reduced, several different reactive oxygen species, both radicals and non-radicals, may be produced

(Slater, 1984 and Halliwell and Chirico, 1993). Examples of reactive oxygen species are hydroxyl radicals ($OH^{\bullet}$), superoxide anion radicals ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2). The hydroxyl radical is an extremely reactive free radical. It is very unstable and attacks a large array of molecules in the nearby environment. Examples of other free radicals are the two gaseous radicals nitric oxide ($NO^{\bullet}$) and nitrogen dioxide ($NO^{\bullet}_2$), the carbon-centred ($R^{\bullet}$), alkoxyl ($RO^{\bullet}$) and peroxyl radicals ($ROO^{\bullet}$) formed during peroxidation of lipids and the trichloromethyl radical ($CCl^{\bullet}_3$) formed by the metabolism of carbon tetrachloride(CCl_4) in the liver.

Radicals can react with other molecules in several ways (Slater, 1984 and Halliwell and Chirico, 1993). When two free radicals meet, their unpaired electrons can form a shared electron pair in a covalent bond and both radicals are lost. When a radical gives one electron to, takes one electron from or simply adds on to a non-radical, that non-radical becomes a radical. Since most molecules present in living organisms are non-radicals, any free radical produced in the body will most likely reactions *in vivo* tend to proceed as chain reactions.

2. Free Radicals Induced Cellular Damage

Free radicals damage cell membranes by catalyzing amino acid oxidation, protein-protein cross-linking, protein strand scission, and attacking polyunsaturated fatty acid in cell membrane leading to the subsequent chain reaction process called lipid peroxidation (Halliwell et al., 1984 and Gutteridge et al., 1990). Lipid peroxidation is a process by which any free radical ($R^{\bullet}$) in the presence of molecular oxygen (O_2), with sufficient energy, abstracts an hydrogen atom from a methylene group ($-CH_2$) of unsaturated fatty acid (LH) (Gutteridge et al., 1990 and Halliwell et al., 1993) (Figure 5).

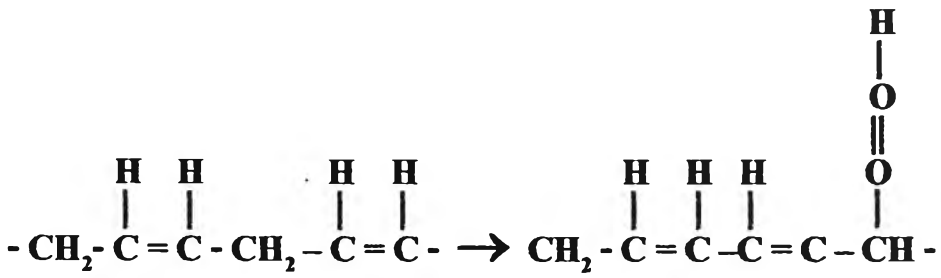


Figure 5. Change in the structure of lipid due to hydrogen abstraction by free radical (Asmus et al., 1994)

Normally, biomembranes contain relatively large amounts of polyunsaturated fatty acid (PUFA). The greater the number of double bonds in the fatty acid side chain the easier the removal of the hydrogen atom. So, PUFAs are the major sites of lipid peroxidation (Asmus et al., 1994 and Sen, 1995). Oxidation of PUFA or lipid peroxidation leads to the disintegration of fatty acid and formation of hydrocarbon gasses (e.g., pentane) and aldehydic compounds, in particular the volatile low molecular weight aldehyde, malondiadehyde (MDA) (Wade et al., 1988 and Ji, 1996) (Figure 6). The deleterious health outcomes associated with accumulation of large amounts of lipid peroxidation byproducts such as MDA (Asmus et al., 1994).

3. Malondialdehyde (MDA)

MDA, a lipid peroxidation byproduct, is a toxic compound capable of reacting with sulfhydryl and amino groups of protein. It is a bifunctional cross-linking agent since it can produce both intra- and intermolecular linkages which can lead to inactivation and polymerization of enzymes such as ribonuclease. Its reactivity toward amino groups can result interaction with the endogenous base of DNA as well as inhibition of DNA, RNA, and protein synthesis thereby affecting their biological function (Tappel, 1973; Lee, 1980 and Siu et al., 1982).

MDA can induce polymerization of membrane components and variety of cross-linking reactions such as lipid-lipid cross-linking and lipid-protein cross-linking (Gregory, 1983) (Figure 7). These effects will result deteriorative changes in cellular membranes such as loss of fluid properties and membrane flexibility due to the alteration in the fluid-mosaic bilayer, a decrease in membrane fluidity, and an inability to maintain ionic gradients (Niess et al., 1996). Cellular swelling, a loss of cell integrity and cell inflammation will follow (Alessio, 1993). Free radicals may also disrupt subcellular membranes of important organelles including mitochondria, microsomes, and lysosomes (Gregory, 1983 and Cotran, 1999). Lipid peroxidation of the mitochondrial membrane leads to swelling, lysis, and disintegration of the mitochondria. Microsomal membrane damage results in disaggregation of polyribosomes and inhibition of protein synthesis. For the lysosomes, since they contain hydrolytic enzymes, the lysosomal membrane damage will be followed by the enzymatic digestion of other cell components (Sen, 1995).

Antioxidant System

The human body is equipped with a sophisticated antioxidant system to deal with the production of reactive oxygen species (ROS). The system includes enzymatic antioxidants and non-enzymatic antioxidants. Antioxidant enzymes or "scavenging enzymes" provide the first line of defense against ROS by converting them to more reduced and more stable species. The three major scavenging enzymes are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) (Ji, 1996) (Figure 8). A second line of defense is provided by non-enzymatic or exogenous antioxidants obtained primarily as nutrients or nutritional supplements such as carotenoids, reduced glutathione, vitamin E, and vitamin C (Sen, 1995 and Margaritis, 1997).

The possible mechanisms by which antioxidants protect against oxygen toxicity are as follows: (Arouma, 1994 and Sen, 1995)

1. decreasing localized oxygen concentration and prevention at ROS formation via a fall in substrate

2. a chain-breaking mechanism whereby intermediate radicals (e.g., $O_2^{\cdot}$) are scavenged to prevent continuation of more deleterious forms (e.g., $OH^{\cdot}$, H_2O_2)

3. interception of ROS attack by scavenging the reactive metabolites and converting them to less reactive molecules and/or by enhancing the resistance of sensitive biological targets to ROS attack

4. facilitating the repair of damage caused by ROS and triggering the expression of genes that encode antioxidant proteins

5. providing a favorable environment for the effective functioning of other antioxidants (e.g., as a cofactor or by acting to maintain a suitable redox status)

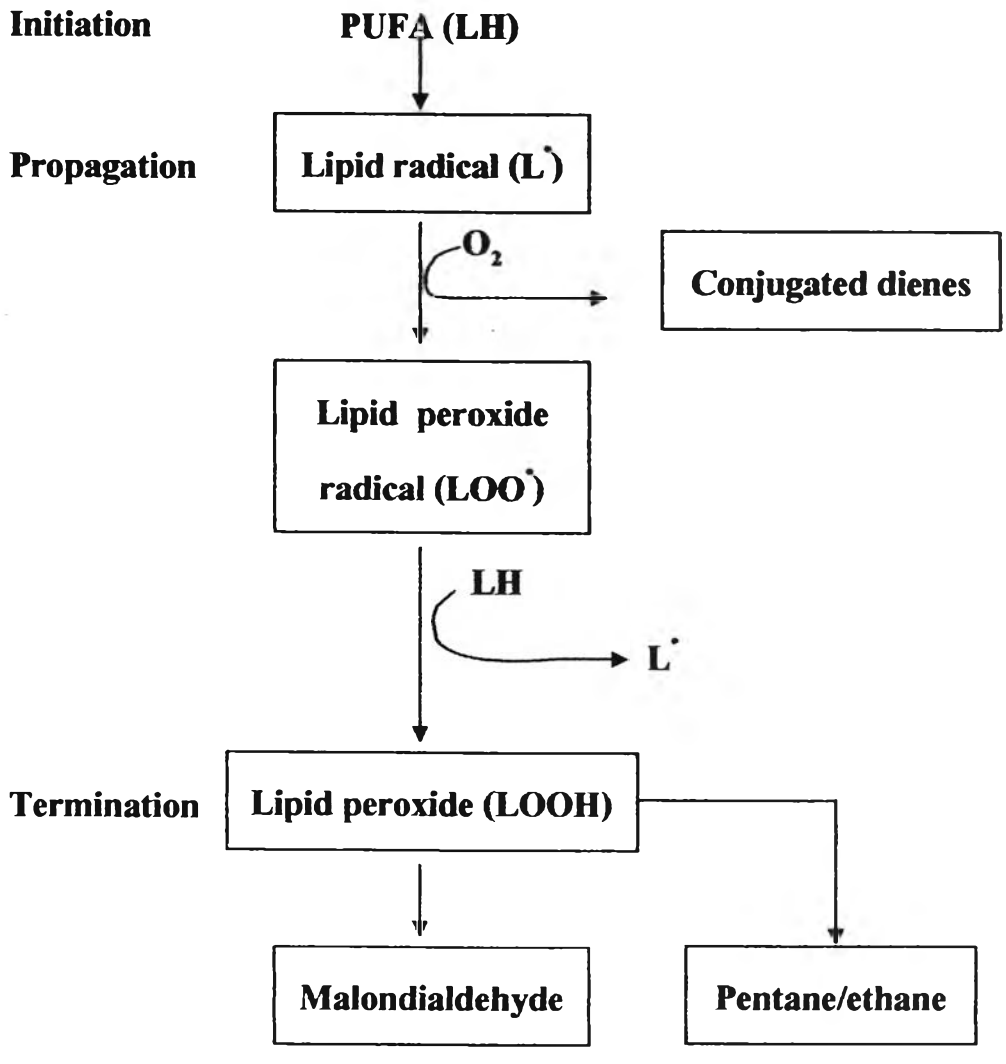


Figure 6. Three steps of lipid peroxidation: initiation, propagation and termination (Jenkins, 1993)

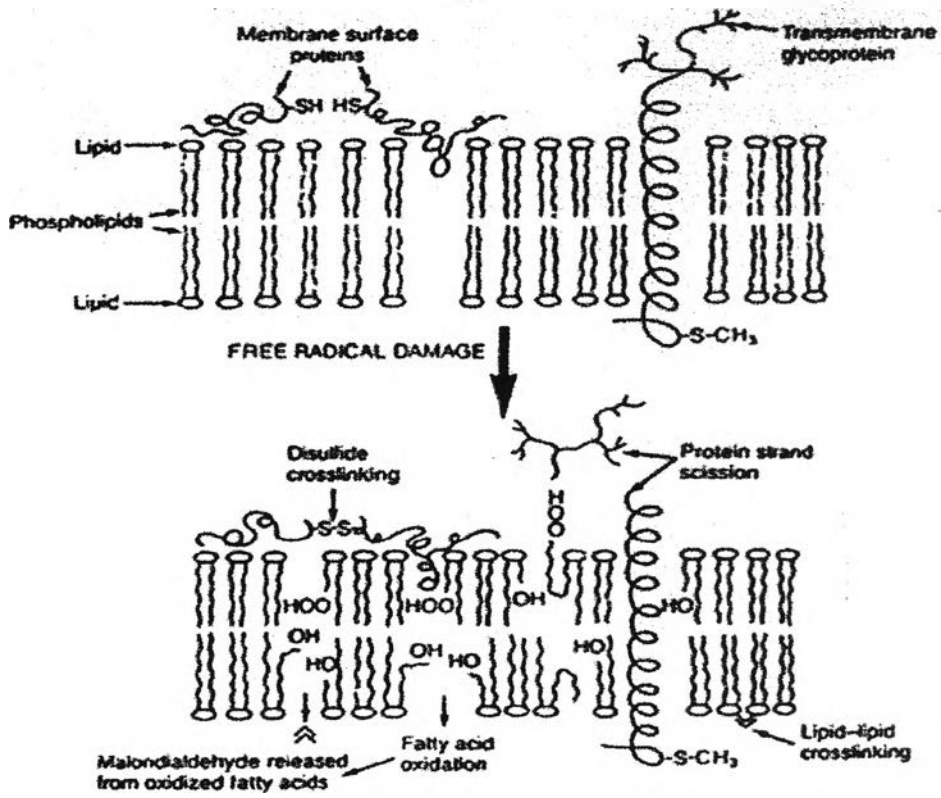


Figure 7. Free radical damage of membranes. Free radical can affect lipids by initiating peroxidation, which leads to short chain fatty acyl derivatives and the byproduct malondialdehyde. A variety of cross-linking reactions can be mediated malondialdehyde reaction. Free radical can also catalyze amino acid oxidation, protein-protein cross-linking, and protein strand scission (Freeman et al., 1982).

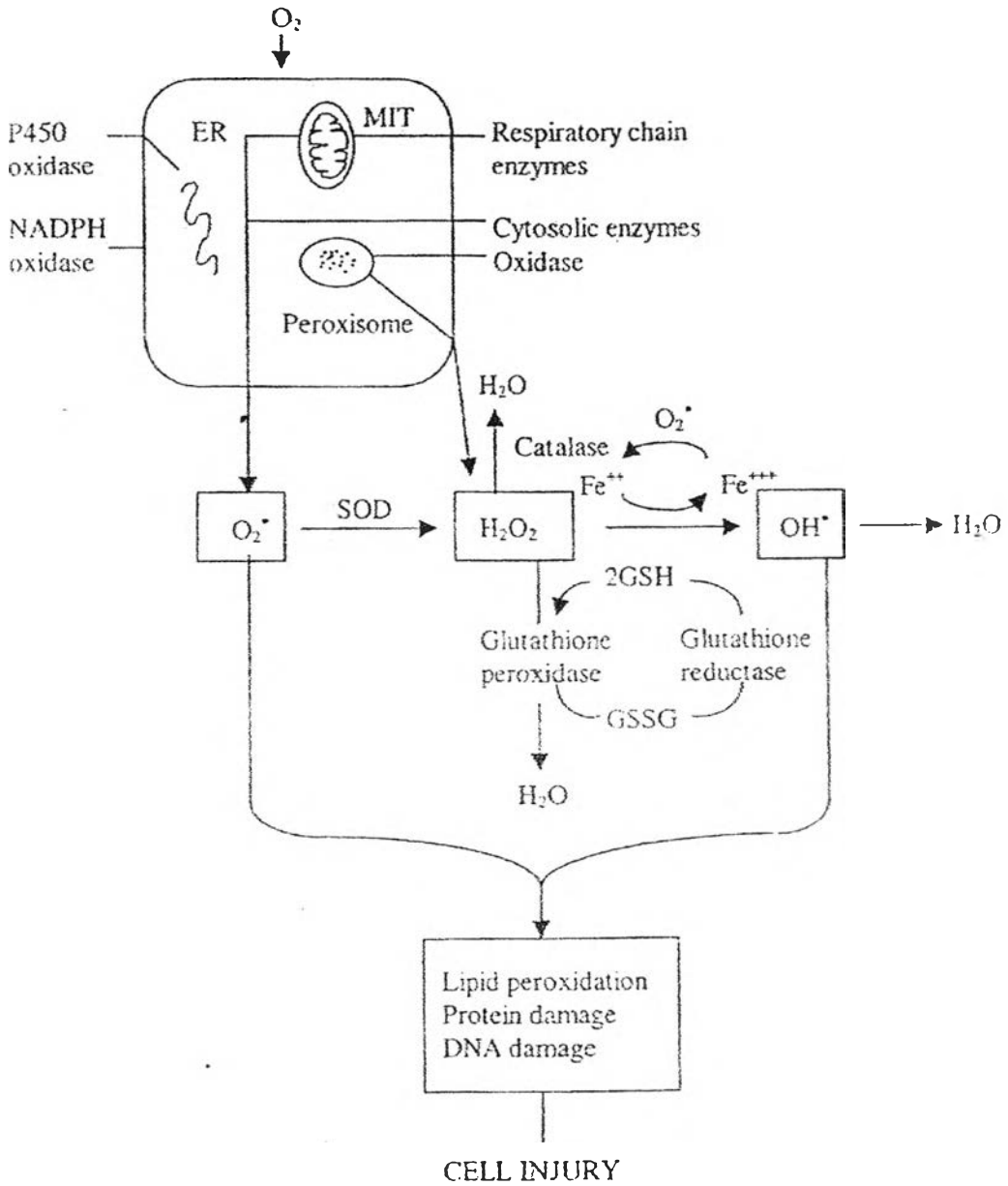


Figure 8. Antioxidant system to deal with the production of reactive oxygen species (ROS) (Halliwell et al., 1984)

1. Glutathione

Glutathione (GSH) is a tripeptide, γ-glutamylcysteinylglycine. It is considered to be the most prevalent and most important intracellular non-protein thiol/sulfhydryl compound in mammalian cells, and the most abundant low-molecular-weight peptide (Meister, 1988; Deneke et al., 1989 and Wu et al., 1994). GSH serves as a nucleophilic co-substrate to glutathione transferase in the detoxification of xenobiotics and is an essential electron donor to glutathione peroxidase in the reduction of hydroperoxides (Arias et al., 1976 and Baillie et al., 1991). It is also involved in amino acid transport and maintenance of protein sulfhydryl reduction status (Inoue, 1985 and Inoue et al., 1987). Concentration of GSH ranges from a few micromolar in plasma to several millimolar in tissues such as liver (Wendel, 1980 and Lash, 1985).

GSH is synthesized by formation of the dipeptide γ-glutamylcysteine and the subsequent addition of glycine. Both reactions require activation of carboxyl groups by ATP (Figure 9) (Devlin, 2002). The first reaction, catalyzed by γ-glutamylcysteine synthetase, is effectively rate-limited by GSH biofeedback (Meister et al., 1983; Meister, 1988 and Deneke et al., 1989). The second step involves GSH synthetase, which is not subject to negative feedback by GSH. When GSH is consumed and feedback inhibition is lost, availability of cysteine as a precursor can become the rate-limiting factor (Deleve and Kaplowitz, 1991).

1.1 Form of GSH

1. Reduced form (antioxidant) is called glutathione (GSH) (Figure 10A). The sulfhydryl (-SH) group, which gives the molecule, its electron-donating character, comes from the cysteine residue. Glutathione is present inside cells mainly in its reduced GSH form (Lomaestro and Malone, 1995).

2. Oxidized form is sulfur-sulfur linked compound, known as glutathione disulfide or GSSG (Figure 10B). In the healthy cell GSSG rarely exceed 10 percent of total cell glutathione. After GSH has been oxidized to GSSG, the recycling of GSSG to GSH is accomplished mainly by the enzyme glutathione reductase. This enzyme uses as its source of electrons, the coenzyme NADPH (Figure 11) (Lomaestro and Malone, 1995).

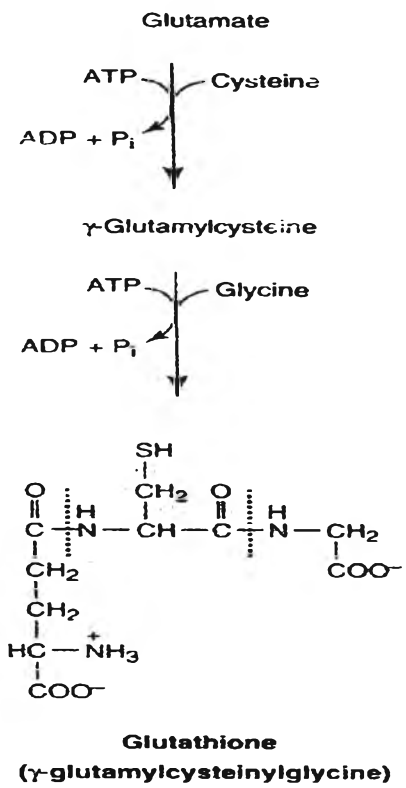
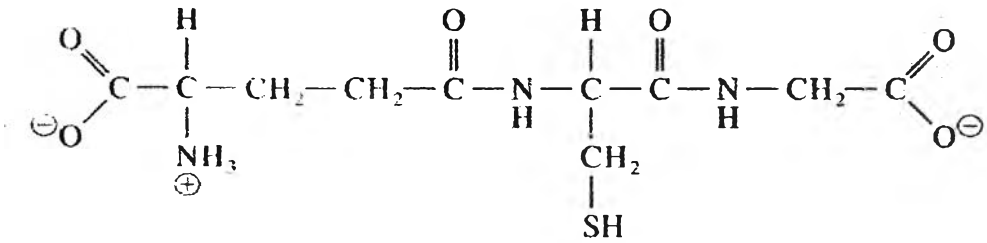


Figure 9. Synthesis of glutathione (Devlin, 2002)



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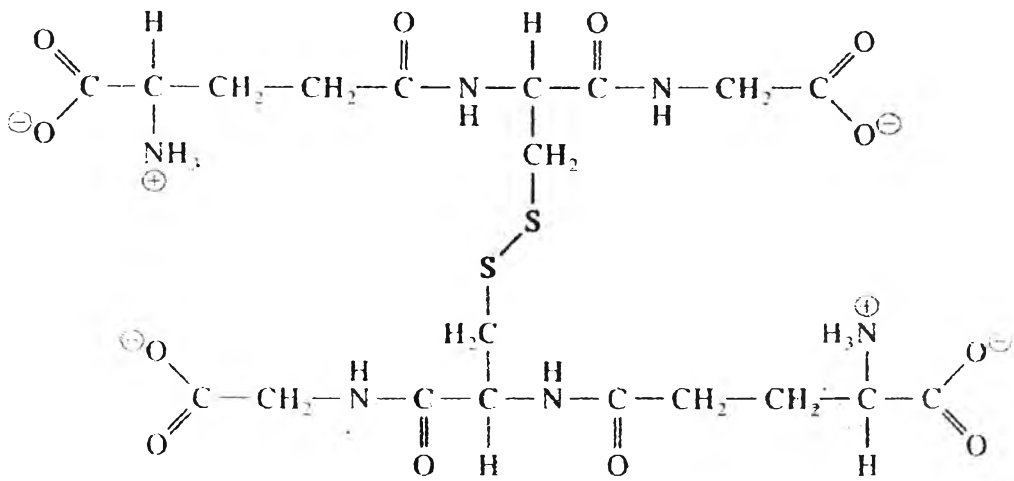

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Figure 10. Structure of GSH (A) and GSSG (B) (Rawn, 1989)

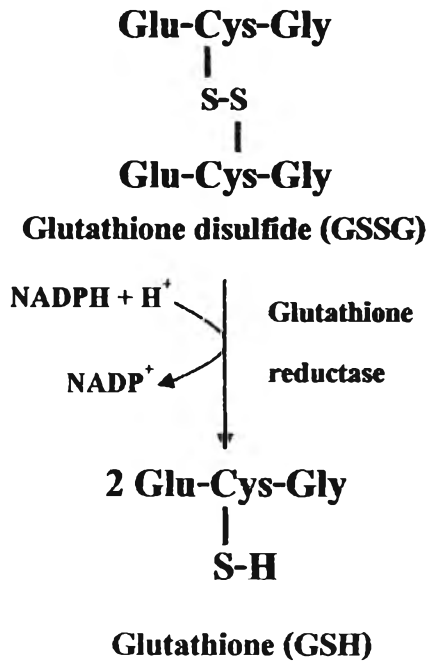

Figure 11. Regeneration of reduced glutathione by glutathione reductase (Lomaestro and Malone, 1995)

Intracellular GSH status appears to be a sensitive indicator of the cell's overall health, and of its ability to resist toxic challenge (Lomaestro and Malone, 1995). Experimental GSH depletion can trigger suicide of the cell by a process known as apoptosis (AW et al., 1991 and Halliwell, 1994). Several reseaches found that GSH (reduced form) in the liver and plasma decreased in pateints with NASH and excess GSSG in postulated to result in alteration of a variety of cell function, including enzyme function and protein synthesis (Altomare et al., 1988; Koruk et al., 2004 and Nobili et al., 2005).

2. N-acetylcysteine

N-acetylcysteine (NAC), a precursor of reduced glutathione (GSH), has been in clinical use for more than 30 years, primarily as a mucolytic. In addition to its mucolytic action, NAC is being studied and utilized in conditions characterized by

decreased GSH or oxidative stress such as HIV infection, cancer, and heart disease. Because of its hepato-protective activity, intravenous and oral administration of NAC have been used extensively in the management of acetaminophen poisoning (Kelly, 1998).

2.1 Chemistry and Pharmacokinetics

NAC is a thiol (sulfhydryl-containing) compound which has the chemical formula $C_5H_9NO_3S$ and a molecular weight of 163.2 (Figure 12) (Rovinsky, 2001). NAC can be present in plasma in free form or bound to protein (Holdiness, 1991). The initial step in the metabolism of NAC is deacetylation to cysteine (low bioavailability). The transformation takes place in the gastro-intestinal tract, in the intestinal mucosa and, to a lesser extent, in the liver. Cysteine is further metabolized by incorporation into GSH, mixed disulfides and protein as well as by degradation to inorganic sulfate (The European Agency for the Evaluation of Medicinal Products). Peak concentrations of NAC typically appear in the plasma in less than one hour following oral administration (Borgstrom et al.,1986 and De Caro et al., 1989). The plasma half-life of free NAC is estimated to be about 2.15 hours, and virtually no NAC is detectable 10 to 12 hours post-administration (De Caro et al., 1989). Between 13 and 38 percent of a radioactive oral dose is recovered in urine within 24 hours (Borgstrom et al.,1986). The sulfhydryl (-SH) group is responsible for a great deal of the metabolic activity of NAC, while the acetyl-substituted amino group makes the molecule more stable against oxidation.

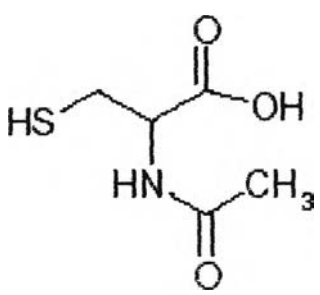

Figure 12. Chemical structure of N-acetylcysteine (NAC) (Rovinsky, 2000)

2.2 Mechanisms of Action

NAC's effectiveness is primarily attributed to its ability to reduce extracellular cystine to cysteine, or to act intracellularly as a source of sulfhydryl groups. As a source of sulfhydryl groups, NAC stimulates glutathione (GSH) synthesis, enhances glutathione-S-transferase activity, promotes liver detoxification by inhibiting xenobiotic biotransformation, and is a powerful nucleophile capable of scavenging free radicals (De Flora et al., 1985 and De Vries et al., 1993). NAC's effectiveness as a mucolytic agent results from its sulfhydryl group interacting with disulfide bonds in mucoprotein, with the mucus subsequently being broken into smaller, less viscous units. NAC may also act as an expectorant by stimulating both ciliary action and the gastro-pulmonary vagal reflex, thereby clearing the mucus from the airways (Zimet, 1988).

2.3 Research Summary of NAC

2.3.1 Acetaminophen and Other Poisonings

Historically the most prevalent and well-accepted use of NAC has been as an antidote for acetaminophen (paracetamol) poisoning. The result of liver toxicity is due to an acetaminophen metabolite that depletes the hepatocytes of glutathione and causes hepatocellular damage and possibly even death. NAC administered intravenously or orally within 24 hours of overdose is effective at preventing liver toxicity; however, improvement is most notable if treatment is initiated within 8-10 hours of acetaminophen overdose. NAC's effectiveness declines when treatment is delayed beyond 10 hours and risk of mortality significantly increases (Smilkstein et al., 1988; Wang et al., 1997 and Perry et al., 1998). NAC has also been effective for heavy metal poisoning by gold, silver, copper, mercury, lead, and arsenic, as well as in cases of poisoning by carbon tetrachloride, acrylonitriles,

halothane, paraquat, acetaldehyde, coumarin, and interferon (Zimet, 1988). Studies involving these poisons are primarily animal studies or single case reports and therefore additional studies are needed to establish NAC's effectiveness in this area.

2.3.2 Respiratory Illness

Several animal and human studies have explored NAC's effectiveness as a therapeutic agent for various types of respiratory illness. While results varied, NAC administration resulted in decreased expectoration difficulty, cough severity,(Jackson et al., 1984), and diaphragm fatigue (Hida et al., 1996). A small study was conducted with 18 patients diagnosed with fibrosing alveolitis; a condition characterized by severe oxidative stress and decreased glutathione levels. NAC was administered at a dose of 600 mg three times daily for 12 weeks and improvement in both pulmonary function and glutathione levels in bronchoalveolar lavage were noted (Behr et al., 1997). In contrast, studies of patients with chronic bronchitis, severe airway obstruction, and cystic fibrosis showed a slight, although not statistically significant, decreased in the exacerbation rate (Gotz et al., 1980 and British Thoracic Society research Committee, 1985).

2.3.3 HIV Infection

Human immunodeficiency virus (HIV)-positive individuals usually exhibit low GSH and cysteine levels, prompting studies on NAC's effectiveness as a therapeutic tool for these patients. Research suggests that NAC is capable of enhancing T cell immunity by stimulating T cell colony formation, (Wu et al., 1989) and blocking NF kappa B expression (Droge et al., 1992 and Breithaupt, 1996). In a double-blind, placebo-controlled trial, Akerlund et al. found NAC positive impact both plasma cysteine levels and CD4+ lymphocyte cell counts (Akerlund et al.,

1996). More studies are needed but it appears that if given to HIV-positive patients early in the course of disease, NAC may help to prevent progression to AIDS.

2.3.4 Cancer/Chemoprevention

Research has shown that NAC has potential both as a chemopreventative agent and a treatment in certain types of cancer, including lung, skin, head and neck, mammary, and liver cancer (De Flora et al., 1992). In vitro studies have demonstrated NAC to be directly anti-mutagenic and anti-carcinogenic as well as inhibiting the mutagenicity of certain compounds in vivo (De Flora et al., 1986). Research also indicates NAC administration in both cell cultures and animal studies selectively protects normal cells, but not malignant ones, from chemotherapy and radiation toxicity (De Flora et al., 1996). Other in vitro studies noted NAC's effectiveness at inhibiting cell growth and proliferation in human melanoma, prostate, and astrocytoma cell lines (Arora-Kuruganti et al., 1999; Chiao et al., 2000 and Redondo et al., 2000).

2.3.5 Heart Disease

Several small clinical studies have demonstrated that NAC may be an effective therapeutic agent in the management of heart disease. Wiklund et al. demonstrated NAC's ability to reduce plasma homocysteine levels by 45 percent, (Wiklund et al., 1996) while Gavish and Breslow demonstrated NAC (2-4 grams daily for eight weeks) was able to reduce lipoprotein(a) by 70 percent (Gavish et al., 1991). Due to its ability to significantly increase tissue GSH, NAC may also be useful in treating the ischemia and reperfusion seen in acute myocardial infarction, and the resultant depletion in cellular sulfhydryl groups (Ceconi et al., 1988). In addition, NAC appears to potentiate nitroglycerin's coronary dilating and anti-platelet properties

and therefore may be a useful combination therapy in-patients with unstable angina pectoris and myocardial infarction (Winniford et al., 1986 and Chirkov et al., 1996).

2.3.6 N-acetylcysteine in the Treatment of Nonalcoholic Steatohepatitis

There were two studies which used NAC for the treatment of NASH. In this uncontrolled study, NAC (1g/day) was administered to 11 NASH patients for 3 months. The results showed that serum transaminases (AST and ALT) and gamma-glutamyl transpeptidase levels decreased significantly following 1st month of NAC treatment and more significantly at the end of treatment period (Gulbahar et al., 2000). After that, in this controlled study, NAC (600mg/day) was administered to NASH patients for 4 weeks, with significant improvements in aminotransferase levels (Gulbahar et al., 2003).

Model of NASH

1. Genetic Model

ob/ob mice have a naturally occurring mutation that prevent the synthesis of leptin, a satiety hormone that inhibits feeding behavior and increases energy expenditure (Campfield et al., 1996). Because *ob/ob* mice lack leptin, they have hyperphagia and become obese (Pelleymounter et al., 1997). The mechanisms for hepatic steatosis in *ob/ob* mice are not well understood, but emerging evidence suggests that increased hepatic lipogenesis is probably involved (Koteish and Diehl, 2001). In the absence of leptin, the activity of the adipocyte differentiation factor, PPARγ, is enhanced in white adipose tissue (fat) (Koteish and Diehl, 2001). Consequently, genes expressed by well differentiated fat cells, including TNF-α and

lipoprotein lipase, are increased (Zhou et al., 1999). The actions of those factors promote the release of fatty acid from fat stores. Fatty acids and TNF-α induce uncoupling protein (UCP)-2 gene expression in hepatocytes (Cortez-Pinto et al., 1998 and Cortez-Pinto et al., 1999). Uncoupling proteins are thought to promote futile fatty acid cycling, reducing the efficiency of mitochondrial oxidative phosphorylation (Skulachev, 1998). In addition, leptin deficiency increases the production of insulin by beta cells of the pancreas (Zhou et al., 1997). Hyperinsulinemia induces the lipogenic transcription factor SREBP-1c in both the pancreas and liver, up-regulating the expression of fatty acid synthase (FAS) and, ultimately, increasing triglycerides synthesis in both organs.

2. Methionine and Choline Deficient Diets

Rats which received a diet devoid of methionine-choline for 4 weeks expressed macrovesicular steatosis and necroinflammation in hepatic acinar zone 3 (Weltman et al., 1996). Methionine and choline are components for lipoprotein synthesis. In the face of methionine-choline deficiency, hepatic triglycerides secretion may be compromised leading to hepatic triglycerides accumulation (Bertics et al., 1999 and Fong et al., 2000).

3. High Fat Liquid Diet

Feeding high fat diet could lead to the accumulation of triglycerides within hepatocytes. Rats which were fed a high-fat liquid diet with 71% of energy derived from fat, 11% from carbohydrates, and 18% from protein for 3 weeks expressed panlobular steatosis, abnormal mitochondria, and mononuclear inflammation (Lieber et al., 2004).

4. 100% fat diet

Rats were fed 100% fat diet for 6 weeks showed moderate steatosis, mild lobular inflammation, hepatocyte ballooning and necrosis. This model provided the same pattern pathological change as in human (Thong-Ngam et al., 2005).