I. INTRODUCTION
Atherosclerosis and Lipid Metabolism - In the last few decades concerted efforts have been made by Biochemists, Pathologists, Nutritionists, Clinicians and Internists to investigate the role of various hypocholesterolemic agents, in combating hypercholesterolemia and atherosclerosis. Atherosclerosis and allied diseases related to hypercholesterolemia and hyperlipemia are major medical problems of the modern age. Since it is believed that hypercholesterolemia and hyperlipemia are prelude to atherosclerosis, the compounds capable of correction of these two abnormalities are considered to be of paramount importance and help in the chemotherapy of atherosclerosis.

The modern name of the disease "Atherosclerosis" is derived from two Greek words: 'athera' meaning "porridge" or "mush" and 'scleros' meaning "hard". This apparently contradictory combination describes the fact that the lesion begins as a soft deposit and hardens as it ages. However, the term "Atherosclerosis" has a variety of meaning depending upon the approach to the problem, for Internists, the diagnosis consists of certain specific signs and symptoms that indicate the disease, for a Pathologist, a person has atherosclerosis if a variable combination of changes in intima
of arteries involving local accumulation of lipids, complex carbohydrates, blood and its coagulation products, fibrous tissue and calcium deposits are present which may be associated with clinical symptoms. Björck (1965) explained the term in many ways in clinical studies. In his opinion:

"The term fatty streak or spot is applied to superficial yellowish grey intimal lesions, which are stained selectively by fat stains. It is not synonymous with 'atheroma'."

"The term 'fibrous plaque' is applied to a circumscribed, elevated intimal thickening which is firm, and grey or pearly white."

"The term 'atheroma' is applied to an atherosclerotic plaque in which fatty softening is predominant. Complicated lesions are defined as lesions with additional changes or alterations such as haemorrhage, thrombosis, ulceration and calcareous deposits."

Hence, for clinicians atherosclerosis encompasses a wide variety of clinical entities which may include myocardial infarction, angina pectoris, cerebro-vascular diseases and occasionally changes which can be detected only on an Electrocardiogram. Conclusively, atherosclerosis is a complex and multifaceted condition which may be a collection of pathological states with overlapping similarities of arterial abnormalities. Generally, atherosclerosis either of the aorta or coronary arteries involves the formation of lipid deposits inside the walls of blood vessels. These fatty
substances appear mainly in smooth muscle cells and foam cells within the arterial linings. In the spaces between the cells small amounts of cholesterol can be detected. Very fine fibers of a material which behaves like fibrin also show up within the lining as well as on its surface. At this early stage the forerunner of atherosclerotic lesion can be recognized in the form of fatty streaks. It is believed that 90% of all genuine forms of angina pectoris and myocardial infarction are associated with sclerotic changes in coronary artery (Schettler, 1961). Arteries afflicted with atherosclerosis exhibit thickening of the arterial wall intima, usually hypertrophy of the media with clearly demonstrable lipid deposits. Since all these factors lead to decrease in the size of the arterial lumen, it results in restriction of flow of blood at reasonable pressures. Thus the restricted supply of oxygen and nutrients to coronary muscle leads to diminished performance and finally heart failures.

Many factors, that are suspected of contributing to atherosclerosis have been investigated. Undoubtedly heredity is an important factor. This includes marked-obesity, hyper-tension (diastolic blood pressure of 95 mm or above), "inborn errors"
of hyperlipidemia and hypercholesterolemia (serum cholesterol 260 mg% or above), diabetes, deranged thyroid functions, malfunction of the adrenals and many others. Environmental factors may be of primary or secondary influence; these include emotional stress situations, diet, climate, detrimental habits (smoking, lack of exercise etc.), contracted diseases, certain drugs, and other metabolic disorders such as elevated serum uric acid (Montoya et al., 1967; Benedek, 1967), lowered serum albumin (Mashev et al., 1967), lowered lipoprotein lipase (Petrova, 1967) and lowered endogenous heparin (Valecon, 1967), etc. At present, no single factor can be exclusively isolated in the etiology of coronary atherosclerosis. High blood pressure, no doubt, is a serious contributor to the atherosclerosis along with hypercholesterolemia. Diabetes, in combination with hypertension, is also a crucial factor responsible for the development of the lesion (Robertson, 1962).

Recently epidemiological aspects of atherosclerosis have been reviewed (Epstein, 1971). Accordingly, serum cholesterol levels are generally low in India but in recent years these may have been rising. Hypertension is probably the commonest of all cardiovascular disorders in the African continent and Western Countries. In India, high blood pressure
is frequent among patients with ischemic disease. In India, as would be expected in a vast country with many populations differing in origin and living conditions, wide variations in the frequency of coronary disease have been reported. It is quite unexpected and unexplained to find the prevalence of coronary disease in Chandigarh, the capital city of Punjab, as high as in the United States. These studies suggest that culture and customs are related to prevalence of the disease. In general, coronary disease is less frequent in India than in more affluent societies.

Besides heredity factors, dietary factors are numerous and studies to produce variations in serum cholesterol level are even more plentiful especially in animals. Schönebeck et al. (1962) concluded that two factors are responsible for the development of atherosclerosis; one is located in serum and the other in the arterial wall. According to Ursprung (1965,66) cholesterol plays a major role in the development of atherosclerosis. Epidemiological studies in man suggested that high fat diet with or without cholesterol plays a major role in the development of atheromata. It is important to remember that the correlation between cholesterol level and heart disease, although soundly based, is statistical and that there is yet no antemortem, diagnostic test for this disease.
Cholesterol is a major constituent of atherosclerotic lesions (Frantz and Moore, 1969) and the routine method of producing atherosclerosis in experimental animals is by feeding cholesterol at levels of 0.5-5% by weight of the diet (Kritchevsky, 1964; Constantinides, 1965). Cholesterol with added fats and oils in the diet has been found more atherogenic than alone. Kritchevsky et al. (1961) showed that cholesterol produces atheromatous plaque in rabbits when suspended in corn oil. These workers further noted that cholesterol in heated corn oil is more atherogenic. This could be due to the presence of higher concentration of free fatty acids in heated corn oil (Kritchevsky and Tepper, 1962). It has been found that saturated fats along with cholesterol produce more severe atheroma than unsaturated fats (Kritchevsky and Tepper, 1967a). Therefore, cholesterol and most probably fatty acids are inevitable suspects, because the formation of the atherosclerotic lesion is essentially an inflammatory response to these parameters. It is of interest to note that after prolonged cholesterol feeding, a slow fall in the cholesterolemia is observed with an increase in the severity of the pre-established atheromatous lesions (Mc Millan et al., 1955; Kritchevsky and Tepper, 1961).
is due to the fact that fatty plaque becomes fibrotic and begins to resemble the advanced human lesion (Constantinides et al., 1960). These observations have been confirmed in rabbits as well (Bailey and Butler, 1967; Kritchevsky and Tepper, 1967b). Rabbits receiving corn oil and coconut oil diets (no added cholesterol) alternately, or as a 50:50 mixture, for 10:10 week periods, developed more aortic and coronary atherosclerotic lesions on the alternate regimen than on the 50:50 mixture (Vles and Kloeze, 1967). Without added cholesterol in the diet, few atheroma are found (Conner et al., 1967). Gresham and Howard (1960) found that rats developed atherosclerosis when fed a diet containing cholesterol, cholic acid, thiouracil and peanut oil. Recently Kritchevsky et al. (1971) have reported that the rabbits fed a diet containing coconut or peanut oil with 2% cholesterol had the most extensive, as well as the most frequent and severe, lesions than that of corn oil or special fat fed rabbits. Recently much more severe atherosclerosis was developed in hypercholesterolemic baboons by an immunological injury (Howard et al., 1971).

**Mechanism of Development of Atherosclerosis** — The deposition of lipids in arteries or the atheroma formation is a complicated process which is not easy to explain. Pathologists
have been puzzled about the factors responsible for the progression of atherosclerosis. There are two prevalent concepts on the genesis of atheromatous plaques, the filtration theory and the thrombogenic theory. They involve different mechanisms and are not easily reconcilable. The filtration theory presumes that plasma constituents enter directly into the arterial wall by a process of diffusion from the lumenal surface of the vessel. Injury to the endothelium and/or intima or a high level of lipoprotein encourages plaque formation. Courtois and Garlick (1962) reported that serum lipoprotein first passes across the endothelial wall, but β-lipoprotein fraction may become held up in the subendothelial space and hence get deposited. It has become necessary, therefore, to postulate that either lipoproteins are altered in some way during the process of deposition or the lipid deposits arise in part by the synthesis in the arterial wall.

According to thrombogenic theory, mural thrombi adhere to the lumenal surface and become incorporated into the wall by an overgrowth of endothelium. The type of plaque formed is dependent upon the ratio of the adhering materials, platelets and fibrin. It has been shown that macrophages also play an important role in deposition and removal of
lipids in atherosclerosis. Lipid removal may be a critical factor (Adams, 1969) in plaque formation and the location of lecithin-cholesterol transacylase in the arterial wall (Abdullah et al., 1969) could play an important role, since cholesterol is rapidly exchanged (Smith, 1969) with the blood while cholesterol ester is not. Day (1964) has suggested four possible ways for the deposition process with macrophages: (a) by the uptake of lipid or lipoprotein by macrophages in the lesion (b) by the subsequent metabolism of such ingested lipid or (c) by primary synthesis of lipids by macrophages which have accumulated in the vicinity of the initial lesion; and (d) alternatively macrophages may ingest lipid at some site distant from the artery and carry it into the arterial wall.

There are several possible ways to explain the pathogenetic patterns of atherosclerosis caused by the various food fats. One possibility is that some types of the lipid may be metabolized more effectively by the liver and the artery walls than others. As shown in peanut oil fed rabbits, a greater gastrointestinal absorption of dietary cholesterol or a relatively retarded catabolism of the exogenous cholesterol may be associated with the consistent accumulation of aorta lipids (Kritchevsky et al., 1971).
A further possibility is that some types of lipids when deposited within cells of the aortic wall may lead to a more severe metabolic reaction than others. This reaction is probably at least partly responsible for the characteristic morphology of the lesions. Some aspects of triglyceride structure must also be important to cholesterol absorption, transport and deposition. Few evidence also indicate that relatively little lipoprotein penetrates from the lumen into the normal rabbit aorta, whereas entry from lumen is very substantially augmented when the endothelium and intima become more permeable in severe atheroma (Adams et al., 1970). These observations indicated that little cholesterol enters the inner layers of the normal aortic wall as lipoprotein, but in severe atheroma direct plasma leak into the wall from the lumen would allow cholesterol to enter in lipoprotein form. Day (1962) showed that in early and advanced lesions the distribution of phospholipids was similar to those of cholesterol and neutral lipids but after few weeks cholesterol level diminished, however, phospholipid remained distributed intracellularly in macrophages scattered throughout the newly organized lesions.

In the coronary diseased groups, both cholesterol and phospholipids were increased in the serum, but the cholesterol
phospholipids (C/P) ratio was likewise increased. This indicated that the rise in phospholipids had not kept pace with that of cholesterol. It is suggested that factors favouring the deposition of cholesterol in the intima are enhanced because of the lack of sufficient phospholipid to act as a colloid stabilizer. Pesters et al. (1970) also believed that the structure of phospholipids may play a role in the stability of lipoproteins and their interaction with the arterial wall. Since the cholesterol to phospholipids ratio is generally believed to be an index of atherogeneity, the rise in C/P in the β-lipoproteins is a pre-requisite for atherogeneity (Gresham et al., 1965).

A correlation between elevated serum triglyceride and cholesterol levels has been shown (Feldman and Wallace, 1964). The aortas of the human and most of the experimental animals are capable of synthesizing some of these lipids which accumulate in the atherosclerotic lesions (Stein and Stein, 1962; Loomwiger et al., 1962). Leofland et al. (1965) showed that in pigeon the coronary disease is characterized by the accumulation of various lipids especially of sterol esters and free sterol. In the latter stages as the aorta becomes relatively more diseased, the synthesis of fatty acids is
enhanced in aorta. The atherosclerotic plaque itself appears to be the chief site of this synthesis. Likewise, as the aorta becomes more diseased, relatively more of the newly synthesized fatty acids become esterified to cholesterol. Newman et al. (1968) showed the incorporation of fatty acids into aortic cholesterol esters in cholesterol-fed animals. They also demonstrated that serum fatty acids penetrate rapidly than cholesterol to the site of stratification in arteries. The exact mechanism by which cholesterol esters accumulate in the arterial wall has not so far been elucidated. Clements and coworkers (1969) have shown the presence in aorta of aldose reductase, an enzyme which they feel provides a mechanism for the alteration of arterial metabolism by hyperglycemia. Another approach to molecular interactions was described by Levy and Day (1969) who concluded from their results that the low density lipoproteins are uniquely polycationic at the surface and that these ions react with the internal arterial macromolecular polyanions.

High concentrations of sphingomyelin have been shown in the aortas and plasmas in experimental monkeys than those of normal monkeys (Portman and Alexander, 1970). Increased
rates of synthesis and of uptake from plasma of sphingomyelin may account for the increased concentrations of sphingomyelin in the atherosclerotic arteries, even though the ability to degrade sphingomyelin is also enhanced in the atherosclerotic aorta. This might be due to synthesis, catabolism and endothelial uptake from, or exchange with the plasma lipoproteins. Recently elevated proportions of phosphatidyl ethanolamine were found to be more closely connected with the occurrence of peripheral occlusive arterial disease than any other of plasma lipid and phospholipid parameters, and hence plasma phosphatidyl ethanolamine is better indicator in the predictability of atherosclerotic complications (Kunz and Stummvoll, 1971).

Some Important Hypocholesterolemic and Hypolipidemic Agents-

It is clear from the previous discussions that atherosclerosis can not be explained in terms of cholesterol alone, although it has been implicated in the etiology of atherosclerosis in animals and man. Certainly, cholesterol is one of the causative factor which is amenable to control by dietary means or by administration of drugs. Because cholesterol is the principal component of the atheromatous plaque, measures to reduce the level of cholesterol in the
blood by drug therapy can reasonably be expected to prevent, or at least retard the development of atherosclerosis. At present the prevailing therapeutic approach to atherosclerosis focuses on the treatment of hyperlipidemia, hypertension, obesity, diabetes and other associated pathologies. Diet is one of the most important factors in the prophylaxis of atherosclerosis. After the disease has manifested itself, diet control is usually a mandatory part of the therapeutic regimen. Generally, diets are directed toward the reduction of obesity and replacement of meat and saturated fat products with food containing unsaturated fats and non-meat high protein substances (Christakis and Rinzler, 1969). If food management does not lower serum lipids sufficiently the levels may be decreased further by administration of hypolipidemic drugs. Nowadays drug use has centred on compounds or substances that will lower specific serum lipids, notably cholesterol and/or triglycerides. There are two major indications for the lowering of serum lipids. One is the reduction of severe hyperlipidemia to (a) prevent lipid deposits (xanthomata) and (b) elimination of abdominal pain and pancreatitis due to high lipid levels. The other rationale to reduce serum lipids is that lowering of these blood constituents will lessen the likelihood of coronary heart
disease and atherosclerosis risk. There are three main possible ways by which most of the cholesterol inhibitors may affect hypercholesterolemia and hyperlipemia:

(i) stimulation of cholesterol catabolism and excretion
(ii) inhibition of cholesterol absorption and reabsorption
(iii) inhibition of its endogenous synthesis.

Administration of large doses of nicotinic acid has been shown to exhibit protective action on hypercholesterolemia and lipid deposition in aorta of cholesterol-fed rabbits (Altschul et al., 1955; Altschul and Hoffer, 1955). Later investigations have shown that it also reduced the triglyceride, phospholipid and free fatty acid (FFA) contents of the blood, together with the level of \( \beta \)-lipoproteins (Altschul, 1964). It has been suggested that nicotinic acid may act prior to the formation of mevalonic acid (MVA) (Gamble and Wright, 1961). Carlson et al. (1968) showed that administration of 1g nicotinic acid per day for five days decreased plasma triglycerides, cholesterol and phospholipids in hyperlipoproteinemimic patients. It was proposed that nicotinic acid inhibited mobilization of free fatty acids from adipose tissues. This reduced the uptake of free fatty acids in liver, which resulted in decreased hepatic formation of very low density lipoprotein (VLDL).
The consequences of these events would be reduced conversion of VLDLP to LDLP and eventually diminution of LDL level in plasma; thus plasma cholesterol and phospholipids would be lowered.

Many derivatives of nicotinic acid were shown to reduce the serum lipids without definite side effects (Tribiano and Spencer, 1962). Blanco et al. (1963) showed that complamin, a derivative of niacin, decreased blood cholesterol as well as other lipids. It was shown that administration of dl-α-tocopherol nicotinate, in hypercholesterolemic subjects caused decrease in serum total cholesterol with improvement of the subjective symptoms (Wakasa et al., 1966). Among nicotinic, nicotinuric and 3-pyridin acetic acids (100 mg - 250 mg/kg) given intraperitoneally to hyperlipidemic and hypercholesterolemic rats nicotinuric acid was found to be most effective (Brus, 1967). Further, a new derivative 2,2,6,6-tetrahydroxynicotinoyloxy- methyl) cyclohexanol (K-31) has been reported as hypocholesterolemic agent (Aso et al., 1969). K-31 suppressed the elevation of serum cholesterol, phospholipid and triglyceride only levels but depressed the accumulation of total cholesterol and phospholipids in liver. The hypocholesterolemic action
of K-31 may be due to inhibition of exogenous sterol absorption. Recently, Barboriac and Meade (1971) showed reduction of usual alcohol-induced enhancement of alimentary lipemia in man by nicotinic acid administration. Similar results were obtained in rats also when they were fed a corn oil-alcohol mixture in the diet. Under these conditions less incorporation of the $^{14}$C-labelled dietary fat into plasma triglycerides was observed. Another new derivative of nicotinic acid, pentraerythritolte-tranicotinate (PETN) has been shown to possess hypolipidemic properties in high fat-high cholesterol fed rabbits (Brattsand and Lundholm, 1971). They showed that PETN reduced the increase of free and estrified cholesterol and triglycerides in serum. PETN appeared generally to be somewhat more effective than nicotinic acid in reducing lipid infiltrated area of the aorta.

Puiyc-Muset et al. (1960) showed a marked reduction of blood cholesterol in humans as well as in experimental animals when an enzyme hepatocatalase was intramuscularly injected. The site of inhibition of this compound was believed to be between MVA and squalene (Caravaca et al., 1963). It has no significant effect on blood triglycerides and phospholipids. Recently, an active peroxidase, subunit of the hepatocatalase markedly prevented cholesterol induced hypercholesterolemia and also lipids accumulation in liver, heart and aorta.
in rabbits without any toxicity and undesirable side effects (Caravaca et al., 1967). It also decreased the serum turbidity.

Blohm et al. (1959) showed that triparanol (M&K-29) lowered serum and liver cholesterol levels, with concomitant accumulation of 7-dehydrocholesterol. De-Oliveira (1964) also found that triparanol caused cholesterol reduction in rats' aorta with a maximum accumulation of 7-dehydrocholesterol in brain. M&K-29 has no clinical value now a days due to toxic side effects (Wong et al., 1966). 1-Dimethylaminoethyl-4-benzyl-piperidine (in 379) has been shown to possess hypolipidemiac and antiatherosclerotic properties (O'Dell et al., 1962), but Kottke et al. (1966) failed to confirm these properties in birds.

Trans-1,4-bis (2-chlorobenzylaminomethyl-cyclo-hexane-diHCl (AY-9944) was shown to be a potent inhibitor of cholesterol synthesis (Chappel et al., 1964; Hill, 1966 and Jones et al., 1966). In almost all tissues especially serum and adrenal long term administration of the compound caused reduction in cholesterol levels but 7-dehydrocholesterol accumulated in lungs. Therefore, it is quite likely that AY-9944 inhibits the conversion of 7-dehydrocholesterol to cholesterol (Dvornik and Hill, 1968). Arnold et al. (1966)
showed that 17-(3-hydroxy-1-propynyl)-3-methoxyestra-1,3,5(10)-trien-17β-ol-17α-hydrocinnamates (HMPE) markedly reduced serum cholesterol in rats maintained on commercial laboratory feed. It has estrogenic property. The estrogens are known to possess hypocholesterolemic properties (Stamler et al., 1963). However, in the extensive studies of Borden et al. (1964) rats given estradiol benzoate parenterally and forced-fed a high fat, low protein atherogenic diet responded with markedly elevated blood cholesterol levels. The effect of estrogen on lipids and protective effect on coronary atherosclerosis has been studied in cockrels (Clarke et al., 1966), and in the cholesterol fed pigeon (Pritchard et al., 1966). Recently, Arnold et al. (1967) showed that HMPE exerted hypocholesterolemic effect in rats fed high fat, low protein diet. Cholestane-triol and its derivatives were found to lower (orally) blood and liver cholesterol and atherosclerosis in cholesterol fed rabbits, while serum and liver cholesterol were lowered in chickens and only the liver cholesterol was lowered in rats (Aramaki et al., 1967; Imai et al., 1967). It has been shown that 2-methoxy methyl-17α-methyl estradiol 3-methylether (P-5780) exerts a hypocholesterolemic effect, improves the plasma C/P ratio and partly prevents the
accumulation of aortic total lipids and cholesterol without any sign of estrogenicity in both sexes of cholesterol-cholate fed rats (Nakamura et al., 1965). Besides cholesterol, accumulation in aorta, liver and adrenals and improvement in plasma C/P ratio, P-5780 did not suppress the incidence of coronary atherosclerosis and hypercholesterolemia in cholesterol and corn oil fed rabbits. Norethynodrel (2mg/day; orally) was found to be an hypocholesterolemic agent in cholesterol fed rabbits and was able to reduce somewhat the aortic atherosclerotic development (Gore et al., 1967).

Kritchevsky (1968) reported that the most popular estrogen preparation, premarin, a mixture of conjugated equine estrogens lowers the serum \( \beta \)-lipoprotein and serum cholesterol levels. Although the mechanism of action is not clear, it seems to involve a shift of cholesterol from \( \beta \) to \( \alpha \)-lipoproteins. Estrogens have been shown to have a marked ameliorative effect on experimental atherosclerosis. The major drawback of estrogen therapy is the frequently observed feminization occurring in man. Recently, Amnaud (1970) studied the effect of premarin in preventing thrombosis & atherosclerosis in hyperlipemic rats. As fed rats an hyperlipemic diet with premarin (8 or 12 mg/kg) upto 15 weeks. The two doses were equally effective in reducing the serum cholesterol and triglyceride levels. However, the lower dosage was most
effective in prolonging coagulation and preventing thrombosis. The higher dosage was responsible for increased corticosterone level in plasma and decreased the serum albumin level, so among estrogens premenin is a better hypolipemic agent.

Several androsterone derivatives were reported as cholesterol inhibitors for a number of animal species (Singer et al., 1959; Abell & Mosback, 1962; Holmes, 1964). However, the site of action of these compounds remains to be established. A protein anabolic steroid 7\alpha-\textit{ethyl}-\textit{thio}-17\beta-hydroxy-17\alpha-methyl-5\alpha-androstane (3, 2-C) Pyrazol [PS-179] has hypocholesterolemic and antiatherogenic properties (Nakamura et al., 1967). This compound has prophylactic effect on aortic atherosclerosis, reduces tissue deposits and improves the plasma non-esterified fatty acids (NEFA) composition, also slightly reduces the incidence of coronary atherosclerosis. Conversely, it was found ineffective on withdrawal of high fat feed i.e. after establishing hypercholesterolemia. Later, Abell and Mosback (1968) observed a marked decrease in serum cholesterol level with 17\alpha-methyl-5\alpha-androstane-3\beta, 17\beta-diol (a metabolite of 17\alpha-methyltestosterone) in dogs. It was also found that about 10 mg/kg (orally) of either parent compound or diol diminishes serum cholesterol
level by 50%. Cortisone acetate has been shown to reduce the development of atherosclerosis in cholesterol-fed rabbits by about 75% (Bailey and Butler, 1966), but failed to regress developed lesions. This indicated that the action of the drug is in the early stage of plaque formation through its anti-inflammatory properties rather than via any lipemic effects.

A series of thyroxine analogues have been synthesized and evaluated for hypocholesterolemic activity (Blank et al., 1966). Kritchevsky and Tepper (1967c) noted a severe reduction in atheroma in cholesterol-fed rabbits with D-thyroxine (0.5 mg/day) or L-thyroxine (0.05 mg/day). D-thyroxine also lowers serum cholesterol as well as β-lipoproteins. The mechanism of its action is not clear. However, there is evidence from animal studies that thyroxine may cause redistribution of cholesterol from serum into muscle and skin.

A very promising method of lowering serum cholesterol appears in the bile acid sequestering agents. Cholestyramine, an ion-exchange resin originally made available for relief of biliary puritis, caused a dramatic reduction in serum cholesterol levels (Hashim and Van Itallie, 1965). Beher et al. (1967) reported that the compound prevented the tissue cholesterol accumulation in rats fed atherogenic diet, and
was effective in inhibiting atherosclerosis in rabbits. In a large number of patients constipation was the side effect of cholestyramine. Several other agents which inhibit absorption of cholesterol have also been studied as cholesterol lowering agents. Linoleamide (α-cyclohexyl-linoleamide) effectively reduced serum cholesterol levels and severity of atherosclerosis in rabbits maintained on cholesterol diet (Iatey et al., 1966; Hutsell and Quackenbush, 1967). Linoleic acid and its ethyl ester in cholesterol containing diet have also been shown to suppress the development of atherosclerosis in various animals (Quidery and Wu, 1966; Hutsell and Quackenbush, 1967). On the other hand, α-cyclohexyl-linoleamide, while showing a good cholesterol lowering effect in cholesterol-fed rabbits suppressed aortic atherosclerotic plaque at high doses (600 mg/day), and N-(α-methylbenzyl-linoleamide (MBLA) was more effective both ways (Kritchevsky and Tepper, 1967d and Toki et al., 1967). Later, Fukushima et al. (1969) compared the hypocholesterolemic activity of N-cyclohexyl-linoleamide (CHLA), MBLA and β-sisterol. They noted that β-sisterol has lesser activity than the former compounds, and the reduction of serum and liver phospholipids, triglycerides and cholesterol was more with MBLA than CHLA.
The inhibitory effect on experimental atherosclerosis was in the order:

D- MBLA > MBLA > L-MBLA

Recently another optically active (-) N-[<\text{-}\text{phenyl-}\beta-\text{-(\text{toly}l)} \text{ethyl}] \text{linoleamide (PTLA) was found to have a remarkable hypocholesterolemic effect in rabbits (Nakatani et al., 1970). Administration of PTLA (5-10 mg/day/animal) also significantly lowered serum and liver cholesterol and prevented formation of aortic atheromatous changes in rabbits that had been given 1.6g cholesterol daily. In comparison to MBLA, PTLA showed a more pronounced lipid lowering effect in cholesterol fed rabbits and severity of atherosclerosis was also lowered markedly in PTLA treated than MBLA treated animals.

Fisher et al. (1964) showed significant retardation in the developments of aortic lesions following the feeding of a diet containing citrus pectin without cholesterol supplementation in mature cockrels. Pectin with cholesterol in the diet reduced blood cholesterol in rabbits and caused less aortic atherosclerosis without any effect on severity of coronary lesions (Fisher et al., 1967). This suggested that pectin acts by accelerating the transit of food through
the alimentary tract resulting in an increased excretion of possible atherogenic substances as well as of total nutrients (calories). Other agents which inhibit absorption of cholesterol have also been studied as cholesterol lowering agents. Dextran and cellulose when administered orally were also shown to possess hypolipidemic properties in cockrels and dogs (Parkinson, 1967). Dextran (6%, 30ml/day) given intravenously to cholesterol-fed rabbits reduced the rise in serum cholesterol, phospholipids and triglycerides, apparently due to expansion of plasma volumes (Brahmanker and Connor, 1967). Dextran-40 was also found antiatherosclerotic in patients (Ditzel and Dyeberg, 1969). Recently a new apparently nontoxic naturally occurring acid mucopolysaccharide from a mammalian source has been isolated and purified, which has good lipoprotein lipase activating effects. The polysaccharide, heparitin sulphate has been shown to produce a reduction in serum cholesterol levels in rabbits fed a 2% cholesterol diet. It also reduced the formation of dietary induced atheroma in the aortic wall of rabbits kept on a 2% cholesterol diet (Grossman et al., 1971).

CPIB, familiarly known as clofibrate was introduced 10 years ago for treatment of hyperlipidemia (Thorpe and Waring 1962, Atromid symposium, 1963). Some important actions
attributed to this drug include inhibition of triglyceride release from liver (Asarnoff et al., 1965; Mishkel and Webb, 1967 and Gould et al., 1967), inhibition of hepatic cholesterol synthesis (Gould et al., 1966) interference with lipoprotein synthesis (Gould et al., 1967), inhibition of fatty acid synthesis (Maragoudakis, 1969), stimulation of lipoprotein lipase activity in adipose tissue (Tolman et al., 1970), enhancement of triglyceride uptake by adipose tissue (Nestel and Austin, 1968), stimulation of adenyl cyclase activity (Greene et al., 1970), and lowering of basal glycerol release from epididymal fat (Carlson et al., 1972). It has also been found that the hypolipidemic action of CPZB is dependent upon the nature and type of diet (Zakim and Herman, 1969).

Activity of a number of clofibrate analogues was described for use in the treatment of atherosclerosis. Hess and Bencze (1968) have reported a marked reduction in serum cholesterol and triglycerides of male rats receiving the new tetralin phenoxy isobutyrate (CIBA, Su-13437 or TP1A). In humans a reduction in serum triglycerides and to a lesser extent cholesterol and phospholipids was observed on the administration of Su-13437 (Best and Duncan, 1969). The most extensive study was that of Hartman and Forster (1969), who
found that in 88 patients Su-13437 was effective in reducing the serum triglycerides and cholesterol levels of Types III, IV and V hyperlipidemia while the Type II patients responded to a lesser degree. Su-13437 was found more potent than clofibrate (Best and Duncan, 1970). It was effective and well tolerated compound with sustained hypolipidemic activeness. However, a decrease of fibrinolytic activity during the treatment was observed (Mannucci et al., 1971). In preliminary findings Berkowitz (1969) reported that SaH 42-348, 1-methyl-4-piperidyl bis (p-chlorophenoxy) acetate was effective in reducing serum triglyceride and serum cholesterol. Timms et al. (1969) who worked with rats also found that this clofibrate analogue was a more active hypolipidemic agent than the parent compound. Another important compound of this series CDJB (potassium 2-methyl-2-[p-chlorophenyl]-phenoxy-propionate) has been reported to be an effective hypolipidemic agent which does not cause liver enlargement in rats (Leigh et al., 1968).

Control of Lipogenesis - It has been known for many years, that dietary and hormonal conditions may exert pronounced effect on lipogenesis (Fritz, 1961; Masoro, 1962). Starvation, fat-feeding or diabetes, induced by the injection of alloxan, decrease hepatic fatty acid synthesis greatly (Bruggen et al., 1952; Medes et al., 1952; Fritz, 1961; Whitney and Roberts, 1955;
and Brice and Okay, 1956). These results were also confirmed with adipose tissue (Leveillé and Monson, 1966; and Leveillé, 1967a). Refeeding of starved animals restores fatty acid synthesis, if the diet is not high in fat, while lipogenesis is restored on giving insulin to the alloxan diabetic animals. In experiments with rat liver extracts it was found that fasting leads to a strong depression of acetyl CoA carboxylase and to smaller depression of fatty acid synthetase (Noma et al., 1961; Korchak and Masoro, 1962). Similar findings were also obtained with diabetic rats (Wieland et al., 1963) as well as with rats fed on a fatty diet (Borts et al., 1963).

On the other hand, the specific activities of both enzymes rise to very high levels in liver during high-carbohydrate, fat-free refeeding of starved rats (Gibson et al., 1966).

A marked increase of long chain acyl CoA under all conditions of depressed fatty acid synthesis was evident (Borts and Lynen, 1963; Tubbs and Garland, 1964 and Wieland et al., 1965). A large number of enzymes have been shown to be inhibited by palmitoyl CoA (Ager-Nielsen et al., 1965; Taketa and Pogell, 1966).

The other regulating factor of acetyl CoA carboxylase is its activation by citrate. Brady and Gurin (1952) first
observed that homogenate systems which incorporate acetate into long chain fatty acids required citrate for optimum activity. Vajelos et al. (1963) clearly demonstrated that citrate as well as other Krebs cycle intermediates (Gregolin et al., 1968) had a striking effect on the degree of aggregation of acetyl-CoA carboxylase. There is now evidence that the inhibitory action of acyl-CoA's is related to disaggregation of active carboxylase trimer structure to the inactive monomers in the presence of acyl-CoA (Gregolin et al., 1966a,b,; Numata et al., 1966; and Lenginger, 1969). It is considered now that acyl-CoA is a negative effector of acetyl-CoA carboxylase while Krebs cycle intermediates are positive modulators of this enzyme.

Citrate is of course, primarily synthesized by citrate synthetase from acetyl-CoA and oxaloacetic acid. This enzyme is inhibited by long chain acyl-CoA thioesters (Tubbs, 1963; Wieland and Weiss, 1963; Wieland et al., 1964; and Lynen et al., 1963). Moreover, citrate cleavage enzyme primarily responsible for the breakdown of citrate to acetyl-CoA and OAA, was found in low concentrations in starved rats and at high concentrations in fed rats (Ball, 1966). Recent work with a chloroplast fatty acid synthetase strongly suggests that, at least in plants,
a rate-limiting step in the synthetase system may be at ACP level. But no evidence is available concerning the role of acyl-ACP in regulating acetyl CoA carboxylase activity. There is also evidence that palmitoyl CoA is an obligatory intermediate in the transfer of palmitoyl groups from ACP to glycerol (Kuhn and Lynen, 1965). A quite different possibility, in view of the participation of ACP derivatives in fatty acid biosynthesis and of CoA esters in fatty acid oxidation, is that an increase in the level of acyl CoA esters may signal an increase in fat breakdown. At a time when the actions of hormonal and regulatory systems are causing the degradation of fats, it is appropriate that fat synthesis should be blocked. Conceivably this is accomplished in part through inhibition of first step in lipid synthesis by long chain acyl CoA esters, which are early intermediates in fat breakdown. It may well be not only ACP but also systems which remove long chain acyl CoA thioesters such as triglyceride synthesizing systems would be additional partners responsible for the control of lipid synthesis in the cell.

Lipid synthesis is also increased above normal when the animals are on a high carbohydrate diet, the increase being especially large if the starved animals are refed diet
high in carbohydrate and low in fat (Kornacker and Lowenstein, 1965). Those changes which occur relatively slow are associated with alterations in tissue levels of lipogenic enzymes. Such adaptive phenomena are probably to be distinguished from short term effects of change of diet in which rate control of lipogenesis may be mediated through the action of modifiers on acetyl coA carboxylase (Lowenstein, 1968). Furthermore, the ratio of NADPH/NADP also plays an important role in the control of lipogenesis. Hence a variety of enzymatic systems and dietary conditions are responsible for the control of lipid synthesis.

The activity of both the lipogenic and glycolytic enzymes are important determinants of rate of hepatic fatty acid synthesis (Zakim et al., 1967; Ballard and Hanson, 1967; and Hill et al., 1958). The activities of a number of hepatic enzymes are effected markedly by changes in the carbohydrate content of diet (Tepperman and Tepperman, 1965; Hill et al., 1960). Fasting, high fat diets and high carbohydrate diet lead to parallel changes in the activity of various hepatic enzymes including glucose-6-phosphate dehydrogenase, NADP-malic enzyme and citrate cleavage enzyme (Ball, 1966; Fitch and Chaikoff, 1960). Baldwin et al. (1966)
showed that activities of various enzymes involved with Embden-Meyerhof pathway, EMP shunt and fat metabolism were decreased by giving high fat diets. Leveille (1967a,b) showed the activities of glucose-6-phosphate dehydrogenase, malic enzyme, and 6-phosphogluconate dehydrogenase in liver as well as in adipose tissue were decreased by giving corn oil diet to rats. He also observed decrease in malic enzyme activity in adipose tissue of animals fed high protein diet which was low in carbohydrate along with lipogenesis. The various hepatic enzymes also showed a pronounced adaptive behaviour toward hormonal influences (Olsen, 1966; Strere, 1965; Ballard and Hanson, 1967; Kornacker and Lowenstein, 1965; and Atkinson, 1969).

It is thought that the depression of enzyme activities during starvation is due to the repression of enzyme synthesis. Thus the rise in enzyme activities in animals under varying dietary conditions, viz., high carbohydrate, fat free refeeding, could be explained as adaptive synthesis of new enzyme. Evidence supporting this view has emerged from studies of Gibson et al. (1966). As they found, specific inhibitors of protein synthesis, such as puromycin and actinomycin D, injected into rats at the beginning of or
during the refeeding period block the anticipated rise in the activity of acetyl CoA carboxylase, fatty acid synthetase and citrate cleavage enzyme.

The assumption that the changes in enzyme concentration are due to repression or induction of enzyme synthesis is further supported by experiments of Gellhorn and Benjamin (1966) with adipose tissue, who found that the rates of fatty acid production and their desaturation are reflected in the rates of RNA synthesis. That there is a functional relationship between these events is strongly suggested by the fact that the restoration of RNA synthesis with refeeding precedes the repair of the failure in fatty acid synthesis.

Scope of this thesis - Numerous physiological and non-physiological lipid-lowering agents have been investigated but due to side effects their fate in the successful chemotherapy of atherosclerosis and allied diseases is still debated. It is believed that any inhibitor of cholesterol biosynthesis acting prior to HMG-CoA reductase (E.C.1.1.1.34) step, which is considered to be the main regulatory point in hepatic cholesterologenesis (Siperstein and Fagan, 1966; Shapiro and Rodwell, 1971) cannot be successfully used as hypocholesterolemic drug because it
would also effect other important metabolic reactions of the body. 3-hydroxy-3-methylglutariacid an anti-metabolite of MVA for bacterial system (Wright, 1957) is formed in vivo by deacylation of HM3-COA (Dekker et al., 1958) and has been shown to inhibit bacterial HM3-COA reductase (Fimognari and Rodwell, 1965). Its possible inhibitory effect on hepatic HM3-COA reductase is evident from observations that HM3 decreases the conversion of acetate to mevalonate (Fimognari, 1964) and inhibits the in vivo and in vitro incorporation of acetate but not mevalonate into cholesterol (Beg and Lupien, 1972). Earlier studies have indicated that HM3 exerts a potent hypolipidemic action at relatively low doses and is well tolerated in rats (Beg and Siddiqi, 1967, 1968). Histopathological studies also showed that HM3 feeding to hypercholesterolemic rats did not bring any adverse effect at the cellular organization of the tissue (Beg et al., 1968). Recently Lupien et al. (1973) reported that HM3 has a protective action on rabbits with experimental atherosclerosis.

The present work is directed to elucidate the hypolipidemic action of HM3 in normal and hypercholes-terolemic rabbits. The effect of prolonged administration
of this compound on serum, liver and aortic lipids has been investigated in animals fed an atherogenic diet for 54 days. The in vitro and in vivo effect of HMG on hepatic enzymes and its role in the regulation of fatty acid synthesis has also been studied to understand the effect of HMG on various metabolic systems.

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