III. INTRODUCTION
A white crystalline substance discovered in human gallstones, about 200 years ago, was named cholesterine by Chevreul (1816) who showed that it was nonsaponifiable in contrast to other animal waxes. When Berthelot (1859) demonstrated the presence of an alcohol group in the molecule, the more descriptive name cholesterol became generally acceptable.

Although the empirical formula of \( \text{C}_{27}\text{H}_{46}\text{O} \) was proved, in 1888, on the basis of an analysis of cholesterol acetate dibromide (Reinitzer, 1888), it was not before 1932 that the structural formula of cholesterol was first established.

Cholesterol is one of the most widely distributed compounds in the animal kingdom (Doree, 1909). It is always present in vertebrates, often in invertebrates and recently has been discovered in algae. It occurs normally free or combined in tissues and secretions,
notably liver, brain, skin, adrenals, bile, blood and adipose deposits. It is found pathologically in biliary calculi, sebaceous cysts and atheromatus blood vessel walls.

Cholesterol forms the chief component of the non-saponifiable fraction of blood. It is present in the blood both in the form of the free alcohol and in the form of ester. About 75% of the plasma cholesterol is normally esterified in the case of man. In the plasma, cholesterol is ordinarily combined with the unsaturated fatty acids. A large proportion of the cholesterol in human plasma is likewise in combination with protein. Gofman et al. (1949, 1950) separated lipoproteins containing cholesterol from rabbit and human sera by ultracentrifugation. It is believed that the protein combinations with cholesterol represent the forms in which cholesterol can exert its atherogenic action (Gofman et al., 1950). When an excess of dietary cholesterol is fed, it results in hypercholesterolemia (Peters and Van Slyke, 1946) as well as fatty livers in rats (Best and Ridout, 1933; Best et al., 1934; Cook, 1936; Swell and Flick, 1953). Rabbits maintained on a diet containing cholesterol and bile salts not only develop hypercholesterolemia (Swell and Flick, 1953) but
also atherosclerosis (Turner, 1933; Turner and Bidwell, 1935). In the case of guinea pigs, hypercholesterolemia together with fatty infiltration in liver is developed when sterol is given (Cook, 1936; Okey, 1942). Wissler et al., (1953) reported a commercial diet which produced lipomatus lesions in the coronary arteries of rats, if fed for a year. Knudson (1921) reported an increase in free cholesterol in both the plasma and the corpuscles, when either free or esterified cholesterol is fed to dogs. However, ester cholesterol remains unaffected. There is considerable evidence in literature that various lipid components other than cholesterol in the blood may be altered by the ingestion of cholesterol diet containing fat. Page and Bernhard (1935) showed that when cholesterol in olive oil was fed to rabbits, the plasma phospholipids were increased. This was further confirmed by Weinhouse and Hirsch (1940a) who noted that the neutral fat and cholesterol fractions of the blood were increased, when fat added cholesterol diets were fed to rabbits. Vermenlen and coworkers (1942) also reported similar type of rise in non-cholesterol fractions of serum lipids of rabbits fed cholesterol dissolved in sunflower-seed oil. Dubach and
Hill (1946) noticed an increase in all blood lipids, when rabbits were given either lanolin or cholesterol.

In arteriosclerosis, where blood cholesterol together with other lipids is significantly increased, there is loss in the elasticity of arteries, associated with thickening and hardening of their walls. This condition is usually a manifestation of hypertension.

The type of arteriosclerosis produced experimentally in animals is referred as atherosclerosis, where only the intima is involved. The atheromatous lesions (or 'patches') present in this layer contain small droplets of lipid material. The atherosclerotic lesion is a complicated affair. When fully developed, it is comprised of a considerable variety of structures and substances: blood and blood products, fibrous scar tissue, calcium deposits, complex carbohydrates, cholesterol, fatty acids and lipoproteins. Apparently the fatty acids and cholesterol are the crucial substances responsible for the development of lesion, because they provoke inflammation and scarring of the arterial wall. Cholesterol is indeed an inevitable suspect, because the formation of the atherosclerotic lesion is essentially an inflammatory response to this
substance. The involvement of cholesterol in the disease has been demonstrated in many different ways. Experiments have been performed to produce the disease by cholesterol feeding in many animals, including rabbits, rats, guinea pigs, chickens, dogs and monkeys. In almost every case in which the disease is induced experimentally the animal's serum shows a rise in cholesterol as a prelude to the atherosclerosis. In primates the disease exhibits all the features that occur in human beings. It has also been shown that patients suffering from peptic ulcer treated with a diet rich in milk and cream had elevated levels of cholesterol in their serum and suffered twice as high a rate of heart attacks from coronary atherosclerosis as ulcer patients who did not use this diet. Conversely, patients with multiple myeloma, a malignant disease that tends to lower the serum cholesterol level as one of its effects, have an unusually low rate of heart attacks. People dying of so-called wasting diseases (essentially malnutrition) show a low lipid content in their arteries, which suggests that the loss of fat may reduce their atherosclerotic lesions. On the other hand people suffering from diseases or conditions, that are usually accompanied by a high cholesterol, such as, diabetes, nephrosis,
hereditary elevation of lipids in the body, tend to develop atherosclerosis at an earlier age and more extensively, than usual. There is ample clinical evidence that diabetes mellitus is a condition characterized by an excessive incidence of atherosclerosis. However, evaluation of the role of diabetes in atherogenesis is difficult since hypertension is commonly associated with the dysfunction of lipid metabolism as in the diabetic state.

Contrary to expectations, alloxan-induced diabetes was found to reduce atheromatosis in cholesterolized rabbits (Duff and McMillan, 1949; McGill and Holman, 1949). Although cholesterol-induced lipemia was rather enhanced by alloxan, the protective effect on atherogenesis has been attributed to the diabetic state and not to alloxan as such (Cook et al., 1954; Duff et al., 1954). The apparent immunity of alloxanized rabbits to the atherogenic stimulus of high serum cholesterol level has been explained by Pierce (1952). An important contribution to this problem has been made by Fisher et al. (1961), who reaffirmed that alloxan-diabetes inhibits atherogenesis in cholesterolized rabbits and observed that renal hypertension in conjunction with diabetes and cholesterol feeding
leads to more severe aortic atherosclerosis than that in conjunction with cholesterol feeding alone. This effect of hypertension in diabetic animals was not accompanied by any aggravation of lipemia. In studies on alloxanized rats, fed a diet containing high amount of fat, drastic hyperlipemia was observed in the diabetic animal than in the controls (Kalant and Harland, 1961). However, the degree of lipid deposition in the vessels was not significantly altered by the diabetic state.

Morrison and Johnson (1950) observed that the average cholesterol content of the coronary arteries of patients who had died of acute coronary thrombosis was four times the average for a group of control patients. Numerous workers have shown that cholesterol deposition or its accumulation in these atheromatous patches takes place by preference (Schonheimer, 1924a, 1924b; Rosenthal, 1934; Leary, 1941; McArthur, 1942). The amount of cholesterol deposited in the aorta of presumably normal person has been shown to increase with advancing age (Burger, 1928; Rosenthal, 1934). In fact, Bragdon (1952) showed that spontaneous atherosclerosis may be present in aging rabbits. Because of the predominance of cholesterol in
the atheromatous masses, it is believed that deposition of cholesterol in the arterial wall is probably attributable to hypercholesterolemia. Bjorksten (1952) demonstrated that the intima of fresh hog aortas can be made to adsorb and retain cholesterol from a suspension provided that the intima was first treated with certain cross-linking agents, of which lead acetate is most efficacious. On the basis of these results, it has been hypothesized that the primary cause of cholesterol deposition and the subsequent pathologic complications of the arteries may be a chemical process affecting the protein content on the lining of the arteries. Injury may also cause the formation of atheromas (Schlichter et al., 1949). Duff (1935) demonstrated that injury to the intima precedes the deposition of cholesterol in the blood vessels. In this case the primary cause may not be hypercholesterolemia or disturbance in cholesterol metabolism, but a chemical disturbance in the tissue which favours the deposition in a particular area. This disturbance may be exaggerated by high levels of plasma cholesterol and thus acts as a contributory factor of atherosclerosis. Cholesterol deposition in arterial walls was demonstrated in vitro.
by Wilens (1951) who showed that a visible lipid retention occurred if normal human blood serum was filtered through the walls at normal arterial pressures for 24 hours or longer. It was estimated that 2-38% of the cholesterol was retained intramurally. Therefore, it was suggested that atherosclerosis results from filtration of serum through the walls of the artery. Since the major portion of cholesterol fails to enter the arterial intima due to its combination with large protein molecules, it is postulated that only free cholesterol can pass into the arterial walls. Peters and Van Slyke (1946) postulated that presence of cholesterol in atheromatous changes does not prove that hypercholesterolemia or any disturbance in cholesterol metabolism is the only cause of the deposition. Furthermore Chernick et al. (1949) as well as Werthessen et al. (1954) have shown that cholesterol can be synthesized within the arterial wall.

Anitschkow (1925) failed to produce atherosclerosis in cats or dogs by cholesterol feeding. Steiner et al. (1949) obtained positive results when thiouracil is also given. These results would indicate that atherogenesis
may occur more readily when hypofunction of the thyroid gland is there. This result was consistent with the report of Katz and coworkers (1953) that thyroid hormone is able to suppress both hypercholesterolemia and atherogenesis. Hormones other than those associated with thyroid are also active in relation to atherogenesis e.g., 17-hydroxycorticosterone (compound F) intensifies hypercholesterolemia and hyperlipemia in cholesterol-fed chicks, ACTH in large doses duplicates these effects. Estrogens when given with cholesterol diet completely inhibits the atherosclerotic developments. Moreover the pre-established lesions were reversed in chicks under estrogen treatment. Estrogen also causes reduction in total cholesterol/lipid P ratio (Katz et al., 1953).

Pyridoxine (B₆) is also associated with the development of atherosclerosis. Thus in monkeys, Rinehart and Greenberg (1949, 1951) succeeded in producing atherosclerosis by means of a pyridoxine deficient diet, while prolonged feeding with cholesterol failed to produce significant lesions. Later studies (Greenberg and Rinehart, 1951) revealed that a greater degree of cholesterolemia was present in the pyridoxine deficient monkeys
kept on cholesterol than the normal animals receiving larger quantities of cholesterol.

Gould et al., (1951) believed that an equilibrium exists between plasma cholesterol and liver cholesterol. Marx et al. (1951) failed to show any such relationship between the level of liver and plasma cholesterol and their relative susceptibility to atherosclerosis. However, Siperstein and Fagan (1964) have demonstrated maximum increase in serum and liver cholesterol levels of various species when fed cholesterol along with bile salts. The highest levels of plasma cholesterol were found in chickens and rabbits (both are susceptible to atherosclerosis), whereas highest average value have been reported in the livers of hamsters and rats, which are resistant to atherosclerosis (Goldman, 1950; Altschul, 1950; Marx et al., 1951). This may be due to the fact that hamsters and rats have the ability to store the cholesterol more efficiently in the liver than chickens or rabbits.

Although a hypercholesterolemia following cholesterol feeding is associated with the production of an atherosclerosis in rabbits, there are conflicting views regarding the arteriosclerosis prevalent in man. Leary (1934) reported that on autopsy the lesions, obtained
from the blood vessels of rabbits having an experimentally produced atherosclerosis and those obtained from the coronary arteries of arteriosclerotic patients, were similar. Peters and Van Slyke (1946) pointed out that experimental atherosclerosis of rabbits differs from the diseased condition in man by the speed of development and also unusual susceptibility of the former to iodine and to the activity of the thyroid gland.

Similar disagreements, as whether hypercholesterolemia is an invariable concomitant of hypertension in man or hypertension follows the manifestations of hypercholesterolemia, has always been a subject matter of discussion. Several workers have reported that cholesterol, as well as, all blood lipids are increased in hypertension (Fahrig and Wacker, 1932; Wacker and Fahrig, 1932; Harris, 1949). Alford (1949) found that coronary heart disease is associated with hereditary hyperlipemia. Gertler and Garn (1950) observed that serum cholesterol was higher in males having experienced myocardial infarction. In the coronary diseased groups, both cholesterol and phospholipids were increased in the serum, but the cholesterol: Phospholipid (C/P) ratio was likewise increased, indicating that the rise in phospholipid had not kept pace with
that of cholesterol. It is suggested that the factors favoring the deposition of cholesterol in the intima are enhanced because of the lack of sufficient phospholipid to act as a colloid stablizer. It is important to report that hypercholesterolemia exists in a variety of diseases other than hypertension and in certain cases normal cholesterol level may occur in hypertension provided diseases like nephritis is absent (Harris and Lipkin, 1930; Alvarez and Neuschlosz, 1931; Page et al., 1936; Gofman, et al., 1951; Duff and Meissner, 1951). Therefore the occurrence of hypertension is not supposed to be an adequate proof for the existence of atherosclerosis (Peters and Van Slyke, 1946).

It is now well recognized that a number of "risk factors" may be involved in the development of atherosclerotic heart disease. These factors include hypercholesterolemia (serum cholesterol 260 mg/100 ml or above), hypertension (diastolic blood pressure of 95 mm or above), marked obesity, heavy cigarette smoking, diabetes mellitus, and a family history of vascular disease (Kannel et al., 1961; Stamler et al., 1962, 1963; Doyle et al., 1962). Recently there has been growing suspicion that dietary
carbohydrates, by virtue of an influence on the serum triglycerides and phospholipids, may play a role in the development of atherosclerotic disease (Albrink, 1965; Kuo and Bassett, 1965; Ostrander et al., 1965). A correlation between elevated serum triglyceride and cholesterol levels and coronary artery disease has been shown (Albrink and Man, 1958, 1959; Carlson, 1960; Albrink et al., 1961; Havel and Carlson, 1962; Albrink, 1962, 1963; Feldman and Wallace, 1964). Experiments have been performed to demonstrate an increasing C/P ratio in patients suffering from conditions known to be predisposing to atherosclerosis (Ahrens and Hunkel, 1949). The cholesterol fed rats (Berger et al., 1963), chickens (Chaikoff et al., 1948; Orma, 1957), rabbits (Dury, 1957; Wang et al., 1954) and cholesterol-fed thiouracil treated dogs (Davidson et al., 1949) have been shown to possess higher C/P ratios than comparable controls. However, the etiologic significance of serum triglycerides and phospholipids awaits further clarification. In fact, the serum total cholesterol level appears to remain the best single predictor of risk in coronary heart disease (Kannel et al., 1964), prior to the development of myocardial infarction. The survival rate of patients following myocardial infarction,
however, was not influenced by the plasma concentrations of cholesterol (Little et al., 1965). The proportionality between plasma lipids and lipids of atheromata (Schonheimer, 1926; Weinhouse and Hirsch, 1940b)Page, 1941; Mead and Gouze, 1961) strongly suggests that the source of most of the lipids of atheromatous plaques is the serum.

From the foregoing discussion, it is clear that atherosclerosis can not be explained simply in terms of cholesterol. However, from the data so far available one can definitely conclude that involvement of the cholesterol in this disease is of utmost importance. In conjunction with various lipids it may be called as "VILLAIN" in the diseases associated with hypercholesterolemia and hyperlipemia.

Control of Cholesterol Biosynthesis

In normal humans, as well as in other species, the blood cholesterol level remains constant within narrow limits, although this can be raised by addition of large amounts of cholesterol in diet. This relative constancy appears to be maintained by a negative feed-back control, It has been known since the experiments of Gould (1951),
Tofflkins et al. (1953), Langdon and Bloch (1953) and Frantz et al. (1954) that cholesterol feeding to rats causes a marked depression in the rate of hepatic synthesis of cholesterol by such animals. The elucidation of the detail of homeostasis is of obvious importance, both from the biochemical and as well as, perhaps, from clinical standpoint of view. It is now possible to define most of the reactions of cholesterol synthesis from acetate. A simplified sequence of the biosynthesis is given in Fig. 1., more detail information may be found in a number of recent reviews (Kritchevsky, 1958; Gould, 1958; Popjak and Cornforth, 1960; Tchen, 1960; Porter, 1961; Clayton, 1965).

Using rat liver preparations, Gould and Swyryd (1966) were able to demonstrate three sites of inhibition of cholesterol synthesis which is depicted in Fig. 2., as $S_1$, $S_2$ and $S_3$. The maximum capacities of normal rat liver homogenates and slices to synthesize cholesterol from mevalonic acid (MVA) were shown to be far greater than from acetate. Consequently the sites of inhibition after mevalonate ($S_2$ and $S_3$) probably do not have a significant effect on the overall rate of cholesterol synthesis in the intact cholesterol-fed animals. It has
Fig. 1. Scheme showing some of the metabolic functions of acetate and its conversion to cholesterol.
Fig. 2. *Sites of inhibition of Cholesterol biosynthesis produced by cholesterol feeding.*
also been shown that cholesterol feeding results in a marked inhibition of cholesterol synthesis in man (Bhattathiry and Siperstein, 1963) which leaves no question that humans also possess a feed-back system at least as sensitive as that of lower animals. The major site of cholesterol feed-back regulation has now been definitively shown to be the hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase (EC 1.1.1.34) (Siperstein and Fagan, 1964). From the works of Bucher et al. (1960) and Linn (1967) on the assay of HMG-CoA reductase activity in livers from fasted, triton-treated rats and cholesterol-fed rats, it is further confirmed that this enzyme is concerned in the overall regulation of cholesterol biosynthesis. A series of experiments mainly due to Behr et al. (1961) show the operation of a double feed-back mechanism in regulation of cholesterol biosynthesis. The main catabolic pathway of cholesterol, the conversion to bile acids, seems to be controlled by a feed-back similar to that of cholesterol synthesis. As shown in Fig. 3., the two feed-back controls are considered to be complementary (Gould, 1959).
Fig. 3.  Double feed-back inhibition of Cholesterol biosynthesis.
Acetate $\rightarrow$ Cholesterol $\rightarrow$ Bile acids $\rightarrow$ Fecal sterols
Inhibitors of Cholesterol Biosynthesis

In the last two decades concerted attempts have been made by Biochemists, Pathologists, Clinical nutritionists and Internists to investigate the role of various drugs in combating hypercholesterolemia and atherosclerosis. Since hypercholesterolemia and hyperlipemia are prelude to atherosclerosis, drugs or agents capable of correction of these two abnormalities without any evidence of advanced atherosclerosis would be of considerable help. The effect of various hypocholesterolemic and hypolipemic agents, in regressing the pre-established atherosclerotic lesions, would also be of great benefit in the treatment of advanced arteriosclerosis.

Taking blood lipid levels as the main measurable parameter of lipid turnover in the body it has been established that many types of hypercholesterolemia are the result of a prior elevation in plasma triglycerides and phospholipids (Friedman et al., 1962). It is well known, however, that effective inhibitors of cholesterol biosynthesis will lower not only cholesterol levels but also the levels of lipoprotein as a whole. Of the three main possible ways of lowering body cholesterol, interference
with rate limiting step in biosynthesis (HMG-CoA to MVA) seems the most promising one (Gould, 1960; Migicovsky, 1962). The other two possibilities, i.e., stimulation of cholesterol excretion and degradation to bile acids, inhibition of dietary cholesterol absorption or reabsorption of biliary cholesterol appear less feasible. Any change in cholesterol absorption or degradation, at least initially, is compensated for by the liver which is the main source of plasma cholesterol (Friedman et al., 1951). In the following pages some important inhibitors of cholesterol biosynthesis have been discussed. These compounds are either in use as hypocholesterolemic drug or have therapeutic potentiality in the treatment of hypercholesterolemia and hyperlipemia.

A. Inhibitors Acting Between Acetate And Mevalonate

(i) \( \alpha \)-Phenylbutyrate and Related Compounds - In 1953 Redel and Cottet (1953) reported that \( \alpha \)-phenylbutyrate had a hypocholesterolemic effect in normal rats, which was later on confirmed by Bargeton et al. (1954). Cottet et al. (1954) found that this compound was also effective
in hypercholesterolemia of man. It appeared likely, from the work of Steinberg and Fredrickson that α-phenylbutyrate inhibits the first step in cholesterol biosynthesis, i.e., acetate activation. The other derivative of this drug, β-benzalbutyric acid and α-methyl-γ-phenylbutyric acid were shown to inhibit cholesterol biosynthesis (Canonica et al., 1961). The site of action of all these phenyl derivatives seem to be CoA dependent reactions prior to HMG-CoA formation. (Steinberg and Fredrickson, 1955, 1956; Masters and Steinberg, 1958). However, this inhibition may not be of any significance in relation to cholesterol biosynthesis as acetyl-CoA may also be involved in other biosynthetic reactions.

Gallo et al. (1963) found that 2,3-dimethyl-5-phenyl-2-trans-4-trans pentadienoic acid inhibits cholesterol synthesis, presumably at HMG-CoA or MVA level. It was also shown to cause transient lowering of plasma cholesterol levels in rats. Shapiro et al. (1962) observed the hypocholesterolemic effects of α-indan-4-oxybutyric acid.
(ii) **Nicotinic Acid and its Derivatives** — In 1955, Altschul observed that administration of large doses of nicotinic acid protected cholesterol-fed rabbits from the development of hypercholesterolemia and lipid deposition in the aorta (Altschul et al., 1955; Altschul and Hoffer, 1955). He further observed that administration of large doses of this vitamin decreases serum cholesterol in hypercholesterolemic patients (Altschul and Hoffer, 1958). These results were confirmed and extended, in patients (Parsons et al., 1956; Miller et al., 1958; Kottke et al., 1962) and in variety of animals (Merrill and Lemley-Stone, 1957; Gaylor et al., 1960). Rats were found to be unresponsive to niacin (Friedman and Byers, 1959). Clinical studies have indicated that depression of serum phospholipids (Gurian and Adlersberg, 1959), triglycerides (Miller et al., 1960), and \( \beta \)-lipoproteins (Galbraith et al., 1959) may be associated with nicotinic acid administration. The mode of action of nicotinic acid has been subject of a recent review by Goldsmith (1962). Nicotinic acid may act prior to the formation of mevalonic acid (Gamble and Wright, 1961).

Recently, the clinical use of aluminum nicotinate has been advocated as a means of obtaining reduction in
serum cholesterol, without vascular and gastrointestinal side effects. (Tribiano and Spencer, 1962). On feeding Hexanicit, the hexaester of inositol and nicotinic acid to hyperlipemic patients, a decrease in blood lipid was observed (Schulze, 1962). Mesoinositol hexanicotinate caused 28% reduction in total serum cholesterol in normal rabbits. In vitro incorporation studies suggest that mesoinositol hexanicotinate inhibits the conversion of acetate to mevalonic acid (Nakamura, 1963). Blanco et al. (1963) showed that Complamin, a derivative of nicotinic acid decreases blood cholesterol as well as other blood lipids. Recently Wakasa et al. (1966) reported that dl-α-tocopheryl nicotinate causes decrease in total serum cholesterol levels with improvement of the subjective symptoms, when given to hypercholesterolemic patients. Injection of this compound to mice decreased the acetate-1-14C incorporation into hepatic cholesterol, but had no effect on mevalonate-2-14C incorporation, indicating inhibition between acetate and mevalonate.

(iii) Benzyl N-benzylcarbethoxyhydroxamate (W-398) - Berger and Coworkers (1963) have reported that W-398 will reduce serum and liver lipids, in cholesterol-fed
rabbits and hypercholesterolemic weanling rats. However, it significantly elevates liver cholesterol levels in normocholesterolemic rats with no effect on serum levels (Kritchevsky and Tepper, 1966). A marked weight loss was also observed. It is believed that W-398 limits the conversion of HMG-CoA to mevalonate (Douglas, 1964).

(iv) Coenzyme Q — The decreased incorporation of acetate into cholesterol, with no effect on mevalonate incorporation in coenzyme Q-fed animals, suggest that it blocks some biosynthetic step between acetate and mevalonate common to both coenzyme Q and cholesterol (Joshi et al., 1965).

(v) O-benzoylthiamine disulfide (BTDS) — Nakamura (1966) reported that injection of BTDS to mice decreased the incorporation of acetate-1-C\textsuperscript{14} into hepatic cholesterol with no effect on incorporation of mevalonic acid-2-C\textsuperscript{14}. In mice receiving a diet supplemented with 1% cholesterol, administration of 20 mg/Kg BTDS suppressed the increase in blood and hepatic cholesterol levels. These results suggest that hypocholesterolemic action of the compound may be due to inhibition of cholesterol biosynthesis between acetate and mevalonate.
B. Mevalonic Acid Analogs.

In an attempt to interfere specifically with the metabolism of mevalonic acid, the common precursors in the biosynthesis of sterol and other steroid compounds, several antimetabolites of MVA have been discovered. Of a number of structural analogs of MVA synthesized and tested by Stewart and Woolley (1959) in a Lactobacillus acidophilus system, 4-methylmevalonic acid was particularly active in inhibiting MVA dependent growth of the organism. However, this compound as well as some others failed to effect cholesterol synthesis in mice (Stewart and Woolley, 1961).

Mentzer et al. (1956) showed a 30% reduction in hepatic cholesterol levels of rat after administration of Δ^3^-3-methylpentenoic acid. Pertinent to the present discussion, Gey and associates (1957) found that Δ^3^-3-methylpentenoic acid and Δ^4^-3-methyl-3-hydroxypentenoic acid inhibited the incorporation of acetate into cholesterol. Weiss et al. (1961) found that 3-methyl-3-hydroxypentenoic acid, Δ^2^-3-methylpentenoic acid and Δ^3^-3-methylpentenoic acid, all inhibited the conversion of mevalonate to cholesterol by rat liver homogenates, the
hydroxy acid was most effective. The most potent MVA antagonist so far described appears to be 3-hydroxy-3-fluoromethyl velerolactone (3-fluromevalonic acid) (Singer et al., 1959; Tschesche and Machleidt, 1960).

Among fatty acids, acting as MVA antagonists (Wright, 1957), biphenylbutyric acid has been shown to inhibit cholesterol synthesis in vivo (Ranney, 1958). Garattini et al. (1961) claimed that dippenic acid (2-[4-biphenyl] - \( \Delta^4 \) -hexemic acid) influences experimental atherosclerosis as well as reduces serum lipid levels in man and dog. Klimov et al. (1967) showed that 2-phenyl-3-methyl-2-pentenoic acid and 2-phenyl-3-methyl-3-hydroxypentanoic acid respectively inhibited non-competitively and competitively incorporation of MVA into cholesterol. Although 2-phenyl-3-methyl-3,5-dihydroxypentanoic acid had little effect on MVA incorporation, it significantly suppressed cholesterol biosynthesis, presumably by inhibiting acetyl CoA formation.

C. Inhibitors Acting Between Mevalonate And Squalene

(1) Vanadium - Vanadium decreased the incorporation of acetate-1-C\(^{14}\) into cholesterol by rat and rabbit liver
in vitro as well as in vivo (Curran, 1954; Curran and Costello, 1956). Moreover, the levels of serum, liver and aortic cholesterol was lower in animals receiving Vanadium with a high cholesterol diet (Mountain et al., 1956). Vanadium was found anti-atherogenic in cockerels and chickens (Eades and Gallo, 1957; Loustalot et al., 1961). The locus of vanadium action has been assumed at the squalene synthetase level (Azarnoff et al., 1961). According to Holmes (1961) the MVA kinase reaction may represent the actual site of action. The available evidences strongly suggest that in humans the hypocholesterolemic properties of vanadium result primarily from its ability to interfere with cholesterol biosynthesis (Holmes, 1964).

(ii) Terpenes - In 1961 Pletscher et al. (1961) showed that 1-methylnerolidol and 2-dihydroneorolol are active in decreasing Lieberman - Burchard - positive sterols in the serum, but not in the liver, of normal rats. The inhibition of cholesterol biosynthesis, presumably at the mevalonate kinase level by compounds related to farnesoic acid has been extensively reviewed (Holmes, 1964).
(iii) **Benzmalecene** - This compound (N-[^1]-methyl-2,3-di-p-chlorophenyl-propyl maleamic acid) has been shown by Huff and Gilfillan (1960) to non-competitively inhibit MVA conversion to cholesterol in rat liver homogenates. On oral administration, it lowers plasma cholesterol in rats and dogs (Tennent et al., 1960). It is also effective in clinical hypercholesterolemia, but with some side effects (Bergen et al., 1960; Sachs et al., 1960). The site of inhibition of benzmalecene has been shown between isopentenyl pyrophosphate and farnesyl pyrophosphate (Holmes, 1961). According to Lack and Weiner (1963), it interferes with cholesterol metabolism by reducing the ATP levels necessary for phosphate transfer reactions. Although the compound has been shown to effectively reduce serum cholesterol levels of hypertensive patients, the undesirable side effects rule out its usefulness as a therapeutic agent.

(iv) **SKF 525-A, SKF 3301 and SKF 7732-A** - Many ester and ether derivatives of diethyl- or dimethylaminomethanol markedly inhibit the *in vitro* conversion of MVA to cholesterol and also effectively decrease serum cholesterol levels in mice, rats, dogs and monkeys (Dick et al., 1960; Greenberg et al., 1960). Three of the most active compounds
are \( \beta \)-diethylaminoethyl diphenylpropylacetate hydrochloride (SKF 525-A), 2,2-diphenyl-1(\( \beta \)-dimethylaminoethoxy) pentane hydrochloride (SKF 3301-A) and tris (2-dimethylaminoethyl) phosphate trihydrochloride (SKF 7732-A\(_2\)). It appears that these related compounds mainly affect the conversion of isopentenyl pyrophosphate to presqualene polyprenols (Holmes and Bentz, 1960; Holmes, 1964). SKF 525-A and SKF 3301-A were found to reverse fatty infiltration of liver (Rice and Greenberg, 1960).

(v) Sulfonyl Ureas – In rat liver homogenates, conversion of acetate-\( ^{14} \)C to cholesterol has been shown to be inhibited by tolbutamide, chloropropamide, metahexamide and phenethylbiguanide (McDonald and Dalidowicz, 1962). Apparently, the inhibition of sterol biosynthesis by such hypoglycemic agents occurs after the formation of MVA but before the squalene formation.

(vi) Squalenes – Pletscher et al. (1961) demonstrated that among unnatural derivatives of squalene, only 1,1-dimethylsqualene depresses liver cholesterol in rats.
(vii) **Hepatocatalase (Caperase)** - It has been reported that it causes a considerable reduction of circulating cholesterol in humans and experimental animals receiving the enzyme by intramuscular injection (Puig-Muset et al., 1960). Caravaca and associates (1963) showed that caperase inhibition site is between mevalonate and squalene. The caperase-induced lowering of blood cholesterol occurs without any significant effect on the triglycerides and phospholipids.

D. **Inhibitors Acting Between Lanosterol And Cholesterol**

(i) **Triparanol** - MER - 29, \(1-(\text{4-diethylaminoethoxy})_1\) \(\text{phenyl}-1-(\text{p-tolyl})_1-2-(\text{p-chlorophenyl})_1\) ethanol) produces a marked lowering of serum and liver cholesterol levels (Blohm et al., 1959). Blohm and Mackenzie (1959) showed that it inhibits cholesterol synthesis beyond squalene. Evidence is now accumulating that triparanol may inhibit \(\Delta^{24}\) side chain reduction at several points from lanosterol via zymosterol to desmosterol (Steinberg and Avigan, 1960). Although triparanol greatly reduces the level of available cholesterol, it causes accumulation of desmosterol which is definitely atherogenic (Blankenhorn,
Avigan and Steinberg, 1962; Herndon and Siperstein, 1963) in man as well as in experimental animals. Therefore this compound is no longer used in hypercholesterolemia therapy.

(ii) **Role of Diethylaminoalkyl Grouping** - A characteristic part of the triparanol molecule is the \(-\text{OCH}_2\text{CH}_2\text{N(C}_2\text{H}_5\}_2\) side chain. This radical is present in several compounds with a hypocholesterolemic activity, e.g., cyanostilbene derivative bearing the diethylaminoethoxy side chain (Hughes et al., 1962) and 1-dimethylaminoethyl-4-benzylpiperidine (IN 379) (O'Dell et al., 1962). The latter has been shown to be antiatherogenic in rabbits. An ether derivative of dimethylaminoethanol, (3\(\beta\)-dimethylaminoethoxy androst-5-en-17-one) has a significant hypocholesterolemic effect in rats (Gordon et al., 1961). This compound as well as its \(\beta\)-diethyl derivative (Phillips and Avigan, 1963) inhibit both desmosterol reductase and lanosterol reductase activities.

(iii) **Trans-1,4-bis(2-chlorobenzylamino)methylcyclohexane-di HCl (Ay - 9944)** - It inhibits hepatic cholesterol synthesis between lanosterol and cholesterol without
affecting the enzyme involved in the reduction of Δ²⁴-bond. Ay-9944 inhibits the enzymic transformation of 7-dehydrocholesterol to cholesterol in rat liver homogenates (Kraml et al., 1964a). The compound is active in lowering serum sterol levels in rats, pigs, dogs, pigeons, monkeys and rabbits (Chappel et al., 1964).

(iv) N,N'-dibenzylethlenediamine - The compound is effective in lowering serum sterols in hypercholesterolemic rats. The site of action is at the level of the conversion of 7-dehydrocholesterol to cholesterol (Kraml et al., 1964b).

E. Miscellaneous Drugs Affecting Cholesterol Synthesis

(1) 1-p-Chlorophenylpentylsuccinate (AF-425) - Palazzo and associates (1961) reported that AF-425 is a potent hypocholesterolemic agent for Triton-induced hypercholesterolemia in rats. It markedly inhibits the acetate and mevalonate incorporation into cholesterol. All the available evidences suggest that the compound might block cholesterol biosynthesis at more than one site.
(ii) \( \Delta^4 \) - Cholestenone - It has been shown that feeding of several steroids causes marked depression in the capacity of liver slices from different animals, to incorporate acetate-1-\(^{14}\)C into cholesterol (Tomkins et al., 1953; Steinberg and Fredrickson, 1956). Steinberg and Fredrickson showed that \( \Delta^4 \) -cholestenone inhibits the in vivo incorporation of acetate into cholesterol. Cholestenone also induced an appreciable lowering of the serum cholesterol in rats, dogs and chickens (Tomkins et al., 1957). The exact locus of action is not known. \( \Delta^4 \) -Cholestenone has no clinical significance because it causes accumulation of dihydrocholesterol, which has atherogenic properties (Nichols et al., 1955).

(iii) Androstene Derivatives - Large doses of methyl testosterone and 17 -methyltestosterone lower the serum cholesterol levels in dogs, swine and rats (Furman et al., 1957; Cox and Hale, 1960; Abell and Mosbach, 1962). Rubin and White (1959) showed that the rate of incorporation of acetate into cholesterol by liver slices of castrated animals receiving testosterone propionate was significantly lower. \( \Delta^4 \) - androstene-17\( \alpha \)-ol-3-one-17\( \beta \)-Oic acid and \( \Delta^1 \) -testolactone were also effective in
inhibiting the synthesis of cholesterol from acetate and blocked the conversion of MVA to cholesterol (Singer et al., 1959). However, the site of action of these compounds is not yet clear. 4-chloro-17α-methyl-19-nortestosterone (SKF 6612) lowered the rat serum cholesterol level (Holmes, 1964). SKF 6612 was found effective in inhibiting the in vitro conversion of acetate to MVA, MVA to squalene and its conversion to cholesterol. It has been suggested that this compound exerts its hypcholesterolemic effect in rats by interfering at one or more sites of cholesterol synthesis. However, definite mechanism is still obscure.

(iv) Psychotropic Drugs - Recently Yakubovskaya and Kiseleva (1961) reported that chlorpromazine decreased the liver cholesterol levels of pigeons and also inhibited the rate of cholesterol biosynthesis. It seems highly possible that chlorpromazine also inhibits the esterification of fatty acids with α-glycerophosphate.

(v) Ethyl Chlorophenoxyisobutyrate (CPIB) - In 1962 Thorp and Waring (1962) reported that CPIB administration to rats resulted in reduction of serum and liver
cholesterol levels. Oral administration of CPIB produces excellent sustained hypocholesterolemic and hypolipemic effects in man (Hellman et al., 1963). The ability of CPIB, to lower postprandial plasma optical density as well as triglycerides and lipoproteins (Strisower and Strisower, 1964), suggests an action of this drug on lipoprotein lipase. The mechanism is believed to involve either induction or increase of lipoprotein lipase or blockade of an inhibitor of that enzyme. Apparently the compound has an action on the blood clotting mechanism as well as on lipoprotein lipase. When CPIB and androsterone (Atromid) are administered simultaneously, prolongation of the thrombin time, decrease of platelet adhesiveness, and increased fibrinolytic activity was observed. CPIB has no influence on known components of the fibrinolytic system when, given alone (Sweet et al., 1965). The apparent synergistic action of Atromid on the blood clotting mechanism awaits clarification. The effect of Atromid on lipid metabolism and atherosclerosis is extensively discussed in a number of papers (Symposium on Atromid, 1963).
Scope of This Thesis

There is certain rationale to the fact that a compound active as an inhibitor of cholesterol biosynthesis may have utility in the treatment of hypercholesterolemia, atherosclerosis and other allied diseases. However, from the foregoing discussion on the inhibitors of cholesterol biosynthesis, it is evident that none of the inhibitors, so far known can be successfully used without any side effects. 3-Hydroxy-3-methylglutlaric acid (HMG), which in vivo arises from HMG-CoA by the action of HMG-CoA hydrolase (EC 3.1.2.5) (Dekker et al., 1958), is an antimetabolite of mevalonic acid (Wright, 1957). Recently it has been shown to competitively inhibit the HMG-CoA reductase (EC 1.1.1.34) activity in bacterial as well as rat liver preparations (Fimognari and Rodwell, 1965). This may also explain the HMG inhibition of cholesterol synthesis from acetate (Rabinowitz and Gurin, 1954). Although HMG is one of the inhibitors of cholesterol biosynthesis, no attempts have so far been made to evaluate its possible hypocholesterolemic and hypolipemic role.

The present work is directed to investigate the hypocholesterolemic and hypolipemic properties of HMG in
normal as well as in hypercholesterolemic animals. This is the topic of the chapter IV of this thesis. The effect of HMG at cellular level and its role in the reversal of fatty liver has been described in chapter V. A preliminary pharmacological screening of HMG is the subject matter for chapter VI. The effect of dietary cholesterol on hepatic HMG-CoA hydrolase has been studied and discussed in chapter VII.