Discussion
The catalogue of damage caused to various systems of the body by chronic alcohol use is already vast, and yet each year there are added more conditions in which chronic alcohol use may play a part (Rix 1977). One of such additions is diabetes mellitus, a chronic heterogenous group of prevalent disorder (Kowsalya et al. 1996) which is associated with many complications like retinopathy, nephropathy etc., (Elkeles 1983). These problems are further complicated by alcoholism (Franz 1983). The influence of alcohol consumption on diabetics and the precise biochemical mechanism(s) underlying the same observed in chronic alcohol abuse are, at best, partially understood.

In the present study, a beginning has been made to understand the influence of alcohol consumption on diabetics, by generating information on alcohol induced biochemical changes in blood and red cells in particular.

In the present study the extent of erythrocyte membrane lipid peroxidation (Fig.4b) was found to be more in diabetics and alcoholic diabetics when compared to normals, with a maximal hike in alcoholic diabetics indicating the pronounced toxic effect of alcohol consumption on tissues especially the red blood cells. Increased malondialdehyde (MDA) formation was also observed in the intact red cells of alcoholic diabetics confirming the damage caused (Fig.4a). Biomembranes are major sites of lipid peroxidative damage and red cell membranes are liable to lipid peroxidation owing to their lipid content of polyunsaturated lipids and to the fact that they are directly exposed to molecular O₂ and involves the generation of the free radical intermediates and semi-stable peroxides.
The lipids of erythrocyte membrane undergo peroxidation when exposed to H$_2$O$_2$, hyperbaric oxygen or ultra-violet radiations (Kurian and Iyer 1976). Ethanol exerts its deleterious effects by either generation of free radical species during metabolism or by contributing to side mechanisms that in the end promote enhanced oxidative damage. It has been recently demonstrated that chronic ethanol exposure leads to an increased susceptibility of brain microsomes to iron-induced lipid peroxidation without modifying the glutathione (GSH) content or the activities of GSH peroxidase (GSH-Px), GSH-disulphide reductase of the whole brain (Morell et al. 1998). In many tissues the mechanism is not established completely in chronic alcoholics. Hence, the studies are merited. Peroxidation of membrane lipids can lead to damage to the membrane itself resulting in a number of pathological phenomena such as membrane rigidity, increased cellular deformability, reduced erythrocyte survival, lipid fluidity and altered permeability characteristics and lysis (Selvam and Anuradha 1988). Considerable changes in the structural organisation, function of RBC membrane and RBCs were reported in diabetes mellitus (Selvam and Anuradha 1988; Freyburger et al. 1989).

Changes in RBC membrane and lipid peroxidation reported in diabetics in earlier studies were confirmed by the present investigation. Our results further indicate that the severe damage in alcoholic diabetic group is due to alterations in membrane. The increased lipid peroxidation and membrane alterations due to generation of free radicals either extra-membranously or within the membrane itself, may lead to further oxidative damage resulting in susceptibility of RBC to splenic sequestration (Sarala
Kumari and Rao 1991). At the same time, the possibility of hyperglycemia induced lipid peroxidation and cell damage (Jain 1989) also cannot be ruled out here (Tables 1 and 2 and Fig.2a). From the stand point of comparative biochemistry, it is important to know the factors governing the susceptibility of erythrocytes to peroxidation. In this context one has to distinguish between the peroxidation potential and the rate of peroxidation. The peroxidative potential (as reflected by the maximum MDA yield under a given set of conditions) should be basically related to fatty acid composition of erythrocyte lipids (Kurian and Iyer 1976).

Maintenance of stable levels of blood glucose is a finely regulated of all homeostatic mechanisms in which various hormones, enzymes, factors and tissues play a part. Such finely regulated glucose homeostasis is disturbed in diabetics. The disturbance in blood glucose homeostasis seems to be more pronounced in the alcoholic diabetics according to our result. Hyperglycemia observed in the present study in diabetics and alcoholic diabetics was well expected before as the chosen subjects were confirmed diabetics. The degree of hyperglycemia observed in alcoholic diabetics is high in fasting and post-prandial states whereas alcohol induced hypoglycemia in diabetics acutely administered with alcohol was established (Frienkel et al. 1965; Walsh and O'Sullivan 1974; Nikkila and Taskinen 1975; McMonagle and Felig 1975). In the present study, the chosen subjects were confirmed chronic alcoholics associated with diabetes and were consuming alcohol three times a week for the past ten years and above. From the data and the marked hyperglycemia in alcoholic diabetics suggests an impaired glucose homeostatic mechanism in these subjects. Hyperglycemic effect observed in alcoholic diabetics is not
significant when compared to diabetic group indicating the efficiency of glucose homeostatic mechanism even under complications such as chronic alcohol abuse.

Elevated levels of glucose in the medium or blood are known to cause membrane damage or cell death of red cells, cultured pericytes, kidney cells and retinal cells (Jain 1989). However, biochemical mechanism that result in membrane damage and cell death is not known. Hyperglycemia leads to the glycosylation of a number of proteins. Haemoglobin is also glycosylated in aged red cells of normals and diabetics. Glycosylation of enzymes is known to cause changes in their activity, solubility and susceptibility to degradation. The glycosylation of haemoglobin occurs by a non-enzymatic reaction between glucose and amino terminal valine of β-chain. This on rearrangement resulted in formation of 1-deoxy fructose molecule attached to the valine. The resulting protein called HbAic is a good index of uncontrolled diabetes mellitus. Collagen fibres, antithrombin III are also glycosylated. These changes may possibly favour the accelerated blood vessel damage that occur in patients with diabetes (Devlin 1997).

The further increments in the blood glucose levels observed in fasted and post-prandial status in alcoholic diabetic group strongly suggest the possible damage as mentioned above.

The findings related to blood glucose levels in alcoholic diabetics have been discussed in the light of variable results by different workers (Frienkel et al. 1965; Walsh and O'Sullivan 1974; Christiansen et al. 1994) and it is suggested that efforts to throw light on anabolic and catabolic pathways, and
membrane studies would yield more fruitful results on the aetiology of chronic alcoholism.

A 0.5 fold increase in blood lactic acid levels in diabetics as well as alcoholic diabetics points to the inhibition of lactate utilisation primarily through gluconeogenesis (Table 3 and Fig.2b). Generally in alcoholics the extra reducing equivalents generated block the conversion of lactate to glucose and promotes the conversion of alanine to lactate, resulting in considerable lactate accumulation in the blood. Since lactate has no place to go, lactic acidosis can develop although it is mild (Devlin 1997).

No change in the amino acid content in the blood among the three groups was observed. Although there appears to be minor zigzags, those are not significant (Table 4 and Fig.2b). The increased flow of lactate with no change in amino acids may point to either increased operation of Cori’s Cycle or the inability of gluconogenic tissues to utilise lactate.

In the present study the elevated levels of triglycerides, cholesterol, phospholipids and free fatty acids in plasma in diabetic and alcoholic diabetic subjects (Tables 5 to 8 and Fig.3a) can be correlated to clinically apparent complications in both groups. An accelerated lipolysis in adipose tissue might have resulted in the increased free fatty acid levels in diabetics. The increased free fatty acid levels observed in the present study is in agreement with earlier reports (Datta et al. 1969; Bhise and Magar 1969; Thompson 1983). A further increase of the same in alcoholic diabetic is due to much accelerated lipolytic process in adipose tissue. An increased triglyceride and cholesterol content was observed in diabetics (New et al. 1963) and these contents are further elevated in alcoholic diabetics. A rise in plasma
phospholipid content in the diabetics and alcoholic diabetics is an added observation. The overall result is hyperlipemia as reflected by increased formation of triglycerides and accumulation of the other lipid constituents. Ultimately, lipid moiety accumulated in the liver. Deposition of neutral lipid in the hepatocyte is an example of a multifactorial biochemical phenomenon resulting from a combination of both adaptive and injurious responses to chronic heavy ethanol ingestion. The failure to oxidise fatty acids represents their replacement by ethanol as an obligatory hepatic mitochondrial fuel. This is aggravated by the generation of $H^+$ equivalents as byproduct which lead to stimulation of denovo fatty acid synthesis and formation of triglyceride. In the fasted state, the experimental subject may respond to high doses of ethanol by releasing excessive amounts of free fatty acids from adipose tissue. However, it is unlikely that this comprises a significant source of lipid for the liver during chronic ingestion in man. Finally, although the increased rate of fatty acid accumulation is initially accompanied by enhanced lipoprotein synthesis, the progressive injurious effects of alcohol (acetaldehyde) are associated with reduced synthesis of lipoproteins and failure to export endogenously synthesized lipid. It is also apparent that the above series of events cannot be prevented simply by ensuring that dietary deficiencies do not develop during the course of chronic alcohol ingestion. Nevertheless, dietary fat plays an aggravating role in the process, and a low fat diet, particularly one that favours shorter chain fatty acids, does help to reduce the rate of triglyceride deposition in the liver (Isselbacher 1977; Elkeles 1983).
A marked hypercholestremia, hyperphospholipidemia and hypertriglyceridemia observed in the present study need careful considerations. This suggests some defect in transportation, disposal and metabolism of fat accounts for the observed hyperlipemia in diabetics and alcoholic diabetics in particular, the effect being more pronounced in alcoholic diabetics.

Increased activities of GOT and GPT in plasma (Tables 9 and 10 and Fig.3b) were observed in alcoholic diabetic group indicating a hepato-cellular injury of alcoholic aetiology. The increase of the same in the diabetic group is not to the above extent, but the hike is significant in diabetics also suggesting a hepatic failure. A rise in GOT and GPT ratio in plasma was reported earlier in alcoholics (Jain et al. 1988) and the reasons attributed for the same include the striated muscle damage (rhabdomyolysis) in the alcoholic, the higher mitochondrial concentration of GOT relative to GPT and the dependency up on pyridoxine as a co-factor in the synthesis of GPT (Whitehead et al. 1978). This tends to be deficient in the alcoholic, either as a consequence of dietary deficiency or as a result of increased catabolism of pyridoxal phosphate (Whitehead et al. 1978).

Surprisingly, diabetics associated with alcohol consumption showed more elevated activities in GOT and GPT in plasma strongly indicating the pronounced cell damaging effect of ethanol.

In the present study an increase in the membrane cholesterol is reported in diabetic and alcoholic diabetic groups (Table 11 and Fig.5), being maximum in alcoholic diabetic group. Cholesterol, one of the major lipid constituent of erythrocyte membrane interacts with different classes of
membrane phospholipids and modulates the fluidity and consequently the membrane function (Sarala Kumari and Rao 1991). The observed change of erythrocyte membrane cholesterol increase in diabetics and alcoholic diabetics indicate a compensative mechanism operating in the erythrocyte to resist the fluidising effect of ethanol and disease. High serum cholesterol levels and a dramatic increase in membrane cholesterol content lead to atherosclerosis suggesting these two groups are susceptible for cardiovascular disturbances and hypertension (Houslay and Stanley 1984).

The highest phospholipid content was reported in diabetic group and a dramatic fall in phospholipid content of alcoholic diabetic group is a striking observation in the present study (Table 12 and Fig.5). An increase in cholesterol and decrease in phospholipid content of erythrocyte membrane of alcoholic diabetic group leading to a significant increase in erythrocyte membrane cholesterol and phospholipid ratio indicating that the membrane is resistant to the fluidising effect of ethanol. Chin et al. (1978) have reported an increase in the ratio of cholesterol to phospholipid in erythrocyte and brain membranes in ethanol-tolerant mice. A further indepth study is warranted on individual phospholipids such as phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine and phosphatidylinositol, membrane bound enzymes such as ATPases, acetylcholinesterase and other proteins like channels, receptors, carriers and transporters and cytoskeletal components etc., which influence the behaviour, functioning and structural organisation of the membrane to understand the ethanol induced toxicity and adaptive biochemical changes in membranes.