REVIEW OF LITERATURE
2. REVIEW OF LITERATURE

2.1 Fulminant hepatic failure

Trey and Davidson introduced the term *fulminant hepatic failure* in 1970 to describe a "potentially reversible condition, the consequence of severe liver injury, with an onset of encephalopathy within two weeks of the appearance of jaundice and in the absence of pre-existing liver disease (Trey & Davidson 1970). The lack of prior liver disease is critical to the definition of FHF. Patients who suffer an acute deterioration of previously stable cirrhosis from alcohol or chronic hepatitis may have a life-threatening illness, but they do not have FHF. Unlike such patients with acute or chronic liver disease, patients with FHF have the potential to completely recover normal liver function, although this is far more likely with some causes of FHF than with others (Cillo et al., 2004). Despite its relative rarity, FHF is important because it is associated with high mortality (Mohanty & Schiff 2009). However, the dismal prognosis associated with FHF appears to be improving, at least in the developed world. This trend probably points to the improvement in medical intensive care, and a change in the epidemiology of FHF with a shift from causes with a relatively poor predictability (e.g., hepatitis related causes) to those with a relatively good predictability (e.g. acetaminophen overdose) (Vaquero & Blei, 2003).

2.1.1 Risk factors

A diverse array of insults can cause FHF. Liver failure due to viral attack is currently the most common cause of FHF in developing nations (Poddar et al., 2002) while liver injury from drugs and toxins forms the largest cause in the US and the UK (Alam et al., 2009). Older infants and children are more likely to develop FHF from viral hepatitis. The following is a brief classification of the causes of FHF. Selected etiologies of FHF are discussed below.
Viral infection: Hepatitis A, B & E and Herpes simplex virus

Drugs and toxins: Acetaminophen & other drugs, amanita phalloides, isoniazid, aflatoxin, halothane are associated with development of FHF.

Vascular problems: These causes include portal vein thrombosis (Murad et al., 2006), hepatic artery thrombosis (Montalti et al., 2005), ischemic hepatitis (Gibson & Dudley 1984), vascular shock (Morishita 1984) or tumor infiltration of the liver (Rowbotham et al., 1998).

Metabolic causes: Causes in very young infants include neonatal iron storage disease (Barnard & Manci 1991), certain metabolic disorders such as tyrosinemia (Scriver et al., 1967) and galactosemia (Suchy, 1996) peroxisomal diseases (Roels et al.,1991), and defects in respiratory chain (Goncalves et al., 1995) and synthesis of bile acid (Lee et al., 2001). Family screening is appropriate for many metabolic or genetic causes of FHF.

Indeterminate causes: Approximately 15-20% of adult cases and up to 50% of FHF in children cannot be attributed to a specific cause (Davern 2004).

2.1.1.1 Viruses

In parts of Asia and most of Africa, viruses represent the lead cause of FHF (Capocaccia and Angelico, 1991). In the past, viral hepatitis B (HBV) was one of the leading causes of FHF in the US, but its incidence has been declining. It is now a rare cause of FHF in the US, accounting for about 5% of FHF cases (Polson & Lee, 2005). Acute viral hepatitis is diffuse liver inflammation caused by specific hepatotrophic viruses that have diverse modes of transmission and epidemiologies and each type shares clinical, biochemical, and morphologic features (Chu & Liaw, 1990). Acute infection tends to develop in predictable phases. Infection begins with an incubation period during which the virus multiplies and spreads without symptoms. The pre-icteric phase follows, producing nonspecific symptoms, such as profound anorexia, malaise, nausea and vomiting, and, often, fever or right upper quadrant
abdominal pain. During the icteric phase, the liver is usually enlarged and tender. Most cases resolve spontaneously, but some progress to chronic hepatitis. Occasionally, acute viral hepatitis progresses to fulminant hepatic failure (Vento et al., 1998).

**Etiology and Epidemiology:** At least five specific viruses are known to cause hepatitis (Table 2.1.1.1).

*Hepatitis A virus (HAV):* HAV is a single-stranded RNA virus. It is the most common cause of acute viral hepatitis and is particularly common among children and young adults (Dmochowski, 1976). Infection with hepatitis A virus is usually transmitted by a fecal-oral route and thus may occur in areas of poor hygiene. Waterborne and food-borne epidemics occur, especially in underdeveloped countries. Eating contaminated raw shellfish is sometimes responsible.

*Hepatitis B virus (HBV):* HBV is the most thoroughly characterized and complex hepatitis virus. The infective particle consists of a viral core that contains circular double-stranded DNA and it replicates within the nuclei of infected hepatocytes (Jilbert 1992). HBV is the second most common cause of acute viral hepatitis (Joshi 1984). Routine screening of donor blood for hepatitis B surface antigen (HBsAg) has nearly eliminated the previously common post transfusion transmission, but transmission through needles shared by drug users remains common. Prevalence varies widely according to several factors, including geography (eg, < 0.5% in North America and northern Europe, > 10% in some regions of the Far East) (Dmochowski, 1976, Snyder & Pickering, 2004). Vertical transmission from mother to infant is common in China and other parts of Asia (WHO document, 1998).

*Hepatitis C virus (HCV):* HCV is a single-stranded RNA virus. Six major HCV subtypes exist with varying genotypes; these subtypes vary geographically and in virulence and response to therapy (Alter, 1997). Infection is most commonly transmitted through blood, primarily when parenteral drug users share needles (Ridzon, 1997) but also through tattoos.
Table 2.1.1.1: Characteristics of Hepatitis Viruses

<table>
<thead>
<tr>
<th></th>
<th>Hepatitis A Virus</th>
<th>Hepatitis B Virus</th>
<th>Hepatitis C Virus</th>
<th>Hepatitis D Virus</th>
<th>Hepatitis E Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleic acid</td>
<td>RNA</td>
<td>DNA</td>
<td>RNA</td>
<td>*</td>
<td>RNA</td>
</tr>
<tr>
<td>Major transmission</td>
<td>Fecal–oral</td>
<td>Blood</td>
<td>Blood</td>
<td>Needle</td>
<td>Water</td>
</tr>
<tr>
<td>Incubation(days)</td>
<td>15–</td>
<td>40–180</td>
<td>20–120</td>
<td>30–180</td>
<td>14–60</td>
</tr>
<tr>
<td>Epidemics</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Chronicity</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Liver cancer</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

*Incomplete RNA; requires presence of hepatitis B virus for replication.

(Haley and Fischer 2001). Up to 20% of patients with alcoholic liver disease harbor HCV (Coelho–Little, 1995); both HCV and alcohol act to exacerbate liver damage.

**Hepatitis D virus (HDV):** HDV, or delta agent, is a defective RNA virus that can replicate only in the presence of HBV (Smedile 1994). It occurs uncommonly as a co-infection with hepatitis B. Prevalence of HDV varies widely geographically, with endemic pockets in several countries (Jacobson 1995).

**Hepatitis E virus (HEV):** Hepatitis E virus (HEV) is associated with a high incidence of FHF in women who are pregnant in regions like Mexico and Central America, India and the subcontinent, and the Middle East (Navaneethan et al., 2008). It is the most common cause of FHF in endemic regions of the world, with high mortality (25%) in pregnant women (Kumar et al., 2004). HEV is an enterically transmitted RNA virus. Outbreaks of acute HEV infection, often waterborne and linked to fecal contamination of the water supply, have
occurred in China, India, Mexico, Pakistan, Peru, Russia, and central and northern Africa (Corwin, 1996).

2.1.1.2 Acetaminophen

Acetaminophen poisoning is the leading cause of FHF in the developed countries like US and the UK and is responsible for nearly 50% of all cases (Larson et al., 2006). FHF due to acetaminophen poisoning may result from the intentional or unintentional overdosing. There are genetic and environmental factors that affect a given person’s threshold of toxicity (Vesell, 1991). For example, alcohol abuse and prolonged fasting may be associated with enhanced susceptibility to acetaminophen toxicity. Alcohol acetaminophen syndrome is emerging as an important cause of FHF in the United States. The syndrome is characterized by extremely high transaminase levels and a poor prognosis (Bray et al., 1991). Early treatment with an antidote called N-acetyl cysteine (NAC) is life-saving.

2.1.1.3 Idiosyncratic drug reactions

These are drug reactions which occur rarely and unpredictably amongst the population. Symptoms of idiosyncratic drug reactions are different than the pharmacological effect of the drug. Reactions resulting in FHF include non-steroidal anti-inflammatory drugs (Lewis, 2005) and antitubercular drugs (Smith et al., 1998). Uses of drug combinations which result in FHF are of greater concern than single agents. These include trimethoprim-sulfamethoxazole, rifampicin-isoniazid and isoniazid-acetaminophen.

2.1.1.4 Mushrooms

Amanita phalloides, a species of mushroom also called the “death cap”, contains very potent liver toxins that cause severe liver damage (Lionte et al., 2005). Eating Amanita
phalloides results in a syndrome of severe nausea, vomiting, diarrhea and abdominal pain that typically begins about 8-16 hours after eating the mushrooms following which fulminant liver damage occurs.

2.1.1.5 Other conditions

Several other conditions that affect the liver can cause FHF. In autoimmune hepatitis the immune system of the affected individual attacks the liver (Alvarez et al., 1999). Autoimmune hepatitis usually responds to immunosuppressive therapy, but treatment may not be successful when the patient has advanced liver failure. FHF may rarely occur in pregnancy (Patra et al., 2007), usually during the last trimester. Most patients with pregnancy-related FHF recover following prompt delivery of the infant.

2.1.2 Symptoms

Most patients who develop FHF become ill very rapidly, and within a week or less from the onset of illness near total liver collapse may occur. The early symptoms of liver failure are nonspecific and similar to symptoms of many other conditions. Some of the most common initial symptoms of liver failure are (Yoshiba, 1998):

- Nausea, Loss of appetite, Malaise, Fatigue, Abdominal discomfort, Diarrhea
- Jaundice and scleral icterus (yellowing of the eyes) are often present, but may not be initially noted by patients or their families until relatively late in the course of the illness.
- Encephalopathy, the cognitive dysfunction that is an end result of FHF, is initially subtle. Minor changes in personality, particularly irritability, inattention, mild memory lapses, and insomnia may be the first signs. However, encephalopathy may
dramatically and suddenly worsen, culminating in hepatic coma in a relatively short time.

- Other symptoms of fulminant hepatic failure include: a build up of fluid in the abdomen, which causes swelling called ascites, oedema, a tendency to bruise or bleed easily, fever, itchy skin, dark urine, sleepiness, coma
- As the condition progresses, it causes confusion and erratic behavior as the build up of toxins in the blood affects brain function. Liver failure can also cause kidney failure, coma, and death.

2.1.3 Diagnosis and evaluation

Obtaining a detailed and accurate medical history from patients with FHF is challenging, due to the presence of an altered mental status. Thus, the clinician is usually forced to rely on others to obtain information about recent symptoms, use of medication, risk factors for viral hepatitis (e.g., intravenous drug use, recent tattoos, recent travel, exposure to other ill individuals), and any significant past medical problems. Physical examination is done focusing primarily on determining the stage of encephalopathy, excluding chronic liver disease, and establishing the etiology of FHF (Subramanian et al., 2005). Measurement of prothrombin time and careful evaluation of mental status is carried out in patients with clinical or laboratory evidence of moderate to severe acute hepatitis. If the prothrombin time is prolonged by 4–6 seconds or more and there is any evidence of altered sensory response, the diagnosis of FHF is suspected (O’Grady et al., 1989). Initial laboratory examination must be extensive so that the etiology and severity of liver failure are efficiently evaluated.

Initial laboratory analysis includes the following tests or estimation. Prothrombin time; Complete blood count; Liver function test: AST, ALT, alkaline phosphatase, GGT, Total bilirubin, Albumin; Creatinine, Urea/blood urea nitrogen; Sodium, potassium, chloride,
bicarbonate, calcium, magnesium, phosphate; Glucose; Lipase; Lactate; Blood type and screen; Paracetamol (Acetaminophen) level; Toxicology screen; Viral hepatitis serologies: anti-HAV IgM, HBSAg, anti-HBc IgM, anti-HEV; Autoimmune markers; Immunoglobulin levels; Ceruloplasmin Level (when Wilson's disease suspected); Pregnancy test; Ammonia.

Depending on the purpose of the laboratory tests, they may be classified into 3 groups (Table 2.1.3.1).

Table 2.1.3.1 Classification of laboratory tests for fulminant hepatic failure

<table>
<thead>
<tr>
<th>Purpose</th>
<th>Tests</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Determine severity of liver failure</td>
<td>Prothrombin time</td>
<td>The prothrombin time is the single most useful test to determine how the patient recovers from FHF.</td>
</tr>
<tr>
<td></td>
<td>Bilirubin, Albumin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Liver transaminases</td>
<td></td>
</tr>
<tr>
<td></td>
<td>And other enzymes</td>
<td></td>
</tr>
<tr>
<td>Determine cause of FHF</td>
<td>Viral serologies</td>
<td>Determining the etiology quickly and accurately is important as prognosis of recovery depends in part on the cause of FHF. Also some causes FHF may respond to specific therapy.</td>
</tr>
<tr>
<td></td>
<td>Autoimmune serologies</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acetaminophen levels</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ceruloplasmin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serum copper</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pregnancy test</td>
<td></td>
</tr>
<tr>
<td>Predict complications of FHF</td>
<td>Serum Creatinine</td>
<td>Various lab tests are done to determine whether renal failure, anemia, infection or other problems have complicated FHF. The ammonia level in blood may help in determining the risk of developing cerebral edema.</td>
</tr>
<tr>
<td></td>
<td>Blood urea</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hemoglobin, Blood culture</td>
<td></td>
</tr>
<tr>
<td></td>
<td>White blood count</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Urine culture, Glucose</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Electrolytes, Ammonia</td>
<td></td>
</tr>
</tbody>
</table>

2.1.3.1 Blood tests and serum liver markers:

A series of blood tests can determine the presence of liver disease. Bilirubin is produced by the liver and excreted in the bile. Elevated levels of serum bilirubin often indicate an
obstruction of bile flow or a defect in the processing of bile by the liver (Yoshiba, 1998). A low level of serum albumin is associated with FHF apart from other liver ailments like chronic liver failure and cirrhosis (Baichoo & Samson, 2006). Elevated levels of serum alkaline phosphatase, the enzyme found in bile usually indicate an obstruction to bile flow and liver injury (Green & Flamm, 2002). Serum aminotransferases, AST and ALT are released into circulation from damaged liver cells (Kew, 2000). The enzyme gamma glutamyl transaminase is liver specific and is released into circulation when the liver is damaged (Karan, 2009). The majority of the coagulation factors are manufactured by the liver and hepatic failure will lead to the depletion of these factors (Blonski, 2007). In addition to impaired hepatic metabolism the absorbance of fat soluble vitamin K through the bile acid system can be impaired creating an effective vitamin K deficiency and inadequate activation of the vitamin K dependent clotting factors (Kowdley et al., 1997). Liver cell damage as seen in FHF interferes with blood clotting that enhances the prothrombin time, the time it takes for blood to clot. Liver health and function should be optimal for production of urea through urea cycle that takes place in liver. Thus urea level in blood gives an indication of liver function and is used to assess prognosis in FHF (Jalan & Lee 2009).

2.1.3.2 Viral serology tests:

Viral serology tests are performed to check if the FHF is caused by viral hepatitis (Gimson et al., 1986). HAV IgM, HEV IgM detect active infection by hepatitis A or E virus. HCV antibodies detect evidence of infection by Hepatitis C virus. HBsAg, HBeAg, and HBV DNA detect infection with hepatitis B virus (Gimson et al., 1983). Anti HBs provides evidence of good immunity to hepatitis B virus infection.

2.1.3.3 Imaging tests
2.1.3.3.1 Abdominal ultrasound: A diagnostic ultrasound is performed to diagnose an injury or disease of the liver, gallbladder, spleen, pancreas, kidneys, or other organs inside the abdomen. In FHF, an abdominal ultrasound is performed to assess the liver, to verify that its vascular supply is intact, and to rule out masses or other problems.

2.1.3.3.2 Computerized tomography scan (CT scan): CT scan is a way to examine internal organs without surgery through which cross-sectional images of structures can be visualized inside the body.

2.1.3.3.3 Liver tissue biopsy: A liver biopsy involves removal of sample of liver tissue for diagnostic purposes. Through a small incision and a hollow-tube needle, the sample is drawn in a quick and painless method.

2.1.4 Pathology

In majority of FHF cases there is widespread hepatocellular necrosis beginning in the centrilobal distribution and progressing towards portal tracts (Boyer and Klatskin 1970). The degree of parenchymal inflammation is variable and is proportional to duration of disease. Most frequently, in FHF, liver shows confluent necrosis, though there is considerable variability in the extent of liver injury. There are no differences in the histopathology corresponding to different etiologies (Hanau et al., 1995). Kuramoto et al., 1991 described a case of FHF following congestive heart failure in which the liver was severely atrophied with massive centrilobular necrosis following which manifestations of hepatic failure like jaundice, encephalopathy, haemorrhagic rashes etc were seen within a week. In FHF due to tumor infiltration, liver histology showed widespread hepatocellular necrosis where the clinical course was of rapid deterioration and death from multiorgan failure (Rowbotham et al., 1998). Histology of liver in FHF developed as an idiosyncratic drug reaction with antiepileptic medication shows hepatic necrosis, with hepatic lobules showing inflammatory
infiltrate with lymphocytes (Albataineh & Siddiqui, 2007). Acute fatty liver of pregnancy is characterized by FHF associated with characteristic pathologic changes of hepatocytes, i.e., microvesicular fatty degeneration with severe mitochondrial swelling (Saibara et al., 1994). The pathogenesis in viral-induced hepatitis - including cell necrosis, inflammation, fibrosis, and cirrhosis formation - is reasonably well understood, and this knowledge assists both in prognosis and in monitoring of therapy (Popper 1975).

2.1.5 Clinical consequence

2.1.5.1 Cerebral oedema and encephalopathy

In FHF, cerebral oedema leads to hepatic encephalopathy, coma and eventually death (Wijdicks et al., 1995). Detection of encephalopathy is central to the diagnosis of FHF. It may vary from subtle deficit in higher brain function (e.g. mood, concentration in grade I) to deep coma (grade IV) (de Carlis et al., 2001). The causative factors remain unclear but are likely to be a consequence of several phenomena (Hazell et al., 1999). There is a build up of toxic substances like ammonia in the brain that affects neurotransmitter level and neuroreceptor activation (Shawcross et al., 2010). Autoregulation of cerebral blood flow is impaired and is associated with anaerobic glycolysis and oxidative stress (Stamelou et al., 2009). Neuronal cell are susceptible to these changes which swell (Larsen and Wendon 2002), resulting in increased intracranial pressure (Koutsilieri et al., 2002). Cerebral edema in FHF results primarily from astrocyte swelling rather than a leaky blood brain barrier (Ranjan et al., 2005).

2.1.5.2 Coagulopathy

Coagulopathy, a disorder in which blood is either too slow or too quick to coagulate, is another cardinal feature of FHF (Gotthardt et al., 2007). Liver has central role in synthesis of coagulation factors. Hepatocellular necrosis leads to impaired synthesis of many coagulation
factors (Kaul and Munoz, 2000). This prolongs prothrombin time which is widely used to monitor severity of hepatic injury. Electron microscopy of platelets from patients with fulminant hepatic failure show structural abnormalities including numerous pseudopods, vacuoles and blurred plasma membranes. In a study by Rubin et al., 1977, it was shown that as patients recovered from fulminant hepatic failure, platelet function improved and platelets with normal ultrastructure appeared amongst the abnormal ones.

2.1.5.3 Renal failure

Renal failure is common, present in more than 50% of FHF patients, (Ring-Larsen and Palazzo, 1981) either due to original insult such as paracetamol resulting in acute tubular necrosis (Cobden et al., 1982) or from impaired circulation leading to hepatorenal syndrome (Barada, 2004) or functional renal failure (Fernández et al., 2003). Because of defective production of urea in liver, blood urea does not represent degree of renal impairment but is an indicator liver function.

2.1.5.4 Inflammation and infection

About 60% of all FHF patients fulfil the criteria for systemic inflammatory syndrome (Leithead et al., 2009, Rolando et al., 2000) irrespective of presence or absence of infection. This often contributes towards multi organ failure (Bown et al., 2003). Impaired host defence mechanism due to impaired opsonisation, chemotaxis and intracellular killing substantially increase risk of sepsis (Larcher et al., 1982). Bacterial sepsis (Dirix et al., 1989) mostly due to gram positive organisms and fungal sepsis (Kung et al., 1995) are observed in up to 80% and 30% patients respectively.
2.1.5.5 Metabolic derangements

Hyponatraemia (Bernstein & Tropodi 1998) is a constant feature due to water retention and shift in intracellular sodium transport from inhibition of Na/K ATPase (Papadakis et al., 1990). Hypoglycaemia due to depleted hepatic glycogen store and hyperinsulinaemia (Martin & Pappas, 1990), hypokalaemia, hypophosphataemia and metabolic alkalosis (Calvo & Park, 1996) are often present. Lactic acidosis occurs predominantly in paracetamol overdose (Zabrodski & Schnurr 1984).

2.1.5.6 Haemodynamic and cardio-respiratory compromise

Hyperdynamic circulation with peripheral vasodilatation due to low systemic vascular resistance leads to hypotension (Fernández-Rodriguez 1998). Adrenal insufficiency has been documented in 60% of FHF (Harry et al., 2002) and is likely to contribute in haemodynamic compromise. There is also abnormal oxygen transport and utilization (David et al., 1985). There is a decrease in tissue oxygen uptake, resulting in tissue hypoxia and lactic acidosis (Zabrodski & Schnurr 1984). Pulmonary complications occur in up to 50% patients (Mostafa et al., 2006). Severe lung injury and hypoxemia result in high mortality. Pulmonary haemorrhage, pleural effusions (Trewby, 1978), and intrapulmonary shunts also contribute to respiratory difficulty (Fordham et al., 1998).

2.1.6 Treatment options

Treatment for FHF often involves admission to an intensive care unit and is based upon the cause and the symptoms. Supportive treatment is with adequate nutrition, mechanical ventilation and intracranial pressure monitoring (in severe encephalopathy) (Bernuau & Durand, 2006), and treatment aimed at removing the underlying cause such as acetylcysteine for paracetamol poisoning (Pol & Lebray 2002). The administration of intravenous fluid
restores glucose levels and fluid and electrolyte balance (Russell et al., 1987). Medications, blood transfusions (Ramos & Almario 1990), and hemodialysis (Berger et al., 2000) can be used to remove toxins from the body. While many people who develop FHF recover with supportive treatment (Yoshiba, 1998), liver transplantation (Sass & Shakil 2005) is required in people who continue to deteriorate or have adverse prognostic factors (Bernuau et al., 1986). Various measures to replace normal liver function are evolving as a treatment modality and is gradually being introduced in the care of patients with liver failure. Use of hepatoprotective drugs is increasingly being used to restore liver function (Gong, 2010 (article in press), Kim et al., 2008 and Sinha et al., 2007).

2.1.7 Prognosis

The liver performs myriads of vital functions including processing proteins, sugars, fats, and vitamins; removal of toxic substances (e.g., ammonia); production of bile acids, required for normal digestion; synthesis of clotting factors that prevent bleeding. Consequently, when the liver fails suddenly, the result is a devastating illness. The overall survival for FHF patients in the pre-transplant era was less than 10% (Rakela et al., 1985) and mortality has been very high, being in excess of 80%. In recent years the advent of liver transplantation and multidisciplinary intensive care support has improved survival significantly (Ostapowicz et al., 2002). Though transplantation has improved mortality, the procedure is ridden with issues, which calls for alternate methods of treating FHF.

2.1.8 Prevention

Since there are multiple causes of FHF that all lead to essentially the same syndrome, no single measure is likely to be effective in preventing all cases. However, several measures can be envisioned that, if successfully executed, should significantly decrease the incidence
of FHF. For example, vaccination for hepatitis A and B has probably contributed to the declining incidence of FHF from viral hepatitis (Mathur & Arora, 2008). Public health initiatives, including guidelines regarding appropriate food handling, (Fitzsimons et al., 2010) also have contributed by reducing the incidence of food-borne hepatitis A.

**What does the future hold for FHF?**

FHF is potentially reversible (Kobayashi et al., 2009). The FHF patient’s outcome depends on the balance between liver injury on the one end and liver regeneration and repair on the other. If the liver injury can be attenuated, or the liver repair and regenerative responses can be enhanced, then recovery is likely. Use of hepatoprotective drugs to counteract the manifestations of FHF is a promising field and a lot of research is currently on in this area. Also recent advances in molecular and cell biology have resulted in the identification of molecular targets that might be manipulated achieve this goal (Mor, 2001).
2.2 Taurine

Taurine, or 2-aminoethanesulfonic acid, is an organic acid. It is also a major constituent of bile (Bellantini 1987) and can be found in the lower intestine and in small amounts in the tissues of many animals, including humans. Taurine is a derivative of the sulfur-containing (sulphydryl) amino acid, cysteine. It is one of the few known naturally occurring sulfonic acids. Taurine is named after the Latin taurus, which means ox, as it was first isolated from ox bile in 1827 (Tiedemann & Gmelin, 1827) Taurine is not a typical amino acid as it lacks a carboxyl group and in its place contains a sulfonate group and may be called an amino sulfonic acid. Small polypeptides have been identified which contain taurine, but no aminoacyl tRNA synthetase has been identified as specifically recognizing taurine and capable of incorporating it into a tRNA. Taurine plays an important role in the functions of the body (Huxtable, 1992). Absence of taurine does not result in immediate deficiency and disease, but long-term deprivation can cause many health problems.

2.2.2 Structure

The taurine molecule (H2N-CH2-CH2-SO2H) is small and consists of hydrogen (H), nitrogen (N), carbon (C), sulfur (S) and oxygen (O) (Fig 2.2.2.1). Most amino acids have a L- or D-configuration, which means the molecule when put into a solution will rotate light either to the left (Levo=L) or the right (Dextro=D). Taurine, like the amino acid glycine does not polarize light and consequently it does not have an L- or D-configuration. It occurs in the body as a free molecule and is never incorporated into muscle proteins. The taurine molecule
is water soluble and thus doesn’t easily cross the mostly fatty membranes of the body’s cell but it is present in all membranes (López-Colomé & Pasantes-Morales 1981).
2.2.3 Occurrence in nature

Taurine is a phylogenetically ancient compound with a disjunct distribution in the biosphere. It is present in high concentration in algae and in animal kingdom, including insects and arthropods (e.g. Allen & Garrett, 1971; Huxtable, 1992; Yin et al., 2000). It is generally absent or present in traces in the bacterial and plant kingdoms. In many animals, including mammals, it is one of the most abundant of the low-molecular-weight organic constituents. A 70-kg human contains up to 70 g of taurine. Taurine is found in greater concentrations in all animal products. Meat, poultry, eggs, dairy products, and fish (Allen & Garrett 1971) are good sources of taurine. Table 2.2.3.1 shows the level of taurine content present in some seafood. In the animal kingdom taurine is found abundantly in tissues that are excitable, rich in membranes, and that generate oxidants. Thus, it is the most prevalent of all the amino acids in the tissues comprising the skeletal and cardiac muscles and the brain. It is critical to the proper function of the brain, heart, lungs, kidney and blood. Because it performs key functions in cholesterol metabolism related to bile acids, it is essential to the role of the liver, pancreas, and gall bladder.

2.2.4 Properties of taurine

2.2.4.1 Physio-chemical properties

The physio-chemical properties of taurine are given in Table 2.2.4.1.

2.2.4.2 Physiological properties

For a long time, taurine was considered a nonessential nutrient for humans. However it is increasingly recognized that taurine plays several important roles in the body being involved in a number of metabolic processes and is essential to newborns of many species. Gaull
### Table 2.2.3.1 Taurine content of some aquatic organisms

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Aquatic organism</th>
<th>mg/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Spiral shell</td>
<td>520</td>
</tr>
<tr>
<td>2.</td>
<td>Conch</td>
<td>851</td>
</tr>
<tr>
<td>3.</td>
<td>Scallop</td>
<td>332</td>
</tr>
<tr>
<td>4.</td>
<td>Blood clam</td>
<td>439</td>
</tr>
<tr>
<td>5.</td>
<td>Mussel</td>
<td>349</td>
</tr>
<tr>
<td>6.</td>
<td>Prawn</td>
<td>143</td>
</tr>
<tr>
<td>7.</td>
<td>Crab</td>
<td>279</td>
</tr>
<tr>
<td>8.</td>
<td>Cuttle fish</td>
<td>673</td>
</tr>
<tr>
<td>9.</td>
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</tr>
<tr>
<td>10.</td>
<td>Ray</td>
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</tr>
<tr>
<td>11.</td>
<td>Conger pike</td>
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<tr>
<td>12.</td>
<td>Flat fish</td>
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</tr>
<tr>
<td>13.</td>
<td>Journje fish</td>
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</tr>
<tr>
<td>14.</td>
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<tr>
<td>15.</td>
<td>Yellow Croaker</td>
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</tr>
<tr>
<td>16.</td>
<td>Spotted maigre</td>
<td>225</td>
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<tr>
<td>17.</td>
<td>Baby croaker</td>
<td>64</td>
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<tr>
<td>18.</td>
<td>Silver pomfret</td>
<td>41</td>
</tr>
<tr>
<td>19.</td>
<td>Hairtail</td>
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<tr>
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<td>Yellow crucian carp</td>
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<tr>
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<td>Black snapper</td>
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<tr>
<td>22.</td>
<td>Grass carp</td>
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<tr>
<td>23.</td>
<td>Silver carp</td>
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<tr>
<td>24.</td>
<td>Gucian carp</td>
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<tr>
<td>25.</td>
<td>Variegated carp</td>
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<tr>
<td>26.</td>
<td>Shell fish</td>
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<td>27.</td>
<td>Eel</td>
<td>91</td>
</tr>
<tr>
<td>28.</td>
<td>Inkfish</td>
<td>672</td>
</tr>
</tbody>
</table>

Table 2.2.4.1 Physio-chemical properties of taurine

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular formula</td>
<td>C_2H_7NO_3S</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>125.15</td>
</tr>
<tr>
<td>Physical state</td>
<td>Large monoclinic prismatic rod shaped crystals.</td>
</tr>
<tr>
<td>Colour</td>
<td>White crystals</td>
</tr>
<tr>
<td>Odor</td>
<td>Odorless</td>
</tr>
<tr>
<td>Solubility</td>
<td>Soluble in water and insoluble in absolute alcohol.</td>
</tr>
<tr>
<td>Melting point</td>
<td>300°C</td>
</tr>
<tr>
<td>pH (0.5M in water, 25°C)</td>
<td>4.5-6</td>
</tr>
<tr>
<td>Optical rotation</td>
<td>Nil</td>
</tr>
</tbody>
</table>

(1989) suggests that since man has low cysteinsulfinic acid decarboxylase, an enzyme necessary for the formation of taurine from cysteine, people are dependent upon dietary taurine. Under certain conditions of high stress or in disease states the need for taurine may increase (Boelens et al., 2003). Several studies reported that plasma taurine concentrations decrease in response to surgical injury (Chirala et al., 1989), trauma (Neary et al., 1997), sepsis, and critical illnesses (Paauw & Davis 1990). As metabolic patterns progressively deteriorate, plasma taurine concentrations decrease severely, suggesting an increased expenditure and possibly an increased requirement for taurine. Taurine along with GABA and glycine is an inhibitory type of amino acid (Saransaari & Oja, 1998), contrary to the excitatory amino acid like aspartate, glutamate and glutamine. Taurine is important in the visual pathways, the brain and nervous system, cardiac function, and it is a conjugator of bile acids. Basically, its function is to facilitate the passage of sodium, potassium, calcium and magnesium ions into and out of cells and to stabilize the charged cell membranes. Another important function of taurine is detoxification. Taurine is required for efficient fat absorption.
& solubilization. Studies also showed that dietary taurine supplementation ameliorates experimental renal disease including models of nephrotic syndrome and diabetic nephropathy. The beneficial effects of taurine are mediated by its antioxidant action. (Trachtman et al., 1995). Taurine may also have an important role in renal development. One study with rats showed protective effect of taurine on induced inflammatory bowel disease. With all these discoveries and more on the horizon taurine research is accelerating rapidly.

2.2.5 Synthesis

Mammalian taurine synthesis occurs in the pancreas via the cysteine sulfenic acid pathway (Fig 2.2.5.1) from the amino acids methionine and cysteine (Beetsch & Olson, 1998). Vitamin B-6 (pyridoxal-5' phosphate) is a key cofactor in this process. In this pathway, the sulfhydryl group of cysteine is first oxidized to cysteine sulfenic acid by the enzyme cysteine dioxygenase. Cysteine sulfenic acid, in turn, is decarboxylated by sulfinoalanine decarboxylase the rate-limiting step in the taurine synthesis to form hypotaurine. Hypotaurine is then spontaneously or enzymatically oxidized to yield taurine.

![Taurine synthesis and physiological roles in mammalian cells](image)

Fig 2.2.5.1 Taurine synthesis and physiological roles in mammalian cells
2.2.6 Biological roles

Taurine availability can be affected by the reduction of plasma taurine transport to the cell, restricting dietary supply or using transport inhibitors or antagonists (Braghiroli et al., 1990). Studies of taurine knockout mice have been useful to unravel its role in the development and maintenance of normal organ functions (Warskulat et al., 2007). Such studies have demonstrated significant effects on retinal regeneration (Militante & Lombardini 2004) cardiac dysfunction, cardiac myopathy (Schaffer et al., 2000) and platelet hyperaggregation (Mc Carty, 2004). The decline of tissue taurine content during aging has been demonstrated and could exacerbate age-related increase of oxidative stress and related morbidity (Dawson, 2004). Studies on taurine deprivation have also shown their effect on growth and development. Taurine is essential for growth and survival of mammalian cells as well as fetus development, development of the newborn and during childhood (Kim et al., 2006).

2.2.6.1 Osmoregulation and cellular tonicity

Cell volume is an essential parameter in the cellular regulation of secretion, metabolism, cell growth and programmed cell death (Zonia & Munnik, 2007). Taurine plays an important role as an organic osmolyte in cell volume control in mammalian cells and a change in the cellular taurine content is an indication of a shift in the cell volume (Lambert, 2004). Cellular volume changes in response to insults like infection, disease, and trauma; restoration of cell volume is essential to recovery from illness (Koshy et al., 1996). Taurine, an osmolyte, helps to regulate osmolarity without causing additional perturbations of cellular tonicity. When cells are hypo-osmotic, they would swell and lyse; taurine is thus extruded to prevent such severe osmolar changes. In hypernatremia, cells are usually shrunken; taurine uptake is thus increased to help regulate osmolarity to prevent possible cell death. This phenomenon was
illustrated by Trachtman et al., 1990, where taurine exerts a protective, osmoregulatory effect on cerebral and extra-cerebral tissues during extreme hyponatremia.

2.2.6.2 Brain health

2.2.6.2.1 Free radicals

Free radicals are highly reactive atoms that wreak havoc in the body by converting stable molecules into unstable ones (Harman, 1992). They can oxidize macromolecules of healthy tissue, causing cell death, mutagenic changes or an increase in unstable substrates such as oxidized LDL, which can readily stick to the lumen of arteries. Free radicals are particularly detrimental to brain tissue, which contains a high concentration of lipids (Evans, 1993). Recently, the role of taurine's precursor hypotaurine as a potent antioxidant has been discovered (Fontana et al., 2004; Aruoma, 1998). The sulfinyl group in the hypotaurine molecule is responsible for its efficiency as a radical scavenger. The process by which hypotaurine converts to taurine has been shown to effectively scavenge free radicals and it increases cell viability.

2.2.6.2.2 Hypoxia and ischemia

The central nervous system is least tolerant to hypoxic conditions. Brain death usually occurs in three to five min in an anoxic state. Glucose is one of the basic energy molecules that cells utilize to produce energy, with rapid death of neurons in the hypoglycemic state. A compromise in supplies of both oxygen and glucose results in the condition known as ischemia. Taurine has been shown to prevent the disturbances associated with hypoxia (Michalk, 1997). Taurine modulates the enzymes involved in energy metabolism in the brain, restoring adenine and ATP while reducing ADP and AMP levels.
2.2.6.2.3 Hepatic encephalopathy

Hepatic encephalopathy (HE) is a condition whereby the brain is poisoned by ammonia (Kilburn, 2000). It occurs in association with severe liver damage, where the organ fails to efficiently convert ammonia to urea for excretion (Chatauret & Butterworth, 2004). In addition, concentrations of amino acid precursors to urea that function as excitatory substances such as aspartate and glutamate build up in the body, particularly in the brain (Kojic, 2005). Taurine is redistributed to adjacent cells located in the central nervous system, an attempt to protect those cells from damage (Butterworth, 1996). By its ready availability to CNS cells, taurine's role in cell volume regulation and neuro-protection may be particularly valuable in those suffering from HE.

2.2.6.2.4 Excitotoxicity

Excitotoxicity is the term for the presence of excess amounts of the excitatory amino acids, especially glutamate and aspartate, such that they create an intracellular and extracellular toxic environment resulting in cell death (Chen et al., 1999). To combat this, cells release extra quantities of taurine, known for its volume regulatory ability, thereby buffering the dramatic changes in osmolarity (Wu et al., 2005). Taurine thus acts as both an osmoregulator and neuromodulator (Idrissi & Trenkner, 1999).

2.2.6.2.5 Brain aging

Taurine is found in high concentrations in the brain, though levels decline with age (Idrissi, 2008). Researchers showed that the learning ability of older rats was impaired, impairment correlated to the reduction in taurine levels (Dawson et al., 1999). In cerebrospinal fluid of patients with Parkinson's disease, levels of a few amino acids including taurine are lowered (Engelborghs et al., 2003). Taurine is reported to promote release of
dopamine, a neurotransmitter lacking in Parkinson’s disease, from the neuronal pool. Alzheimer's disease, is due to in part, an increase in the generation of nitric oxide (Togo et al., 2004). The supplementation of taurine, along with magnesium inhibits NO production (Chaekyun et al., 2006).

2.2.6.3 Heart health

Taurine makes up nearly 50% of the free amino acids in the heart cells (Huxtable, 1980). Taurine level is depleted in the failing heart (Azuma & Schaffer, 1995). Taurine’s electrophysiological actions in cardiac cells are brought about by modulation of ion channels (Satoh, 1998). Calcium homeostasis is critical to stable myocardial contractile function. Changes in the intracellular taurine pool modulate calcium transport and taurine exerts a cardioprotective action (Punna et al., 1994). Through the sodium-calcium transport exchanger in cardiomyocytes, taurine permits the entry of sodium which favors a co-transport of calcium (Harada et al., 1988). It also modulates the activity of calcium channels to promote sodium influx. In these regards, taurine has an essential function in ensuring stable calcium levels, which thereby promotes proper contractile function of the heart tissue. Likewise, potassium is also an important ion in heart cells. Taurine directly modulates the potassium ion current by increasing the current's action potential duration (Satoh, 1998).

In arrhythmia, irregular heartbeat patterns are caused by abnormal extracellular calcium concentration in heart cells. In a research study, the addition of taurine attenuated this response of myocytes to varying calcium concentrations (Takahahsi et al., 1998). Taurine is valuable for its role in protection of heart from oxidative stress and post-ischemic injury (Chapman et al., 1993). It reduces lipo-peroxidation. The ability to scavenge free radicals is a potent cardioprotective role. The quantity of lactate, a marker of ischemic challenge was reduced and quantity of glutathione was enhanced with taurine. ATP levels are also
suppressed in ischemia which was restored by taurine. Through the modulation of lactate, glutathione, and ATP, taurine influences the ability and extent of recovery in heart (Man’kovs’ka et al 1998, Timbrell et al., 1995).

2.2.6.4 Effect on skeletal muscle

Taurine is necessary for normal skeletal muscle functioning. This was shown by a study, using mice with a genetic taurine deficiency (Ito et al., 2008). They had a nearly complete depletion of skeletal and cardiac muscle taurine levels. These mice had a reduction of more than 80% of exercise capacity compared to control mice (Geny et al., 2006).

2.2.6.5 Effect on liver health

2.2.6.5.1 Bile

The liver is the site of synthesis of bile which is essential for proper emulsification and thus digestion of fats. Taurine is conjugated via its amino terminal group with chenodeoxycholic acid and cholic acid to form the bile salts sodium taurochenodeoxycholate and sodium taurocholate. The low pKa of taurine's sulfonic acid group ensures that this moiety remains ionized and negatively charged even at the high acidity that occurs at the upper intestine and thus improves the surfactant properties of the cholic acid conjugate (Mende et al., 1999).

In addition to its role in bile salts formation, taurine has considerable importance in cell maintenance functions (Nakashima et al., 1990). As with the neurons and neuroglia, taurine exerts cyto-protective effects when hepatocytes are exposed to hypoxia (Nakashima et al., 1996). According to Nakashima et al., when conjugated with bile acids, taurine increases membrane mobility as well as fluidity. Without proper levels of taurine, the liver cells would be susceptible to osmotic changes and their membranes would become less
permeable (Schaffer et al., 1998). The resulting impairment to the liver would significantly compromise its ability to detoxify blood, allowing toxins to spill into the body.

2.2.6.5.2 Effects on cholesterol

Elevated low density lipoproteins (LDL) are implicated in a range of heart and vascular diseases, including myocardial infarction and artherosclerosis (Holvoet et al., 2003). High density lipoproteins (HDL) are recognized for their protective function on both the heart and vasculature (Karádi et al., 1987). Taurine can attenuate increases in total and LDL cholesterol in people consuming a high fat, high cholesterol diet (Wen et al., 2004) and help reach the favorable lipids ratio. Rats fed a high cholesterol diet plus high doses of taurine demonstrated significant reductions in plasma levels of total cholesterol (32% reduction), LDL cholesterol (37% reduction), and triglycerides (43% reduction) when compared to rats fed a high cholesterol diet without taurine (Park & Lee 1998). Taurine conjugates of all bile acids suppress very low density lipoprotein (VLDL) secretion (Lin et al., 1996). With regard to HDL cholesterol, taurine enhances serum HDL concentration in a dose-dependent manner. In mice, taurine administration lowered serum LDL and VLDL by 44% while elevating HDL by 25% (Murakami et al., 1999). Taurine also decreased the content of cholesterol in the liver by 19%. The cholesterol-lowering action of taurine may lie in its ability to promote the conversion of potentially harmful cholesterol to relatively harmless bile acids. From the studies relating to the ability of taurine to conjugate bile acids and thereby promote fat absorption, a new drug sodium tauroursodeoxycholate to treat cholestasis has been synthesized (Ishizaki et al., 2001).
2.2.6.5.3 Exposures to solvents

Exposure to solvents is common hazard for industrial workers in chemical and petroleum refinement, the plastics and automotive industries, among many others. Solvents have a deleterious effect on the function of the liver and have been linked to birth defects, sterilization, chronic fatigue, etc (Xiao & Levin 2000). Carbon tetrachloride, adversely affects liver function. In a lab study that produced degenerated hepatocytes and necrosis damage from exposure to carbon tetrachloride, researchers discovered that the concurrent administration of taurine could ameliorate the damage (Miyazaki et al., 2005). Taurine moderated the extent and severity of lesions and reduced the number of cancer-antigen positive hepatocytes (Cetiner et al., 2005). Taurine was also shown to protect against DNA (Messina & Dawson 2002) damage which may have prevented the hepatocyte degeneration, lesions, and necrosis characteristic of carbon tetrachloride exposure.

2.2.6.6 Effects on kidney

Taurine is essential for proper kidney function and in its absence, renal capacity is diminished such that the process of excretion of toxic substances from the blood is grossly impaired (Mozaffari, 2003). Taurine acts in the kidney, as an organic osmolyte. Depending on the tonicity of the urine emerging from the kidney, the cells modify their tonicity. When the fluid in medulla is hypertonic, its cells accumulate taurine and similar osmolytes, thus exerting a conservatory effect upon taurine (Fugelli et al., 1995). Trachtman et al., 1995 demonstrated the therapeutic effects of taurine on kidney function of diabetic rats. Taurine administration reduced the total proteinuria and albuminuria by approximately 50%, prevented glomerular hypertrophy, diminished glomerulosclerosis and tubulointerstitial fibrosis, overall ameliorating diabetic nephropathy by reducing renal oxidant injury.
2.2.6.7 Vision

In the healthy eye lens, taurine is found in very high concentration among other amino acids (Heinämäki et al., 1986). Lenses subjected to oxidative stress exhibit characteristic changes in their amino acid profile, with taurine levels greatly depressed and cause changes in lens transparency (Fris et al., 2006). Cataract, the clouding of the clear eye lens, is due to oxidation and glycosylation of proteins in the lens. A lack of the antioxidant nutrients (taurine, vitamins A, C and E and carotenoids) is a major causative factor for the development of cataracts. Taurine acts as an antioxidant by preventing changes in the levels of glutathione, ATP, and insoluble proteins-factors that predispose the formation of cataracts (Devamanoharan 1997).

Taurine plays a critical role in the structure and function of the photoreceptors (Rentería et al., 2004). Through its osmoregulatory function, taurine makes the rod outer segment of the retina resistant to injury. A high-affinity, taurine-specific uptake system is present in the rod outer segment system (Militante & Lombardini, 1999). Through modulation of membrane ion channels, taurine increases calcium uptake to promote the transmission of visual signals from the retina to the brain. Also taurine is important for the regeneration of damaged cells in the retina. It functions to phosphorylate specific proteins and increase cellular outgrowth (Lima & Cubillos 1998).

2.2.6.8 Gastroenterology

Inflammatory bowel disease is a chronic condition characterized by diarrhea, low-grade fever, fatigue, weight loss, and abdominal cramps (Podolsky, 2002). It is frequently associated with colon ulceration and/or inflammation, which cause an increase in colon weight -- a reflection of tissue edema. Taurine by defending against oxidative damage ameliorates IBD (Mi et al., 1998). Non-steroidal anti-inflammatory drugs (NSAIDs) can
cause gastric ulceration (Wallace, 2000). Taurine by inhibiting neutrophil activation and preventing their adhesion to the gastric lining (Zeybek et al., 2006) and lipid anti-peroxidation effects exerts a protective effect on the intestinal tract.

### 2.2.6.9 Pulmonary function

The depletion of taurine is harmful to pulmonary tissue. Alveolar macrophages on the surface of lung alveoli become susceptible to oxidative stress when deprived of the antioxidant protective capacity that taurine provides (Minko et al., 2002). In cystic fibrosis (CF), respiratory and digestive systems are affected and are characterized by steatorrhea, indicative of fat malabsorption (Balinsky & Zhu 2004). In children with CF and steatorrhea, taurine supplementation resulted in improved fat digestion, decrease of fecal fatty acid and total sterol excretions and modified lipid profile (Carrasco et al., 1990). CF is also marked by liver disease. In a year-long study of CF patients with poor liver function, taurine supplementation caused an increase in serum pre-albumin and restored fat absorption, with no severe side (Colombo et al., 1996). Finally, taurine significantly attenuates endothelial cell apoptosis and necrosis due to oxidative stress (Zhang et al., 2008). These functions are due to antioxidant activity and regulation of intracellular calcium flux. This has great implications for the therapeutic value of taurine in inflammatory-type lung conditions.

### 2.2.6.10 Inflammatory disorders

In inflammatory disease, plasma taurine becomes depleted, signifying a greater demand by the body in this state (Xu et al., 2008). Taurine prevents the tissue damage that may otherwise result from inflammation. The mechanism involves taurine monochloramine, formed in the leukocytes that inhibit the production of tissue-damaging pro-inflammatory factors like nitric oxide, prostaglandin PGE2, and tumor necrosis factor (Fallahzadeh et al.,
The taurine derivative N-chlorotaurine is a weak oxidant produced by leukocytes in response to bacterial and fungal exposures (Neher et al., 2008).

2.2.6.11 Diabetes

In both forms of diabetes - insulin dependent (Type 1) and non-insulin dependent (Type 2), taurine exerts a multitude of beneficial actions. Platelet aggregation in Type 1 diabetes results in an increased risk of cardiovascular incidents. When taurine is supplemented, an increase in both plasma and platelet taurine levels occur that raise the threshold at which aggregation can be triggered (Fianconi et al., 1995). Taurine changes the abnormal blood lipid profile that is associated with the diabetic condition. Researchers found that elevated plasma triglycerides and LDL cholesterol in diabetics were lowered through administration of taurine (You & Chang 1998). In models of diabetic mice, researchers found that taurine supplementation lowered levels of malondialdehyde (MDA), a marker of lipid peroxidation in liver and islets (Lim et al., 1998).

In Type 2 diabetics, the impaired glycemic control is largely due to peripheral insulin resistance, hepatic insulin resistance, and a failure of beta cell function. New complementary therapies including dietary changes, exercise programs, weight loss and supplementation with chromium, vitamin E, magnesium, and soluble fiber can produce improvement in peripheral insulin resistance (Mc Carty, 1997). Recently, taurine has found a role as well, correcting the metabolic anomalies in Type 2 diabetes. Recognizing that these approaches may be adequate but not optimal measures, they are worthy of consideration as adjuncts to drug therapies.

2.2.6.12 Cancer

In cancerous conditions, taurine is a potent cytoprotective agent and immune enhancer (Redmond 1998). Researchers have discovered that taurine inhibits tumors and extends
survival of mice (Schuller-Levis & Park, 2003). Taurine has been shown to be depleted in people taking chemotherapy (Desai et al., 1992). When combined with chemotherapy, taurine extended survival with no tumor visible -- an inhibition rate of 100% (Zhang et al., 1997). Tumor cell membrane fluidity was much improved with taurine supplementation. Recombinant interleukin-2 immunotherapy, in certain types of cancers produces cytotoxic effect on both tumor cells and vascular endothelial cells. When added to the cancer therapy program, taurine acts to reduce endothelial cell death and actually increases the tumor cytotoxicity. The calcium homeostatic mechanism of taurine was found to be the critical feature in these anti-cancer functions (Finnegan et al., 1998).

Hepatocarcinogenesis is marked by changes in lipid peroxidation. Rats exposed to carcinogenic substances without pre-supplementation with taurine, showed depressed glutathione peroxidase activity and membrane stability (You & Chang 1998a). Membrane stability and enzyme activity were restored when taurine was supplemented prior to exposure.

2.2.6.13 Prenatal and childhood development

As a key organic osmolyte, membrane stabilizer, and antioxidant, taurine facilitates cellular function right from the first stages of embryonic development. Taurine plays an indirect role in cell division as the process is associated with changes in cell number and cell volume (Fugelli et al., 1995). Deprivation of taurine to embryos proves to be disadvantageous on cellular development, because they rely on inorganic osmolytes for volume regulation and thus are deprived of various cytoprotective functions of taurine. Pregnant women consuming diets that are deficient in taurine place their fetuses at risk for retarded growth (Wu et al., 1998). Taurine is critical during development to produce normal fetal beta cell function (Reusens and Remacle 2006). Taurine has a dose-dependent trophic effect on the human fetal brain cell, promoting proliferation and differentiation. Taurine is indispensable to proper
neurological development and neuromuscular function (Rice, 2000). Taurine is necessary for the proper retinal development in children. Its presence prevents granulation of the retina (Chesney et al., 1998). In the postnatal infant and during subsequent development, taurine continues to dynamically influence the activity of the retina. In neonatal cardiomyocytes, taurine functions as an organic osmolyte (Ying et al., 2009). When taurine is lost, these cardiac cells reduce in size and change in shape as well as configuration to protect against tonicity fluxes.

2.2.6.14 New applications

2.2.6.14.1 Alcoholism

From studies of correlation of taurine with alcohol consumption (Quertemont et al., 1998), the drug acamprosate, calcium salt of N-acetyl-homotaurinate was synthesized. It is specifically designed to maintain abstinence in alcohol-dependent patients and interacts with glutamanergic neurotransmission channels (NMDA receptors) to reduce calcium flux, resulting in a depressed interest in alcohol consumption (Dahchour et al., 1998).

In a study of rats, researchers induced hepatic steatosis and lipid peroxidation by administering alcohol for a period of 28 days (Kerai et al., 1998). However, in the group in which taurine was co-administered, hepatic steatosis was greatly reduced and lipid peroxidation completely prevented.

2.2.6.14.2 To combat effects of ageing

Decline in taurine levels of the spleen, kidney, eye, cerebellum, and serum are associated with age in rats (Dawson et al., 1999). Taurine supplementation effectively corrects these deficits. Taurine may prove to be important in preserving normal muscle function that is ordinarily compromised with age. In a study of aging rats, depletion of taurine in skeletal
muscle tissue causes decreases in both the electrical and contractile properties. Taurine supplementation significantly raised muscle taurine level, enhancing performance to that of a young rat. Taurine also improves the mechanical threshold for contraction (Pierno et al., 1998). These findings may become applicable for the development of future novel therapies to combat age-related muscular decline.

2.2.6.14.3 Effect on migraine

The underlying biological basis of migraine is yet to be understood. Many of the conditions associated with migraine - neuronal hyperexcitation, vasospasm, hypoxia, platelet activation, and sympathetic hyperactivity (44th Annual Scientific Meeting, American Scientific Society, 2002) - are expected to be countered by increased tissue levels of taurine and magnesium. Some studies state that magnesium taurate may become a valuable drug to reduce migraine incidents (McCarty, 1996).

2.2.6.14.4 Taurine as a marker of disease

In asthmatics, researchers found increased taurine content in bronchoalveolar lavage fluid. Thus, the profile of amino acids in the fluid may serve as a potential diagnostic tool (Hofford et al., 1997). It may be possible to develop specific treatments targeted at modulating the profile of asthmatic bronchial fluid.

2.2.6.14.5 Protection against radiation

Taurine is useful in countering the damaging effects of low levels of gamma radiation. A combination of taurine with vitamin E & C, and alpha lipoic acid has been shown to protect against radiation-associated protein leakage (Bantseev et al., 1997).
2.2.7 Uptake of taurine

The intracellular taurine concentration is a balance (Fig 2.2.7.1) between (i) active taurine uptake via the Na\(^+\), Cl\(^-\)-dependent, pH-sensitive and high affinity taurine transporter TauT, (ii) synthesis from cystein/methionine, and (iii) release via either a transport process that resembles TauT working in reverse or a volume-sensitive taurine leak pathway (Ballatori & Boyer 1992). The total body taurine pool in humans is controlled by TauT located at the brush border of the renal proximal tubule and in the basolateral membrane of the distal nephron (Han et al., 2002).

**Fig 2.2.7.1 Taurine uptake and release systems in mammalian cell**

2.2.8 Post absorptive metabolism

Taurine reacts non-enzymically with hypochlorous acid (HOCl) to form N-chlorotaurine (taurine chloramine) and this is then converted to (Fig 2.2.8.1 & Fig 2.2.8.2) sulfoacetaldehyde and isethionic acid (8th International Congress on Amino Acids and Proteins, 2003, Cunningham et al., 1998).

\[
\begin{align*}
&\text{HO}_3\text{SCH}_2\text{CH}_2\text{NH}_2 \quad \text{Taurine} \\
&\quad \xrightarrow{\text{Spontaneous}} \text{H}_2\text{O} \\
&\text{HO}_3\text{SCH}_2\text{CH}_2\text{NHCl} \quad \text{Taurine chloramine} \\
&\quad \xrightarrow{\text{Spontaneous plus enzyme}} \text{H}_2\text{O} \quad \text{NH}_3 + \text{HCl} \\
&\text{HO}_3\text{SCH}_2\text{CHO} \quad \text{Sulfoacetaldehyde} \\
&\quad \xrightarrow{\text{NADPH} + \text{H}^+} \text{Aldehyde reductase} \quad \text{NADP}^+ \\
&\text{HO}_3\text{SCH}_2\text{CH}_2\text{OH} \quad \text{Isethionic acid}
\end{align*}
\]

Fig 2.2.8.1 Formation of isothenic acid
Fig 2.2.8.2 Breakdown of taurine

2.3 D-Galactosamine (D-GalN)

2.3.1 Experimental model

FHF is a severe liver injury accompanied by hepatic encephalopathy which causes multiorgan failure with an extremely high mortality rate, even if intensive care is provided (Fernandez et al., 2003). Management of severe FHF continues to be one of the most challenging problems in clinical medicine. Liver transplantation has been shown to be the most effective therapy. However, the lack of donors combined with the high costs, technical difficulties, viability issues and the disadvantage of needing life-long pharmacological immunosuppressant treatment (Nasseri & Vacanti 2002) following surgical intervention with the added complication that the immunosuppressant agents used themselves produce side effects in the kidneys, liver and other organs (Khan, 2009), mean that liver transplantation is not always an option. For these reasons, other therapeutic options to help patients recover or stabilize have to be considered. Although a number of clinical trials testing different liver assist devices are under way (Tilles et al., 2002, Patzer II 2001, Detry et al., 1999, Chen et
al., 1997), these systems alone have no significant effect on patient survival and are only regarded as a useful approach to bridge patients with FHF to liver transplantation. As a result, reproducible experimental animal models (Newsome et al., 2000) resembling the clinical conditions are very relevant. The three main approaches (van de Kerkhove et al., 2004) used to create an animal model for FHF (Table 2.3.1.1) are: surgical procedures (total/partial hepatectomy, complete/transient devascularization), toxic liver injury by the use of hepatotoxic drugs (acetaminophen, D-GalN, thioacetamide, and others) and infective (viral models) procedures.

Table 2.3.1.1 Main FHF animal models in different species

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Species</th>
<th>Advantages/disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total/partial hepatectomy</td>
<td>Pig, dog, rabbit, rat, mouse</td>
<td>Hepatic encephalopathy (HE); reproducible/no reversibility; no long-term survival</td>
</tr>
<tr>
<td>Total/partial devascularization</td>
<td>Pig, dog, rabbit, rat</td>
<td>HE; reproducible/no reversibility; no long-term survival</td>
</tr>
<tr>
<td>Chemical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>Pig, dog, rabbit, rat, mouse</td>
<td>HE; no hazard/non-reproducible; variable interval between damage and death; species and age variability</td>
</tr>
<tr>
<td>Amanitin</td>
<td>Pig</td>
<td>HE; specific toxic effects; large animal</td>
</tr>
<tr>
<td>Azoxymethane</td>
<td>Mouse</td>
<td>HE; reproducible/small size; hazard</td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td>Pig, rabbit, rat, mouse</td>
<td>HE /non reproducible; extrahepatic toxicity; small time window before death</td>
</tr>
<tr>
<td>Concanavalin A</td>
<td>Rat, mouse</td>
<td>HE /small size</td>
</tr>
<tr>
<td>D-Galactosamine</td>
<td>Pig, dog, rabbit, rat, mouse</td>
<td>biochemical markers/non-reproducible; hazard; variable interval between damage and death; species differences, HE</td>
</tr>
<tr>
<td>Lipopolysaccharide</td>
<td>Rat, mouse</td>
<td>HE /non-reproducible; small size; hazard; small time window before death</td>
</tr>
<tr>
<td>Thioacetamide</td>
<td>Rabbit, rat, mouse</td>
<td>HE; reproducible; large time window before death/hazard</td>
</tr>
<tr>
<td>Viral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemorrhagic disease</td>
<td>Rabbit</td>
<td>HE; reproducible; no hazard</td>
</tr>
</tbody>
</table>

The use of chemical agents such as acetaminophen, thioacetamide or D-GalN (Blei et al., 1992) may reproduce a number of important FHF clinical characteristics, such as hypoglycemia, encephalopathy, and increased blood levels of hepatic enzymes and hepatotoxic chemical agents are frequently used as a model for FHF. Criteria for an FHF animal model are shown in Table 2.3.1.2

<table>
<thead>
<tr>
<th>Criteria for an FHF animal model Terblanche and Hickman (1991)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reversibility</td>
</tr>
<tr>
<td>Reproducibility</td>
</tr>
<tr>
<td>Death from liver failure</td>
</tr>
<tr>
<td>Therapeutic window</td>
</tr>
<tr>
<td>Adequate animal size</td>
</tr>
<tr>
<td>hazard to personnel</td>
</tr>
</tbody>
</table>

D-GalN is a molecule which, when metabolized via the galactose pathway in the liver, causes serious metabolic alterations and hepatic necrosis through depletion of different uridine intracellular mediators (Kim et al., 2004), and has therefore been used to develop FHF models. This model also displays the characteristic effects of human FHF (Hung et al., 2007), such as an increase in blood levels of liver enzymes, bilirubin, ammonium or lactate and the associated coagulopathy, hypoglycemia, coma and increase in intracranial pressure. A reproducible model has been developed with pigs (de Groot 1987) which, because of their size, are suitable for the assessment of different support systems designed for treating FHF in humans. Significant differences in D-GalN sensitivity across different species exist (Galanos et al., 1979). Furthermore, the interval between damage caused and death is not uniform, the agent is expensive to use in large-scale models, and lastly, it carries health risks. D-GalN
models have been used to investigate the renal damage which accompanies FHF and the liver metabolic pathways involved. D-GalN models have also allowed testing of different extracorporeal hepatic support devices and bioartificial systems (Frühauf et al., 2004), including hepatocytes transfected with the human gene interleukin-1 receptor antagonist in rats, the use of a nonwoven fabric bioreactor containing porcine hepatocytes, or the study of the potential effects of cerebrospinal fluid drainage and cranial decompression in rats.

2.3.2 Structure and properties of D-galactosamine

![Fig 2.3.2.1 Structure of galactosamine](image)

Chemically D-GalN is 2-amino-2-deoxy-hydrochloride with a molecular formula of C₆H₁₄ClNO₅.HCl and molecular weight is 215.63206 g/mol. The hydrochloride salt of D-GalN is a stable white powder soluble in cold water with a melting point 178-180°C. In presence of conditions like excess heat and incompatible materials, the compound is explosive and it is reactive with oxidizing agents (Fig-2.3.2.1).

2.3.3 Pathophysiological mechanism of D-galactosamine
D-GalN has been shown to produce a liver damage closely related to human viral hepatitis both biochemically and histologically (Shi, 2008). The mechanism of the hepatotoxicity of D-GalN is as yet unknown, although certain biochemical defects have been reported. These include depletion of liver glycogen and adenine nucleotides (Keppler & Decker, 1969), and depletion of uridine phosphates with accumulation of UDP-hexosamines (Keppler et al., 1970), as well as a decrease in protein synthesis. D-Galactosamine-1-phosphate and UDP-galactosamine were identified as the predominant early metabolites of D-GalN in rat liver (Beckwith-Hall et al., 1998).

Galactosamine-1-phosphate accumulation is enhanced by the strongly reduced levels of UDP glucose. The influence of the strongly diminished UDP glucose levels on the UDP glucose-linked syntheses of glycogen, heteropolysaccharides and glucuronides as well as the trapping of uridine phosphates by formation of UDP-hexosamines may play an important role in the induction of galactosamine hepatitis. Within thirty min of D-GalN administration in rats, there occurs a high accumulation of UDP-galactosamine derivatives in the liver, leading to a depletion of hepatic UTP (Galanos et al., 1979). As a result biosynthesis of macromolecules (RNA, proteins, glycoproteins, glycogen etc) ceases. These alterations lead to eventual cell damage and cell death which at later stages of the reaction may be identified by the increase of liver enzymes in the blood and by histology. The D-GalN-induced liver injury is intimately connected with the alterations in the structure and function of the plasma membrane due to impaired glycoprotein synthesis (Sinha et al., 2007).

### 2.3.4 Metabolic alterations

Administration of the amino sugar D-GalN to rats causes liver damage, which morphologically resembles acute hepatitis (Lehmann et al., 1987, Keppler et al., 1970). D-GalN treatment of rats results in a marked increase of liver specific enzyme activity in the
blood of animals (Sugiyama et al., 1999). These enzymes include aspartate aminotransferase, alanine aminotransferase, alakaline aminotransferase, lactate dehydrogenase, gamma glutamyl transferase and glutamate dehydrogenase. Bilirubin concentration, predominantly unconjugated type is elevated making the plasma of galactosamine-treated rats (Lo et al., 1987). Gluconeogenesis from lactate is decreased (Yokoyama et al., 2005) which may be due to the lower activity of the key enzyme, phosphoenolpyruvate carboxylase (EC 4.1.1.32) resulting in hypoglyceamia. Effect of D-GalN on the concentrations of metabolites in blood includes a significant elevation of lactate, pyruvate and 2-oxoglutarate all of which indicate impaired gluconeogenesis (Banta et al., 2005). The activity of several hepatic enzymes is lowered which may be a result of membrane damage (Sugiyama et al., 1998), reduced protein synthesis (Geng et al., 2005), increased rates of intracellular degradation of the enzymes or conversion into inactive forms.

2.3.5 Morphological and structural changes

It is known that administration of D-GalN in large quantities to the rat induces liver injury which shows morphological and functional features similar to those of acute human viral hepatitis. D-GalN produces foci of hepatocellular necrosis scattered throughout the lobule (Sielaff et al., 1995) and accompanied by an inflammatory infiltrate of polymorphonuclear leukocytes and lymphocytes. The picture of progressive inflammatory response with severe liver cell degeneration and necrosis as well as lobular distortion and increased fibrous tissue closely resembled those in human active hepatitis (Tsuji & Shinohara 1981). D-GalN produces acute hepatocellular lesions in rats (Taniguchi et al., 2002) and other animals (Braun et al., 2000, Nayyar & Koenig 1974). Light microscopically, cirrhotic changes were observed (Dhanabal et al., 2006) in most of the animals characterized by the proliferation of the connective tissues from portal triads into hepatic lobules. Features like edema of
hepatocellular microvilli and widening of sinusoidal endothelial fenestrae (SEF); massive hepatic necrosis with hemorrhage are commonly seen. A single intraperitoneal injection of 800 mg/kg or 1500 mg/kg D-GalN-induced remarkable histological and cytological changes in the rat liver (Sinha et al., 2007). Light microscopically, the liver showed diffuse parenchymal damage, (Wan et al., 2008) in which hepatic cell cords were disorganized and a marked accumulation of lipid droplets (Sasaki et al., 1996) were found in the hepatocytes.

2.3.6 Ultrastructural changes

Electron microscopical studies of hepatocytes of mice treated with D-GalN showed-dilation of ER of both rough and smooth type with swollen mitochondria (Datta & Bhattacharya, 2001; Shigeta, 1997). Nuclear changes showed increase in size and striking anisonucleosis, condensation of chromatin, fragmented and dispersed nucleoli in D-GalN-induced hepatotoxic mice (Trump et al., 1973, Boyer & Klatskin 1970).

In an ultra structural study (Takenaka et al., 2007) of D-GalN induced hepatic damage, edema of the hepatocellular microvilli, widening of the smooth endoplasmic reticulam and transmigration of red blood cells (RBC) and platelets to the space of Disse without exfoliation and necrosis of the sinusoidal endothelial cells were observed. Transmigration of RBCs and platelets to the space of Disse resulted in massive hepatic necrosis due to occlusion of the microcirculation (Yi et al., 2006). In a study, (Arai, 2001) transient injury to the rat liver was induced by a single intraperitoneal injection of D-GalN. After the administration of the drug, most of the liver cells showed synchronous morphological alteration of the plasma membrane accompanied by changes in the histochemical localization of several enzymes in the liver cell. In a study involving administration of a single dose of D-GalN (Kouta et al., 1985) liver ultrastructure showed disruption of lamellar arrangement of rough endoplasmic reticulum, dissociation of intrahepatic cell space and an increase in the number of autophagic
vacuoles. Histo- and cytochemical detection of 5'-nucleotidase and alkaline phosphatase activities revealed disruption of the bile canaliculi system and a disturbance of plasma membrane.

An electron microscopic study (Funatsu et al., 1978) of mitochondria demonstrated mitochondrial proliferation and irregularities with crenated membranes, focal hypertrophy of the smooth endoplasmic reticulum, and decrease of the rough endoplasmic reticulum with partial detouchment of ribosomes, loss of compactness of nucleoli and accumulation of lipid droplets in the cytoplasm. The proliferation of collagen fibers was observed around the hepatocytes and acid mucopolysaccharides were seen in the space of Disse and partly in the sinusoids histochemically using electron microscope. D-GalN, injected into the lumbar theca in a dose of 1 mg or 5 mg (Nayyar & Koenig, 1974), produced a lumbosacral myelopathy with hindlimb weakness or paralysis and loss of sphincter and sensory function. Important early changes visible in the light microscope were a perineuronal and perivascular edema and a condensation of glial chromatin. On electron microscopic examination glia were more severely damaged than neurons. The early glial lesions included: (1) distension of the cisternae of the rough endoplasmic reticulum, including the perinuclear cisternae; (2) mitochondrial swelling; (3) astroglial edema with partial uplifting of nerve endings at synapses; (4) nucleolar changes, including loss of granular constituents and fragmentation; and (5) nuclear changes, notably, a clumping of chromatin, and aggregation of nucleoplasmic granules.
2.4 Liver

The liver is the largest organ in the body, weighing 1.2 to 1.8 kg in the adult (Mitra & Metcalf 2009). The gallbladder is attached to the inferior surface. The liver is divided into the right lobe (larger) and left lobes. The connective tissue septa between the lobules hold branches of the hepatic artery and the portal vein, as well as bile ducts. The liver is composed primarily of hepatocytes which are arranged in cords that extend from the central vein to the portal triads. Each hepatocyte is a polygonal cell with a large, centrally located nucleus. In healthy liver cells, deposits of glycogen are seen (Leander et al., 2000). Adjacent liver cells form tight junctions. Each liver lobule is surrounded by a number of portal triads, each consisting of a single branch of the portal vein, a branch of the hepatic artery, and a bile duct. The liver cell cords are separated from each other by sinusoids which contain blood. Numerous Kupfer cells, which are macrophages by lineage, are also present along the sinusoidal space. The liver cells themselves are separated from the sinusoid by a narrow space called the space of Disse (Selden et al., 1999).

2.4.1 Liver cell injury

In case of liver injury hepatocytes swell via increased water content (Farrell et al., 2008). This is termed hydropic change and is a result of membrane damage and impaired mitochondrial function. Fat mainly the neutral fat of triglycerides accumulates, a phenomenon referred to as steatosis. It indicates some defect in lipid metabolism or lipoprotein synthesis or unusual quantities of adipose or dietary lipid brought to liver. There is loss of cellular fine structure or microvilliFrom the nucleus point of view (Dancygier & Schirmacher, 2010), karyolysis (DNA degradation), pyknosis (nuclear shrinkage), karyorrhexis (nuclear fragmentation) occur (Hooser, 2000). Cell injury can be reversible or irreversible (Fig 2.4.1.1).
Reversible & irreversible injury

Reversible injury is characterized by generalized swelling of the cell and its organelles, blebbing of the plasma membrane (Myagkaya et al., 1984) detachment of ribosomes from the endoplasmic reticulum and clumping of nuclear chromatin (Trump et al., 1996). Transition to irreversible injury is characterized by enhanced swelling of the cell, swelling and disruption of lysosomes, presence of large amorphous densities in swollen mitochondria, disruption of cellular membranes and profound nuclear changes (Trump et al., 2001). The latter include...
nuclear condensation, fragmentation and dissolution of the nucleus. Irreversibly injured cell becomes a dead cell either by apoptosis or by necrosis (Table 2.4.1.1 & Fig 2.4.1.1).

<table>
<thead>
<tr>
<th>Feature</th>
<th>Necrosis</th>
<th>Apoptosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell size</td>
<td>Enlarged</td>
<td>Reduced</td>
</tr>
<tr>
<td>Nucleus</td>
<td>Pyknosis/ karyorrhexis/ karyolysis</td>
<td>Fragmentation</td>
</tr>
<tr>
<td>Plasma membrane</td>
<td>Disrupted</td>
<td>Intact</td>
</tr>
<tr>
<td>Cellular contents</td>
<td>Enzymatic digestion</td>
<td>Intact</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Frequent</td>
<td>None</td>
</tr>
<tr>
<td>Physiologic/pathologic</td>
<td>Pathologic</td>
<td>Physiologic</td>
</tr>
</tbody>
</table>

**Table 2.4.1.1: Features of Necrosis and Apoptosis**

2.4.2 Causes of liver injury or hepatitis

Hepatitis is an inflammation of the liver characterized by diffuse or patchy necrosis (Danet *et al.*, 2003, Semelka *et al.*, 2001). Major causes are specific hepatitis viruses, alcohol, and drugs (Ungo *et al.*, 1998). Less common causes include other viral infections (eg, infectious mononucleosis, yellow fever, cytomegalovirus infection (Stern, 1972) and leptospirosis (Datta & Christopher 2005). Various systemic infections and other illnesses may produce small focal areas of hepatic inflammation or necrosis.

2.4.2.1 Viruses

*Acute viral hepatitis* is diffuse liver inflammation caused by specific hepatotropic viruses that have diverse modes of transmission and epidemiologies and each type shares clinical, biochemical, and morphologic features. Acute infection tends to develop in predictable phases. This topic has been described in Introduction.
2.4.2.2 Drug-induced hepatotoxicity

This is a chemical-driven liver damage. Hepatotoxicity can be broadly considered to occur in two forms, type A *symptomatic* or type B *idiosyncratic*. Drugs that have a *symptomatic* hepatotoxicity (Thiim & Friedman, 2003) are those that are common, related to the pharmacological action of the drug and have predictable dose-response curves and well characterized mechanisms of toxicity. In contrast, the second type, *idiosyncratic* hepatotoxins (Deng *et al*., 2009) are uncommon and cause liver damage in only a small fraction of the population that is exposed to the agent, does not have a clear dose-response.

Most of the drugs are lipophillic substances and they cannot be excreted in bile or urine. They are transformed into hydrophilic metabolites (that may be stable or unstable) by cytochromes, oxygenases present in liver. The unstable and reactive metabolites covalently bind to hepatic macromolecules and cause type A toxicity (Park *et al*., 2001) (Fig 2.4.2.1).

Fig 2.4.2.1 Drug-induced hepatotoxicity
The activity of cytochromes is widely influenced by environmental factors such as alcohol, genetics and other drugs, giving rise to potential drug interactions (Zanger et al., 2000). In the hepatocytes, there are various protective mechanisms explaining the fact that only a huge dose of a xenobiotic or a high metabolic activation rate (Slikker et al., 2004) will cause direct type A toxicity.

In addition, the reactive metabolites covalently bind to hepatic proteins resulting in the formation of a protein adduct or an “alkylated protein” which forms a complex with the major histocompatibility complex (Robin et al., 1997). This neo-antigen expressed on the liver cell membrane may then be recognized by an immunocompetent T cell which will be responsible for cytotoxicity and cell necrosis. This phenomenon is likely involved in type B indirect type of hepatotoxicity as seen in acetaminophen toxicity (Mumoli et al., 2006). Some reactive metabolites can also bind to hepatic proteins giving rise to a hypersensitivity reaction (Naisbitt 2001). Finally, some reactive metabolites can induce genotoxicity, giving rise to carcinogenicity and teratogenicity (Luch 2005).

Among the different targets for drug toxicity in the liver, the first target is the hepatocyte and drug-related hepatitis can be acute or chronic (Fig 2.4.2.2.2) (Liu, 2009). Some drugs induce prolonged inflammation and even cirrhosis of the liver. In the hepatocyte, mitochondria can be involved in some toxicities and this gives rise to microvesicular steatosis (Pessayre et al., 2007, Fromenty & Pessayre, 1997). Some drugs cause inflammation of the endothelial cells of blood vessels of the liver giving rise to a condition called veno-occlusive disease (Schouten et al., 2008). Finally, some substances can induce an activation of the stellate cells. These cells will be transformed into myofibroblasts giving rise to an extensive fibrosis of the liver (Paik et al., 2009). Many drugs, including isoniazid, methyldopa, nitrofurantoin and, rarely, acetaminophen, can cause chronic hepatitis. The mechanism varies
with the drug and may involve altered immune responses, cytotoxic intermediate metabolites, or genetically determined metabolic defects (Park et al., 2000).

2.4.2.3 Alcohol-induced liver injury

Alcoholic liver injury is a form of toxicity, with an immune component to some of the injury (Hines & Wheeler, 2004). Pathophysiology involves fat accumulation (steatosis), inflammation, and fibrosis (Brunt 2007). It has a number of very specific, reproducible and easily recognizable features.
2.4.2.3.1 The Fatty Liver of the Alcoholic Chronic consumption of alcohol causes the liver cells to accumulate triglycerides (steatosis) resulting in enlarged livers with rounded edges (Enomoto, 2000). Alcohol and its metabolites affect virtually every aspect of lipid metabolism in the liver. Possible mechanisms for steatosis include reduced synthesis of very low density lipoprotein (VLDL) and increased hepatic triglyceride synthesis (Mensenkamp, 2001). Alcoholic livers tend to accumulate protein as well, presumably as a result of a defect in protein secretion by the hepatocytes (Kharbanda et al., 2007).

2.4.2.3.2 Ultrastructural Changes Chronic alcohol intake causes mitochondrial enlargement and distortion of cristae (Lewis, 2000). Alcoholic mitochondria have defects in Kreb's cycle enzymes and are sluggish energy producers (Hoek et al., 2002). The mitochondria may reach sizes, larger than the nucleus. Expansion of the smooth endoplasmic reticulum, (Gariot et al., 1987), results in enlargement of the liver cells.

2.4.2.3.3 Alcoholic Hepatitis A few alcoholics develop a central zone hepatitis characterized by cellular swelling, spotty necrosis (Sherlock, 1990). Alcoholic hepatitis progresses to more severe forms of chronic alcoholic injury, including cirrhosis (Teli et al., 1995)

2.4.2.3.4 Central Sclerosis Some alcoholics deposit collagen in the spaces of Disse along the central zone sinusoids and around the central vein (Taguchi and Asano, 1998), eventually obliterating that vein. This type of injury is a precursor of alcoholic cirrhosis.

2.4.3 Types of liver injury

Liver diseases are defined by the types and patterns of injury, inflammation, and cholestasis they induce. The basic patterns of injury, as presented below, are those which are continually used by tissue pathologists to make diagnoses of liver disease.
2.4.3.1 Hepatic necrosis

Hepatic necrosis is a severe and rapidly progressing form of hepatitis accompanied by hepatocellular death and the signs and symptoms of hepatic failure (Goodman, 2002). Massive and submassive hepatic necroses are the histopathological manifestations of fulminant hepatic failure (Kirsch et al., 2009). Causes are diverse and may be due to infections, intoxications, severe hepatic ischemia, hepatic metabolic disease like Wilson’s disease, acute auto-immune hepatitis and others. Grossly, the liver with large areas of necrosis may appear shrunken and yellowish. Typically necrotic cells go through a phase of hydropic degeneration whereby the cell enlarges and its outline becomes irregular, with resultant swelling of the organ followed by rupture of the cell (Levine & Saltzman, 2004). Focal necrosis is followed by an inflammatory infiltrate which results in removal of the necrotic debris and restructuring of the lobular structure, by division of existing cells within the connective tissue framework (Gerlach et al., 2008). Repeated bouts of necrosis and repair may result in disruption of the structure of the liver and result in cirrhosis. Massive necrosis, on the other hand, can result in liver failure and death (Ekici et al., 2005).

2.4.3.2 Hepatic fibrosis

Hepatic fibrosis is an accumulation in the liver of connective tissue in response to hepatocellular damage of nearly any cause (Youseff & Tavill, 2002). It results from excessive production or deficient degradation of the extracellular matrix. Hepatic fibrosis can regress if the insult is reversible (eg, viral clearance). More commonly, however, injury is chronic or repeated, leading to progressive distortion and dysfunction of liver architecture (Christidis, 2001). Fibrosis itself causes no symptoms but can lead to portal hypertension, hepatocyte ischemia or cirrhosis (Levison et al., 1982). Kupffer cells, injured hepatocytes, platelets, and
leukocytes aggregate, releasing reactive O$_2$ species and inflammatory mediators which accelerate fibrosis.

2.4.3.3 *Hepatic cirrhosis*

Cirrhosis is a leading cause of death worldwide (Bosch *et al.*, 2008, Krige *et al.*, 2006). Cirrhosis of the liver is the terminal sequel of prolonged repeated injury to the hepatic parenchyma (Fang *et al.*, 2003). It may be due to alcoholic liver disease or viral hepatitis (Fang *et al.*, 2003). Cirrhosis is fibrosis that progresses to produce diffuse disorganization of normal hepatic structure, characterized by regenerative nodules surrounded by broad bands of fibrotic tissue (Brandão *et al.*, 2006). The liver is misshapen, nodular and shrunken weighing less than 1 kg in some extreme cases of cirrhosis. Cirrhotic patients have some evidence of hepatic cell dysfunction, including jaundice, anorexia, easy bruising and fatigue. The loss of normal liver tissue slows the processing of nutrients, hormones, drugs, and toxins by the liver. Also slowed is production of proteins and other substances made by the liver. In response to injury, growth regulators induce hepatocellular hyperplasia and arterial growth. Angiogenesis produces new vessels through which blood flow becomes distorted and along with compression of hepatic venules by regenerating nodules contributes to portal hypertension (Bosch *et al.*, 2008). Terminal consequences of liver cell necrosis are the accumulation of ammonia resulting in encephalopathy, liver failure and ascites (Rothuizen, 2009). Therapy of underlying liver disease is primarily supportive and no specific therapy can be instituted, other than liver transplantation.

2.4.3.4 *Fatty liver*

Fatty liver is an excessive accumulation of fat especially triglyceride inside hepatocytes, the most common liver response to injury (Adachi *et al.*, 2005). The most common causes of
fatty liver are alcoholism, obesity, diabetes, and elevated serum triglyceride levels. Other causes include malnutrition, hereditary disorders of metabolism and drugs (such as corticosteroids, tetracycline and aspirin. The mechanism by which these diseases or factors cause fat to accumulate within liver cells is not known (Sanyal, 2005). One possible explanation is these factors slow the rate at which fat is metabolized and excreted by the body. The resulting buildup of fat within the body is then stored inside the liver cells. In some cases fatty liver progresses to scarring (fibrosis) and cirrhosis, because of underlying inflammation (Friedman 2007).

2.4.3.5 Hepatocellular carcinoma (HCC)

Hepatitis B infection is strongly linked to the prevalence of hepatocellular carcinoma (HCC) (Kumada et al., 2010). Also HCC, very often occurs on a background of cirrhosis (Brancatelli et al., 2003). In many parts of the Asia, particularly in areas where viral hepatitis is common, primary cancers of the liver can represent up to 40% of all reported malignancies (Malcom, 2005). However they are extremely uncommon in Western countries and represent around 1% of all reported cancers (Perilongo et al., 1990). Microscopically, HCC includes a well differentiated form with cells that are recognizably hepatocyte in origin. As the tumor becomes more anaplastic, the liver cells can be bizarre and often sufficiently undifferentiated to become spindle. The tumor shows a distinct tendency to invade vascular channels (Nagase et al., 2000).

Hepatocellular necrosis occurs under a wide range of pathological conditions. In most cases, toxic cell death takes place over a finite span of time, accompanied by homeostatic counterresponses that are varied and complex. The present strategies for discovering critical steps in cell death recognize that (1) different types of injuries produce similar morphologic changes that precede killing in widely varied cell types, and that (2) lethal events are likely to involve one or more compartmentalized functions that are common to most cells.
Investigations of the lysosomes, plasma membrane, endoplasmic reticulum, cytoplasm, mitochondrion, and nucleus have greatly advanced our understanding of acute hepatocellular injury.

2.4.4 Subcellular responses of hepatocytes to injury

If hepatocytes are not significantly injured they may undergo proliferation - replication to replace dead cells or hypertrophy - enlargement of organelles produces enlargement of cells probably to increase function of individual hepatocytes (Colman et al., 1983).

2.4.4.1 Lysosomes (heterophagy; autophagy)

The protection of cells from the activity of acid hydrolases by inclusion of the enzymes within lysosomes was first described by de Duve et al., 1955. Since then it has been shown that rupture of the lysosomal membranes causes extensive damage to cell contents and that in the advanced stages of hepatocellular necrosis these enzymes are released into the circulation. Thus Slater and Greenbaum, 1965 found increased serum acid phosphatase activity hours after the oral administration of the hepatotoxin carbon tetrachloride to rats. Liver ischaemia and hypoxia increases the activity of plasma lysosomal enzymes (Grek et al., 2003). In acute and chronic liver injury, many changes occur in the metabolism of lipids, one of them being unusually high levels of tissue free fatty acids (Mavrelis et al., 1983). These fatty acids cause translocation of proapoptotic proteins into lysosomes (Feldstein et al., 2006). This causes lysosomal destabilisation with release of lysosomal enzymes into the cytosol. Lysosomal destabilisation results in NFκB dependent TNF-α expression, which promotes triglyceride accumulation and hepatic steatosis (Boya et al., 2003). Moreover, TNF-α can induce further lysosomal destabilisation and cathepsin B dependent apoptosis in a feed forward loop that exacerbates liver damage (Guicciardi & Gores, 2005).
2.4.4.2 Smooth ER (induction)

In a type of liver injury caused by drugs, many ultrastructural changes take place. Alcohol is an inducer of smooth endoplasmic reticulum (Rubin et al., 1968). Thus, there is likely to be expansion of this membrane system. Expansion of the SER results in enlargement of the liver cells and relative clearing of the cytoplasm, producing a picture very much like cellular swelling due to increased water and electrolytes (Dombrowski et al., 2000).

2.4.4.3 Mitochondria (number, size and shape)

Oxidative stress and mitochondrial injury play a role in the mechanisms of liver injury in viral infection (Choi & Ou, 2006). Liver tissue from virus-infected patients shows evidence of glutathione depletion, morphologic changes in mitochondria, and the presence of lipid peroxide-protein adducts (Zocco et al., 2005). The viral core protein alters mitochondrial function and results directly in an increase in the cellular abundance of ROS with consequent increases in cellular lipid peroxidation. Mitochondrial reactive oxygen species production is induced by hepatitis virus core (Otani et al., 2005) resulting in a reduction of mitochondrial antioxidant capacity and sensitivity to oxidants and TNF α. Alcohol abuse in hepatitis further depletes mitochondrial reduced glutathione, which exacerbates oxidative stress and causes cell death (Li et al., 2007). Ethanol also produces mitochondrial structural abnormalities and decreases adenosine triphosphate synthesis in hepatic mitochondria (Otani et al 2005). It increases electron flux and ROS generation through the mitochondrial electron transport chain. All these effects lead to impaired mitochondrial function and an inability to cope with further oxidative insults. Viral core protein interacts directly with mitochondria where it causes oxidation of the glutathione pool, inhibition of complex I activity, increased reactive oxygen species production and enhanced sensitivity to oxidant-induced cell death (Korenaga et al., 2005).
2.4.4.4 Cytoskeleton

The hepatic cytoskeleton is not an inert, rigid structure but that it is labile and in a state of continuous turnover (Lemasters et al., 1983). There are several reports of surface bleb formation or apoptosis following various types of toxic and metabolic injury to tissues including liver (Lemasters, 1998). Hepatic injuries are characterized by prominent alterations of the hepatocellular cell surface (Gores et al., 1990). The alterations include bleb formation on the sinusoidal surfaces of injured hepatocytes, loss of microvilli and disruption of microfilamentous structures of the cytoplasm. Hepatic enzymes may be released from viable tissues by shedding of cell surface blebs that are mostly cytosolic in origin into the circulation resulting in their appearance in the blood after liver injury (Thurman et al., 1984). Enzyme release from injured liver tissue occurs even in the absence of outright hepatic necrosis. In mild or moderate cellular injury, cytosolic enzymes such as glutamate-pyruvate transaminase and lactate dehydrogenase can be released selectively but larger organelles such as mitochondria are excluded (Morales-Gonzalez et al., 1999). In severe liver injury leading to necrosis, both mitochondrial and cytosolic enzymes appear in the blood (Solter, 2005). This occurs through a generalized breakdown of the plasma membrane permeability barrier leading to indiscriminate release of cellular contents.

2.4.4.5 Nucleus

Accumulating knowledge about two distinct modes of cell death, necrosis and apoptosis, indicates that loss of Ca2+ regulation and subsequent damage to DNA may be critical steps in lethal damage to liver cells (Ray et al., 1991). Host genetic factors are more important than environmental factors in determining the severity of liver diseases (Zeng et al., 2008). Liver injury is typically characterized by lipid peroxidation in the nuclear fraction along with mitochondrial fraction. The lipid peroxidation in turn is considered to be closely
related to the induction of liver cell death and to the production of alterations in DNA. In liver cell necrosis, hydropic changes and cytoplasmic swelling takes place. In the enlarged cells nuclear changes become more prominent. Liver cell shows necrotic nuclear shrinkage, absence or loss of normal structural organization into nuclei, granular chromatin and extreme clumping of nuclear components (Corcoran & Ray, 1992).