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4. DISCUSSION

4.1 ISCHAEMIC HEART DISEASE AND SOME ASSOCIATED FACTORS

4.1.1 Biochemical and pathophysiological changes in atherosclerosis and the resultant myocardial infarction and stroke can be classified into three broad heads namely (a) abnormalities in lipid metabolism leading to the production of atheroma and the associated changes in proteoglycans, (b) excessive blood clotting, precipitated by overproduction of coagulation components and progressive loss of thrombolytic activity and (c) the antioxidant deficiencies leading to lipid peroxidation and cell and tissue damage. While all three aspects have been investigated in experimental atherosclerosis, their extrapolation and verification in the human system is limited to non availability of the aortic and myocardial tissues for biochemical assays. However, abnormalities in the tissues are often reflected in the extracellular fluids and blood analysis which become the mainstay for mirroring tissue abnormalities.

4.1.2 A consistently higher incidence of CHD, diabetes mellitus, and hypertension is observed in the family members of the AMI patients (Table 3.1), when compared to the healthy subjects, confirming a higher incidence of genetic predisposition to coronary heart disease in the patients studied. However, non-genetic differences such as increased incidence of smoking and consumption of animal foods are also observed in patients with CHD suggesting that these modifiable environmental factors may be significant risk contributors to the development of ischaemic disease in them. It has to be remembered that while habits on the use of
tobacco and fleshy foods are not genetically transmitted, they are influenced by familial factors such as customs, practices and habits.

4.1.3 Our observations confirm that in the South Indian population also, serum cholesterol by itself is not a good predictor of CHD, and that there is a significant overlap between the AMI and the healthy population (Table 3.2). The plasma cholesterol level in the healthy population is $211 \pm 25$ mg/dl which is low compared to the plasma cholesterol level of healthy North American population ($230 \pm 55$) reported by Castelli and Anderson (1986). Plasma cholesterol in IHD and AMI are $248 \pm 49$ mg/dl which shows clearly that in this population also, plasma cholesterol cannot independently predict the development of AMI. The dictum, "whenever the cholesterol level is high, the incidence and prevalence of AMI is also high (Rifai, 1986)" has applicability only while dealing with large groups, but not to individual cases.

4.1.4 Epidemiological studies in Japanese have also revealed lower serum cholesterol level and Kukita et al (1982) have concluded that this lowered total cholesterol when compared to the Western population may be due to low dietary fat intake. According to the WHO report (1976), dietary fat intake in Japan is about one third of that in U.S.A. and most of the European countries.

4.1.5 Coronary heart disease (CHD) is relatively common in the affluent classes in India (Padmavathi, 1959). In recent years reports from England, South Africa and West Indies have suggested that Indian immigrants in these countries are hypersusceptible to CHD (Walker, 1963; Tunstall-Pedoe et al, 1975; Miller et al, 1982) i.e., they have higher mortality values than other population groups such as the
Whites and Blacks living in the same regions. The value of serum cholesterol is higher in Indian born physicians living in U.S.A. than U.S. born physicians (Thomas et al, 1986).

4.1.6  In the Framingham study in U.S.A., Castelli and Anderson (1986) reported that the level of total cholesterol proved to be an excellent predictor of CHD in those aged less than 50 years. It was observed that this predictive value declined after 50 years of age. Rose (1985) observed that the majority of cases of coronary heart disease in a population arise from among the large number of people with mild elevation of risk factors and not from among the minority at high risk. These slight elevations tend to be inconspicuous; indeed the critical values are so common that they may even be "average" for their population.

4.1.7  Brown and Goldstein (1986) observed that cholesterol is absolutely insoluble in water, a property that makes it essential in cell membranes where it modulates fluidity and maintains the barrier between the cells and the environment. However, its water insolubility means that when cholesterol accumulates in tissues such as the artery, its presence eventually leads to the development of an atherosclerotic plaque with potentially dangerous consequences. Brown and Goldstein (1986) stated that abnormal cholesterol deposition is favoured by the dangerous tendency of cholesterol to passively exchange between the plasma lipoproteins and cell membranes. Hence, levels of plasma cholesterol must also be kept significantly low to minimise passive exchange.
4.2 HDL BOUND CHOLESTEROL AND IHD

4.2.1 Our observations on the reduced HDL cholesterol in AMI (Table 3.2) confirms once again the negative correlation that exists between HDL and the incidence of AMI. Since the antiatherogenic action of HDL was first suggested by Barr et al (1951), a great number of clinical and epidemiological studies demonstrated a negative association of HDL with incidence of atherosclerotic diseases (reviewed by Eisenberg, 1984).

4.2.2 The putative role of HDL as a protective lipoprotein against premature development of atherosclerotic disease was confirmed by Miller and Miller (1975). Based on a number of studies carried out in different countries, in the normal men (of 30-60 years age), the mean HDL levels in the European countries was 53.0 mg/dl (Lewis et al, 1978), Israel 47.1 mg/dl (Brunner et al, 1979), Africa (Nigeria) 79.2 mg/dl (Ononogbu, 1979), North America 45.5 mg/dl (Heiss et al, 1980), Canada 45.4 mg/dl (Jones et al, 1980), Japan 55 mg/dl (Yano et al, 1980), China 61.8 mg/dl (Jiangcai et al, 1982). In our studies mean HDL bound cholesterol observed is 51.7 mg/dl.

4.2.2.1 Cigarette smoking, sedentary behaviour, diabetes mellitus, use of beta adrenergic blockers and antihypertensives and obesity are conditions which are associated with lowering HDL levels (Levy, 1986; Dieh et al, 1988).

4.2.3 Hypertriglyceridemia is often accompanied by reduced HDL cholesterol levels, the combination of the two apparently providing the most severe profile of risk in coronary patients. (Nikkila et al, 1990; Calabresi et al, 1990). The physiological
and clinical significance of reduced HDLc levels both in coronary patients and in hypertriglyceridemia had been earlier reported by Moberg and Wallentin (1981).

4.2.4 Significantly elevated levels of Total/HDL cholesterol ratio (CRI) are observed in AMI (Table 3.2). The coronary risk index (CRI) is an important factor in the assessment of the severity of MI.

4.2.4.1 When compared with the observations made earlier in our laboratory on 999 cases of symptomatic IHD (Angina, history of MI and ischaemic changes in ECG; but not during an acute episode of MI), the total cholesterol in AMI is no different. However, the coronary risk index (Total/HDLc ratio) is slightly higher in those with AMI (compare 5.15 ± 1.25 and 5.36 ± 0.59 for IHD and AMI respectively).

4.2.4.2 An inverse relationship between the incidence of CHD and the ratio Total/HDL cholesterol was first reported by Miller and Miller (1975). Framingham study and several other studies confirmed that coronary heart disease developed with great consistency in subjects with a ratio of total cholesterol to HDL cholesterol of more than 4.5 (Castelli and Anderson, 1986 and others).

4.2.5 Steinberg (1978) proposed two mechanisms to explain the antiatherogenic activity of high density lipoprotein. They are (a) inhibition of LDL uptake by cells of the arterial wall, and (b) facilitated reverse cholesterol transport from cells of the arterial wall.

4.2.5.1 The first mechanism is based on the observation that HDL competes with binding and uptake of LDL studied in cultured cells (Carew et al, 1976; Miller et al, 1977). This inhibition is observed with a great excess of HDL. Inhibition of LDL
binding to the cell surface receptors and consequent internalisation is considered the best defence against atherosclerosis (Goldstein and Brown, 1982).

4.2.5.2 Eisenberg (1984) observes that the reverse cholesterol transport is undoubtedly an important regulator of cell cholesterol homeostasis.

4.2.6 The levels of HDL_2 bound cholesterol are significantly lower in AMI, when compared to the normal (Table 3.2). Several studies indicate that changes in total HDL concentrations are primarily reflected by fluctuations in HDL_2 values (Gidez et al, 1982). HDL_2 accounts to a major extent for the negative correlation established between HDL and vascular disorders. Low HDL_2 levels are associated with several conditions such as sedentary life style (Nye et al, 1981), obesity (Albrink et al, 1980) and hypertriglyceridemia (Taskinen and Nikkila, 1981).

4.2.6.1 Fellin et al (1985) state that the HDL reduction was due to a fall in both HDL_2 and HDL_3 bound cholesterol, nonetheless, an analysis of the HDL_2C/HDL_3C ratio disclosed that HDL_2C was reduced most. According to Anderson et al (1979), HDL_2 cholesterol defines (the coronary risk profile with 50% more accuracy than the HDL cholesterol level. HDL_2 may be the major antiatherogenic component of the two HDL subfractions. This is supported by the observation that premenopausal women (who are generally resistant to atherosclerosis) have higher HDL_2 levels than age matched males (Shepherd et al, 1980).

4.2.7 In the present study we find that HDL_3 is significantly elevated in AMI (Table 3.2). It is worth mentioning here that in a study carried out in our laboratory on the disturbances in plasma lipoprotein pattern during isocaloric high fat diet
intake, it was observed that HDL$_2$ levels decreased with a simultaneous increase in HDL$_3C$ in adult male volunteers within ten days, and this was reversed by low fat diet administration only after a period of 180 days (Shanmugasundaram et al, 1986). The intake of fat also appears to play a rapid and major role in the cholesterol distribution among HDL subfractions.

4.3 CHOLESTEROL BOUND TO LDL AND VLDL

4.3.1 The variations in the LDL cholesterol levels between the AMI patients and healthy population are significant (Table 3.2). The plasma concentration of LDL is now recognised as a major risk factor in the premature development of coronary heart disease (LRCP, 1979).

4.3.1.1 The most convincing evidence that these lipoproteins are causative factors in this disease is the genetic disorder, familial hypercholesterolemia, in which homozygous patients develop massive increase in LDL and frequently die within the second decade of life from complications of CHD (Fredrickson and Levy, 1972).

4.3.2 Available data suggests that several properties of LDL are important in the development of atherosclerosis, including molecular weight, the surface of LDL, physical state of LDL core, cholesterol esters, the concentration of apo-B and the presence of other apoproteins. These factors may function in atherosclerosis by altering the binding of LDL to cell surface receptors and to proteoglycans (Rudel et al, 1986) confirming that the low density lipoproteins are important in the initiation and/or exacerbation of coronary artery atherosclerosis.
4.3.3 Goldstein et al (1977) and Goldstein and Brown (1979) demonstrated three hereditary defects in LDL receptor function in familial hypercholesterolemia namely (1) inability of the receptor to bind to LDL-particle, (2) reduced affinity of the receptor and (3) normal binding but defective internalisation of LDL. As a consequence of defective function of LDL receptors, heterozygous and homozygous patients exhibit reduced catabolism of LDL and a secondary increase in the concentration of LDL circulating in plasma (Goldstein et al, 1977; Goldstein and Brown, 1979).

4.3.4 LDL is a heterogeneous complex and recent studies by Austin et al (1988) and Luc et al (1988) have revealed that the LDL subclass pattern characterised by a preponderance of small, dense LDL particles was significantly associated with a three-fold increased risk of myocardial infarction independent of age, sex and relative weight. Similarly, Jurgens et al (1987), demonstrated that plasma LDL can undergo free radical oxidation resulting in structural and functional abnormalities. Further studies on the genetic defect in the patients with a family history of MI are needed to characterise the genetic make up of this ethnic group.

4.3.5 VLDL cholesterol accounts for about 22% of the total cholesterol in the healthy population while it accounts for more than 27% in AMI (Table 3.2). In IHD, VLDL cholesterol accounts for 23% total cholesterol. An interesting point to note is that in the IHD cases studied (Table 3.2) with and without MI, mean value for total cholesterol is unaltered at 248 mg/dl. The cholesterol bound to the atherogenic lipoprotein (LDL + VLDL) account for 200 mg cholesterol in both cases. But with an episode of AMI, there is a shift in the distribution. VLDL cholesterol increase with a relative reduction in LDL bound cholesterol. This shift may be the
consequence of one or more of the following factors: (a) increased synthesis of VLDL by increased hepatic metabolism for coping with the shock, (b) reduction in the lipoprotein lipase activity in the vascular endothelium affecting VLDL catabolism and (c) increased uptake of LDL for internalisation in the peripheral cells. One or more of the process may be mediated through (a) the action of stress hormones, epinephrine and cortisol released in response to AMI and (b) the heparin therapy initiated at the time of hospital admission.

4.3.5.1 Patsch et al (1978) and Taskinen et al (1982), showed that during the catabolism of VLDL and chylomicrons, HDL₃ is converted to HDL₂ and they demonstrated an inverse relationship between VLDL and HDL₂. Based on this assumption, the elevated levels of VLDL with reduced HDL₂ levels in MI observed in our study (Table 3.2) may reflect slow VLDL catabolism. In view of the fact that the patients had already been treated with heparin, it will be interesting to assay the lipase activities in plasma.

4.3.6 Cholesterol in plasma VLDL in our population is considerably higher than the data reported in the West (Heiss et al, 1980). In the healthy population in LRC programme in North America (Lipid Research Clinics Programme Epidemiology Committee, 1979), VLDL cholesterol amounts to 24.2 ± 8.1 mg/dl which accounts to about 10% of total cholesterol. In the European cities (London, Naples, Uppsala and Geneva), the VLDL₃ levels were reported by Lewis et al (1978), in the range of 15-20 mg/dl. In New Zealand, the levels were found to be around 12.0 mg/dl (Blackburn, 1980) and in Canada 24.0 mg/dl (Tan et al, 1980). In Japan the level of VLDL₃ was reported to be 27.0 mg/dl (Hosaki et al, 1985). In our study the level of VLDL cholesterol was found to be 48.3 mg/dl (Table 3.2)
4.3.6.1 VLDL is synthesized endogenously by liver and is released into the circulation. The Indian diet is loaded with cereals and carbohydrate provide 65 to 75% calories. With 10 to 15% calories obtained from dietary protein, fat derived calories are between 10 and 20%. Protein and fat rich food stuffs being more expensive influence the dietary patterns. In the target group, we have studied (the urban middle class), 15 to 20% fat derived calories and 10-15% protein derived calories are staple unlike the high fat diet in the Caucasians.

4.3.6.2 During digestion glycerides and fatty acids are absorbed into the lymph and enter the systemic circulation at the thoracic duct. Digestion of starch is followed by the uptake of glucose into the portal circulation and enhanced activity of the liver for energy purposes. As a corollary, VLDL is synthesized in the liver and released into the circulation excessively and provides the source of fatty acids for vascular energy process. The role of dietary carbohydrate in the VLDL biosynthesis in rat liver was shown first by Schonfield and Pfleger (1971). Ruderman et al (1971) showed that VLDL in high carbohydrate diet fed men are richer in triacylglycerols. The higher triglyceride levels found in our population (Table 3.3) is due to the dietary carbohydrate content.

4.4 OTHER PLASMA LIPIDS IN ACUTE MYOCARDIAL INFARCTION

4.4.1 Triglyceride levels which are already high in the Indian subjects is further elevated (p<0.001) in AMI (Table 3.3).

4.4.1.1 The predictive value of plasma triglyceride in CHD is slowly being accepted by epidemiologists. In the 12 year follow up of 5,919 middle aged men free of clinical
coronary heart disease in the Honolulu Heart Program, Benfante et al (1989) observed that serum triglyceride in men under 60 years is an independent predictor of AMI. This predictive value tends to decline after 60 years of age. Case control studies in Framingham (Brunner et al, 1977; Castelli et al, 1977) have clearly demonstrated triglyceride as a risk factor independent of total cholesterol or LDL cholesterol levels. In most but not all prospective studies such as Honolulu heart study (Rhoads et al, 1976), Evans country study (Heyden et al, 1980), triglyceride is independently and closely associated with the development of heart disease.

4.4.2 The triglyceride levels in both the healthy subjects and in patients are higher than the levels in the West. In the American population normal triacylglycerol levels are reported to be $92 \pm 32$ mg/dl (Fredrickson et al, 1967; Vega and Grundy, 1985). In Geneva (Europe), the mean triacylglycerol level was found to be 103.6 mg/dl plasma (Lewis et al, 1978). In the Far East in Japan, it is 134.0 mg/dl (Yano et al, 1980) and in China the level was found to be 108.0 mg/dl (Jiangcai et al, 1982). This is similar to the findings (110.4 mg/dl) observed in our normal subjects reported in Table 3.3. The patients have significantly higher mean value (161 mg/dl) (nearly 50% higher) and the triglycerides appear to have greater predictive value than cholesterol.

4.4.2.1 Austin (1989) who reviewed data from several studies draws two conclusions: - (1) the case control studies clearly demonstrate an apparent univariate association between hypertriglyceridemia and coronary heart disease and (2) this association is seen less consistently in large, long term follow up studies.

4.4.3 Sailer (1979) observed that VLDL triglycerides are increased after a carbohydrate diet. Falko et al (1980) observed that when healthy volunteers are
given a high carbohydrate diet, VLDL rose. These changes are associated with apo-C changes.

4.4.3.1 Jiangcai et al (1982) have observed that in China, where people consume rice as staple food providing upto 75% calories had higher levels of triglyceride in plasma. Contribution of dietary carbohydrates to the origin of serum triglyceride has been confirmed by studies made in our laboratory (Shanmugasundaram et al, 1986) which showed that with increase in fat content with isocaloric diet, plasma triglyceride was significantly lowered.

4.4.4 The patients suffering from AMI had elevated levels (p<0.001) of plasma phospholipids when compared to the healthy population (Table 3.3).

4.4.4.1 Portman (1970) observed that total phospholipids are increased in atherosclerotic arteries both in man and experimental animals. Stimulation of phospholipid synthesis has been observed during the genesis of atherosclerosis (St.Clair, 1976). In cholesterol fed pigs, increases in blood cholesterol and phospholipids were reported by Narendra and Mukherjee (1982).

4.4.4.2 Recent studies by Bovet et al (1989) reported that the serum phospholipid and their fractions differ significantly in CAD, when compared with healthy subjects. The total phospholipids was increased at a statistically significant level as in the case of total cholesterol.

4.4.5 Few and divergent data are available on the levels of blood phospholipid subfractions in relation to CHD, in contrast to numerous reports on phospholipids (Jackson and Gotto, 1974). Bose et al (1984) reported a transient modification of
plasma phospholipid sub-fractions in the acute phase of myocardial infarction. Significant changes in the absolute amounts of phosphatidylserine, phosphatidylethanolamine and lysolecithin have been reported by Nothman and Proger (1962). Kunz and Stumvoll (1971) suggested thromboplastic properties of phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol, while lysolecithin may modulate transmembrane diffusion of LDL (Wells et al, 1986).

4.4.6 Free fatty acids in plasma of the patients with AMI are significantly higher than those observed in the healthy population (Table 3.3). This may be due to the action of epinephrine on the adipocytes, as a response to the stress following the infarction.

4.4.6.1 Elevated levels of free fatty acids may play an important role in the pathogenesis of coronary vascular diseases. Free fatty acids precipitate cardiac arrhythmias, which led Nestel et al (1978) to postulate that individuals who maintain high circulating levels of free fatty acids are at a high risk for developing CVD. Burstein et al (1978) proposed that increases in plasma free fatty acid levels increases the intensity of platelet aggregation which plays a major role in the evolution of atheroma and influences the basic disease process.

4.4.7 Elevated free fatty acid levels also increase myocardial oxygen requirements (Simonsen and Kjekshus, 1978) which may cause serious ventricular arrhythmias. Free fatty acids taken up by the liver are reformed into triacylglycerols and secreted as component of VLDL (Schonfeld and Pfleger, 1971). The rate at which the liver secretes VLDL is determined partly by the rate at which it synthesized fatty-acids from carbohydrates and partly by the rate free fatty acid are taken up from the blood
(Basso and Havel, 1970). The elevated levels of VLDL observed in AMI (Table 3.2) may also arise from excessive lipolysis mediated through catecholamine as a sequel to the infarct. Norepinephrine levels in plasma is found to be elevated in AMI. (Gazes et al, 1959; Christensen and Videback, 1974).

4.4.7.1 Free fatty acids levels in plasma have been reported to increase the intensity of platelet aggregation (Burstein et al, 1978), which plays a major role in the evolution of atheroma and it influences the basic disease process.

4.5 APSm AS A LIPID LOWERING AGENT IN THE TREATMENT OF ISCHAEMIC HEART DISEASE

4.5.1 Effect of APSm tested for longer durations (2 to 36 months) in 50 clinically defined IHD patients with a variety of complications (Table 3.11) confirms the effectiveness of this drug in combating IHD. Many of the patients had already tried other lipid lowering medications like clotibrate, guggulipids, gemfibrozil and probucol. Some had found them ineffective in controlling lipids, while others developed undesirable side effects like GI disturbances which had led to discontinuation of therapy.

4.5.1.1 The lipid lowering effect of APSm is found to be sustained and gradually increased during its long term administration (Table 3.13). Earlier workers in our laboratory had observed that the lipid lowering during APSm therapy is reversed, when the drug was withdrawn subsequently (unpublished).

4.5.2 Modified Anna Pavala Sindhooram (APSm), has already been proved to be a potent hypolipidemic agent in experimental hypercholesterolemia and
atherosclerosis (Shanmugasundaram and Marita, 1982; Shanmugasundaram et al, 1983; Marita and Shanmugasundaram, 1988), and a protective agent against beta agonist (IPH) induced myocardial infarction (Srinivas and Shanmugasundaram, 1987). APSm was successfully tested for its lipid lowering activity in a double blind cross over design on 30 patients with IHD (Shanmugasundaram et al, 1991) which confirmed its effectiveness in lowering both cholesterol and triglycerides in Types IIa, IIb and IV hyperlipoproteinemia.

4.5.2.1 APSm reduces total cholesterol LDL, VLDL and coronary risk index (TC/HDL_c ratio) while increasing the cholesterol bound to HDL especially in the HDL_2 subfraction and thereby the HDL_2/HDL_3 ratio (Table 3.12 and 3.12a). In 18 months, APSm reduces total cholesterol by 22% and LDL and VLDL by over 25% (Table 3.12). CRI is reduced by 35%, while HDL_2/HDL_3 ratio is nearly doubled at the end of 18 months therapy.

4.5.3 VLDL is formed in the liver and is released into the circulation, where it is acted upon by the lipoprotein lipases on the vascular endothelium, and is converted to the cholesterol rich LDL in a stepwise manner (Krauss et al, 1973). LDL disappears from the circulation by internalisation into the peripheral cells. The LDL content at any time is the balance between these two opposing forces - namely VLDL catabolism and LDL binding. In some of the patients, VLDL cholesterol rose in the first two months of APSm therapy (Fig. 3.3) while LDL was lowered. It will be useful to assay the lipoprotein lipase and hepatic lipase activities before and during APSm therapy to assess the effect of the drug on these important enzymes. However, in both TYPE-IV and TYPE-II b HLP (Table 3.12c). APSm therapy is associated with a significant reduction in both VLDL bound cholesterol and serum triglycerides and
over 35% reductions in VLDLc at the end of 12 months therapy (Table 3.12c). Considering the fact that when all the 50 patients are taken together (Table 3.12) the mean reduction in VLDL is about 20% at the end of 12 months APSm therapy. In those with elevated VLDL cholesterol as in TYPE IIb HLP, VLDLc is reduced by 38% in 12 months therapy. It may be inferred that VLDL catabolism by lipase may not be inhibited by APSm therapy. This is further confirmed by the observation that in the hypertriglyceridemia of TYPE II b HLP (Table 3.12c), triglycerides are also lowered by 30%.

4.5.4 APSm reduces LDL cholesterol (Table 3.12 and 3.12b) and it may be inferred that the risk from coronary heart disease may be reduced by this drug.

4.5.4.1 The reduction in LDL cholesterol may be through a shift in the dynamic equilibrium between cholesterol biosynthesis and elimination. Dietary contribution of cholesterol in the population studied is negligible, and the circulating cholesterol originates primarily from biosynthesis. Animal experiments carried out earlier (Marita and Shanmugasundaram, 1982) has shown that incorporation of 14C-acetate, into mevalonate and cholesterol is significantly reduced by Anna Pavala Sindhooram therapy suggesting that the drug acts also by controlling cholesterol biosynthesis.

4.5.4.2 Lipoprotein lipase and hepatic lipase were also found to be elevated by Anna Pavala Sindhooram therapy (Shanmugasundaram and Marita, 1982). Extrapolating these observations to human atherosclerosis, the reduction in LDL cholesterol observed in our studies may be attributed to both increased catabolism by binding on the hepatic B-E receptor and internalisation and reduced cholesterol biosynthesis.
Lipoprotein lipase and hepatic lipase in plasma and LDL receptor density in the white blood cells are factors which need monitoring during APSm therapy in future.

4.5.5 Epidemiological studies made in Framingham (Castelli, 1984), in Scandinavia (Miller and Miller, 1975) and others have shown the protective role of HDL in coronary heart disease. Lern (1987) has convincingly shown that increase in HDL cholesterol is associated with a reduction in the incidence of CHD. By increasing this form of cholesterol (Table 3.12 and 3.12a) APSm appears to provide protection by control of atherosclerotic disease.

4.5.6 Considering the positive and negative associations of LDL and HDL with IHD, APSm induced changes in the concentration of these lipoproteins should be highly favourable with regard to the management of IHD.

4.5.6.1 The increase in the HDL cholesterol is mainly observed in the HDL₂ subfraction after APSm therapy, thereby increasing the HDL₂/HDL₃ ratio (Table 3.12a).

4.5.6.2 Gidez et al (1982) have observed that changes in total HDL cholesterol concentrations are primarily reflected by fluctuations in HDL₂ values. HDL₂ accounts to a major extent for the negative association seen between HDL₂ and vascular disorders. According to Anderson et al (1979), the HDL₂ cholesterol defines the coronary risk profile with 50% more accuracy than the HDL cholesterol levels.

4.5.6.3 Tall and Small (1978) observed that the cholesterol carried by plasma HDL and HDL₂ originate from the peripheral cells. The increased cholesterol observed
in HDL\textsubscript{2} fraction after APSm therapy gives indirect evidence of cholesterol removal from the peripheral cells. The increased HDL\textsubscript{2} also may denote the increased conversion of HDL\textsubscript{3} to HDL\textsubscript{2} (Assman, 1982). As a result, HDL\textsubscript{2}/HDL\textsubscript{3} is increased when compared to the pretreatment levels.

4.5.6.4 The reduction in total cholesterol and increase in the HDL bound cholesterol reduce the ratio Total/HDL\textsubscript{c} (CRI) after APSm therapy (Table 3.12 and 3.12a). In the Framingham study it was observed that coronary heart disease developed with greater consistency in patients with ratio of total cholesterol to HDL cholesterol of more than 4.5 (Castelli and Anderson, 1986). Hence, they suggested that intervention should include therapy aimed at reducing this ratio. APSm reduces this ratio and effectively provides protection from the risk of ischaemic episodes.

4.5.7 APSm therapy lowers the increased levels of triglycerides, phospholipids and free fatty acids to near normal levels (Table 3.13).

4.5.7.1 Plasma triglyceride is independently and closely associated with the development of heart disease. Regulatory mechanisms of plasma triglyceride levels appear to involve several determining factors. In simple terms, plasma triglyceride levels are linked directly to the influx of triglycerides into the plasma and to the clearance of triglycerides (Nakamura, 1975).

4.5.7.2 Cenedolla \textit{et al} (1968) reported that reduction in plasma triglyceride level was mediated through the activation of VLDL triglyceride catabolism. Carlson \textit{et al} (1972) reported that clofibrate stimulate lipoprotein lipase and brings down serum triglyceride levels. Marita and Shannugasundaram (1982) have shown that APSm
administration in atherosclerotic rabbits is associated with increase in hepatic, vascular and heparin induced lipolytic activities measured in serum.

4.5.8 Hyperlipaemia is associated with increased levels of phospholipids (Portman, 1970). Using $^{14}$C-acetate studies, Marita and Shanmugasundaram (1988) have convincingly shown that APSm reduces plasma and aortic phospholipid synthesis in atherosclerotic rabbits. Phospholipids are significantly reduced by APSm therapy (Table 3.13). Similar reduction in plasma phospholipids was observed with gemfibrozil therapy (Cuvelier et al, 1985; Sorisky et al, 1987).

4.6 LEUCOCYTE LIPIDS AND CHOLESTEROL ESTERIFYING ENZYMES IN ACUTE MYOCARDIAL INFARCTION

4.6.1 Effect of dietary fat modifications have been investigated on the lipid composition in plasma, liver, aortic smooth muscle cells and fibroblasts in animals but the studies on man generally have been restricted to plasma lipids only. However, in the last few years it has been convincingly proved that mononuclear leucocytes consisting predominantly of lymphocytes show similarity to hepatocytes and fibroblasts in the regulation of cholesterol biosynthesis (Fogelman et al, 1975; Mc Namara et al, 1980) and their enzymes respond to effectors such as cholesterol feeding, etc. in a manner parallel to hepatocytes (Young and Rodwell, 1977).

4.6.1.1 Leucocytes both polymorphonuclear lymphocytes (PMNL) and monocytes have been observed in the earliest lesion of atherosclerosis in animal models and man (Jorgenson et al, 1972; Gerrity et al, 1979) Leucocytes were also found in clusters
in focal areas of atherosclerosis in the aortas of rabbits fed with a high cholesterol diet (Duff et al, 1957).

4.6.1.2 Gerrity and Schwartz (1977) stated that a direct correlation was found between intimal swelling, a marker of the early atherosclerotic lesions and PMNL and monocyte infiltration of the intima. Endothelial cell damage and platelet-fibrin thrombi were also present in these regions. Polymorphonuclear lymphocytes can adhere to vascular endothelium and damage endothelial cells in vitro (Sachs et al, 1978). Increased attention needs to be paid to the metabolism of leucocytes which is believed to play a vital role in atherogenic process and is also readily accessible for analytical purposes.

4.6.2 Elevated levels of leucocyte cholesterol, phospholipids and triglycerides observed (Table 3.9) in patients with MI is associated with an increase in C/P ratio, confirming the relative abundance of cholesterol over the polar lipid, namely, phospholipids.

4.6.2.1 This cholesterol accumulation in leucocytes of the patients is associated with a significant reduction in the CEH activity (Table 3.10) favouring cholesterol ester accumulation. There is a concomitant decrease in CEH/CES ratio in AMI when compared to the healthy population (Table 3.10). The enzyme activity measured are the lysosomal enzymes namely the LAL which hydrolyse cholesterol ester at acid pH, and the ACAT which is the major contributor for intracellular cholesterol esterification.
4.6.3 Cholesterol is generally metabolized and excreted in quantitatively important amounts by the liver (Nestel and Poyser, 1978). Cholesterol must therefore be transported from the tissues to the liver and HDL is important for the normal clearance of cholesterol from the tissues (Miller and Miller, 1975). The accumulation of cholesterol in the leucocyte may be accelerated by reduced clearance of cholesterol from the cell, secondary to a reduction in the plasma concentration of HDL-cholesterol.

4.6.4 The increased quantities of cholesterol in the leucocytes may reflect an elevated cholesterol level in other tissues including the aortic smooth muscle cells, if observations on animal models are extrapolated into human atherosclerosis. This cholesterol accumulation could be due to slowing down of the clearance of cholesterol, as seen by reduced cellular CEH and plasma HDL-cholesterol levels which has been already discussed.

4.6.5 Esterification of cholesterol and hydrolysis are balanced by the relative activities of CES and CEH (Brockman, 1979). Takano et al (1974) and Takano and Imanaka (1978) showed that in the spontaneous atherosclerosis in the aorta of man, a deficiency of CEH activity is the potential cause of the intracellular accumulation of cholesteryl esters. A parallel finding in the leucocytes as seen in Table 3.9 suggests that CEH and CES activities in the leucocyte fractions may be used for diagnostic and prognostic purposes.

4.6.5.1 Yatsu et al (1980) observed a reduced CEH activity in human asymptomatic atherosclerosis in mononuclear cells and strengthened the theory which postulates a role of depressed CEH levels in atherosclerosis.
4.6.6 Increase in C/P ratio, if present in the cell membrane of leucocyte might influence the specific activities of the cell such as aggregation and phagocytosis in which the membrane function is involved (Mc Murchie and Runson, 1979). The C/P ratio is a major determinant for the fluidity of each membrane since cholesterol increases microviscosity, thus reducing the fluidity (Cooper, 1977).

4.6.7 APSm administration leads to a significant increase in the cholesterol ester hydrolase (Lysosomal acid lipase, LAL) leading to an increase in the relative activity ie, CEH/CES ratio (Table 3.14) studied in the leucocytes. APSm has already been shown to increase the cholesterol ester hydrolases in experimental atherosclerosis in rabbits (Shanmugasundaram and Marita, 1982) and rats (Parthasarathy and Shanmugasundaram, 1983), studied in the aorta, liver and intestine.

4.6.8 In the cell, cholesterol is stored in its ester form, while it is transported to the cell membrane only in the free state. Deposition of cholesterol in cells and tissues is balanced by opposing forces namely (a) cholesterol esterification mainly by cholesterol ester synthetase or ACAT in the cells (Proudlock and Day, 1972) and (b) hydrolysis of cholesterol ester hydrolase (CEH) or LAL (Brecher and Chobanian, 1973). The former favours cholesterol deposition while the latter favours its removal into blood, and in atherosclerotic conditions, ACAT is elevated (Hashimoto and Dayton, 1977). This increase in CEH (Table 3.14 and Fig.3.9) clearly shows that APSm should be leading to regression of atherosclerosis.
4.7 LIPID PEROXIDATION AND THE PATHOLOGY OF ATHEROSCLEROSIS

4.7.1 Significantly elevated ($p<0.001$) levels of malondialdehyde (MDA), the end product of lipid peroxidation in plasma, HDL, erythrocytes and in erythrocyte membrane (Table 3.5) are observed in AMI.

4.7.1.1 Several risk factors in the development of atherosclerotic cardiovascular disease are closely associated with high levels of circulating lipid peroxides in MI. This is in good agreement with the studies reported in other populations (Yagi, 1985; Ledwozyw et al, 1986). Several investigators have confirmed the atherogenic role of circulating lipid peroxides (Kibata et al, 1977; Yagi, 1984). Proxidants and free radicals cause tissue damage by reacting with polyunsaturated fatty acids in cellular membranes, by a process called lipid peroxidation (Machlin and Bendich, 1987). Oxygen derived radicals, especially superoxide ($O_2^-$) and the hydroxyl radical ($OH^-$), have been implicated as possible mediators in the development of myocardial damage induced by ischaemia (Burton, 1985).

4.7.1.2 Significantly elevated levels of serum lipid peroxides are observed in conditions predisposed to IHD such as, hyperlipidaemia (Uzel et al, 1985), hypertension (Pickering, 1974), cigarette smoking (Nadiger et al, 1987), diabetes mellitus (Nishigaki et al, 1981) and aging (Hagihara et al, 1984).

4.7.2 Yagi (1987) suggested three possible routes by which lipid peroxides may promote atherogenesis namely (a) high levels of lipid peroxides injure the blood vessels causing increased adherence and aggregation of platelets to the injured sites.
This is known to be the initial event in the process of atherogenesis, (b) lipid peroxides indirectly increase platelet aggregation by inhibiting the biosynthesis of prostacyclin, an inhibitor of platelet aggregation (through inhibition of prostacyclin synthetase) (Sasaguri et al, 1985), (c) lipid peroxidation accelerate the incorporation of LDL into the arterial smooth muscle cells and promote the formation of lipid-laden foam cells which contribute to the development of atheromatous plaques (Nishigaki et al, 1984).

4.7.3 Peroxidation takes place on polyunsaturated fatty acids to give rise to free radicals and endogenous peroxides which are highly reactive and have chemotactic and cytotoxic properties (Loeper, 1991). The observations of Hiramitsu et al (1976) that atheroma contains a high level of lipid peroxides suggested that the high level of lipid peroxide in plasma would initiate the formation of atherosclerosis (Sato et al, 1979).

4.7.3.1 Loeper et al (1983; 1984) showed that plasma MDA levels can be correlated with plasma lipid concentration and arterial injury. While macrophages lack receptors for LDL (Brown and Goldstein, 1983), they have been shown to take up avidly oxidised LDL (Koster et al, 1986), by specific receptors known as the acetyl-LDL receptors or the scavenger receptors. LDL bound to these receptors is taken up with enhanced efficiency so that cholesterol rapidly accumulates within the macrophage and may convert it into foam cells (Steinberg et al, 1989).

4.7.4 In plasma HDL, lipid peroxides are elevated in AMI (Table 3.5). Similar increases were reported by Maseki et al (1981) and Nishigaki et al (1981) in the
HDL fractions of pre-eclamptic (toxaemia of pregnancy) and diabetic patients with lipid and lipoprotein abnormalities.

4.7.4.1 Eisenberg (1984) has reviewed data on HDL and confirmed that most of the circulating HDL particles are the end products of lipoprotein metabolism. They are formed in the plasma through the interaction of its precursors with VLDL and chylomicron remnants and in the lipids of the cell membrane. Consequently, it might be possible to consider that lipid peroxides produced in cell membrane are transferred to the HDL fractions together with lipids.

4.7.5 In erythrocytes and erythrocyte membrane also, levels of lipid peroxidation products are elevated in AMI patients (Table 3.5), while in patients with IHD, APSm administration reduces the MDA bound to the membrane (Table 3.16).

4.7.5.1 Increased lipid peroxidation of erythrocytes were demonstrated in experimental hypercholesterolemia (Bulur et al, 1986) and in beta agonist (isoproterenol hydrochloride) induced myocardial infarction in rats (Kumari and Menon, 1987).

4.7.5.2 The presence of highly unsaturated fatty acids and hemoglobin and a rich supply of oxygen are the factors favouring the increased susceptibility of erythrocytes to lipid peroxidation (Chiu et al, 1982). Considering the fact that erythrocyte lipids are elevated in hyperlipaemic conditions (Cooper et al, 1980; Bhandaru et al, 1982) may be related to the increased lipid peroxidation of erythrocytes observed in the present study.
4.7.6 Lipid peroxidation process requires substrates with conjugated double bonds and phospholipids provide a ready source for peroxidation products. The abundance of phospholipids in HDL and cell membrane make them easy target for peroxidation. AMI is associated with over two-fold increase in MDA in whole plasma and HDL, three-fold increase in red cells and four-fold increase in the erythrocyte membrane. Considering the patients with symptomatic IHD (Table 3.5), as precursors or having the potential to develop AMI, the subsequent increases in lipid peroxidation is important, a 15% rise in plasma MDA and HDL bound MDA and a 30% increase in erythrocyte membrane bound MDA is observed with the development of MI, suggesting rapid and augmented lipid peroxidation an immediate cause or result of the episode.

4.7.7 The herbomineral Siddha drug APSm administration lowers plasma, HDL, RBC and cell membrane bound MDA in a progressive manner (Table 3.16). Statistically significant alterations are seen in the first 6 months of therapy and 20% reduction is seen in all the fractions at the end of 12 months therapy. The reduction is 30% in 3 years, but had not reached the normal values. It appears that the inherent abnormalities in the metabolic control in patients with IHD cannot be fully reversed by APSm therapy alone. Longer follow up periods are needed to assess the time needed to obtain the full control (if any) of lipid peroxidation. It has to be noted that the participants in the APSm trials are not identical to those studied as healthy controls as can be seen from Table 3.11 in their family background, social status or habits, working in competitive environments (a sizeable number are professionals and executives) and smoking cigarettes, which are factors enhancing the beta adrenergic pathway. Epinephrine triggers free radical release (Misra and
Fridovich, 1972) and hence the exposure levels to free radicals in the two groups differ significantly. Measuring the levels of catecholamines and other stress hormones will be able to throw further light on these aspects.

4.8 PROTECTIVE MECHANISMS AGAINST LIPID PEROXIDATION

4.8.1 In healthy cells, lipid peroxidation is controlled \((in\ vivo)\) by the extremely efficient protective antioxidant systems and their impairment is often associated with increased susceptibility of red cells to peroxidation (Smith and White, 1974).

4.8.1.1 Cardiovascular diseases including atherosclerosis and the cardiac tissue injury after myocardial infarction have been shown to result, in part, from an over-production of free radicals generated at the site of damage (Machlin and Bendich, 1987).

4.8.1.2 Babior (1981) stated that the destruction of erythrocytes by oxidative mechanism is the end result of two process namely (a) denaturation of hemoglobin and (b) oxidation of the cell membrane.

4.8.2 Although the mechanism is not completely understood, it is apparent that denaturation of hemoglobin and precipitation inside the red cell as "Heinz bodies", is a part of the phenomenon. These bodies bind to the erythrocyte membrane and reduce the red cell deformability (Reinhart et al., 1986) and alter membrane permeability (Platterott et al., 1982).

4.8.3 The aerobic cells are able to handle and survive the continuous oxygen free radical production because of the existence of a delicate balance between cellular
system that generate various oxidants and those that maintain the antioxidant defense mechanism. In the cardiac muscles, these defense mechanisms include the enzymes SOD, catalase and glutathione peroxidase and the antioxidant scavengers such as vitamin E, ascorbic acid and cysteine (Diplock and Lucy, 1974, Ferrari et al, 1988).

4.8.3.1 The reduced levels of the protective mechanisms, namely the antiperoxidative enzymes glutathione peroxidase, superoxide dismutase and catalase (Table 3.6) observed in the patients with AMI run parallel to the increased levels of MDA in plasma, erythrocyte lysate and membrane.

4.8.4 The mechanism for clearance of superoxide anions is SOD. This enzyme catalyzes the dismutation of superoxide anions to H_2O_2 and O_2. Two enzymes are important in the metabolism of H_2O_2 produced by the univalent reduction of superoxide anion (Chance et al, 1979; Roos et al, 1980). The first is catalase, an enzyme mainly present in cytosol, which catalyzes the reduction of H_2O_2 to H_2O. Catalase, however, is present only at very low concentrations in the myocardium, where as the alternative enzyme glutathione peroxidase (a selenium dependent enzyme) is present at significant concentrations in the cytosol of cardiomyocytes (Lawrence and Burk, 1978).

4.8.4.1 Looper et al (1991) observed that peroxidation involved in cardiac diseases, myocardial infarction and unstable angina are accompanied by increase in the production of MDA and transient inhibition of protective enzymes especially superoxide dismutase.
4.8.4.2 The substrate for GPx is the reduced form of glutathione (GSH) (described under section 1.8.4) which is regenerated by the supply of the reducing equivalents of NADPH produced in the pentose phosphate pathway of glucose metabolism. Reduced glutathione is in dynamic equilibrium with other thiol groups in the internal milieu.

4.8.5 Fridovich (1978) observed that the enzymes SOD, GPx and catalase present in the erythrocytes catalyse the removal of superoxide and hydrogen peroxide formed in the erythrocytes under normal conditions. However, in ischaemia, these enzymes are structurally altered or functionally impaired thereby allowing the overproduction of superoxide free radicals (Guarnieri et al, 1982).

4.8.5.1 Burton (1985) reported that rabbits with interventricular septa pretreated with SOD can withstand ischaemia with little structural damage, when compared to control septa confirming the ability of SOD to protect against ischaemia induced free radical mediated injury.

4.8.5.2 It is interesting to note that with Anna Pavala Sindhooram (APSm) therapy, in patients with IHD (Table 3.15), a statistically significant rise in superoxide dismutase activity is observed at the end of 12 months, while GPx and catalase in the red cells show a significant rise at the end of 6 months. It can be concluded that this therapeutic method not only lowers cholesterol and triglycerides in plasma, but also may be providing protection to the cardiac myocytes against superoxide, peroxide and possibly other reactive radicals. Parallel studies on the erythrocyte antioxidant enzymes and cardiac enzymes should be made in animals to test whether the enzyme changes in the myocytes and erythrocytes do run parallel to each other. Even
assuming the absence of improvement in the cardiac antioxidant enzymes by APSm therapy, the increased levels of SOD, Catalase and GPx in the RBC should be able to flush out the damaging oxygen radicals in the myocytes during the passage of blood through the capillaries, and help in combating ischaemia mediated pathophysiology.

4.8.6 Although there is progressive rise in SOD, Catalase and GPx with APSm therapy, it has to be noted that even at the end of 36 months therapy, these levels have not reached those found in healthy adults. While APSm therapy over a period of 36 months brings plasma cholesterol to 209 ± 21 mg/dl and CRI to 3.7 ± 0.43 which are lower than that observed in age matched controls, MDA in plasma, HDL and the erythrocytes remains more than two-fold than controls, confirming that the potential or inherent metabolic defect in the antioxidant armamentarium in the body of the patients with IHD remain unaltered ie, the lipid lowering effect may not be parallel to its protection from oxidative damage. It would be interesting to study the effect of APSm with increased dietary intake of vitamin E,C, carotene, selenium and methionine, the dietary antioxidant scavengers and study SOD, GPx and Catalase changes. Such studies are warranted in the light of the following observations.

4.8.6.1 Guarnieri et al (1982) established that the protective enzymes SOD, CAT and GPx are structurally or functionally affected in IHD thus allowing the over production of lipid peroxides. Fiohe et al (1985) has successfully shown that therapeutic use of vitamin E, SOD and catalase leads to lowering of the oxidative insult after ischaemia. It may be inferred that by raising the cellular SOD, CAT and GPx, APSm therapy provides some degree of protection against the development of myocardial intarction in the patients with IHD. Further studies are needed to confirm the suggestions on vitamin supplements.
4.9 IMMUNOGLOBULINS IN AMI

4.9.1 Serum immunoglobulins are significantly elevated in AMI (Table 3.7) when compared with healthy subjects.

4.9.1.1 There is growing evidence that immunological mechanisms can influence the development of atherosclerosis (Poston and Davis, 1974). Significantly elevated levels of IgG, IgA and IgM were observed in patients with mixed angina pectoris (Muscari et al, 1987) and in patients with multiple atherosclerotic lesions (Muscari et al, 1988).

4.9.2 Circulating immune complexes appear to be initiators in the arterial lesions (Sharma and Geer, 1977) possibly playing a pathogenic role in atherosclerosis. According to the autoimmune theory of pathogenesis of atherosclerosis, the formation of lipoprotein-antibody (Lp-Ab) complex is one of the key moments for the initiation of a lesion in the arteries (Klimov 1986; Klimov et al, 1987). Fust et al (1978) and Klimov (1987) detected these complexes in the blood and aorta of 70% of CHD patients. Klimov et al (1985; 1987; 1988) demonstrated that Lp-Ab complex may be taken up by the macrophages. Such uptake may accelerate the accumulation of cholesteryl esters in macrophages and their transformation into foam cells.

4.9.3 Beaumont et al (1988) have convincingly shown that IgM-Lp complex may be used as a marker for familial hypercholesterolemia and IgA-Lp may be used as a marker for the risk of atherosclerotic ischaemic disease. Klimov et al (1988) studied the interaction of naturally occurring Lp-Ab autoimmune complexes isolated for the first time from the blood of IHD patients. They found that these autoimmune
complexes could produce transformation of macrophages into foam cells under in vitro condition. More detailed studies are needed to monitor the Lp-Ab complexes during APSm therapy, and in vitro studies are envisaged to assess the modifications if any on the transferability of macrophages.

4.10 BLOOD CLOTTING AND THROMBOSIS IN AMI

4.10.1 Plasma fibrinogen is significantly higher in AMI and in IHD than in the age matched controls (Table 3.8).

4.10.1.1 Fibrinogen and platelet count are raised by 10% in AMI when compared to the cases of symptomatic IHD studied (Table 3.8). In symptomatic IHD, 60% rise in fibrinogen and 35% rise in platelet count is already noticed. In spite of heparin administration, prothrombin time is not brought back to the normal suggesting that thrombosis is not wholly arrested during therapy.

4.10.2 Coronary thrombosis occurs in most patients with acute myocardial infarction (Davis and Thomas, 1984). Small et al (1987) reported that fibrinogen levels showed a positive association with the extent of coronary atheroma. In addition, Fletcher et al (1981) observed high levels of fibrinogen in patients with hyperlipoproteinaemia.

4.10.3 The atherogenic role of fibrinogen is mediated through its promotion of red cell and platelet aggregation (Schmidt-Shonbein et al, 1976; Diminno et al, 1983). Increased rheological stasis and amplification of the coagulation cascade at higher concentrations (Dormandy, 1983) are also pathological.
4.10.4 In the North Wick Park Heart Study, Meade et al (1980), observed that the survivors of MI had a significantly lower fibrinogen levels (293 mg/dl) while those who suffered cardiovascular deaths showed a higher mean fibrinogen level of 332 mg/dl. In one of the prospective studies, Meade et al (1986) observed that among the smokers the association of plasma fibrinogen with MI was stronger than that of cholesterol with MI. In the White Hall Study made by Rose (1985) the socio-economic class with greater CAD mortality showed significantly higher plasma fibrinogen levels confirming the predictive value of this component. Recently, Handa et al (1989) have reported that plasma fibrinogen levels increase progressively with the severity of coronary atherosclerosis.

4.10.5 These studies and ours show a predictive value of fibrinogen higher than that of cigarette smoking, serum cholesterol, hypertension and other major risk factors. This prognostic significance is seen from the frequency distribution curve (Fig. 4.1).

4.10.5.1 Fibrinogen is present in the intimal lesions (Smith and Staples, 1983) and deposition of fibrinogen in the arterial intima precedes LDL deposition. Handa et al (1989), reported a significant association between plasma fibrinogen and serum cholesterol in both men and women, and that plasma fibrinogen and LDL may well have a combined effect in promoting coronary atherosclerosis.

4.10.6 The significant increase observed in the levels of platelet counts in AMI (Table 3.8) is in agreement with the following reports from other part of the World.

4.10.6.1 Increased level of platelet mass and reduced platelet survival were reported in patients with atherosclerosis (Corash et al, 1981; Martin et al, 1985) and
Fig. 4.1

% Frequency

Fibrinogen mg/dl

Control  
IHD  
AMI

% Frequency

Prothrombine time in secs.

% Frequency

Platelet count $10^5$ cells/mm$^3$
hyperlipoproteinaemia (Harker and Hazzard, 1979). Mazoyer et al (1988) reported significantly elevated platelet counts in hypercholesterolemic rabbits. It has been shown that atherogenic diets high in cholesterol induce intrinsic morphological and kinetic abnormalities of platelets in rabbits (Mazoyer et al, 1988).

4.10.6.2 In conjunction with these observations, the significantly higher platelet counts in the present study may be considered as a response secondary to the lipid abnormalities observed in AMI.

4.10.7 Our observation that prothrombin time is lowered in MI is supported by studies made on other populations and also with the concurrent observations of increased fibrinogen and platelet counts in blood.

4.10.7.1 Epidemiological studies (Baker et al, 1982; Yarnell et al, 1985) reported shorter clotting time in MI patients (O'Brien, 1974; O'Brien et al, 1974; 1975 and several others).

4.10.8 Significantly higher levels of (within the normal range) blood sugar and urea (Table 3.1) are due to the higher incidence of diabetes among the patients. While levels of serum uric acid do not show any difference between MI and healthy populations, plasma protein levels are elevated significantly as can be observed from Table 3.1. Immunoglobulins and other protein mediated injury to the arterial wall which cause the development of atherosclerosis have been observed in a number of clinical and experimental studies (Howard et al, 1971; Klimov et al, 1988; Muscari, et al, 1988). The level of plasma proteins in our control group is $7.9 \pm 0.96 \text{mg/dl}$, which is higher than the normals reported in European literature. Earlier studies in
our laboratory also have shown a higher level of gamma globulins possibly due to immunisation obtained during childhood by exposure to a variety of infectious agents.

4.10.9 Vlaicu et al (1985) reported the preferential retention of 14 different proteins including immunoglobulins and complement components in the fibrous plaque in the intima of human aorta with atherosclerosis. They considered that the higher concentration of these proteins in the diseased intima might be due to an increased permeability of the diseased vessel to the plasma proteins. The increased plasma proteins in the patients may be a contributory factor in the aggravation of the atherosclerotic lesions. Its contribution in increasing blood viscosity and its effect on the rheological properties need further elaboration.

4.10.10 The enzyme assays reported in Table 3.4 can be used only for a qualitative verification of (a) the episod al MI and (b) the recovery. No attempts were made to authenticate the tissue specificity of the enzymes, since this was not the main thrust of the studies.

4.11 COMPARISON OF APSm WITH OTHER LIPID LOWERING DRUGS

4.11.1 The study presented envisages a holistic approach to the treatment and control of atherosclerotic disease and APSm was administered, without altering the other drug regime followed by the patients for correction of hypertension, coagulation defects etc., involving β-blockers, calcium channel blockers, diuretics, anticoagulants (dipyrimole, warfarin) m-dopa, allopurinol and the different hypoglycaemic agents.
4.11.2 For effective lipid lowering, the fibric acid derivatives clofibrate, fenofibrate and benzofibrate were administered at 4x5 mg/day, 3x100 mg/day and 3x200 mg/day respectively (reviewed by Monk and Todd, 1987). Benzofibrate produced 50% reduction in triglyceride, 15% in total cholesterol and 20% in LDL cholesterol, while, HDL cholesterol rose by 20%. Other fibrates showed lesser effectiveness. Probucol (Heller and Harvengt, 1983) raised LDL cholesterol with a reduction in HDLc while triglycerides were unaffected. There was good (100%) acceptance of APSm in the patients, and many of them could reduce their antihypertensive and antianginal drug discussed (Table 3.20) showing the beneficial effects of APSm as therapy of choice.

4.11.2.1 It may be observed from the Table 4.1 even though fairly effective in reducing plasma lipoprotein abnormalities, ciprofibrate produced peptic ulcer in 2 out of 20 cases studied. The reduction in triglyceride is not consistent because in another study made by Olsson and Oro (1982), ciprofibrate therapy did not reduce serum triglycerides.

4.11.3 In the case of fenofibrate, it may be seen that it could not reduce total cholesterol, LDL and triglycerides or increase HDL and HDL3 to the same extent as APSm. Moreover, there is a significant reduction in the HDL2 fraction with a concomitant decrease in the ratio HDL2/HDL3 which may favour cholesterol accumulation or deposition. In addition, Rossner and Oro (1981) showed that fenofibrate possess some cytotoxicity.

4.11.4 Gemfibrozil is a drug which is mainly used in patients with hypertriglyceridemia which shows a significant reduction in HDL2/HDL3 ratio, while lowering plasma triglycerides. It has no effect on total cholesterol while increasing
Table 4.1 Lipid lowering effect of APSm compared with other drugs

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Number of patients</th>
<th>Treatment period (months)</th>
<th>Total cholesterol (Reduction)</th>
<th>HDL (Increase)</th>
<th>HDL₂ (Increase)</th>
<th>LDL (Reduction)</th>
<th>HDL₂/HDL₁ (increase)</th>
<th>Triglyceride (reduction)</th>
<th>Infavourable or other side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>APSm 300 mg/day</td>
<td>50</td>
<td>2</td>
<td>12%</td>
<td>8.5%</td>
<td>29%</td>
<td>16%</td>
<td>24%</td>
<td>11%</td>
<td>Nil</td>
</tr>
<tr>
<td>Clofibrate 500 mg/day</td>
<td>12</td>
<td>36</td>
<td>23%</td>
<td>19%</td>
<td>33%</td>
<td>30%</td>
<td>102%</td>
<td>38%</td>
<td>Nil</td>
</tr>
<tr>
<td>(1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fenofibrate 300 mg/day</td>
<td>20</td>
<td>3</td>
<td>11%</td>
<td>8%</td>
<td>13%</td>
<td></td>
<td></td>
<td>22%</td>
<td>Produced peptic ulcer in 2 cases TG is increased in another study (5)</td>
</tr>
<tr>
<td>(2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gemfibrozil 600 mg/day</td>
<td>33</td>
<td>12</td>
<td>14%</td>
<td>9%</td>
<td>16% Reduction</td>
<td>15%</td>
<td>33% Reduction</td>
<td>30%</td>
<td>HDL₂ decreased, cytotoxic</td>
</tr>
<tr>
<td>(3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prindolol 15 mg/day</td>
<td>20</td>
<td>3</td>
<td>2%</td>
<td>25%</td>
<td>15%</td>
<td>11% reduction</td>
<td>Reduction</td>
<td>48%</td>
<td>Decreased HDL₂/HDL₁, GI disturbances like diarrhoea, constipation, etc</td>
</tr>
<tr>
<td>(4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atenolol 100 mg/day</td>
<td>30</td>
<td>2</td>
<td>No change</td>
<td>15%</td>
<td>-</td>
<td>4%</td>
<td>-</td>
<td>2%</td>
<td>Minimal lipid lowering effects</td>
</tr>
<tr>
<td>(5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

LDL (11%), the atherogenic lipoprotein (Sorisky et al, 1987). Vega and Grundy (1985) and others (Manninen et al, 1982; Seed et al, 1982) also observed increase in LDL during gemfibrozil therapy.

4.11.5 Medical Research Council Working Party (1985) has concluded that most of the hypertension reduction trials using diuretics and/or beta blockers have failed to reduce the mortality and morbidity from IHD and this is confirmed in the reports of Amery et al (1985). Increase in atherogenic lipoproteins (LDL and VLDL) and triglycerides with a decrease in HDL during use of antihypertensives over long periods have been reported by Ballantyne and Ballantyne (1983).

4.11.5.1 An ideal drug in the management of IHD should be capable of reducing the atherogenic lipoproteins while simultaneously increasing HDL (Criqui, 1986). On this score, treatment with betablockers might increase the risk of developing coronary atherosclerosis (Northcote et al, 1986; Northcote, 1988). The two beta blockers namely, pindolol and atenolol, (unpublished - Table 4.1) show very little lipid lowering effect. There is increase in triglycerides level by 34% with atenolol group while there is neither reduction nor increase in triglyceride with pindolol.

4.11.6 Several studies have shown that HDL$_2$ accounts to a major extent for the negative association seen between HDL and IHD (Anderson et al, 1979 and Gidez et al, 1982). HDL$_2$ is the protective form of cholesterol in HDL. If this is taken into consideration, gemfibrozil and fenofibrate show some signs of increasing the risk of IHD.
4.11.6.1 Several other intervention trials have questioned the hypolipidemic efficacy of many older drugs such as clofibrate, nicotinic acid, cholestyramine (CDP, Research group, 1975; 1977; Marmot, 1979; La Rosa, 1982). They have also reported that these drugs produce side effects such as constipation, nausea, vomiting, abdominal discomfort, reduced glucose tolerance, etc. In addition, lithogenicity is considered to be a potential side effect of clofibrate and its derivatives (CDP Research group, 1977; Oliver et al, 1978).

4.11.6.2 The superiority of APSm over the other drugs in lipid lowering as well as the absence of any undesirable side effects may be realised from the data provided in the text. APSm is a better lipid lowering drug with 100% safety which is required for the long term prevention of ischaemic heart disease.

4.11.7 The increase in the CEH or LAL activity in leucocyte (Table 3.14) during APSm therapy leads to an increase in the relative activity of CEH/CES in this sample of peripheral cells, suggesting removal of cholesterol from cells. With increased CEH activity in the leucocytes in viewed in conjunction with the significantly elevated HDL and HDL₂ levels after APSm therapy, APSm induced cholesterol mobilisation appears to be the probable pathway operating in clearing the arteries and other peripheral cells of accumulated cholesterol.

4.11.7.1 The reduction in plasma fibrinogen by APSm therapy (Table 3.17) should provide improved control of blood clotting and thrombus formation. In addition to its role in augmenting blood clotting, increased concentration of fibrinogen and platelets may affect rheological factors and influence blood flow (Nirot et al, 1988).
The reduction in plasma fibrinogen and platelet counts may provide some protection from thrombosis and blood flow abnormalities.

4.11.7.2 APSm therapy prolonged the prothrombin time which was shortened in IHD (Table 3.17). There are reports that bleeding time is increased by high intakes of fatty fish or fish oil supplement which has a beneficial effect on proven or presumed cardiovascular risk factors (Sinclair, 1980; Saynort and Verel, 1982). Rogers et al (1987) observed an increase in the bleeding time after fish oil supplementation for 6 weeks. This is believed to be mediated to the effect of ω-3 fatty acids in fish oil.

4.11.7.3 APSm induced reduction in platelet counts (Table 3.17) is similar to that observed with other lipid lowering drugs such as fenofibrate (Mellies et al, 1987). Platelet survival is reduced in patients with atherosclerosis (Corash et al, 1981) or hyperlipoproteinaemia (Harker and Hazzard, 1979). This induces the hyperactivity and aggregation of the platelets (Corash et al, 1981).

4.11.7.4 Mazoyer (1988) showed that atherogenic diets high in cholesterol induce intrinsic morphological and kinetic abnormalities of platelets, suggesting that changes in lipid constitution and composition may partly be responsible for their abnormal number or aggregation associated with atherosclerosis. APSm probably reduces (to some extent) the overproductions of platelets and thus leads to reduced platelet aggregation by normalising the lipid abnormalities. In depth study on the coagulation factors and platelet aggregation are needed to understand the relationship between plasma lipids and coagulation abnormalities. APSm therapy (Table 3.18) leads to a marginal reduction in Immunoglobulins G,A, and M. The immunological mechanism
involved in the development of atherosclerotic lesion beginning with formation of antibodies for apo-B (already discussed) needs further investigation during APSm therapy.

4.11.7.5 Long term intake of APSm does not show any unfavourable side effects, as usually encountered with many lipid lowering drugs. The reduced blood urea, serum uric acid together with improved glycaemic control (in the diabetics) are the favourable side effects. APSm does not show nephrotoxicity or hepatotoxicity. The latter is evidenced by the unaffected serum transaminase levels. ($T_{\text{c.b.L.}}$ 3.19)

4.11.7.6 In addition to correcting plasma lipid and lipoprotein abnormalities, APSm produces other favourable changes including weight reduction in obese patients, blood pressure reduction in 7/16 hypertensive patients, improvement in ECG in 3 cases etc. ($T_{\text{c.b.L.}}$ 3.20)

4.11.7.7 It is worth mentioning here that most of the patients entered the trial because of poorly controlled blood pressures, plasma lipids and ECG abnormalities despite medication. Guggullip, clofibrate, probucol and gemfibrozil are some of the lipid lowering drugs tried unsuccessfully by them. L-methyldopa, atenolol, nifedipine, verapamil, dithiazide and isosorbide are the common drugs the patients took during APSm therapy. During APSm therapy, some of the patients slowly cut down on their antihypertensives and other medicines. Those who had completed 18 months APSm therapy have stopped one or more drugs after consulting the cardiologist (Table 3.20).
4.11.7.8 All the patients felt "Comfortable" when they were on APSm. Nifedipine was discontinued by one patient. While all the hypertensives on L-methyl dopa and propranolol, atenolol etc. reduced this drug confirming that APSm therapy improves cardiovascular resilience by regulating blood pressure through action on the beta adrenergic receptors and the vascular tone. The reductions seen in FFA levels already can be due to reduced β-adrenergic activity. More detailed investigations on echocardiographic monitoring and coronary angiographic studies are proposed to be made.

4.11.7.9 It is felt that the hypolipidemic action of APSm must be due to the presence of calcium, iron and a variety of unidentified substances having pharmacological activity. In the experimental animal models, APSm reduced cholesterol deposition produced regression of aortic atherosclerosis (Dhandapani et al, 1984), increased cholesterol and biliary excretion (Kareem, 1983), and HDL bound cholesterol in addition to lowering LDL, VLDL, triglyceride etc. In epinephrine and IPH induced myocardial lesions, prior treatment with APSm protected the laboratory rats against beta adrenergic hyperfunction (such as lipolysis, cholesterologenesis increased heart rate, ST elevation in ECG and the onset of myocardial infarction) suggesting that continuous administration of the drug may lead to modification of the beta adrenergic receptors resulting in decreased receptor number or binding.

4.11.7.10 The observations reported in the thesis provides indirect evidence to APSm induced modulation of beta adrenergic receptor response. Further work on the receptor study and binding studies with labelled catecholamine is needed to confirm the inferences.