I. Introduction
Although the relationship between elevated blood cholesterol levels and increased incidence of coronary heart disease (CHD) has been recognized for many years, concerted efforts to identify and treat individuals with hypercholesterolemia have emerged recently. In recent years evidence has accumulated to indicate that cholesterol plays a major role in the development of atherosclerotic coronary disease. Various factors linking cholesterol to atherosclerosis have been reviewed (Roberts, 1988) which provide evidence that elevation of total blood cholesterol (specifically LDL-cholesterol) is the cause for the development of atherosclerosis. These include:

(1) Feeding high cholesterol diets to certain nonhuman animals produces atherosclerotic plaques similar to those occurring in humans.

(2) Cholesterol is found in both experimentally induced atherosclerotic plaques in nonhuman animals and in plaques in human.

(3) Atherosclerotic plaques, large enough to produce clinical problems, occur only in persons having plasma total cholesterol levels > 150 mg/dl for long periods of time.

(4) The higher the blood total cholesterol level the greater the chance of having symptomatic and fatal atherosclerotic disease.
(5) The higher the serum total and LDL-cholesterol levels, the greater the extent of the atherosclerotic plaques.

(6) Lowering the blood total cholesterol and LDL-cholesterol levels decreases the chance of fatal or nonfatal atherosclerotic disease.

(7) Atherosclerotic plaques regress when high blood cholesterol levels are lowered.

Moreover, hyperlipoproteinemia, abnormal platelet function (thrombosis) and macrophage uptake of modified lipoproteins contribute to the progression of the atherosclerotic process. Therapeutic agents which interfere or reverse one or more of these pathological processes should reduce clinical manifestations in the form of myocardial infarction, ischaemia, variant angina and thromboembolic disorders (Hiluiaj et al., 1988). Hence, atherosclerosis is generally considered to be multifactorial disease, as such, the prevention or cure of the disease may be approached at different levels, control of plasma cholesterol and or atherogenic lipoproteins (VLDL and LDL) being an important one. Excessive plasma VLDL and LDL are generally implicated as at least one of the causative factors in this major disease problem.

Evidence that treatment of hypercholesterolemia reduces the incidence of primary CHD was first presented in 1984, with publication of the results of the LRC-CPPT (Lipid Research
Clinica Program. 1984 a, b). For every 1% reduction in cholesterol level, there was approximately a 2% reduction in CHD risk in participants of these studies.

At present physicians are frequently reluctant to prescribe cholesterol lowering agents for a number of very good reasons. The bile acid sequestrants and nicotinic acid frequently cause side effects. Gastrointestinal problems such as nausea, bloating, constipation and flatulence are the most frequent side effects of cholestyramine and colestipol. Flushing occurs in most patients who receive the high doses of nicotinic acid that are required to reduce blood cholesterol levels. Poor adherence to treatment has also been a problem in LRC-CPPT with cholestyramine and with nicotinic acid in Coronary Drug Project (The Coronary Drug Project Research Group, 1975). In addition, treatment of hypercholesterolemia with a bile acid sequestrant is quite costly. Thus, there is a great need for more effective and better-tolerated cholesterol lowering drugs.

Recently, clinical trials testing the safety and efficacy of a new class of lipid lowering drugs, which are competitive inhibitors of the enzyme HMG-CoA reductase, have been reported (Moł et al, 1986; Nakaya et al. 1986; Grundy and Vega, 1985). This enzyme catalyzes the rate limiting step in endogenous cholesterol synthesis in liver. Available informations suggest that the HMG-CoA reductase inhibitors
are well tolerated and quite effective in reducing elevated total and particularly LDL-cholesterol levels.
In the future, data from treatment trials may provide clearer guidelines for use of an HMG-CoA reductase inhibitor to manage hypercholesterolemia.

A. Atherosclerosis and Lipoproteins

To describe the mechanism of action of HMG-CoA reductase inhibitors, it is necessary to understand the mechanism of lipid and lipoprotein metabolism. Fat absorbed from the diet and lipids synthesized by the liver and adipose tissue must be transported between various tissues and organs for utilization and storage. Since lipids are insoluble in water, the problem of their transport in an aqueous environment, the blood plasma, is solved by associating nonpolar lipids (triglycerides & cholesteryl ester) with amphipathic lipids (phospholipids and cholesterol) to proteins to make water miscible macromolecular complexes. These complexes are known as lipoproteins. Abnormalities of lipid metabolism occur at the sites of production or utilization of lipoproteins, causing various kinds of hypo- or hyper-lipoproteinemias. The most common of these is diabetes mellitus, where insulin deficiency causes excessive mobilization of FFA and under-utilization of chylomicrons and VLDL, leading to hypertri-
glyceridemia. Most other pathologic conditions, affecting lipid transport primarily, are due to inherited defects in synthesis of the apoprotein portion of the lipoprotein, of key enzymes or of lipoprotein receptors. Some of these defects cause hypercholesterolemia and premature atherosclerosis.

Four major groups of lipoproteins have been identified that are important physiologically as well as in clinical diagnosis. These are, (i) chylomicrons, derived from intestinal absorption of triglycerides; (ii) VLDL derived from the liver for the export of triglycerides; (iii) LDL representing a final stage in the catabolism of VLDL; and (iv) HDL involved in VLDL and chylomicron metabolism and also in cholesterol metabolism.

Except for LDL which is thought to be primarily the end product of VLDL lipolysis (Shaefer et al., 1978), the lipoproteins are formed either by the intestinal mucosal cells (enterocytes) or by the liver cells (hepatocytes). The major lipoprotein of the intestinal origin is the chylomicron, however, both VLDL and HDL may also be produced by the intestine (Hu and Windmueller, 1978). In the gut, dietary fat is hydrolyzed to monoglyceride and free fatty acids which are then transported by an energy independent process into the enterocytes. Triglycerides are reformed in the smooth endoplasmic reticulum and packaged along with other
lipids and proteins by the golgi apparatus into large triglyceride rich chylomicrons. The chylomicrons are expelled from the golgi apparatus through exocytosis into the intercellular space at the basal aspect of the cell (Sabesin and Frase, 1977). The chylomicron reach the lymphatic system and eventually enter the blood stream through the thoracic duct. In the circulation 75-80% of the chylomicron triglyceride is hydrolyzed by the enzyme lipoprotein lipase (Chajek and Eisenberg, 1978) present in the vascular endothelium. As a result of hydrolysis, the free fatty acids are liberated and taken up into the tissue, esterified, stored and oxidized for future energy needs. After the hydrolysis of chylomicrons, monoglyceride as well as excess surface components are also removed from the chylomicrons (Eisenberg and Levy, 1975) leaving cholesterol ester rich triglyceride poor remnant particles which bind with high affinity to receptors in the liver (Sherrill and Dietschy, 1978; Cooper and Yu, 1978). The transport of exogenous triglyceride in this manner via chylomicrons is highly efficient so that chylomicronemia ensues within few hours after fatty meal. This process can account for the transport of as much as several hundred grams of triglycerides daily. In either genetic or acquired deficiency of lipoprotein lipase the capacity to transport triglycerides is quite limited and chylomicronemia may persist from meal
to meal. This condition is also termed as exogenous hyperlipidemia.

The remaining three lipoprotein classes are of endogenous origin. In the fasting state majority of plasma triglycerides are contained in the triglyceride rich VLDL and hypertriglyceridemia, often but not always, means an increase of VLDL, sometimes also associated with an increase of both HDL- and LDL- triglycerides. VLDL and HDL are the primary contributions of liver to circulating lipoproteins. VLDL is secreted from the liver in much the same way as the chylomicrons are formed by the intestine (Glauman, et al, 1975). Nascent HDL formed by liver is much different from its circulating counterpart, however, cholesterol and triglyceride content of nascent VLDL is same as that of plasma VLDL (Mahley, 1978). Several evidence indicate that the surface of the nascent lipoproteins is modified once they enter the extracellular environment. The metabolism of VLDL and production of LDL has been shown in Fig. 1. The cholesterol content on the surface also increases while that of phospholipid falls (Hamilton, 1973). Hepatogenous VLDL acquires C-apoproteins (apoC) from HDL when comes in the circulation. The apoC increases the affinity of the particle for lipoprotein lipase and enhances the catalytic rate of the enzyme on component triglycerides (Fielding, 1973; Nilsson-Ehle, 1980). The hydrolysis of triglyceride core
Nascent VLDL

B-100

TG C

E

Apo-C, Apo-E

Fatty acids

Lysosome

EXTRAHEPATIC TISSUES

VLDL

B-100

TG C

E

Apo-C

HDL

Apo-E receptor

Fatty acids

Cholesterol

LIVER

LDL receptor

LDL

C

B-100

( VLDL remnant )

Glycerol

Lysosome

EXTRAHEPATIC TISSUES

LIPOPROTEIN LIPASE

Fatty acids
of VLDL normally occurs rapidly at the surface of blood capillaries in several tissues (most notably adipose tissue and heart) where most of the triglyceride fatty acid is taken up into the tissues for storage or oxidation. The partially degraded particles (remnants) contain only a fraction of triglycerides, phospholipids and apoC, originally present, but they retain all of the cholesterol esters and apoB and the bulk of cholesterol. The liver accounts for removal of almost all the remnant cholesterol ester and cholesterol. Upon hydrolysis of VLDL, surface components including cholesterol are released in proportion to triglyceride hydrolysis. Extensive hydrolysis of triglyceride produces the smaller and denser LDL particles. Also, during VLDL hydrolysis in vivo, the surface components (apoprotein, phospholipid, unesterified cholesterol) are transferred to HDL (Shen et al., 1977), preserving the basic structure of the lipoprotein (Fig. 2). Most LDL appears to be formed from VLDL, but there is evidence for some production directly by the liver (Noel and Rubinstein, 1974; Thompson and Myant, 1976).

Since cholesterol esters and cholesterol are major components of atherosclerotic lesions, the interaction of the cholesterol carrying lipoproteins in plasma with the cells of the arterial wall seems to be important. An increased level of cholesterol carrying lipoprotein, LDL, is
associated with an increased risk of developing atherosclerotic cardiovascular disease. As LDL level increases more LDL presumably filters into subintimal regions and the cholesterol carried in LDL accumulates within the arterial wall (Vega et al., 1985).

The contribution of LDL to atherosclerosis, however, may not be determined solely by plasma LDL concentrations. The disturbances in metabolism of LDL may also affect the atherogenecity of this lipoprotein. For example, it has been proposed that normal LDL must be modified before it can induce the development of foam cells (Brown and Goldstein, 1983). Other metabolic abnormalities may also render LDL more atherogenic. There are several reports of such abnormalities (Sniderman et al., 1980; Brunzell et al., 1983; Teng et al., 1983; Vega and Grundy, 1984). It has been shown that the cholesterol of atherosclerotic plaques is derived from LDL particles that circulate in the blood stream. The more LDL there is in the blood, the more rapidly atherosclerosis develops (Goldstein and Brown, 1984a).

There is also considerable evidence that elevated level of plasma triglycerides is also a risk factor for ischaemic heart disease. Since LDL and VLDL both are considered to be atherogenic, it seems desirable to develop therapeutic agents which specifically lower these atherogenic lipoproteins. The removal of HDL from the circulation seems
to be accomplished primarily by the liver (Rachmilewitz et al, 1972). Therefore, cholesteryl ester carried by HDL or in remnants of triglyceride rich lipoproteins is transported to the liver where cholesterol, released on hydrolysis, is mainly converted to bile acids and thus excreted from the body. The cholesteryl ester that enters LDL, either from catabolism of VLDL or by transfer from HDL, may have a different fate. LDL serve to supply cholesterol to the metabolic needs of the cells, accomplished by the receptor mediated endocytosis of LDL particles. The work of Brown and Goldstein (1977, 1979, 1983) on the cellular metabolism of LDL elucidated the "LDL pathway". The high affinity receptors bind LDL particles and extract them from the fluid that bathes the cells. The LDL is taken into the cells, rapidly followed by hydrolysis of the lipoprotein, yielding its cholesterol for cellular needs. In supplying cells with cholesterol, the receptors perform a second physiological function which is critical to the development of atherosclerosis; they remove LDL from blood stream. The number of receptors displayed on the surface of cells varies with the cellular demand for cholesterol. When the need is low, cells make fewer receptors and take up LDL at a reduced rate. This protects cells against excess cholesterol but at a higher price, the reduction in the number of receptors decreases the rate of removal of LDL from the circulation,
blood level of LDL rises and atherosclerosis is accelerated. In WHHL rabbits, with a genetic deficiency of LDL receptor function, extremely high plasma LDL-cholesterol levels are observed with the development of atherosclerosis early in life (Goldstein et al., 1983). LDL receptor activity is under metabolic regulation in vivo, such that receptor activity can be increased or decreased by appropriate interventions with diet and/or drugs (Mahley and Innerarity, 1983; Brown and Goldstein, 1983).

Stimulation of receptor activity in vivo has been reported in rabbits (Slater et al., 1980) and in patients with heterozygous FH (Shepherd et al., 1980) after cholestyramine treatment. Kovanen et al. (1981) first demonstrated the stimulation of hepatic LDL receptor activity, by pharmacologic manipulation using mevinolin to treat Beagle dogs and later on same observations were made in FH heterozygotes (Bilheimer et al., 1983). Therefore, as shown in Fig. 3, Bilheimer (1984) proposed a rationale for the use of an inhibitor of HMG-CoA reductase alone or in combination with bile acid depletion to stimulate increased hepatic LDL receptor expression and thus to lower plasma LDL levels. This central metabolic position of LDL receptors particularly in the liver has changed the focus for therapeutic lowering of plasma LDL-cholesterol. When the cells of the liver have a high demand for cholesterol they produce high level of m-RNA
Fig. 3  Rationale for the use of HMG-CoA reductase inhibitor alone or in combination with bile acid depletion to stimulate receptor expression and to lower plasma LDL levels [Bilheimer (1984)]
NO DRUGS

HMG CoA -> CHOLESTEROL -> BILE ACIDS

HMG COA REDUCTASE INHIBITOR

HMG CoA REDUCTASE INHIBITOR + BILE ACID DEPLETION

HMG CoA REDUCTASE INHIBITOR + BILE ACID DEPLETION

HMG CoA -> CHOLESTEROL -> BILE ACIDS
for receptors. The opposite phenomenon takes place when excess cholesterol accumulates in cells (Russel et al. 1983). The hepatic demand for cholesterol leading to increased expression of LDL receptors can therefore be achieved by inhibiting the intestinal absorption of bile acids and the synthesis of cholesterol. A powerful effect can be obtained through inhibitors of hepatic cholesterol biosynthesis, as reported for example, with compactin (Endo, 1985) and other competitive inhibitors of HMG-CoA reductase.

In contrast to LDL, as shown in Fig. 2, HDL prevents or reverses atherosclerosis by mediating reverse cholesterol transport. In this process cholesterol is removed from sites of deposition, such as arterial wall or tendon xanthomas and delivered to the liver for biliary excretion (Glimset, 1968). Thus, reverse cholesterol transport renders HDL or some of its subfractions to be antiatherogenic. Because of the inverse association of HDL with the incidence of CHD, as demonstrated by a number of clinical and epidemiological studies (Diehl et al. 1988; Bahler et al. 1980; Tyroder, 1980; Miller, 1980) major interest has now been focussed on this lipoprotein. HDL form a heterogenous group of particles which can be divided on the basis of density, into two subfractions HDL$_2$ and HDL$_3$. The concentrations of these subfractions vary independently and they have separate
metabolic pathways (Nikkila, 1981). HDL₂ is larger, less dense and contains more phospholipid and cholesterol than HDL₃. Their concentration is influenced by many physiologic variables such as hormones, diet, sex and physical activity. In contrast to other lipoproteins, HDL is not turned over as a single compound. Its constituents may enter and leave the mother particle at different rates and undergo exchange or bidirectional shuttling between HDL and other lipoproteins or cell membranes. HDL precursors relatively poor in cholesterol would appear to be excellent candidates for reverse cholesterol transport by virtue of their considerable capacity for uptake of cholesterol and LCAT induced cholesteryl ester core formation. As shown in Fig. 2, HDL₃ acts as an acceptor of cholesterol, phospholipids and apoproteins that are released during degradation of VLDL and chylomicron by lipoprotein lipase at extrahepatic capillary beds. Upon this process HDL₃ is transformed into HDL₂ (Patsch et al. 1978). It has been speculated that HDL₂ could lose only part of its surface lipid into the liver and be converted back to HDL₃ which is returned to plasma and starts the circle again. The process of conversion of plasma HDL₃ to HDL₂, involving neutral lipid exchange and role of triglyceride lipases, has recently been documented by Deckelbaum et al (1986).

The incidence of CHD has been correlated positively with
high levels of LDL-cholesterol and negatively with high levels of HDL-cholesterol (Li et al., 1988). However controversy exists regarding the relative importance of HDL subfractions. The relationship of HDL subfractions to alcohol consumption, other life style factors and CHD was recently evaluated in a study made by Dichl et al (1988). These findings suggested that HDL-cholesterol as well as HDL₂-cholesterol may be related to coronary risk and indicated that the protective effects of alcohol consumption may be mediated via this subfraction. In another study, distribution of HDL subfractions was determined in two groups of subjects with different coronary risk indices (Griffin et al., 1988) and it was suggested that the level of these subfractions might provide a better index of coronary risk than that of total HDL-cholesterol, as conventionally employed in many epidemiological studies. It has also been documented that HDL₂ subfraction has a stronger negative relationship with atherosclerosis risk than total HDL or HDL₃ (Eisenberg, 1984).

Plasma lipoprotein abnormalities are also common in patients with diabetes mellitus (Nikkila, 1984). They are of considerable clinical interest because of their relationship to atherosclerosis. The excess of CHD and other atherosclerotic vascular diseases associated with diabetes has often been attributed to the disordered lipoprotein
metabolism (Ganda, 1980). Diabetes is most commonly associated with hypertriglyceridemia and elevation in VLDL levels (Behr and Kraemer, 1988). Macrophages from insulin deficient mice have also shown increased activity of HMG-CoA reductase and subsequently an increased rate of cellular cholesterol synthesis (Kraemer, 1986) and subnormal HDL cholesterol levels are reported in untreated diabetic patients (Nikkila, 1981). A profound decrease in the activity of lipoprotein lipase is also observed in diabetes mellitus and a decreased secretion of lipoprotein lipase by murine macrophages has been reported (Behr and Kraemer, 1988).

B. PLATELET AGGREGATION AND ATHEROGENESIS

It is well known that the excessive deposition of components of the blood including fibrin and platelets, onto the vascular intima is responsible for the development of atherosclerotic plaques. The association of platelets with the atherosclerotic lesions has been repeatedly documented (Faggiotto et al, 1984; Faggiotto and Ross, 1984). The lesions may develop as a result of blood vessel injury, platelet adhesion to the injured site, the release of platelet derived growth factors, and the ensuing migration and proliferation of smooth muscle cells (Mustard et al, 1988).
Numerous constituents released by platelets such as vasoactive amines, lysosomal enzymes, thromboxanes, prostaglandins and nucleotides and nucleosides may be injurious to endothelium. Diet induced changes in plasma lipids and intrinsic platelet alterations have been investigated in several studies (Joist et al, 1976; Zucker et al, 1988).

In CHD patients increased sensitivity of platelets to aggregating agents (Yamazaki et al, 1976; Gormsen et al, 1977) or a tendency to disaggregate more slowly following ADP-induced aggregation (Davis et al, 1978) have been reported. Platelet activation is thought to be important in the generation of atherosclerotic disease (Weksler and Nachman, 1981). Takagi et al (1988) demonstrated that thrombin activated platelets or substances shed by activated platelets increase both the rate of esterification of cholesterol and accumulation of cholesteryl ester in macrophages. These observations suggested that platelets may contribute to lesion progression by enhancing foam cell formation. The atherogenic properties of lipoproteins are not restricted to their role in cholesterol and lipid metabolism but also involve their effect on blood platelet activity (Aviram, 1988). The possible effect of plasma lipoprotein concentration on the function of circulating platelets becomes of importance in the light of accumulating
evidence that hyperlipidemia, platelet function, and thrombosis are interrelated and directly influence the atherosclerotic process. Considerable evidence suggest that hypercholesterolemia, particularly the increase in LDL-cholesterol fraction, is associated with increased platelet aggregation (Carvalho et al., 1974). Patients with atherosclerotic disease are also reported to have enhanced platelet activity (Knapp et al., 1986). Platelets from patients with FH have been found to be hypersensitive to aggregation (Carvalho et al., 1974), to contain increased amounts of cholesterol and phospholipid (Shastri et al., 1980; Norday and Rodest, 1971) and to produce increased amounts of thromboxane A₂ (Tremoli et al., 1979). The high levels of circulating LDL, responsible for hypercholesterolemia in these patients, could result in increased platelet activation in vivo, which may in turn potentiate arterial injury and allow for cholesterol accumulation in the arterial wall. Incubation of platelets in vitro with cholesterol rich liposomes has also been shown to increase platelet aggregation and platelet thromboxane production (Stuart et al., 1980; Horner and Patscheke, 1980). It has also been found that platelets possess specific binding sites for LDL (Aviram et al., 1981) and these sites are qualitatively different in FH derived platelets, that are hyperreactive when aggregation and release responses are
tested in vitro. In animals a similar enhancement in platelet function could be induced by a cholesterol rich diet (Oversohl et al. 1975; Joist et al. 1976). Enhancement of thrombin-induced platelet aggregation by LDL from diabetic patients was reported recently by Watanabe et al (1988) and this may contribute to the accelerated development of atherosclerosis in diabetes mellitus.

Excess LDL may by itself damage endothelium (Heuriksen et al. 1979a) but, in the presence of sufficient HDL no damage ensues (Heuriksen et al. 1979b). One method to reduce early lesion formation in the atherosclerotic process is to either lower LDL or raise HDL levels, and a second less explored possibility might involve use of redox drugs designed to inhibit oxidative processes. Platelet activation may be inhibited by drugs interfering with various biochemical events. These include inhibitors of arachidonic acid metabolism, thromboxane A₂ antagonists, adenylate cyclase activators, phospholipase C inhibitors and calcium channel blockers (Witjak et al. 1988). Clofibrate (Yamamoto et al. 1980) inhibits the activity of phospholipase A₂ possibly through an indirect mechanism. Aspirin inhibits cyclooxygenase and substituted imidazole inhibits thromboxane A₂ synthesis (Moncada et al. 1985). Several other antilipidemic compounds have been found to be antiaggregatory and their mechanisms of action have recently been discussed (Witjak
et al. 1988). In view of these findings, the possibility exists that inhibition of platelet function might retard atherogenesis when such agents are given over very long periods of time.

C. MECHANISM OF ACTION OF OTHER HYPOLIPIDEMIC COMPOUNDS

Although our studies are specifically concerned with the mechanism of hypolipidemic action of HMG - an inhibitor of cholesterol biosynthesis, we shall briefly discuss the action of other agents, widely used as lipid lowering drugs. This might help to understand the mode of action of HMG. Probucol has been reported to effectively reduce plasma cholesterol in human and a number of animal species (Miettinen and Toivonen, 1975; Martin, 1979; Simson et al., 1981). It also affects the composition and in vitro catabolism of LDL in types IIa hypercholesterolemia (Baudet et al., 1986). It increases the activity of plasma lipoprotein lipase and decreases HDL- and LDL-cholesterol concentration in rats (Strandberg et al., 1981). Probucol prevents the development of macrophages into foam cells by inhibiting the lipid storage in macrophages (Yamamoto et al., 1986a). These results coincided with the clinical findings that probucol causes a more marked regression of xanthomas than would be expected from the extent of lowering of
LDL-cholesterol. Probucol seems to act by increasing LDL removal from plasma by an LDL receptor independent mechanism (Kesaniemi and Grundy, 1984), as it causes moderate reduction in LDL-cholesterol in non familial hypercholesterolemics (Sinason et al., 1984) and a smaller decrease in FH patients (Durrington and Miller 1985; Fellin et al. 1986). A marked decline in HDL-cholesterol has been a constant finding (Kesaniemi and Grundy, 1984; Fellin et al. 1986) and circulating HDL in probucol treated patients are smaller than in controls (Yamamoto et al., 1986b). Analysis of LDL composition demonstrated that LDL cholesterol lowering effect of probucol in FH was entirely due to reduction in the proportion of cholesterol in LDL with no reduction in LDL mass, whereas in non FH a reduction of both LDL mass and apoB was achieved. A decrease in HDL₂ fraction was also observed by probucol which is not mediated by lipoprotein lipase or hepatic lipase (Helve and Tikkanen, 1988).

Fibrates are orally active compounds with a relatively long plasma half life. Among fibrates, clofibrate (CPIB), which combined maximal effectiveness and minimal toxicity in the initial screen, was widely used in the management of hyperlipoproteinemia in man. More recently, however, due to questionable activity in the secondary prevention of atherosclerosis (Coronary Drug Project, 1975) and to the observation of untoward effects in primary prevention
(Committee of Principal Investigators, 1978), the use of CPIB has become increasingly circumscribed. Among side effects, proliferation of peroxisomes in rodents has been a key target for chemical and pharmacological studies. A number of clinical trials have constantly shown that CPIB reduces plasma triglyceride levels substantially, affecting both VLDL and LDL associated triglycerides. This reduction is more evident in hypertriglyceridemic patients but less marked in normotriglyceridemic subjects (Hunninghake et al. 1981; Crouse and Grundy, 1981).

The cholesterol lowering activity of CPIB is less dramatic and seems to depend on individual basal levels. In large series of hypercholesterolemic patients, a 10-15% reduction of total cholesterol and LDL-cholesterol has been reported (Crouse and Grundy, 1981; Hunninghake et al. 1981). Besides inhibiting cholesterol biosynthesis at the step involving conversion of acetate to mevalonate, CPIB also affects several other metabolic pathways. Inhibition of fatty acid synthesis (Maragoudakis, 1969) and stimulation of adipose tissue lipoprotein lipase (Tolman et al. 1970), adenyl cyclase (Green et al. 1970) and hepatic acyl-CoA hydrolase (Borreback, 1979) has been reported. Increase in LPL activity results into increased catabolism of VLDL and elevated HDL-cholesterol levels, thus it helps in mobilization of tissue cholesterol. Hazzard et al (1984) demonstrated
significant decrease in plasma cholesterol and triglycerides, VLDL-cholesterol and triglycerides and an increase in HDL-cholesterol upon clofibrate feeding. However, it was associated with increase in reports of nausea. The mechanism by which CPIB exerts its effects on plasma lipoproteins is most likely related to an increased catabolism of triglyceride rich lipoproteins with the increase in LPL in adipose tissue and postheparin plasma without affecting a hepatic lipase (Goldberg et al. 1979). CPIB exerts, most likely, a primary effect on the production of LPL by tissues. On the other hand, the increased LPL activity may also result from the elevation of the apoC-II content of VLDL, the physiological activator of the enzyme (Bengtsson and Olivecrona, 1980), after treatment with CPIB and other fibrates in hypertriglyceridemic patients (Naruszewiez et al. 1980; Franceschini et al. 1985).

Halofenate, structurally related to clofibrate is hypolipidemic and hypouricemic (Sirtori et al. 1972) and effective in lowering serum triglycerides in rat (Kritchevsky and Tepper, 1972). Bezafibrate, a fibric acid derivative, has been reported to lower plasma triglycerides in hypertriglyceridemic patients (Eisenberg et al. 1984) and was effective in reversing most, if not all, of the abnormalities in lipoprotein composition, structure and functionality detected in these patients. It decreased plasma total- and VLDL-tri-
glycerides by about 40%-60% (Schwandt et al., 1982; Vessby et al., 1980). In hypertriglyceridemic patients bezafibrate treatment often resulted in a rise of circulating LDL levels (Olsson et al., 1977). These different responses seem to reflect in part the effect of bezafibrate on LPL-induced VLDL-LDL conversion (Vessby et al., 1982) and on LDL catabolism (Stewart et al., 1982). LDL particles modified by bezafibrate bind to LDL receptor and down regulate the LDL receptor activity better than the pretreatment lipoproteins (Klieman et al., 1985).

Fenofibrate, another analogue, is more potent than the previously described compounds, being fully active at a daily dose of 300 mg (Rosaner and Oró, 1981). It is markedly effective in patients with type II and IV hyperlipidemias (Franceschini et al., 1985) as well as in subjects with familial combined hyperlipidemia (Weisweiler et al., 1984) in decreasing total plasma cholesterol and triglyceride levels. However, HDL-cholesterol levels were unchanged in type II and in combined hyperlipidemia (Malmendier and Delcroix, 1985). The mechanism of hypolipidemic effect of fenofibrate is different from that of parent compound involving its effect on lipoproteins as seen in subjects with FH as well as combined hyperlipidemia. An increase of apoC-II content in VLDL (Franceschini et al., 1985) was consistent with the observed increase in LPL activity after fenofibrate
treatment (Heller and Harvang, 1983), which may result in increased HDL-cholesterol levels particularly in hypertriglycerideremic condition. In a recent study (Olivier et al., 1988) using mice as the model for drug screening, decrease in plasma triglycerides and VLDL-triglycerides was associated with an increase in HDL-cholesterol in animals receiving standard diet. On the other hand in hypercholesterolemic mice, fenofibrate lowered total plasma cholesterol at the same time increasing HDL-cholesterol.

Ciprofibrate is, apparently, the most potent available fibrate, being fully active at 100 mg/day (Olsson and Oro, 1982). It is effective in all kinds of hyperlipidemias (Illingworth et al., 1982). Detailed studies on the mechanism of action of ciprofibrate have not been carried out but reports indicate that it differs essentially in dynamic and kinetic properties (Sirtori and Franceschini, 1988).

Gemfibrozil, another fibrate, also affects triglyceride rich lipoproteins. It has been found to be effective in both men (Shepherd et al., 1985) and mice (Olivier et al., 1988). As with other fibrates, a rise of LDL-cholesterol has been detected with a decrease of apoB/cholesterol ratio. As plasma apoB levels were unchanged during treatment, a cholesterol enrichment of LDL, rather than an increase in the number of LDL particles has been suggested (Vega and Grundy, 1985). The influence of etofibrate on LDL metabolism
was recently reported in a group of hypercholesterolemic individuals (Gries et al., 1988) with a reduction of plasma cholesterol as well as cholesterol in VLDL and LDL fractions. Cholesterol content of HDL did not, however, change although its total mass rose by 29%. But the catabolism of LDL was reported to be increased without affecting its synthesis. Emilo et al. (1988) demonstrated the action of etofibrate involving the enhancement of esterification of fatty acids and glycerol in adipose tissue and increased utilization of triglyceride precursors in other pathways causing a decreased production of triglycerides. The reported findings are indicative of multiple mechanisms responsible for lipid regulating effects of CPIB and its analogues.

The development of specific inhibitors of HMG-CoA reductase has considerably widened the therapeutic opportunities in hypercholesterolemic patients. Compactin and lovastatin (mevinolin) are potent competitive inhibitors of HMG-CoA reductase. A part of their structure closely resembles the HMG moiety of HMG-CoA and the enzyme, HMG-CoA reductase, binds both the compounds with high affinity (Paoletti and Poli, 1987). These drugs have been used to reduce plasma cholesterol levels in many animal species (Alberta et al., 1980; Kovanen et al., 1981; Tobert et al., 1982). In clinical studies mevinolin and compactin effectively reduced plasma
LDL in normal (Tobert et al., 1982) as well as in subjects with heterozygous FH (Bilheimer et al., 1983) and a compensatory increase in the receptor mediated catabolism of LDL was found (Grundy and Bilheimer, 1984). In patients with type IIa and IIb hypercholesterolemia a mean reduction of 32% in serum cholesterol was reported afterLovastatin treatment (The Lovastatin Study Group II, 1986). Goldstein and Brown (1984b) proposed that statins (compactin,lovastatin and simvastatin) lower plasma LDL-cholesterol by inhibiting cholesterol biosynthesis and by increasing the number of LDL receptors. Addition of bile acid sequestrants further activates receptor mediated catabolism of LDL (Vega and Grundy, 1987). An increase in HDL levels has been reported in several studies (Hoeg et al., 1986; Mol et al., 1988) or the levels have remained unchanged (Mabuchi et al., 1981; Illingworth and Sexton, 1984).

The mechanism of action of lovastatin differs from that of probucol (Helve and Tikkanen, 1988). Lovastatin therapy resulted in the increase of both HDL- and HDL₂-cholesterol whereas with probucol HDL-cholesterol was markedly decreased mainly because of reduction in HDL₂. Triglycerides remained unaltered during probucol treatment. Also, no significant changes in lipase activities were observed during lovastatin therapy indicating that these enzymes were not involved in its action. A new specific inhibitor of cholesterol bio-
synthesis, monacolin M, structurally related to mevinolin was isolated from cultures of a strain of Monascus ruber but the inhibition of HMG-CoA reductase was slightly less than that of mevinolin (Endo et al. 1986). Recently, simvastatin (MK-733) was found to be a potent inhibitor of cholesterol synthesis in heterozygous FH (Mol et al. 1988). It inhibited the absorption of cholesterol from the gastrointestinal wall in cholesterol fed rabbits (Fumiaki et al. 1988). Triparanol (Bhattacharyya and Egen, 1984), tibric acid (Pereira and Holland, 1974) and tiadenol (Baggio et al. 1979) are also cholesterol lowering agents. Tiadenol is remarkably effective in inhibiting fructose induced hypertriglyceridemia but its mode of action differs from that of clofibrate and related compounds (Franceschini et al. 1981). Nicotinic acid is possibly the oldest lipid lowering drug. Large doses of this drug rapidly reduced plasma triglycerides by lowering VLDL in normal and sucrose diet fed mice (Olivier et al. 1988). The reduction in triglycerides was reported to be due to decreased production of VLDL (Grundy et al. 1981).

To overcome the difficulty of obtaining adequate compliance with nicotinic acid treatment, because of the drug's numerous side effects, several analogues and derivatives have been tested but no clear advantages have been gained over the parent drug (Crepaldi et al. 1988). Acipimox, a nicotinic
Acid analogue, was found to inhibit adipose tissue lipolysis thereby reducing the FFA flux to the liver, resulting into less production of VLDL (Stirling et al., 1985). In recent study, LDL-cholesterol decreased in type II whereas a rise was observed in type IV patients after acipimox treatment (Crepaldi et al., 1988). Acipimox did not increase LPL activity in men unlike clofibrate (Stuuyt et al., 1985). However, adverse events like skin reactions, gastric disturbances, heart burn, epigastric pain etc. were reported during the treatment (Crepaldi et al., 1988).

An alternate approach towards the control of cholesterol level is the use of bile sequestrant resins. The mode of action of bile acid binding resins is apparently fairly simple: bile acids, which are bound to these resins in the intestinal lumen, are not reabsorbed and are excreted with feces. These sequestrating agents, thus, interfere with the entero-hepatic circulation of bile acids. Since bile acids are synthesized in the liver from cholesterol, as a result, cholesterol catabolism is enhanced. After prolonged treatment hypocholesterolemia is observed. However, there are some events that do not fit in this simple scheme. Homozygous type II patients are insensitive to cholestyramine inspite of increased level of fecal bile acids (Moutafis et al., 1971). The LDL receptor pathway plays a role in the action of bile acid binding resins (Shepherd et al., 1980).
D. ACTION OF 3-HYDROXY-3-METHYLGLUTARIC ACID (HMG)

The implication of blood lipids as a contributing factor in the pathogenesis of atherosclerosis has led to widespread search for compounds which safely and effectively lower the concentration of lipids in blood and, hopefully, in tissues. Since more than 70% of the total input body cholesterol is derived from de novo synthesis in human (Dietschy and Wilson, 1970), it is expected that compounds inhibiting cholesterol biosynthesis may prove useful in combatting hypercholesterolemia. The most suitable target for this inhibition would be HMG-CoA reductase, the rate limiting enzyme of cholesterol biosynthesis (Rodwell et al., 1976). HMG is known to inhibit cholesterogenesis between HMG-CoA and mevalonate (Rabinowitz and Gurin, 1954) and competitively inhibits the enzyme HMG-CoA reductase (Fimognari and Rodwell, 1965).

HMG-CoA hydrolase is known to catalyze in vivo formation of HMG in a number of species (Dekker et al., 1958). The hypolipidemic activity of HMG has been studied in rats (Yousufzai and Siddiqi, 1976a, 1977a; Francesconi et al., 1987) rabbits (Yusufi and Siddiqi, 1974; Lupien et al., 1973), hamsters (Padoya et al., 1982) and humans (Yousufzai et al., 1976b; Lupien et al., 1973). A support to the hypolipidemic action of HMG is also obtained from the observation that low
incidence of CHD in Maasai tribesman is related to their high consumption of cow's milk (Richardson, 1978), known to contain HMG (Mann, 1977). It is now considered a potential hypolipidemic compound often named as 'Mevalon' (Arca et al., 1986) or 'Meglutol' (Corregeloune, 1985). Most of the work on the hypolipidemic action of HMG showed that it had no hepatotoxic effect at the microscopic level (Beg et al., 1968). Lack of clinical and laboratory signs of pathological effects at hepatorenal level was reported (Bonavita et al., 1984) and no change in the liver function occurred (Arca et al., 1986).

HMG effectively counteracted Triton WR-1339 induced hyperlipidemia in rats (Yousufzai and Siddiqi, 1976c) and hyperlipidemic effects of heated corn oil and cholesterol (Yousufzai and Siddiqi, 1977a). Since consumption of saturated fat is known to contribute to the development of CHD, it was of more practical value to find the effect of HMG on tissue lipids of animals kept on high levels of different dietary fats. HMG also prevented the rise of serum and liver lipids of rats fed with high percentage of lard, butter and hydrogenated vegetable oil (Yousufzai and Siddiqi, 1977a). The marked lipid lowering effect of HMG in serum and liver of butter-fed rats is interesting in view of the fact that affluent persons, more prone to CHD, consume larger amount of butter. As decrease in lipid parameters in
one tissue was not accompanied by an increase in other tissue, the effect of HMG on catabolism of lipids is more likely. HMG offered total protection against the lipemic effect of ethanol both in men and rats (Yousufzai and Siddiqi, 1976b), possibly by inhibiting the mobilization of FFA from endogenous lipid stores and it was also effective against the lipemic and atherosclerotic response of massive doses of Vitamin D$_2$ (Yousufzai and Siddiqi, 1976a). High carbohydrate diets are known to produce hypertriglyceridemia and enhanced synthesis of VLDL as well as secretion of VLDL-triglycerides (Shiff et al. 1971) and different kinds of dietary carbohydrates are important in relation to hyperlipoproteinemia (Antar et al. 1970). Hence it was desirable to carry out studies on the effect of HMG in rats given high carbohydrate diet. With all types of dietary carbohydrates, HMG had a significant cholesterol and triglyceride lowering effect (Yousufzai and Siddiqi, 1977b). The action of HMG seems to be independent of the type of carbohydrate in the diet in preventing hypertriglyceridemia and hyperlipoproteinemia produced by dietary carbohydrates. Lupian et al (1979) found usefulness of HMG in the treatment of FH patients without any evidence of adverse clinical or biological effects. All patients maintained excellent compliance to medication. A unique experimental model for FH has now been developed in the form of WHHL rabbits. Van Niekerk
et al (1984) reported that HMG appears to be equally effective in this animal model similar to compactin which is also effective in heterozygous patients (Mabuchi et al., 1983).

In recent years much interest has been developed in the use of hypocholesterolemic drugs as a protective measure against cholesterol gall stone formation (cholelithiasis). Observations have been made in hamsters showing that the addition of HMG to a fat free lithogenic diet resulted in a marked reduction of cholesterol gall stone formation (Kritchevsky et al., 1978). Antilithogenic effect of HMG was further supported when it was found to be effective in reducing both bile cholesterol supersaturation and hypercholesterolemia in hamsters (Padova et al., 1982). The potentiality of the use of HMG against hyperlipidemia gets further support from several recent clinical studies. It appears to be useful for the treatment of most patients with diet resistant polygenic hypercholesterolemia and of some patients with heterozygous FH (Arca et al., 1986). HMG treatment of hyperlipidemic patients for 8 weeks resulted in a significant reduction of total plasma cholesterol but not always a significant reduction of HDL-cholesterol (Bonavita et al., 1984).

Since most of the lipid lowering drugs currently in clinical use (nicotinic acid, clofibrate, etofibrate) induce lithogenic bile secretion, effect of HMG was ascertained on
the bile lipid composition. In a double blind, placebo controlled study, carried out in 16 normolipidemic patients with asymptomatic radiotransparent gall stones, HMG exerted no adverse effect on bile lipid composition differing from other drugs (Padova et al. 1984).

Since hypolipidemic therapy is generally instituted for longer period and since some types of hypolipoproteinemias by themselves carry an increased risk of cholesterol gall stone formation (Ahlberg et al. 1980), the knowledge of the possible lithogenic effect of each drug may influence the therapeutic choice of the physician. In this respect HMG seems to be protective and safe, since its administration does not alter bile cholesterol saturation index even in subjects with pre-existing high risk of a lithogenic bile secretion.

E. SCOPE OF THE WORK

For the first time work from this laboratory indicated that HMG has hypocholesterolemic activity in rats (Dog and Siddial, 1967; 1968) resulting in grant of a US patent No. 3,629,449 dated 21.12.71 for a Process of Combatting Hypercholesterolemia. Since then work from different laboratories has unequivocally established the hypolipidemic and anti-lithogenic action of HMG (Castaner and Paton, 1978;
Ijarar and Siddigí, 1987) resulting in grant of several patents (Ijarar and Siddigí, 1988). Therefore HMG could be a potential hypolipidemic drug of the future. In the present work attempt has been made to understand the possible mechanism of lipid lowering action of HMG. It involves the study of effect of intraperitoneal administration of HMG on the concentration of plasma lipids, lipoproteins and the composition of different lipoprotein classes. Three different groups of rats namely normolipidemic, hyperlipidemic and diabetic groups were used as the experimental animal models. To confirm whether the action of HMG involves its interference with the metabolism of plasma lipoproteins, activities of post-heparin plasma lipases as well as tissue lipases were measured in HMG treated and control rats. A part of the work also consists of the effect of HMG administration on the aggregation of blood platelets.