

## Alcohol-Induced Liver Injury

The liver is a vital organ, involved in the processing of fats, sugars, proteins, and vitamins and in the regulation of blood clotting. It plays a central role in the body's defenses, filtering toxins and microbes from the blood and marshaling an array of responses to trauma, stress, or inflammation (Hill et al. 1997).

Although the liver is capable of regeneration and repair, severe liver disease must be viewed as life threatening. Long-term heavy alcohol use is the leading cause of illness and death from liver disease in the United States. The number of persons with alcoholic liver disease is conservatively estimated at more than 2 million (Dufour et al. 1993).

There are three phases of alcoholic liver disease: fatty liver, which is usually reversible with abstinence; alcoholic hepatitis, or liver inflammation; and cirrhosis, or scarring of the liver. Patients frequently have more than one type of liver disease, such as coexisting fatty liver and alcoholic hepatitis or alcoholic hepatitis together with cirrhosis. Patients with both cirrhosis and alcoholic hepatitis have a death rate of more than 60 percent over a 4-year period, with most of those deaths occurring within the first 12 months of diagnosis (Chedid et al. 1991). This prognosis is bleaker than the outlook for many types of cancer.

The major problem in developing new therapies for alcoholic liver disease has been a lack of understanding of the mechanisms for liver injury. While many people who drink heavily do not develop liver disease, others seem to be highly susceptible to the disease, suggesting a probable role for nutritional, environmental, and hereditary factors.

This section examines current research on one class of potential initiators of liver cell injury and repair, the cytokines. It also discusses recent studies on nutritional variables associated with liver disease and on three other liver disorders

that may affect the course of alcoholic liver disease. Researchers are hopeful that these current investigations will lead to new insights into the mechanisms of alcoholic liver disease and will provide a basis for the development of new forms of therapy.

### Alcoholic Liver Disease

#### The Role of Cytokines

One of the liver's most important responses to trauma or stress is the production of cytokines. These small molecules act as chemical messengers, regulating cellular interactions and functions. Cytokines play an important role in cell-to-cell communication during normal metabolism, and they are the primary chemical messengers during periods of inflammation or infection. The signaling pathways are extremely complex, but current studies are progressing toward a better understanding of these pathways.

Cytokines are produced by many different cell types. Specialized cells within the liver, the Kupffer cells, are major producers of certain cytokines. Other types of liver cells, producing different cytokines, are the hepatocytes, stellate cells, and endothelial cells. The Kupffer cells are macrophages, one of several types of phagocytic white blood cells that engulf and destroy foreign substances; stellate cells are fat-storing cells; hepatocytes are the major functional cells of the liver, acting to process and store nutrients, secrete bile, and remove toxins from the blood; and endothelial cells line the liver's blood vessels.

The Kupffer cells produce the cytokines interleukin-1 (IL-1), tumor necrosis factor alpha (TNF- $\alpha$ , or TNF), and interleukin-6 (IL-6), all of which promote inflammatory responses (Hill et al. 1997). The stellate cells produce and are activated by transforming growth factor beta (TGF- $\beta$ ), which plays an important role in production of collagen (a connective tissue

protein) and in liver fibrosis (the formation of scar tissue) (Hill et al. 1997). This process leads to the scarring of the liver in cirrhosis. Hepatocytes produce the cytokine interleukin-8 (IL-8), which attracts neutrophils to the site of an infection, where they adhere to cell surfaces. Neutrophils, which circulate in the blood, are another type of phagocytic white blood cell. Endothelial cells produce adhesion molecules, which act by increasing the adhesion of phagocytes to the outer surface of cells (Hill et al. 1997). These phagocytes eventually enter liver tissue and injure hepatocytes.

### Cytokine Imbalance

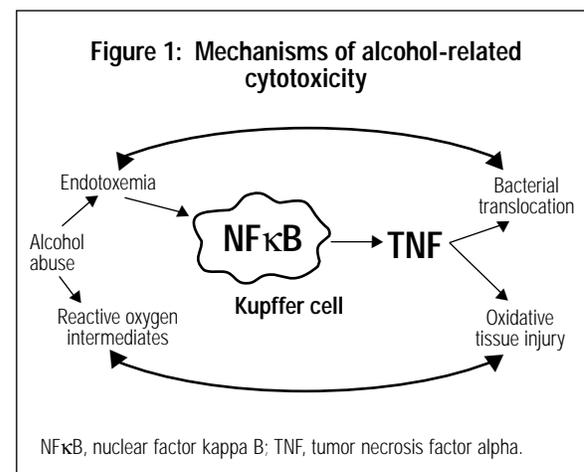
In response to injury or infection, cytokines can initiate a variety of responses, including attracting and activating phagocytes, promoting fibrosis, and stimulating the production of additional chemical messengers, including more cytokines. However, if the increased levels of cytokines do not subsequently return to normal, they can cause chronic inflammation. Even though cytokines probably evolved as protection against injury, infection, or inflammation, overproduction can lead to cell injury or cell death. For example, if there is overproduction of the cytokine TNF or if defenses against TNF are inadequate, liver injury can occur and, in fact, elevated levels of TNF appear to correlate with increased mortality (Rodriguez-Rodriguez et al. 1995).

Alcoholic liver disease is marked by increased levels of cytokines. Increases in IL-1 and TNF were first noted a decade ago in patients with alcoholic hepatitis (McClain et al. 1986, 1989). Investigators initially suspected that these cytokines played a role in the development of hepatitis because of a similarity in metabolic effects: both cytokines and alcoholic hepatitis cause symptoms of fever, increased levels of neutrophils, anorexia (loss of appetite), muscle wasting, and altered mineral metabolism (McClain et al. 1993). As mentioned previously, elevations in TNF levels are correlated with mortality, as are blood levels of neopterin, a macrophage product that is an indicator of macrophage activation (Rodriguez-Rodriguez et al. 1995).

Recent studies have reported that a rare genetic polymorphism (genetic variant) associated with increased production of TNF is also associated with susceptibility to steatohepatitis, or fatty liver (Grove et al. 1997). This suggests that genetic abnormalities in cytokine metabolism may predispose certain individuals who drink alcohol to the development of alcoholic liver disease.

One stimulus for the excessive production of potentially toxic cytokines seen in alcoholic liver disease is endotoxin, or lipopolysaccharide, a complex molecule found in the cell wall of many bacteria. Heavy alcohol consumption has been reported to increase intestinal permeability to endotoxin, allowing endotoxin produced in the intestine to leak into the blood vessels that carry blood to the liver. In the liver, endotoxin is taken up by the phagocytic Kupffer cells. Endotoxin then activates nuclear factor kappa B (NF $\kappa$ B), a regulatory complex of molecules that, when activated, initiates gene-directed synthesis of specific proteins. NF $\kappa$ B causes the production of certain pro-inflammatory cytokines, such as TNF (Schreck et al. 1992). TNF further increases gut permeability, thus creating and perpetuating a destructive cycle (figure 1).

Another stimulus for excessive cytokine production is the generation of reactive oxygen species (ROS) as a by-product of alcohol metabolism in the liver (Hill et al. 1997). ROS are highly reactive molecular fragments capable of inflicting serious damage on cells. Normally, ROS are quickly inactivated by antioxidants,



protective molecules such as glutathione and vitamins A and E. However, if these defenses are impaired or if there is an overproduction of ROS, the result is oxidative stress, which can result in cell death. Lipid peroxidation is a reaction between ROS and components of cell membranes, which results in membrane injury and eventual cell death. ROS also can activate the transcription factor NF $\kappa$ B (Sen and Packer 1996), causing a further increase in production of the cytokine TNF.

The cytokine IL-8, generated by hepatocytes and Kupffer cells, attracts and activates neutrophils (Strieter et al. 1996). The neutrophils contribute to liver cell injury by releasing toxic substances (ROS, cytokines, and proteases, enzymes that break down proteins). Researchers have noted increased levels of chemoattractant cytokines such as IL-8 and growth-related oncogene alpha in alcoholic liver disease, with the highest levels in patients with acute alcoholic hepatitis (Huang et al. 1996; Maltby et al. 1996). Levels were highest in patients who died, and levels correlated with biochemical and histologic evidence of liver disease (Huang et al. 1996). Patients with alcoholic liver disease also had increased levels of cell adhesion molecules, which may explain why certain cells “stick” in the liver and release toxic products that can lead to liver injury (Diez-Ruiz et al. 1996).

Recent studies have found that alcoholic cirrhosis is associated with elevated levels of cytokines that induce inflammation, but at the same time there is an underproduction of the cytokine interleukin 10 (IL-10), which has anti-inflammatory effects (Le Moine et al. 1995). This decreased secretion of IL-10 may play a role in the imbalance of cytokine levels seen in alcoholic liver disease.

### **Apoptosis: Cell Suicide**

Traditionally, injured liver cells have been thought to die by necrosis, a process in which the cells swell and break open, releasing their contents, thereby initiating inflammation that further damages the liver (Hetts 1998). However, a second mechanism called apoptosis, or pro-

grammed cell death, has recently been the subject of intense investigative attention. In this type of cell suicide, the cell shrinks and the entire cell, including the nucleus, breaks into numerous fragments, called apoptotic bodies. These bodies are then ingested and digested by macrophages or by neighboring cells. Apoptosis is considered “physiologic” and is important both in cell development and in removal of senescent cells (Hetts 1998). A disruption in the balance between apoptosis and cell proliferation, however, can cause harm. Initial studies suggest a role for apoptosis in liver cell death in animal models of alcoholic liver disease as well as in human alcoholic liver disease (Goldin et al. 1993; Kawahara et al. 1994; Nanji 1998; Yacoub et al. 1995; Zhao et al. 1997).

Apoptosis may be initiated by a receptor-ligand interaction. Hepatocytes have docking sites, or receptors, on the cell surface. Each specific type of receptor interacts with a corresponding molecule or ligand. The Fas receptor, a member of the TNF receptor family, is normally found in modest levels on hepatocytes (Galle et al. 1995; Muschen et al. 1998); Fas ligand is also present on hepatocytes in alcoholic liver disease (Galle et al. 1995). The Fas receptor-ligand interaction, as well as the interaction between TNF and TNF receptors, can activate pathways leading to cell death by apoptosis (Baichwal and Baeuerle 1997; Galle et al. 1995; Tewari and Dixit 1996).

Recent research indicates that mitochondria are involved in apoptosis (Kroemer et al. 1997; Zamzani et al. 1996). Mitochondria are intracellular bodies that generate energy for the cell’s metabolic processes. They respond to specific signals by releasing two proteins, cytochrome c and apoptosis-inducing factor (AIF), which in turn are involved in the activation of caspases (Kroemer et al. 1997). Caspases are substances that play a role in activating the apoptotic death pathway (Salvesen and Dixit 1997). The mitochondrial release of cytochrome c and AIF and the subsequent interaction of these proteins with caspases represent critical steps in the control of apoptosis.

Alcohol has long been known to influence mitochondrial function. Several recently reported effects of alcohol on mitochondria may be relevant to mitochondrial involvement in apoptosis. Among these alcohol effects are a decrease in the activity of ATP synthase (Marin-Garcia et al. 1995, 1996), an enzyme involved in the production of adenosine triphosphate (ATP), a critical component of the cell's metabolic processes. Other effects of alcohol on mitochondria are reduction in antioxidant levels (Colell et al. 1997; Kurose et al. 1996), enlargement of mitochondria (Mateos et al. 1995), and changes in mitochondrial membranes (Garcia-Ruiz et al. 1995). TNF is thought to mediate at least part of its toxicity through oxidative stress and alteration of mitochondrial function (Stadler et al. 1992).

### Experimental Models

Studies in rats, mice, and tissue culture are evaluating the role of cytokines, especially TNF, in experimental models of liver disease. TNF has been shown to play a role in many forms of experimental liver disease, including that induced by a variety of toxins (Hill et al. 1997). The liver is normally resistant to the toxic effects of TNF. However, as noted previously, when TNF is produced in excess or when defenses against TNF are inadequate, liver injury may result.

Because mice and rats have a natural aversion to drinking alcohol, researchers developed a method of alcohol feeding by infusing it directly into the stomach (gastric infusion) (Tsukamoto et al. 1986). With this experimental model, researchers were able to produce blood alcohol levels high enough to cause liver injury. Research using this animal model demonstrated that liver damage coincided with increased levels of TNF messenger ribonucleic acid (mRNA) in the liver (Nanji et al. 1994*d*), indicating an increase in the message level directing synthesis of TNF. Other work with the same feeding model in rats showed that isolated Kupffer cells had increases in secretion of TNF, both spontaneously and after endotoxin administration, as well as increases in TNF mRNA (Kamimura and Tsukamoto 1995).

A decade ago, studies showed that the livers of chronically alcohol-fed rats, compared with controls, were more sensitive to the toxic effects of injected bacterial endotoxin (Bhagwandeem et al. 1987). Subsequent studies showed that alcohol-fed rats had much higher levels of TNF than did controls after exposure to endotoxin, but that liver injury could be lessened by administration of a prostaglandin-like drug that decreased TNF production (Honchel et al. 1992). Prostaglandins, substances that have diverse physiologic effects, have a protective effect on liver cells, partly as a result of their ability to control inflammation.

Rats fed alcohol by gastric infusion had high blood endotoxin levels and evidence of induction of cytochrome P450 2E1, an enzyme that metabolizes alcohol and causes the generation of ROS (Koop et al. 1996; Nanji et al. 1994*c*). Indeed, markers for oxidative stress and for lipid peroxidation were noted in these animals, confirming the probable presence of ROS (Li et al. 1997; Nanji et al. 1997*a*).

An animal model for liver regeneration is provided by surgically removing a portion of the liver in rats. Researchers have noted elevated levels of TNF and IL-6 in rats following such partial hepatectomy. They also showed that anti-TNF antibody inhibited regeneration (Akerman et al. 1992; Diehl and Rai 1996). Other studies showed that regeneration was inhibited in IL-6 knockout mice, a strain of genetically engineered mice in which the gene for IL-6 has been inactivated (Cressman et al. 1996). Although hepatectomized rats chronically fed alcohol had increased levels of TNF, signaling was depressed in important liver regeneration pathways (Zeldin et al. 1996). It appears that these rats have an impaired regenerative response to a TNF signal. This impairment may partially explain the depressed liver regeneration seen in patients with alcoholic liver disease and in rats chronically fed alcohol. These studies demonstrate the complexity of cytokine signaling in alcoholic liver disease: excessive amounts of TNF can result in liver injury, whereas insufficient levels can result in an impaired healing response. The fine line

between injury and health highlights some of the important issues that will have to be resolved concerning potential anticytokine therapy for liver injury in alcoholic liver disease.

### Preventing Liver Injury

In an attempt to prevent or minimize liver injury, researchers devised several strategies to decrease oxidative stress, decrease endotoxin levels, or decrease cytokine production and activity. In one set of experiments, researchers fed antibiotics to rats in an attempt to sterilize the intestine and thus decrease the source of endotoxin. These experiments were successful in reducing alcohol-induced liver injury (Adachi et al. 1995). Other workers attempted to decrease endotoxin levels by changing the intestinal flora, feeding the rats a “good” strain of bacteria, *Lactobacillus* (Nanji et al. 1994a). This strategy was also successful at lessening liver injury.

Another approach was to administer gadolinium chloride to rats in an attempt to destroy the Kupffer cells in the liver, which are a likely source of toxic cytokine production (Adachi et al. 1995; Koop et al. 1997). This strategy was also successful, significantly diminishing alcohol-induced liver injury. In an attempt to decrease the generation of ROS, researchers administered agents to decrease cytochrome P450 2E1 (Nanji et al. 1994b). This approach also decreased alcohol-induced liver injury in rats. More recently, anti-TNF antibody prevented liver injury in alcohol-fed rats, providing compelling evidence of the relationship of TNF to alcohol-induced liver injury (Iimuro et al. 1997b).

The concept of anticytokine therapy is not unique to alcoholic liver disease. It is, in fact, the current focus of many biotechnology companies. Successful human clinical trials are already under way in other disease processes, such as rheumatoid arthritis and inflammatory bowel disease, where there are increased levels of proinflammatory cytokines such as TNF (MacFarlane et al. 1994; McSweegan 1997; van Dullemen et al. 1995).

### Nutritional Factors

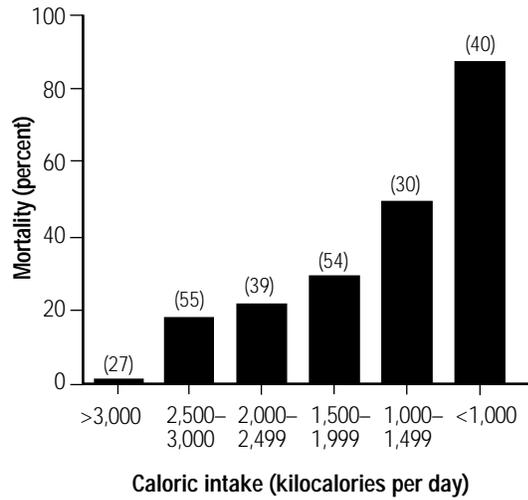
Changes in nutritional status and nutrient metabolism are associated with the development and progression of alcoholic liver disease. Researchers have used specific nutrients as well as total nutritional improvement in an attempt to reduce alcohol-induced liver injury in both humans and experimental animals. Results have shown that individual nutrients can either improve or aggravate liver injury.

#### Malnutrition

The most compelling evidence for a relationship between nutritional status and alcoholic liver disease comes from two large studies undertaken by the U.S. Department of Veterans Affairs. The subjects of these studies were inpatients with liver injury serious enough to cause symptoms of jaundice. All of them suffered from malnutrition (Mendenhall et al. 1995). The degree of malnutrition correlated with the development of serious liver complications such as ascites (fluid retention); encephalopathy (brain injury or damage causing abnormal mental function), which causes changes in mental status; and impaired kidney function. Malnutrition also correlated with death rate. The controls for these studies were patients who were alcoholics but had not yet developed detectable liver disease. However, the control patients also showed some evidence of malnutrition, which suggests that malnutrition might precede serious liver injury.

There was an inverse correlation between caloric intake and mortality. Almost all patients who consumed more than 3,000 calories a day survived, while more than 80 percent of those who took in less than 1,000 calories a day died (figure 2). Patients were strongly encouraged to increase their food consumption, with a goal of 2,500 calories a day. In spite of this, 67 percent of patients consumed insufficient calories, many of them because of severe anorexia. The cause of this anorexia is not clear, but it could be related to elevated levels of the cytokine TNF or to abnormalities in the metabolism of leptin, a hormone that controls appetite.

**Figure 2: Six-month mortality associated with 1-month daily caloric intake in 245 patients**



Numbers in parentheses denote number of patients. A highly significant relationship was observed ( $p = 0.0001$ ;  $\chi^2$  test).

Source: Mendenhall et al. 1995. Reprinted with permission from *Alcoholism: Clinical and Experimental Research*, Vol. 19, pp. 635–641, 1995. Copyright 1995, Lippincott Williams & Wilkins, Baltimore, MD. Publisher web site: <http://lww.com>.

In one of the two Veterans Affairs studies, patients were given an anabolic steroid along with a nutritional support product designed specifically for patients with liver disease. Those with only moderate malnutrition improved significantly. However, those who were severely malnourished did not benefit from this regimen, possibly because their condition had already progressed beyond the point at which they could be helped by nutritional therapy (Mendenhall et al. 1993a, 1995).

A frequent complication of alcoholic liver disease is hepatic encephalopathy (brain disease), in part due to the effects of waste products that the liver is not able to detoxify. Traditionally, the condition has been treated by restriction of protein intake, a treatment designed to reduce toxin production. However, long-term protein restriction inevitably leads to more serious malnutrition. Indeed, these studies showed that low protein intake was associated with a worsening of encephalopathy (Mendenhall et al. 1993a). Recent studies showed that most patients could be treated successfully with anti-encephalopathy medications, obviating the need for protein restriction.

## Fats and Fatty Acids

Investigators have long believed that fat intake correlates with the development of cirrhosis, and that increased body weight may be a risk factor for alcoholic liver disease. Recent research on more than 1,600 alcoholic patients in France has confirmed that obesity is indeed a risk factor for the development of alcoholic liver disease (Naveau et al. 1997). In those patients who had been overweight for 10 years, there was an increased risk of developing fatty liver, alcoholic hepatitis, and cirrhosis. These observations are supported by animal studies. When rats were fed alcohol by gastric infusion and also received a diet high in polyunsaturated fats from fish oil, the severity of liver injury was increased (Nanji et al. 1994c, 1997b). On the other hand, a diet high in saturated fats protected the rats against liver injury (Nanji et al. 1995). These studies suggest that not all fats are alike; some are clearly harmful in the development of alcoholic liver disease, while others may actually have a protective effect. Other diseases, especially autoimmune disorders in animal models, have been accelerated by consumption of high-fat diets (Leitinger et al. 1999; Shimabukuro et al. 1997).

## Iron

There is an association between increased iron in the liver and alcoholic liver disease. Alcohol intake generally increases the iron content in hepatocytes and Kupffer cells (Valerio et al. 1996), and supplemental iron intake exacerbates alcoholic liver injury (Tsukamoto et al. 1997). One effect of iron is that it promotes alcohol-induced injury by ROS (Tsukamoto et al. 1997).

Recent experiments have used cultured stellate cells to study the effects of iron. Stellate cells play a major role in the production of the tissue protein collagen, a step in fibrosis, or scarring. Iron appears to prime Kupffer cells for their role in stimulation of cultured stellate cells. Kupffer cells are a source of the cytokines TNF and IL-6 in liver injury (Kamimura et al. 1995). These cytokines stimulate stellate cells and their activity is regulated by NF $\kappa$ B, which is iron dependent (Lin et al. 1997). Indeed, Kupffer cells of rats

with alcohol-induced injury contain 70 percent more iron and have a two- to threefold increase in NFκB activity (Lin et al. 1997). Treatment of the cells with an iron chelator, a substance that binds the iron, resulted in a return of iron content and NFκB activity to normal levels (Lin et al. 1997). Future studies will attempt to determine the exact roles of iron and iron chelator in the development of injury and fibrosis in alcoholic liver disease.

### Other Nutrients

Experiments with a soybean extract, polyenylphosphatidylcholine (PPC), showed that it can prevent fibrosis and cirrhosis in alcohol-fed baboons. It also stimulates the activity of collagenase, an enzyme that breaks down collagen, in cultured stellate cells (Li et al. 1992; Lieber et al. 1994). These observations led researchers to the conclusion that PPC prevents alcohol-induced fibrosis by way of its anticollagen activity (Lieber et al. 1994). Researchers then studied the effects of several related compounds but found only one, dilinoleoylphosphatidylcholine (DLPC), that had the same anticollagen effect. Experiments with DLPC showed that it not only decreased the severity of liver fibrosis, it actually accelerated its regression (Ma et al. 1996). Other observations of the action of DLPC in alcohol-fed baboons led to the conclusion that it may have antioxidant properties (Lieber et al. 1997). These properties could help prevent fibrosis and protect cells from injury. Because of these encouraging results with DLPC, a current Veterans Affairs study is now evaluating the effects of this drug in humans with early alcoholic liver disease.

Abnormal metabolism of the amino acid methionine is well documented in alcoholic liver disease (Marchesini et al. 1992). An enzyme, methionine adenosyltransferase (MAT), is responsible for conversion of methionine to *S*-adenosyl-L-methionine (SAM) (Lu 1998). SAM is important for its role in a variety of cellular processes. Recent studies have indicated that oxidative stress and depletion of the antioxidant glutathione play a role in MAT inactivation (Sanchez-Gongoro et al. 1997). Low oxygen levels in the liver also can cause a decrease in MAT activity (Avila et al.

1998). Both oxidative stress and low oxygen levels are prominent features of alcoholic liver disease (Chawla and Jones 1994). A reduction in levels of the products of methionine metabolism, such as SAM, thus may be associated with alcoholic liver disease. Depletion of mitochondrial glutathione appears to be an important factor in the development of alcoholic liver disease in rats (Hirano et al. 1992), but other studies have shown that therapy with SAM lessens this depletion (Colell et al. 1997).

Choline is a substance derived from methionine and SAM metabolism in the liver. Choline deficiency causes severe fatty deposits in the liver that are similar to the fatty liver seen in alcoholic liver disease. Recent studies showed that rats fed a diet low in methionine and choline developed SAM deficiency and a marked fatty liver (Chawla et al. 1998). Choline-deficient rats also showed increases in cytochrome P450 2E1 activity, which is believed to play a role in the development of liver injury (Weltman et al. 1996). Rats deficient in methionine and choline were highly susceptible to endotoxin-induced liver injury and had elevated levels of the cytokine TNF. Antibodies to TNF helped protect against endotoxin-induced liver injury in choline-deficient rats (Eastin et al. 1997). Administration of SAM before injection with endotoxin reduced liver injury and decreased TNF levels, an observation that supports the concept that SAM may have protective effects on the liver. Studies are currently under way in both animal models and human subjects with alcoholic liver disease to determine the effectiveness of supplementation with products such as SAM.

### Other Liver Diseases

Recent work has highlighted three liver diseases that may be important in understanding the mechanisms of alcoholic liver disease. Acetaminophen toxicity may be enhanced by alcohol consumption. Hepatitis C has important and complex interactions with alcohol abuse. Fatty liver, often associated with obesity, can mimic alcoholic liver disease and may provide important clues to the mechanisms involved in alcoholic liver disease.

### Acetaminophen Liver Toxicity

Acetaminophen is a widely used over-the-counter pain medicine that until recently had been marketed as an entirely safe product. It is sold in more than 200 formulations in the United States, including brand names such as Tylenol. Two decades ago, reports described an association between chronic alcohol consumption and acetaminophen liver toxicity (Goldfinger et al. 1978; McClain et al. 1980). Acetaminophen overdose can cause very acute, sudden liver failure, and death can occur in less than 1 week (Makin et al. 1995). If the patient survives the severe acute phase of toxicity, the liver will eventually return to normal. One means of maintaining patients through the period of acute toxicity is the use of an artificial liver system, which can keep patients alive until they either recover spontaneously or obtain a liver transplant (Watanabe et al. 1997).

Researchers reviewed cases of 67 patients who regularly consumed alcohol and who developed liver injury after taking acetaminophen for therapeutic reasons (Zimmerman and Maddrey 1995). Acetaminophen doses were within what is generally considered a nontoxic range in 60 percent of the patients and were within the recommended range for the other 40 percent. Patients developed severe liver damage, and 18 percent of them died. In another study of 71 patients hospitalized for acetaminophen liver toxicity, 21 had taken an accidental overdose of acetaminophen as a pain reliever and 50 had taken it in a suicide attempt (Schiodt et al. 1997). Sixty-three percent in the accidental overdose group and 25 percent in the suicidal group were chronic alcohol abusers. The accidental overdose group had much more severe liver injury (52 vs. 14 percent) and a greater number of deaths (19 vs. 2 percent). Acetaminophen ingestion accounted for approximately 40 percent of patients with acute liver failure during the study period. This study highlights the importance of accidental acetaminophen overdose as a major cause of acute liver failure. Patients with sudden and severe liver failure may go to the hospital too late to benefit from the only currently available antidote, *N*-acetylcysteine. Both of these reports

stress the need for greater awareness of the risks of acetaminophen liver toxicity, both in the medical community and among the general public. Acetaminophen itself does not cause liver toxicity. Instead, a highly reactive metabolite, *N*-acetyl-*p*-benzoquinonimine (NAPQI), generated through the alcohol-metabolizing cytochrome P450 2E1 system, is believed to be the cause of liver cell death. Activity of 2E1 is greatest in the area of the liver in which acetaminophen toxicity is most severe (Cohen et al. 1997). NAPQI normally binds to a protective substance, glutathione. However, if liver glutathione stores are depleted, this reactive metabolite is free to cause liver cell injury. Alcoholics are more predisposed to glutathione depletion for several reasons, including poor nutrition.

Animal studies have confirmed the link between alcohol intake and increased risk for acetaminophen toxicity. Cytochrome P450 2E1 is increased in the hepatocytes of alcohol-consuming rats, and recent studies show that it is also increased in Kupffer cells, where inflammatory cytokines and ROS are generated (Koivisto et al. 1996). Investigators found that cytochrome P450 3A, another enzyme system, can be induced by alcohol in humans and in rats (Hoshino and Kawasaki 1995; Kostrubsky et al. 1997*a*). An important additional finding was that an inhibitor of cytochrome P450 3A protected against alcohol-enhanced acetaminophen liver toxicity in rats (Kostrubsky et al. 1997*a,b*).

Researchers have reported the presence of activated macrophages at the site of injury in acetaminophen toxicity. Also, increased levels of the cytokines IL-1 and TNF are present in acetaminophen liver toxicity in mice (Blazka et al. 1995). The same symptoms, Kupffer cell activation and cytokine production, are caused by chronic alcohol consumption. Recent research demonstrated that blocking Kupffer cell function totally prevented acetaminophen liver toxicity (Laskin et al. 1995).

The studies reported here tend to confirm the interaction between alcohol consumption and the

risk of developing acetaminophen liver toxicity. Acetaminophen liver toxicity has been established as the major cause of sudden and severe liver failure in the United States and in many European countries. Fortunately, warning labels are now being placed on acetaminophen-containing products concerning alcohol intake and risk of liver injury.

### **Hepatitis C**

Hepatitis C virus (HCV) is an RNA virus that causes about 30,000 new infections each year (National Institutes of Health [NIH] Consensus Statement 1997). In the United States, almost 4 million people are infected with hepatitis C, four times the number infected with the human immunodeficiency virus (HIV). This infection is more prevalent in minority populations. About 85 percent of HCV-infected individuals do not become virus free within 6 months and are highly likely to develop chronic hepatitis.

There are usually few symptoms during the first 20 years of infection, and therefore the United States is currently entering an era in which individuals infected decades ago are beginning to show symptoms. The disease may be fatal and is, in fact, the leading cause of liver transplantation in the United States. Between 1 and 5 percent of patients with chronic HCV for 20 years will develop liver cancer (NIH Consensus Statement 1997).

A recent study of 100 alcoholics in a rehabilitation program found that 23 percent were positive for antibodies to HCV (anti-HCV). Of those who had liver disease, 43 percent tested positive for anti-HCV, while only 10 percent of those without clinically apparent liver disease tested positive (Coelho-Little et al. 1995). Thus, data from this and other studies strongly suggest that, for as yet unknown reasons, actively drinking alcoholic patients are more likely to have HCV infection.

Patients with alcoholic liver disease are at very high risk of having hepatitis C. A study of 288 patients with alcoholic hepatitis found that 18 percent of them had anti-HCV (Mendenhall

et al. 1993*b*). Previous intravenous drug abuse was a risk factor, but more than 40 percent of these patients had no known risk factor for hepatitis C other than alcohol abuse. Another study of patients with alcoholic liver disease showed that those who were HCV positive had more severe liver disease and were younger than HCV-negative patients (Befrits et al. 1995). A study of individuals with chronic HCV found not only that those individuals developed the disease at a younger age but that those who drank alcohol heavily had higher liver enzyme levels, a clinical marker for liver disease (Cromie et al. 1996). Although abstinence from alcohol is associated with a decrease in liver enzyme levels, the presence of HCV interferes with this improvement (NIH 1997).

There are several possible mechanisms for the association of alcohol consumption with greater severity of HCV. The interaction of alcohol and HCV may impair immune responses to the virus (Geissler et al. 1997; NIH 1997; NIH Consensus Statement 1997). Iron levels in the liver are higher in HCV patients who drink alcohol heavily, and iron accelerates several forms of liver injury (Geissler et al. 1997). In addition, the inflammatory response in the liver is greater in individuals with HCV who drink alcohol. A further complication is that alcohol depresses the response to interferon therapy, which is the therapy of choice for HCV (Izumi et al. 1996). Interferons are proteins that provide an important defense against viral infections by making noninfected cells virus resistant.

### **Nonalcoholic Steatohepatitis**

Nondrinkers may develop a disease that is virtually indistinguishable from alcoholic fatty liver (Pinto et al. 1996; Sheth et al. 1997). As in alcoholic liver disease, nonalcoholic steatohepatitis (NASH) results in inflammation, fibrosis, and cirrhosis. Most studies show that NASH is more frequent in women, although one study reported a higher frequency among men. Most patients are in their 40's and 50's, and most are obese (Sheth et al. 1997). NASH is generally present in greatly obese patients who have abdominal surgery for weight reduction.

Complications of NASH include non-insulin-dependent diabetes, hyperglycemia, and hyperlipidemia, a risk factor for heart disease. Often the liver is enlarged. Up to one-sixth of NASH patients eventually develop cirrhosis (Sheth et al. 1997). Recent studies show that increased iron content or changes in drug metabolism in the liver may play a role in the development of NASH (Bacon et al. 1994; George et al. 1998; Weltman et al. 1998). Nearly one-third of patients have, or carry a gene for, hemochromatosis, a disorder of iron metabolism.

No established therapy currently exists for treating NASH patients, although some physicians recommend use of the drug ursodeoxycholic acid (Laurin et al. 1996). However, because the disease is so closely associated with obesity, a frequent recommendation is simply that patients gradually lose weight (Sheth et al. 1997).

Researchers have developed strains of rats and mice that become obese due to a disruption in the action of leptin, the hormone that controls appetite. Both of these strains develop severe fatty liver. These animals are highly sensitive to endotoxin, developing severe fatty liver after exposure (Yang et al. 1997). Animals chronically fed alcohol exhibit a similar reaction following endotoxin injection (Honchel et al. 1992). In the obese animal strains, females are more sensitive than males to endotoxin injury. Females are also more sensitive to alcohol-induced liver injury, and endotoxin may play a role in that injury (Iimuro et al. 1997a). The obese animals showed abnormalities in the levels and actions of cytokines as well as impaired immune function (Loffreda et al. 1998). Further studies with these genetically obese rodents, as well as with obese humans, may provide new insights into the mechanisms of fatty liver in alcoholic liver disease.

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