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Alcoholism has a detrimental impact on society and is currently recognized as amongst the most prevalent known causes of abnormal human development (Begleiter, 1980). Ethanol ingestion affects a wide range of organ systems. Some of its effects are directly due to the action of ethanol or its metabolites (Lieber, 1980) while some others result from the modifications of metabolism of endogenous substances or drugs which shared a common metabolic pathway of ethanol, whereas others are related to nutritional deficiencies associated with alcohol intake (Lieber, 1991).

2.1 Ethanol Absorption and Metabolism

Chronic and excessive use of alcohol leads to bizarre physiological changes including tolerance/resistance, addiction, organ damage and a whole range of biochemical and neurological lesions (Lieber, 1980). Chronic alcoholism is associated with numerous degenerative and inflammatory disorders in many organs, including liver (Lieber, 1991), brain, heart, skeletal muscle, pancreas, stomach and small intestine (Lieber, 1991), as well as in the endocrine system (Hardt, 1991). Alcohol abuse is also associated with an increased incidence of upper alimentary and respiratory tract disorders (Lieber, 1987). The general influence of ethanol intake enhances the carcinogenicity, mutagenecity and hepatotoxicity of a variety of chemicals (McCay et al., 1981).
It is generally accepted that ethanol, due to its physical properties (hydrophobicity), accumulates in membranes, thus influencing their physicochemical characteristics and induce in vivo changes in membrane lipid composition and fluidity (Lieber, 1988), which may affect cellular functions (Waring et al., 1981). Short-term ethanol administration renders membranes more fluid, whereas long-term administration alters membrane lipid composition, thus resulting in decreased fluidity, which is considered an adaptation to the initial fluidizing effect of ethanol. It has been suggested that increased rigidity impairs mitochondrial function, but such a correlation has not been verified (Gordon et al., 1982). Also due to its ability to diffuse across biological membranes, ethanol exerts its effect on the absorption of various exogenous compounds and has been implicated in potentiating the toxic effects of many chemicals and heavy metals.

Ethanol is rapidly and completely absorbed from the gastrointestinal tract (Lieber, 1976). Although there is some absorption of ethanol through gastric wall, 80% of the ingested ethanol is absorbed from the small intestine (Beck and Dinda, 1981). After ingestion, alcohol disappears rapidly so that 24h time period is usually sufficient to clear from the blood and further rate of ethanol elimination is enhanced among chronic alcoholics (Salaspuro et al., 1978; Pikkarainen and Lieber, 1980). The absorbed ethanol is rapidly metabolized to acetaldehyde and ultimately to carbon dioxide, water and energy,
particularly in the liver (Garban et al., 1985; 1986) - the organ which principally bore the brunt of alcohol intake. The higher levels of peak blood ethanol are found in alcoholics than in controls. This most probably indicates faster distribution of ethanol in controls (Nuutinen et al., 1983). Also the absorption rate of ethanol is known to be an important determinant of blood alcohol concentrations (Wilkinson, 1980).

After absorption, ethanol diffuses very rapidly across capillaries and other membranes so that it ultimately distributes uniformly throughout the whole body (Flora and Tandon, 1987) and is not stored in tissues. It is not blocked by blood brain barrier or even by placental barrier (Begleiter, 1980), hence causing widespread damage to practically almost all organs (Edmondson, 1980).

The metabolism of ethanol in the body is complex. It occurs primarily in the liver, more specifically in the endoplasmic reticulum (Behrens et al., 1988), and this organ ultimately sustains the greatest damage from excessive ethanol ingestion. Three enzyme systems are thought to be responsible for metabolism and elimination of ethanol in vivo, each is located in a different subcellular compartment: (i) the main alcohol dehydrogenase (ADH) pathway of the cytosol or the soluble fraction of the cell (Julkunen et al., 1985); (ii) the microsomal ethanol oxidizing system (MEOS) located in the endoplasmic reticulum (Lieber, 1991); and (iii) catalase located in the peroxisomes (Garban et al., 1986;
Lieber, 1987). For many years, cytosolic zinc containing metalloenzyme, alcohol dehydrogenase was thought to be the predominant pathway for alcohol metabolism (Berry et al., 1980). However, it has been reported that the peroxidative action of calatase (Bradford et al., 1993) and inducible microsomal ethanol oxidizing system (Lasker et al., 1987; Lieber, 1990) also contribute to ethanol oxidation (Teschke and Gellert, 1986).

The three enzyme systems work in concert with aldehyde dehydrogenase (Lieber, 1982; Teschke and Gellert, 1986), since they all produce acetaldehyde and the resulting aldehyde gets oxidized by the action of aldehyde dehydrogenase to acetate which is the first degradation product of alcohol metabolism (Koivisto and Salaspuro, 1996). This enzyme protects the individual against alcoholism because of the adverse nature of this reaction which limits the quantity and frequency of alcohol consumption. The basic pathways of alcohol metabolism (Peters, 1982) are summarized below:
The rate of ethanol metabolism by the liver depends on the rate of mitochondrial reoxidation of the cytoplasmic NADH produced in the oxidation of ethanol. Both alcohol and acetaldehyde oxidation utilizes NAD and prolonged use of alcohol results in the generation of excess of reducing equivalents in the liver, primarily as NADH, and hence in increased NADH/NAD ratio. The large amounts of reducing equivalents produced overwhelm the hepatocyte's ability to maintain redox homeostasis, thus resulting in several metabolic disorders (Lieber, 1991).

Chronic ethanol feeding is associated with an increased rate of ethanol metabolism (DiPadova et al., 1987) but the mechanisms underlying this increase are contradictory. Chronic ethanol consumption is also associated with changes in mitochondrial functions (Matsuzaki and Lieber, 1977), particularly at the energy coupling site I of mitochondrial respiratory chain. Structural and functional changes of mitochondria in alcohol-abusing subjects and experimental animals chronically exposed to ethanol suggest that mitochondria are a prime target of ethanol toxicity. However, the processes leading to mitochondrial dysfunction and damage are not very clear. It has been suggested that administration of ethanol results in an increased generation of reactive oxygen species and mitochondria, in particular,
may be exposed to an oxidative stress during metabolism of ethanol (Kukielka et al., 1994).

2.2 Ethanol and Acetaldehyde Effects

The rate of acetaldehyde metabolism is quite rapid (Nuutinen et al., 1984), almost five times that of ethanol. It is revealed that blood acetaldehyde levels are much higher at elevated than at low concentrations of ethanol. The higher acetaldehyde levels in the alcoholic subjects may be due to either increased production or decreased catabolism (DiPadova et al., 1987). Increased rates of alcohol clearance have been found in alcoholic patients shortly after long term consumption of alcohol (Misra et al., 1971). However, ethanol elimination is probably one factor responsible for the elevation of blood acetaldehyde in alcoholics (Lindros et al., 1980), but reduced hepatic aldehyde dehydrogenase may also contribute (Jenkins and Peters, 1980; Salaspuro et al., 1981).

Acetaldehyde levels in vivo fluctuated between 50mM in the blood of alcoholics (Korsten et al., 1975) to 200mM in rat liver tissue (Eriksson, 1973). It has now been established that in non-alcoholic controls, blood acetaldehyde levels during ethanol oxidation are very low, i.e. less than 2 μM (Pikkarainen et al., 1979; Lindros et al., 1980).

The half life of acetaldehyde in the blood is estimated as 3.1 minutes (Hobara et al., 1985). Several studies showed that the
metabolism of acetaldehyde via aldehyde oxidase may play a role in ethanol-induced free radical injury (Shaw and Jayatilleke, 1990).

There is now experimental evidence that acetaldehyde could be involved in many of the typical manifestations of alcohol abuse (Lieber, 1988). Cederbaum and Rubin (1976) showed that acetaldehyde inhibits several mitochondrial functions (Cederbaum et al., 1974), respiratory functions (Lieber, 1991) and fatty acid oxidation (Cederbaum et al., 1974), especially since the liver mitochondria usually become susceptible to the toxic effects of acetaldehyde after long-term alcohol consumption (Matsuzaki and Lieber, 1977). High intra-cellular levels of NADH resulting from the metabolism of ethanol and acetaldehyde may be involved in alcohol toxicity.

The key to the prevention of alcoholic liver diseases lies in influencing acetaldehyde metabolism. The oxidation of acetaldehyde is largely a cytoplasmic process and if the rate of acetaldehyde removal exceeded its formation, the concentration of acetaldehyde during ethanol oxidation would remain low. The binding of acetaldehyde to cell protein is responsible for irreversible liver damage. The prevention of this binding would be the appropriate way of preventing liver damage due to alcohol (Kulkarni, 1992).

Acetaldehyde concentration may limit the rate of the conversion of ethanol to acetate. Acetaldehyde binding to the plasma membranes of rat liver cells has been shown by Barry et al. (1983). At concentrations of
acetaldehyde up to 10 mM, this binding does not affect cellular metabolism.

2.3 Effects of Ethanol on Liver

Liver is the major site of ethanol oxidation (Lieber, 1982). The association between chronic alcoholism and serious liver disease including cirrhosis is well known (Lieber, 1991).

The alcoholic liver injury appears to be generated by the effects of ethanol metabolism and the toxic effects of acetaldehyde (Ishak et al., 1991). It seems that consumption of alcohol is capable of generating a state favourable to the development of oxidative stress which, in turn, could be responsible for damage to hepatocytes (Suematsu et al., 1981). The increased availability of NADH resulting from ethanol metabolism may turn the NADH oxidation by antioxidant into an important pathway of ethanol dependent free radical generation, contributing to ethanol induced hepatotoxicity (Williams and Barry, 1987; Mira et al., 1995).

The metabolism of ethanol is known to interfere with a number of hepatocellular functions related to mitochondria, such as oxidation of fatty acids and chylomicrons and the activity of the citric acid cycle. These alterations are thought to result from the lowered redox state produced by oxidation of ethanol.

It is well known that chronic ethanol intake both in man and in animals enhances the capacity of liver to oxidize ethanol (Videla et al., 1973). Ethanol consumption also affects drug metabolism in the liver...
itself which is independent of changes in drug excretion or distribution or hepatic blood flow (Lieber, 1994). The induction in the activity of microsomal ethanol oxidizing system after chronic alcohol consumption spills over to various other drug-metabolizing systems in liver microsomes, thereby accelerating drug metabolism in general. Repeated ethanol administration results in increased activities of a variety of microsomal drug detoxifying enzymes. Cytochrome P\textsubscript{450} induced ethanol ingestion offers a likely explanation for the observation that ethanol consumption enhances the rate of drug clearance \textit{in vivo} (Lieber, 1994).

### 2.3.1 Role of Alcohol dehydrogenase

The conversion of ethanol to acetaldehyde is catalyzed by alcohol dehydrogenase. This enzyme is found in many organs of the body, but its greatest concentration is in the liver, the primary site of alcohol metabolism (Moreno and Pares, 1991). Therefore, liver is acknowledged to be the site of removal of virtually all ethanol reaching the peripheral circulation (Levitt \textit{et al.}, 1994). There have been several studies in which the amount of enzyme has been determined as an indication of the ability of the liver to metabolize ethanol. The stomach possesses the highest alcohol dehydrogenase activity in the digestive tract (Julkunen \textit{et al.}, 1985).

The activity of liver alcohol dehydrogenase is maximal at low concentrations of ethanol (Lieber, 1991) and is inhibited by high substrate concentrations.
Many of the toxic effects of ethanol on liver cell function have been ascribed to a shift in free NAD+/NADH system in the cytosol towards a more redox state (Lieber, 1991) as a consequence of the oxidation of ethanol by alcohol dehydrogenase (Fridovich, 1989). The accumulation of NADH results from ethanol oxidation by alcohol dehydrogenase to acetaldehyde, which requires NAD+ as a cofactor, and from acetaldehyde oxidation into acetate by aldehyde dehydrogenase (Peters et al., 1986; Lieber, 1988) responsible for several metabolic disturbances (Lieber and Savollainen, 1984).

Chronic ethanol consumption is associated with an increase of ethanol metabolism in man and in experimental animals (Pikkarainen and Lieber, 1980). This phenomenon was first explained on the basis of an adaptive increase in alcohol dehydrogenase activity. Whether ethanol consumption affects activities of hepatic alcohol dehydrogenase, with increases (Flora and Tandon, 1987), no changes (Caballeria et al., 1997) or even decreases reported (Julkunen et al., 1985), remains to be established.

Oxidation of ethanol by alcohol dehydrogenase generates NADH, and NADH dependent production of reactive oxygen species by various organelles is increased after chronic ethanol treatment. These acute metabolic interactions coupled to induction by chronic ethanol treatment may play an important role in the development of a state of oxidative stress in the liver by ethanol (Kukielka et al., 1994).
Alcohol dehydrogenase is a zinc-dependent metallo-enzyme, and several studies in rats have demonstrated that zinc-deficient diets produce a lower hepatic alcohol dehydrogenase activity and a decreased ethanol elimination rate (Das et al., 1984; Indo et al., 1985). Besides liver, several investigators have shown that zinc deficiency lowers the activity of alcohol dehydrogenase in many other organs also e.g. in bones, testes, kidneys and oesophagus of rats and pigs (Huber and Gershoff, 1975). Until now, the effects of the administration of zinc supplements on hepatic alcohol dehydrogenase activity and in ethanol metabolism have not been investigated.

2.3.2 Role of Aldehyde dehydrogenase

Aldehyde dehydrogenase is a NAD(P)^+ -dependent enzyme that catalyzes the oxidation of acetaldehyde to acetic acid. Although this enzyme is present in numerous body tissues, including brain, yet the liver appears to be the major site of its action. 80% of the acetic acid formed from ethanol metabolism can be recovered in the hepatic venous drainage (Koivisto and Salaspuro, 1996). Therefore, these are the major alcohol-removing enzymes in liver (Nuutinen et al., 1984).

The microsomal aldehyde dehydrogenase is tightly bound to the membrane and cannot be solubilized by sonication or extensive washing (Nakayasu et al., 1978). So it is most susceptible to oxidative damage (Hu and Tappel, 1992). The -SH groups are essential for the activity of aldehyde dehydrogenase (Woenckhaus et al., 1987). The sensitivity of
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Microsomal aldehyde dehydrogenase to lipid peroxidation was discovered only recently (Dillard et al., 1991). Aldehyde dehydrogenase is believed to detoxify potentially harmful aldehydes such as those derived from lipid peroxidation (Lindahl, 1992).

Chronic feeding of alcohol to rats decreases the activity of hepatic low Km aldehyde dehydrogenase (Jenkins and Peters, 1980; Nuutinen et al., 1983). Inhibition of aldehyde dehydrogenase due to ethanol feeding may accentuate cytotoxic effects of reactive aldehydes generated during oxidative stress. Indeed, aldehyde dehydrogenase has been suggested as a protective enzyme (Mitchell and Petersen, 1989) against acetaldehyde effects.

2.3.3 Ethanol and Drug Metabolizing Enzymes

Following the ingestion of alcohol, the metabolism of ethanol proceeds not only via cytosolic alcohol dehydrogenase but also in the microsomal fraction of the hepatocyte which comprises the endoplasmic reticulum (Lieber, 1991). Chronic ethanol consumption results in proliferation of the smooth endoplasmic reticulum of the hepatocyte which is associated with an induction of various microsomal enzymes including the microsomal ethanol oxidizing system and other drug metabolizing enzymes (Lieber, 1994). Contrasting with the inducible effect of long-term administration of ethanol is the inhibition of hepatic drug metabolism seen with short term administration (Lieber, 1982).
One mechanism of interaction of ethanol with microsomal drug metabolism system is direct competition for a common metabolic process involving cytochrome P\textsubscript{450} (Lieber, 1982). Ethanol may also interfere indirectly with microsomal drug metabolism by decreasing the supply of NADPH.

Chvapil et al. (1975) have shown that the mixed-function oxidase system is highly sensitive to Zn\textsuperscript{2+}, exhibiting an inhibition of the rate of drug oxidation and a decrease in the rate of oxidation of NADPH in the presence of micromolar quantities of Zn\textsuperscript{2+}. They suggest that the inhibition of drug metabolism by Zn\textsuperscript{2+} might be due to Zn\textsuperscript{2+}-NADPH complex formation (Ludwig et al., 1980).

### 2.3.3.1 Ethanol and Cytochrome P\textsubscript{450}

Evidence has accumulated for the participation of cytochrome P\textsubscript{450} in the hepatic oxidation of ethanol (Ingelman-Sundberg and Johansson, 1984). Cytochrome P\textsubscript{450} represents a major pathway of ethanol metabolism (Lieber, 1994). Cytchrome P4502E1 (CYP2E1) is the ethanol inducible form of cytochrome P\textsubscript{450}. This enzyme system can be involved in the generation of active oxygen species, including hydrogen peroxide, the superoxide anion radical and the hydroxyl radical (Aust et al., 1985) as well as free radicals derived from xenobiotics (Mason et al., 1982). Since liver is the primary site of metabolism of xenobiotics, it is the most susceptible organ for active oxygen and free radical-induced tissue damage.
Ethanol administration increases the activity of drug-metabolizing enzymes, cytochrome P_{450} content, and activity of the microsomal ethanol-oxidizing system (French et al., 1993; Lieber, 1994) which occurred preferentially in the smooth microsomes.

In isolated microsomes, zinc inhibits NADPH-dependent drug metabolism (Jeffery, 1983). Zinc appears to bind to cytochrome P_{450} and/or to the cytochrome P_{450}/cytochrome P_{450} reductase complex. Zinc has been proposed to exert its effect either by altering the oxidation-reduction potential of the reductase or by altering electron flow in the flavoprotein/cytochrome P-450 complex (Jeffery, 1983). Dietary zinc deficiency caused an apparent uncoupling of the NADPH-dependent cytochrome P_{450} electron transport chain (NADPH-dependent cytochrome P_{450} reductase and cytochrome P_{450}) in liver microsomes. This uncoupling has been linked to NADPH-dependent active oxygen generation in microsomes (Oprian et al., 1983; Aust et al., 1985). Active oxygen generation by uncoupling of the cytochrome P_{450} enzyme system and accumulation of iron are the possible mechanisms by which zinc deficiency causes microsomal lipid peroxidation (Hammermueller et al., 1987).

### 2.3.3.2 Ethanol and Cytochrome b_{5}

Cytochrome b_{5} is a heme protein which receives one electron from b_{5}R or NADPH-cytochrome P_{450} reductase and transfers it to other
proteins such as β-ketoacyl CoA reductase, cytochrome P<sub>450</sub> (Reddy et al., 1977), etc.

It has been shown that mitochondria are a primary target of ethanol intoxication and chronic ethanol administration to rats has been able to induce defects in the electron transport chain, coupling site I and decrease in the cytochrome level (Thayer and Rubin, 1981). However, increase in cytochrome P<sub>450</sub> and cytochrome b<sub>5</sub> contents has been reported in rats exposed to ethanol (Step et al., 1993) but their status remains to be investigated during zinc supplementation.

### 2.3.3.3 Ethanol and Cytochrome C reductases

NADPH bound to NADPH-cytochrome C reductase could be oxidized by O<sub>2</sub> yielding hydrogen peroxide and triggering an autocatalytic process that gives two O<sub>2</sub> and one HO· radicals. Recent experiments have shown that the NADPH-cytochrome P<sub>450</sub> reductase can itself catalyze the oxidation of OH· radical scavengers and ethanol, even in the absence of cytochrome P<sub>450</sub> (Winston and Cederbaum, 1983).

The ethanol oxidation depends on the concentrations of cytochrome P<sub>450</sub> and NADPH-cytochrome C reductase (French et al., 1993). Chronic ethanol feeding strikingly enhances the activity of NADPH-cytochrome C reductase in microsomes (Hammermueller et al., 1987). Other reports suggested that chronic ethanol treatment resulted in no significant changes in the activities of NADPH-cytochrome C reductase or of NADH-cytochrome C reductase (Step et al., 1993).
However, the role of zinc in conditions of ethanol toxicity has not been delineated clearly.

NADH-cytochrome C reductase consists of a flavin-linked reductase (NADH cytochrome b5 reductase) and a cytochrome b5. The enzyme activity has been detected in most intracellular membranes, including microsomes, mitochondria, nuclei, golgi, and plasma membranes (Kuwahara et al., 1978). NADH-cytochrome C reductase can interact with NADH and iron to catalyze the production of reactive oxygen species capable of initiating lipid peroxidation (Dicker and Cederbaum, 1992; McCay et al., 1992).

The activity of NADH-cytochrome C reductase got increased by 40-60% upon ethanol treatment (Kukielka et al., 1994). Intact mitochondria are impermeable to NADH and NADPH, however, the outer mitochondrial membrane contains a flavin enzyme, the rotenone-insensitive NADH-cytochrome C reductase, which is accessible to NADH. Thus NADH active via the outer membrane NADH reductase can catalyze an iron-dependent production of oxygen radicals by rat liver mitochondria which are capable of initiating lipid peroxidation (Cederbaum, 1989; Dicker and Cederbaum, 1992).

2.3.3.4 Ethanol and Glutathione-S-transferase

Glutathione-s-transferase is a family of enzymes involved in Phase II detoxification reactions and catalyze the conjugation of glutathione with products of oxidative stress, electrophiles and DNA reactive
intermediates. It is involved in ethanol metabolism, drug metabolism, free radical scavenging and reduction of peroxides (including lipid peroxides) (Meister and Anderson, 1983). It plays an important role in biotransformation of several xenobiotics (Chasseaud, 1979).

Glutathione-s-transferase is reported to have glutathione peroxidase activity (Burk et al., 1980). It is, in fact, another peroxidase that is involved in reduction of organic peroxides but not of hydrogen peroxide. It has been shown that glutathione-s-transferase is a significant inhibitor of lipid peroxidation (Burk et al., 1980).

Liver glutathione-s-transferase got induced by ethanol consumption (Hambidge et al., 1986). Activity of rat liver cytosolic and microsomal glutathione-s-transferase was increased in ethanol intoxicated group, but the mitochondrial activity was not changed. The response of glutathione-s-transferase to alcohol has been demonstrated to be dose-and duration-dependent. It has been suggested that this is an adaptational change in response to ethanol induced lipid peroxidation. However, the role of zinc, if any, in modulating the action of glutathione-s-transferase during ethanol metabolism remains to be seen.

### 2.3.4 Ethanol and Lipid Peroxidation

In lipid peroxidation, a primary reactive free radical R interacts with a polyunsaturated fatty acid to initiate a complex series of reactions that result in a variety of degradation products e.g. alkenes, malondialdehyde, lipid hydroperoxides and diene conjugates. Lipid
peroxidation may, therefore, be of major significance in cell injury produced by free radical mechanism (Halliwell, 1987; Cederbaum, 1989). A number of mechanisms of lipid peroxidation have been proposed (Aust and Svingen, 1982).

Ethanol toxicity may be associated with elevated production of reactive oxygen intermediates by the liver. The enhancement in the rate of oxygen radical and hydrogen peroxide formation in the hepatocyte cytosol appears as the primary event that leads to a peroxidative stress in chronic alcoholism (Ward et al., 1989; Fridovich, 1989; Albano et al., 1991), concentrations of antioxidants may be inadequate, especially when highly reactive oxidant species are generated in large amounts. Evidence has accumulated regarding the role of oxygen -derived free radicals and lipid peroxidation in various pathological conditions (Tribble et al., 1987), including pathogenesis of alcohol liver injury (Albano et al., 1989; Knecht et al., 1990). It is generally accepted that alcohol can induce in vivo changes in membrane lipid composition and fluidity (Schilling and Reitz, 1980) which may affect cell functions (Rottenberg et al., 1980).

It has been proposed that ethanol and/or acetaldehyde metabolism (Muller and Sies, 1987) could induce an increase in lipid peroxidation (Kamimura et al., 1992; French et al., 1993) either by enhancing the production of free radical species e.g. oxygen radicals as a result of the modification of several enzymatic functions (Ekstrom et al.,
1989; Nordmann et al., 1992) or by inhibiting or exhausting the endogenous antioxidants, involved in the defense against active oxygen species, thus leading to an oxidative stress in the liver (Dianzani, 1985). This increase in the rate of NADPH-dependent lipid peroxidation in animals receiving ethanol indicates the importance of lipid peroxidation in the production of the deleterious effects of ethanol (French et al., 1993).

The susceptibility of a given tissue to peroxidation is a function of the overall balance between prooxidant and antioxidant systems (Fink et al., 1985; Chow, 1987). Recent evidence points to lipid peroxidation as an ethanol induced process that could lead to cell injury and death or that accompanies liver injury in chronic alcoholism (Suematsu et al., 1981; Videla et al., 1982). Thus, free radical generation, lipid peroxidation and a decrease in antioxidant defenses have been implicated in the hepatotoxicity induced by ethanol (Cederbaum, 1989; Knecht et al., 1990).

Peroxidation of microsomal lipids by NADPH in the presence of iron has been shown to depend on the activity of NADPH-cytochrome P450 reductase. The induction of this activity by long-term alcohol consumption (Joly et al., 1973) may thus potentiate peroxidation.

During the metabolism of alcohol to acetaldehyde and the further metabolism of acetaldehyde by cytochrome P4502E1, toxic free radical metabolites are formed (Ingelman-Sundberg et al., 1984). Thus upon
NADH oxidation to NAD⁺, a vicious cycle can be activated leading to high lipid peroxidation observed during ethanol oxidation (Scheme-I).

**Scheme I**

Thus increase in lipid peroxidation results not only from the increased oxygen radical production by the induced 2E1 (Castillo *et al.*, 1992; Dai *et al.*, 1993), but also from the enhanced generation of acetaldehyde, which is capable of causing lipid peroxidation in isolated perfused livers (Morton and Mitchell, 1985). But the concentration of acetaldehyde required is much too high for the mechanism to be of any significance *in vivo*.

Hepatic concentrations of the antioxidant tripeptide glutathione may be reduced by ethanol consumption (Montoliu *et al.*, 1994), either by increased utilization (Shaw *et al.*, 1983) or diminished production (Lauterberg *et al.*, 1984). The decrease in either glutathione levels (Speisky *et al.*, 1985) or GSH/GSSG ratio (Valenzuela *et al.*, 1983) after ethanol treatment correlated with the degree of hepatic lipid peroxidation. Younes and Siegers (1981) reported that an inverse relationship exists between hepatic glutathione concentration and lipid...
peroxidation. The inhibition of peroxidation by glutathione may be due to the formation of conjugates with lipid peroxidation derived aldehydes or to a radical scavenging action (Esterbauer et al., 1991).

The interaction between zinc and oxygen free radicals has been reported. Zinc strongly inhibits lipid peroxidation i.e. zinc administration has a protective effect against lipid peroxidation (Cabre et al., 1995; Mutoh et al., 1995; Dhawan et al., 1995). On the other hand, dietary zinc deficiency caused an increase in liver microsomal lipid peroxidation in rats (Sullivan et al., 1980; Hammermueller et al., 1987) and a 3- to 10-fold increase in the rate of in vitro NADPH-dependent lipid peroxidation from liver (Burke et al., 1985). The role of zinc in containing lipid peroxidation during ethanol administration has not been fully dealt with.

2.3.5 Ethanol and Reduced Glutathione

Cellular oxidant defenses are necessary to prevent peroxidation of membrane polyunsaturated fatty acids by free radicals. The best studied natural antioxidant is glutathione (Meister, 1991). Glutathione is a thiol containing tripeptide (\(\gamma\)-glutamyl-cysteinyl glycine) which, in its reduced state, participates in several functions of vital importance to the cells (Chance et al., 1979). It is present in all types of living cells. Tissues such as mammalian liver normally contain high levels of glutathione (Meister, 1975) in both the mitochondrial and cytosolic compartments (Wahllander et al., 1979).
Glutathione offers one of several mechanisms for the scavenging of toxic free radicals (Meister, 1991; Halliwell, 1994). Glutathione is an important antioxidant and donor substrate for glutathione peroxidase (Chance et al., 1979) and has been reported to be actively engaged in the detoxification of heavy metals (Kang and Enger, 1988; Winterbourn et al., 1994) that involves formation of a variety of potentially harmful electrophilic compounds (Higashi et al., 1985). It maintains the proper redox state and the thiol groups of soluble, structural proteins (Deleve and Kaplowitz, 1990; Meister, 1994) and participates in the detoxification of hydroperoxides and α-oxoaldehydes (Callans et al., 1987). It functions as an intracellular redox buffer (Wendel and Akryt, 1980) and serves as a reservoir of cysteine (Cho et al., 1981). Glutathione also plays an important role in the detoxification of electrophilic metabolites of xenobiotics (Mitchell et al., 1976) and of reactive oxygen species generated during the metabolism of redox compounds (Wong and Klaassen, 1981). Therefore, perturbation of its status can impair the cell defense against toxic compounds (Tateishi et al., 1977).

It has been suggested that glutathione depletion could lead to lipoperoxidation, resulting in cell damage (Hogberg et al., 1977).

Acute ethanol administration produces a significant decrease in glutathione levels (Shaw et al., 1981; Lauterberg et al., 1984), but on the contrary, after chronic ethanol ingestion, glutathione levels were found to increase (Kawase et al., 1989), whereas variable results were reported
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depending, in part, on species (Harate et al., 1983; Morton and Mitchell, 1985). This change could be interpreted as an adaptation to the increase in free radical activity by ethanol.

Under normal conditions, the GSH/GSSG ratio of cells is generally kept high, but when oxidative conditions occur, this ratio decreases (Bartoli and Sies, 1978). Thus, the enhanced oxidation of GSH to GSSG could be the consequence of increased generation of pro-oxidant free radicals leading to an oxidative stress in livers of alcohol fed rats (Videla and Guerri, 1990; Montoliu et al., 1994).

Chronic alcohol consumption is associated with a decrease in the hepatic content of glutathione (Situnayake et al., 1990; Nordmann et al., 1992; Richard et al., 1992). This decrease is concomitant with an enhancement of lipoperoxidative processes (Videla et al., 1980). Vina et al. (1980) reported that this effect is due to acetaldehyde. Contradictory reports are also available which shows that liver glutathione concentration tends to increase (Shaw et al., 1983), decrease (Guerri and Grisolia, 1980) or remain unchanged (Sato et al., 1981) after chronic ethanol consumption.

2.3.6 Ethanol and Antioxidative Enzymes

Antioxidants are needed to scavenge and prevent the formation of free radicals such as superoxide and hence the tissue damage (Halliwell, 1994). Antioxidant protective mechanisms involve both enzymatic and non-enzymatic defense systems (Tribble et al., 1987). Although many
studies have been performed to assess the response of superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase to ethanol challenges, there is no clear consensus concerning the nature of the response and the mechanisms responsible.

2.3.6.1 Ethanol and Catalase

Catalase forms an integral component of the cellular antioxidant defense mechanism. Catalase converts hydrogen peroxide to water and oxygen:

\[ 2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \]

A decrease in catalase activity was observed in the alcoholic liver injury (DeCremer et al., 1991). The observed decrease in cytosolic catalase is the first detectable effect of acute ethanol administration on the protective set of enzymes against oxygen toxicity. In another study, a positive correlation was found between alcohol consumption and catalase activity in humans (Chen et al., 1995). It appears that response of catalase to ethanol administration is variable, depending upon the species of the animal.

2.3.6.2 Ethanol and Superoxide dismutase

Superoxide dismutase catalyzes the dismutation of superoxide free radical (\(O_2^-\)) to hydrogen peroxide and oxygen (Taylor et al., 1988) according to the reaction:

\[ O_2^- + O_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \]
Because superoxide dismutase enzymes generate hydrogen peroxide, they work in collaboration with hydrogen peroxide removing enzymes, i.e., catalase and glutathione peroxidase.

Plasma activities of superoxide dismutase and glutathione peroxidase are higher in alcoholics. However, data from literature concerning alcohol-related variations of antioxidant enzymes are very confusing (Guemouri et al., 1993). Some animal studies have shown that ethanol intake reduces the activities of the enzymatic antioxidant components, such as superoxide dismutase and catalase, in rat liver (Ribiere et al., 1983).

It is clear that cytotoxicity of molecular oxygen is held in check by the delicate balance between the rate of generation of partially reduced oxygen species and the rate of their removal by different defense mechanisms and any shift in this delicate balance can lead to cellular damage. The decreased activity of these enzymes in the hepatic tissue suggests increased damage to this tissue as a result of uncontrolled generation of partially reduced oxygen species.

Both copper and zinc are known to be important prosthetic groups for many metalloenzymes including superoxide dismutase (a Cu, Zn-containing oxygen radical scavenger). Thus, any alteration in the homeostasis of these essential trace metals can also be detrimental to the activity of the enzyme (Sharma et al., 1991). Thus, as a structural component of Cu, Zn-superoxide dismutase, zinc is directly related to
the activity of the free radical destroying enzyme. It was also reported that severe zinc deficiency could lead to a significant decrease in hepatic Cu, Zn-superoxide dismutase (Cao and Chen, 1991).

2.3.6.3 Ethanol and Glutathione peroxidase

Glutathione peroxidase removes hydrogen peroxide by converting reduced glutathione to oxidized glutathione:

\[ 2\text{GSH} + \text{H}_2\text{O}_2 \rightarrow \text{GSSG} + 2\text{H}_2\text{O} \]

\[ 2\text{GSH} + \text{FA-OOH} \rightarrow \text{GSSG} + \text{FA-OH} + \text{H}_2\text{O} \]

Glutathione peroxidase can also destroy fatty acid (FA-OOH) peroxides by converting them to alcohols (FA- OH).

The hepatic metabolism of certain drugs, carcinogens and endogenous lipids may result in the release of reactive free radicals capable of initiating lipid peroxidation in cellular membranes (Mitchell et al., 1982). Lipid peroxidation of cellular membranes would cause extensive cellular damage unless it gets checked by the implication of certain protective agents including enzymes such as glutathione peroxidase. Glutathione peroxidase has been reported to be more efficient than either superoxide dismutase or catalase in preventing lipid peroxidation (McCay et al., 1981). Further, glutathione peroxidase is a more efficient metabolizer of hydrogen peroxide than catalase.

Chronic ethanol ingestion has been reported to result in decrease (Ribiere et al., 1983) or increase (Oh et al., 1997) or no change in hepatic glutathione peroxidase activity.
2.3.6.4 Ethanol and Glutathione reductase

Glutathione reductase (GR), a flavoprotein (FAD-containing enzyme), catalyzes the regeneration of reduced glutathione from oxidized glutathione:

\[
\text{GR} \quad \text{GSSG} + \text{NADPH} + \text{H}^+ \rightarrow 2\text{GSH} + \text{NADP}^+
\]

In another study, liver glutathione reductase activity was found to increase significantly after chronic ethanol intake (Teschke et al., 1977). This rise in glutathione reductase activity was suggested to represent an adaptive change against lipid peroxide toxicity (Videla and Valenzuela, 1982).

2.4 Ethanol Effects on Intestine

Excessive alcohol intake causes injury to gastrointestinal tract mucosa (Lieber et al., 1982). However, the detailed cellular mechanism underlying the damage it causes to the gastrointestinal mucosa remains to be fully elucidated. During ethanol intake, the upper gastrointestinal tract is exposed to concentrations of ethanol several times higher than those attained in other body tissues (Beck et al., 1981). Chronic ethanol ingestion by animals is known to produce a variety of structural and functional alterations in small intestine (Persson, 1991; Kaur et al., 1994) i.e., ethanol ingestion is associated with morphological and biochemical alterations of the small intestine in several mammalian species. Exposure of intestinal surface to ethanol results in
morphological injuries, aberration in brush-border enzyme activities, chemical composition, fluidity of microvillus membrane and an impairment in the absorption of nutrients in intestine (Beck and Dinda, 1981). Both acute and chronic ingestion of alcohol commonly cause gastrointestinal symptoms such as nausea, vomiting, abdominal cramps, and diarrhea (Burbige et al., 1984). Also, there is an association between alcohol misuse and an increased incidence of cancers involving upper alimentary tract (Tuyns, 1983; Lieber et al., 1986) and respiratory tract (Lieber et al., 1986).

It is widely accepted that ingested alcohol is almost completely absorbed from the upper gastrointestinal tract and metabolized in the liver. It has been proposed that the liver is impotent with regard to the first pass metabolism of alcohol (Levitt et al., 1994) and that virtually all such metabolism occurs in the gastric mucosa (Caballeria et al., 1987; 1989). In the last few years, increasing evidences have suggested that a fraction of alcohol ingested is oxidized in the stomach. This phenomenon is known as first pass metabolism of ethanol, and the magnitude of this gastric oxidation may modulate the bioavailability of alcohol and thus, its hepatic and systemic toxicity (Caballeria, 1992). Gastric first pass metabolism of ethanol is an important determinant of blood alcohol concentrations in both experimental animals (Julkunen et al., 1985) and humans (Caballeria et al., 1989).
In the past decade, several studies have demonstrated that a significant fraction of the alcohol ingested is oxidized in the stomach by alcohol dehydrogenase present in the gastric mucosa. Moreover, it has been demonstrated that the human stomach has alcohol dehydrogenase isozymes capable of oxidizing ethanol in the entire range of concentrations likely to occur in the stomach after drinking and that the total activity is sufficient to account for first pass metabolism (Hernandez-Munoz et al., 1990; Moreno et al., 1991). The protective barrier against the systemic toxicity of alcohol has been attributed to oxidation of ethanol in the stomach by the alcohol dehydrogenase activity found in the gastric mucosa (Hernandez-Munoz et al., 1990).

Alcohol dehydrogenase is the conspicuous—although not necessarily the only—system capable of metabolizing ethanol in the gastric mucosa. Alcohol dehydrogenase is abundant in the mucus-producing epithelial cells lining the mucosa of the human stomach, with somewhat less activity in the parietal and chief cells (Maly et al., 1992). In contrast to the activity of liver alcohol dehydrogenase, which is maximal at low concentrations of ethanol and is inhibited by higher concentrations of the substrate, the gastric alcohol dehydrogenase of human subjects shows a second peak of activity at high ethanol concentrations (Hernandez-Munoz et al., 1990). Thus, the activity of gastric alcohol dehydrogenase correlates with the first pass metabolism of ethanol (Frezza et al., 1990). In chronic alcoholics, first pass metabolism of
ethanol is diminished (DiPadova et al., 1987), which may be due to lower gastric alcohol dehydrogenase activity as demonstrated in humans and in rats (Julkunen et al., 1985). The decrease in gastric alcohol dehydrogenase activity is most likely the consequence of gastric mucosal injury (Chey, 1972). This alcohol dehydrogenase activity and the consequent first pass metabolism have been shown to be affected in humans by gender (Frezza et al., 1990), by chronic alcoholism (DiPadova et al., 1987; Seitz et al., 1990) and in males by age (Seitz et al., 1990).

Some studies have shown that zinc-deficient diets produce a lower hepatic alcohol dehydrogenase activity and may result in higher blood ethanol levels and decreased ethanol elimination rate (Das et al., 1984). On the contrary, the effects of administration of zinc supplements on the hepatic and/or gastric alcohol dehydrogenase activities and in ethanol metabolism have not been investigated.

2.4.1 Ethanol and Drug Metabolism

In addition to being the site of absorption, the intestine may play an important role in the first pass metabolism of ingested toxins, and several drugs (Kolars et al., 1991; Kaminsky et al., 1992). Although liver contains the greatest cytochrome P450 content, many extrahepatic tissues such as kidney, lung, and alimentary tract also have significant levels of P450 (Hakkak et al., 1993). CYP2E1 is a microsomal P450 isozyme and is reported also to be present in small intestine (Shimizu et al., 1990). Indeed, small intestinal microsomes from alcohol-treated rats show
enhanced ethanol oxidation (Seitz et al., 1979). Furthermore, intestinal cytochrome P<sub>450</sub> isozymes are important in first pass metabolism of several drugs (Kolars et al., 1991) and may play an important role in the activation of and detoxification of dietary carcinogens (Kaminsky et al., 1992). The capacity of the ethanol to increase cellular levels, and hence the activities of oxidative drug-metabolizing enzymes in endoplasmic reticulum, has been evoked to explain the injury and cancers noted.

2.4.2 Ethanol and Antioxidants

Free radicals may be common biochemical mediators of gastric mucosal injury induced chemically (Pihan et al., 1987). With regard to the susceptibility of gastrointestinal damage induced by ethanol, the body of the stomach is particularly vulnerable to injury, while the intestinal mucosa is considerably more resistant (Soll, 1989). It has been demonstrated that ethanol affects rat gastric and duodenal mucosal antioxidants in a dose-dependent manner. Generally, the high concentration of ethanol was capable of inducing gastric mucosal lesion formation that was associated with decreases in endogenous antioxidant components, notably the levels of reduced glutathione (Miller et al., 1985; Speisky et al., 1985), and the activity of the glutathione-regenerating enzyme, and increases in the activity of the glutathione-utilizing enzyme, glutathione peroxidase. Gastric glutathione level was reduced following ethanol consumption (Shaw et al., 1990). The role of glutathione as an endogenous gastric antioxidant in mucosal protection,
however, remains controversial since recent evidence has indicated an inverse correlation between gastric mucosal glutathione levels and mucosal protection (Robert et al., 1985). The observed increases in the activities of glutathione peroxidase in gastric mucosa of rats treated with ethanol make it unlikely that the decrease in glutathione content was a consequence of generalized tissue necrosis.

In both humans and animals, it has been shown that ethanol-induced gastric ulceration is associated with a significant reduction in non-protein sulfhydryl group levels in the stomach (Loguercio et al., 1991). One possible mechanism for this reduction may be direct inhibition of glutathione synthesis by alcohol (Speisky et al., 1985). Furthermore, animal studies have shown that inhaled ethanol reduced the activities of enzymatic antioxidant components, such as superoxide dismutase and catalase (Ribiere et al., 1983). In contrast to the situation with high concentrations of alcohol, low concentrations did not lead to formation of mucosal damage (Moghadasian and Godwin, 1996). This lower concentration of ethanol caused increases in gastric and duodenal antioxidant capacities, and this might contribute to the adaptive cytoprotection reported to be associated with low doses of ethanol. The short-term damaging effect of free radicals have traditionally been thought to be due mainly to their interaction and modification of unsaturated fatty acids leading to membrane disorganization (Halliwell and Gutteridge, 1985). More recently, however, attention has been paid
to the possibility that free-radical damage may be mediated by alterations in proteins, mainly enzyme (Comporti, 1985), or by functional damage induced at a distance by soluble, highly toxic products of lipid peroxidation (Comporti, 1985). Lipid peroxidation also plays an important role in ethanol-induced damage to gastric mucosa (Halliwell and Gutteridge, 1989; Esterbauer et al., 1991), as determined by measuring gastric mucosal enaldehyde levels, the biochemical markers of lipid peroxidation.

2.5 Zinc

Trace metals are essential for normal growth and reproduction of animals. Zinc is an essential trace element and is vital to life (Vallee et al., 1993). It has proven to be an essential nutrient for a variety of animal species (Hambidge et al., 1986), plants (Mengel and Kirkby, 1987) and micro-organisms (Cousins, 1985). It is a II B element with a complete d-subshell and two additional electrons. It is distinct from other essential trace metals in that it has only one valence state. When chemically combined it is always in +2 oxidation state, which is retained during biological reactions (Riordan, 1976).

Zinc is relatively non toxic and has many important and diverse functions in human metabolism. This divalent cation is involved in many biological functions (Prasad, 1982).

One of the main biochemical roles of zinc is its influence on the activity of several well characterized enzymes (Fasman, 1977). Till date,
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in micro-organisms, plants and animals, over 300 enzymes have been identified representing more than 50 different types, known to require zinc for their metabolism and growth (Golden, 1989). These include oxido-reductases, transferases, hydrolases, lyases, isomerases, ligases, dehydrogenases, aldolases, peptidases and phosphatases (Dixon and Webb, 1979). Indeed, zinc is the only metal encountered in each of the six enzyme classes established by the International Units of Biochemistry (Hambidge et al., 1986). Some of these enzymes are influenced by dietary zinc status, whereas others are refractory to alterations in the dietary zinc supply (Bettger and O'Dell, 1981). The level of zinc in cells may, therefore, control the physiological processes through the formation and regulation of the activity of zinc dependent enzymes.

Zinc may exert some of its physiological effects by interacting with biomembranes (Pasternek, 1987) and intracellular compartments (Cousins, 1985) i.e. by acting directly or indirectly on cellular membranes either by altering permeability or by modulating the activity of membrane bound enzymes (Rodriguez et al., 1995). The observed physiological effects of Zn\(^{2+}\) may be related to competition between Zn\(^{2+}\) and several other divalent cations, such as Cd\(^{2+}\), Cu\(^{2+}\) and Ca\(^{2+}\) (Hill, 1976). Zinc has also been reported to interact with cell membranes and to stabilize them against various damaging effects, including those of oxidative stress (Bettger and O'Dell, 1981). Thus zinc has a general
stabilizing effect on organelles and macromolecular complexes. Some investigators have argued that reduction in the concentration of zinc in biomembranes leads to some of the disorders (Bettger et al., 1978). Loss of zinc from the membrane results in an increased susceptibility to oxidative damage which further results in structural strains, alterations in specific receptor sites and transport systems.

Involvement of Zn$^{2+}$ in growth and development processes, such as reproduction, respiration, digestion, and on the proper function of the brain, nerves and vision is well documented (Mills, 1989). Zn$^{2+}$ participates in protein, nucleic acid, carbohydrate and lipid metabolism as well as in the regulation of gene expression (Wu and Wu, 1987), as a second messenger (Grumuit et al., 1986), as a protective or trigger agent in molecular storage system and many other fundamental processes. Zinc helps to stabilize the structures of RNA, DNA and ribosomes (Clegg et al., 1989). Zinc is well known to be required for development, differentiation and gene expression. It is an integral component of RNA polymerase and has recently been shown to be an essential component of numerous transcription factors (Vallee and Auld, 1990). The presence of zinc ions are essential to various lymphocyte functions (Srinivas et al., 1988). Zinc has gained wide importance because of its participation as an acidic metal in enzyme catalysis (Frausto and Williams, 1991) and as an intracellular regulator through its participation in protein-oligonucleotide interaction (Klug and Rhodes, 1987). The interactions of
Zn$^{2+}$ with macromolecules appear to suggest its participation in cellular processes such as the cell cycle (Chester et al., 1991), antioxidant defenses (Bray and Bettger, 1990), gene expression and DNA stability (Zalewski et al., 1993).

### 2.5.1 Metabolic Role of Zinc

In biological systems, very little, zinc exists in the free form. It carries out its functions as a divalent cation primarily when bound to enzymes and proteins. The total zinc concentration in serum or plasma amounts to 10-23 μM (Foote et al., 1984). About 99% of zinc in the plasma is protein bound (Parisi and Vallee, 1970), about 85% loosely bound to albumin (Masuoka et al., 1993) and 15% tightly bound to α2-macroglobulin (Faure et al., 1990). The remaining 1% is ultrafilterable, and bound to low molecular weight compounds such as transferrin, ceruloplasmin, heptoglobin and γ-globulins (Giroux and Henkin, 1972).

Because of its unique chemistry, zinc has been associated with three enzymes: catalytic (e.g., carbonic anhydrase), co-catalytic (e.g., Cu, Zn-superoxide dismutase) and structural (e.g., fructose biphosphatase) (Williams, 1984; Vallee and Auld, 1992).

Two classes of zinc proteins, the metallothioneins and the gene regulatory proteins, have been recognized and have been studied extensively. Metallothionein has long been known to have an extraordinary metal content (Vallee, 1987), but its function has
remained elusive. It has been thought to detoxify heavy metals, stabilize membranes, or regulate zinc and copper metabolism and has also been suggested to be a free-radical scavenger (Krezoski et al., 1988).

The normal zinc content of a 70 kg male is 1.5-2.0 g and an average requirement is 1-15 mg zinc per day. Approximately 20-30% of the ingested dietary zinc is absorbed from jejunum and ileum (Lee et al., 1989).

Zinc is present in all organs, tissues, fluids, and secretions of the body. Zinc is primarily an intracellular ion and over 95% of the total body zinc is found within cells. Zinc is associated with almost all organelles of the cell, but about 60-80% of the cellular zinc is present in the cytosol and microsomes (Smeyers-Verbeke et al., 1977). Most tissues contain between 0.01 and 0.2 mg zinc/g wet weight. The largest concentration of zinc has been found in liver, kidney, retina, prostrate and muscle.

The liver plays a special role in zinc metabolism. Zinc metabolism in animals has been shown to be regulated homeostatically. Although the control mechanisms involved have not been established, it is clear that the body stores of zinc influence the amount of dietary zinc that is taken up by the mucosal epithelium, transferred to the plasma, and subsequently deposited in the liver and other tissues to meet cellular needs.
Zinc, as a trace element, is regarded as an essential nutrient for human beings. Deficiency of zinc produces several pathological disorders, both in laboratory animals and humans (McClain et al., 1985), involving abnormalities of its metabolism. This can be due to inadequate dietary intake i.e. poor availability of the metal ion in the diet, increased requirements and excretion, defective absorption, conditioned deficiency or genetic causes. Plasma levels of zinc declines in simple human experimental zinc deficiency. The decrease in plasma zinc concentration represents a redistribution to some tissues, particularly the liver and bone marrow (Dunn and Cousins, 1989). Clinical signs of zinc deficiency are manifested only when supply of zinc is limited and when growth or repair is rapid or has accelerated following tissue damage (Golden, 1989). Growth retardation, hypogonadism in the males, poor appetite, mental lethargy and skin changes are the classical features of chronic zinc deficiency (Underwood, 1977).

Depletion of zinc may lead to metabolic effects, resulting in inhibition of cell growth and proliferation. On the other hand, exposure to high zinc concentrations will affect cell membrane integrity and cellular metabolism (Burch and Sullivan, 1976) and may also result in cell death. Marginal zinc deficiency may be related to the development and/or progression of numerous chronic degenerative diseases,
increased oxidative damage resulting from zinc deficiency has been proposed as a possible mechanism for several pathogenic pathways (Lovering and Dean, 1990; Oteiza et al., 1996).

Wong and Klaassen (1981) found that zinc increased glutathione levels in rat liver. Depletion of hepatic glutathione (Bose et al., 1994) has also been suggested by some workers. Liver is the major site of zinc accumulation. This metal increased the metallothionein concentration and reduced glutathione levels in the liver at non-lethal doses (Bose et al., 1994). Glutathione binds to foreign compounds and their metabolites which are ultimately excreted resulting in decreased concentration of this tripeptide following zinc administration.

Zinc acts as an indirect antioxidant via stabilization of cell membranes and inhibition of free radical production. Chvapil et al. (1972) reported that one of the possible mechanisms by which zinc stabilizes a variety of biomembranes both in vivo and in vitro was related to the inhibition of membrane lipid peroxidation. The antioxidative effect of dietary zinc may involve the fact that zinc status of the animals affects free radical metabolism.

As a structural component of Cu, Zn-superoxide dismutase, zinc may also be directly related to the activity of the free radical destroying enzyme. Recently, extracellular superoxide dismutase activity has been shown to be highly sensitive to dietary zinc intake and plasma zinc
concentration in rats and rhesus macaques (Olin et al., 1995). Others have reported that Cu, Zn-superoxide dismutase activity in zinc deficiency was unchanged in rat liver (Taylor et al., 1988). However, other workers reported that severe zinc deficiency could lead to a significant decrease in hepatic Cu, Zn-superoxide dismutase activity (Cao and Chen, 1991), accompanied by increased production of peroxidated lipids. Seagreave et al. (1983) reported that zinc had no consistent effect on glutathione peroxidase and glutathione reductase activity.

Zinc is known to protect fatty acids from peroxidation by inhibiting the production of reactive oxygen species by transition metals. Therefore, zinc strongly inhibits lipid peroxidation (Camps et al., 1992). However, dietary zinc deficiency caused an increase in liver microsomal (Davies et al., 1985) and mitochondrial (Fields et al., 1984) lipid peroxidation (Faure et al., 1991; Coudray et al., 1991).

Zinc ions also inhibit electron transport in both mitochondrial and microsomal electron transport chains. Zinc inhibits NADPH-dependent drug metabolism (Jeffery, 1983). Zinc appears to bind to cytochrome P450 and/or to cytochrome P450/cytochrome P450 reductase complex. Zinc has been proposed to exert its effect either by altering the oxidation-reduction potential of the reductase or by altering electron flow in the flavoprotein/cytochrome P450 complex. Dietary zinc deficiency has also
been shown to cause an increase in NADPH-dependent hydrogen peroxide production in the microsomes of both lung and liver as a result of uncoupling of the cytochrome P₄₅₀ electron transport system.

2.6 Zinc-Ethanol Interactions

One of the first elements which was related to alcoholic liver disease is zinc. A marked decrease in zinc levels was found in the liver, kidney, heart and lungs of the rats treated with alcohol alone (Sharma et al., 1991). It has also been reported that chronic alcoholics have altered zinc homeostasis. Ethanol also appears to have a direct effect on zinc metabolism (Assadi et al., 1986), although data from different laboratories has been conflicting. Abnormalities in zinc metabolism are common in patients with chronic alcoholism (McClain and Su, 1983). Ethanol may alter zinc metabolism via induction of metallothionein in various tissues (Waalkes et al., 1989).

Patients with alcoholic cirrhosis are often characterized by reduced serum zinc levels, increased urinary zinc excretion and low zinc levels in body tissues (Russell, 1980; Solomons and Jacob, 1983; Dinsmore et al., 1985). But there are also reports demonstrating that alcohol consumption does not affect urinary zinc loss. In addition, decreased hepatic zinc concentration (Mills et al., 1983) and leucocyte zinc content (Keeling et al., 1980) have also been demonstrated, despite an increase in whole body zinc content. Keeling and coworkers (1981)
attributed this to impaired hepatic excretion of zinc. These findings suggest that alcoholic cirrhosis may be associated with a state of zinc deficiency (Weisman et al., 1980). This derangement of zinc metabolism may be a possible factor responsible for the widespread deleterious effects of chronic alcoholism in the body. Thus, alcohol is known to have an adverse effect on zinc nutrition, with hypozincemia associated with hyperzincuria noted in alcoholic patients (Gordon et al., 1981).

The existence of chronic zinc deficiency in liver cirrhosis might lead to pathological changes in the intestinal mucosa. Malabsorption of zinc by the gut in cirrhotic patients might also aggravate the zinc deficiency (Karayalcin et al., 1988).

Possible mechanisms for the ethanol effect on zinc excretion may be an increased release of cellular zinc into the circulation which is subsequently lost at the kidney glomerulus, or a change in the binding characteristics of zinc in circulation resulting in more diffusible zinc which is then lost at the kidney.

It has also been postulated that alcoholism might lead to zinc deficiency, which in turn may result in decreased activity of two hepatic zinc metalloenzymes, alcohol dehydrogenase and glutamate dehydrogenase, thus possibly rendering the liver more susceptible to damage from continued alcohol consumption.
The prolonged ingestion of alcohol sufficient to produce severe liver damage affects the handling of zinc in the intestinal tract. Intestinal absorption of zinc, whole body zinc content and total daily loss of zinc from the body, have all been demonstrated to be increased in alcoholic cirrhosis. The increase in zinc absorption is matched by an increase in the zinc elimination, but no change in the rate of zinc turnover. There are few studies which indicate a significant reduction in the absorption of zinc in the alcoholic patients compared with controls (Dinsmore et al., 1985). This could be caused by a direct reduction in the amount of zinc available for absorption or be related to intestinal epithelial changes that occur with alcohol consumption (Hillman, 1975).

It is known that ethanol exposure leads to zinc mobilization from various tissues (Ahmed and Russell, 1982) and this, in turn, may lead to zinc accumulation in the liver.

Zinc compounds are also known to prevent gastric mucosal lesions against various injurious agents, including ethanol (Cho et al., 1985; Wong et al., 1986). This suggests that zinc ions can stabilize the cell membrane (Pfeiffer et al., 1987; Mutoh et al., 1995) and strengthen the mucosal defensive mechanism. As oxygen free radicals have been demonstrated to play an important role in the formation of gastric lesions in vivo (Parks et al., 1983; Itoh and Gutt, 1984), it is likely that zinc ions could scavenge oxygen free radicals and protect against gland

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damage. The other possibility of the mechanisms by which metal ions protect gastric mucosal cells against ethanol induced gastric cell damage might be that metal ions stabilize various biomembranes by inhibiting lipid peroxidation of biomembranes (Mutoh et al., 1995).

It has been shown that pharmacological intakes of zinc can produce antioxidant-like function. The best evidence for this comes from studies describing the protective effect of zinc against acute ethanol toxicity (Dar et al., 1986; Florescheim et al., 1987). Studies have demonstrated that zinc administration decreased liver fibrosis in ethanol exposed rats (Gimenez et al., 1990).

2.6.1 Trace Metals, Zinc and Ethanol

Currently, excessive consumption of ethanol is recognized as among the most prevalent known causes of abnormal human development (Begleiter, 1980). Metabolism and toxicity of heavy metals may be influenced by ethanol or its metabolites (Goyer et al., 1972), which are capable of producing a secondary nutritional deficiency by interfering with the metabolism of essential nutrients, including trace metals, through effects on absorption, tissue redistribution or excretion. Because ethanol diffuses readily across all biological membranes except skin, it can be absorbed from all parts of the gastrointestinal tract, lungs, urinary bladder and pleural cavities. After absorption, ethanol diffuses very rapidly across capillaries and other membranes so that it
ultimately distributes uniformly throughout the whole body (Flora and Tandon, 1987).

Various divalent metals protect against ethanol-induced gastric mucosal injury. This effect is of long duration with cadmium, copper and zinc. These divalent metals oxidize or bind to SH groups (Friedman, 1973) and decrease ethanol-induced gastric mucosal cell damage in vivo. The possible mechanisms by which metal ions protect gastric mucosal cells are: (i) metal ions stabilize membranes of cultured gastric mucosal cells against ethanol and interfere with ethanol's interactions with the gastric cells; (ii) metal ions suppress the activation of a number of enzymes producing oxygen free radicals by ethanol, and (iii) metal ions stabilize various biomembranes by inhibiting lipid peroxidation of biomembranes (Mutoh et al., 1995).

Zinc, a divalent cation, is involved in many biological functions (Prasad, 1982). It is known to compete with cadmium, lead, copper, iron and calcium for similar binding sites (Hill, 1976). Therapeutic use of zinc is known to produce hypocupremia in human subjects (Prasad et al., 1978). In liver and kidney both zinc and copper concentration decreases rapidly resulting in copper-deficiency anemia (Nomiyama et al., 1984). Increased urinary losses of calcium and reduced absorption of calcium from intestine have also been observed in alcoholics (Bjorneboe et al., 1981). Several studies have also indicated that calcium exerts its
antagonistic effect on zinc absorption (Heth et al., 1965). Sodium ions provides a driving force needed for the active transport which is dependent upon Na⁺/K⁺-ATPase. Previous studies in animals and humans have found sodium and potassium deficiency following chronic ethanol ingestion (Anderson et al., 1980).

In alcoholics, serum concentration of zinc was diminished, whereas level of copper was either increased (Hartoma et al., 1977) or normal (Sullivan et al., 1979) in the alcoholics (Bjorneboe et al., 1988). The reduced levels of these trace elements may have other clinical implications in alcoholics.

2.7 **65Zn Uptake and Retention Studies**

The tissue deposition and mobilization of zinc are affected by a number of pathophysiological processes. The extensive study about the biological half-lives of zinc could be useful for understanding the homeostatic mechanisms of zinc in the body.

The kinetics of zinc metabolism in humans have been studied using radiotracers (Babcock et al., 1982; Wastney et al., 1986). Radiotracer studies using ⁶⁵Zn indicated that the tissue uptake of absorbed zinc varies, with liver storing the maximum amount. The zinc concentrations in liver have been reported to reach peak values within 3-4 h of its administration, while it got accumulated relatively slower in other tissues (Methfessel and Spencer, 1973). ⁶⁵Zn accumulates slowly.
e.g., in the cellular components of the blood, the femur and the muscle. Methfessel and Spencer (1973) also showed that the turnover rate of $^{65}\text{Zn}$ in liver and pancreas is rapid and these tissues exhibit a significant decrease in $^{65}\text{Zn}$ concentration at 24 h.

Wastney et al. (1986) carried out a detailed analysis of the zinc kinetics by oral and intravenous administration of $^{65}\text{Zn}$ in adult subjects and identified five major sites where zinc metabolism was regulated, namely, absorption at the gut, excretion in urine, exchange with erythrocytes and muscles and secretion into the gut.

Lowe et al. (1991), in their experiments, found that $^{65}\text{Zn}$ mainly was distributed between two kinetic pools after injection into the body which included the initial plasma pool ($Q_a$) and second, slowly exchangeable, liver zinc pool ($Q_b$). The dynamic balance between the two pools could be affected by a number of pathophysiological processes that altered the systemic distribution, tissue deposition and mobilization of zinc, thus, stepping up the body's self regulatory defense mechanism.

Zinc absorption in humans can be measured with great precision using $^{65}\text{Zn}$ and subsequent measurements of the retained isotope in different tissues can be done using a whole-body counter (Arvidsson and Cederblad, 1978). When the measurement of absorption is combined with the determination of the body retention of $^{65}\text{Zn}$, it is possible to estimate the initial loss of $^{65}\text{Zn}$ from the body. Payton et al. (1982)
observed that a smaller proportion of zinc is absorbed from high test doses than from low test doses and they concluded that a rate limiting mechanism existed for the absorption of zinc. Studies on rat indicated that body-zinc was regulated to a greater extent by the rate of intestinal excretion than by the rate of absorption of dietary zinc (Weig and Kirchgessner, 1980).

Feeding of a zinc salt accelerated the loss of $^{65}$Zn without affecting the partitioning of the isotope among the organs or the organelles. Moreover, the specificity of the zinc pathway through the body was disturbed when stable zinc was injected and the normal absorption was bypassed. The homeostatic control mechanism of zinc metabolism acting at the sites of absorption and endogenous excretion is very sensitive and operates only when the absorptive as well as the excretory mechanisms are functioning together (Cotzias et al., 1962). The injected radiozinc initially combines with the plasma proteins to form a plasma zinc and then slowly combines to other cellular components of the blood. Zinc is transported initially to the soft tissues of the body and ultimately to feces via small intestine (Pekas, 1966). $^{65}$Zn is also observed to be rapidly transported from the plasma through the placenta.

Abnormalities in zinc metabolism are common in patients with chronic alcoholism (McClain et al., 1983). Milman and coworkers (1983) and Mills et al. (1983) found that the intestinal absorption of an oral dose
of $^{65}\text{Zn}$ was increased in alcoholic cirrhosis. This low intestinal zinc absorption contributes to the low hepatic zinc concentration (Valberg et al., 1985). Thus it appears that the prolonged ingestion of alcohol sufficient to produce severe liver damage affects the handling of zinc in the intestinal tract.