IV. Discussion
Atherosclerotic cardiovascular disease is responsible for 50% of the death in the western world (Robert, 1988). In underdeveloped countries industrialization is also rapidly changing the lifestyle of individuals with the result that death due to coronary heart disease (CHD) is becoming more prevalent in such countries. It is very well known that cholesterol, accumulating in the wall of arteries, form bulky plaques that inhibit the flow of blood until a clot eventually forms, obstructing an artery and causing a heart attack or stroke. The association of hyperlipidemia with the development of atherosclerotic lesions has prompted widespread search for compounds which safely and effectively control the concentration of cholesterol and triglycerides in blood and hopefully in tissues with least or no toxic effects.

Recently Gressman et al (1988) have reviewed the clinical trials and testing of the safety and efficacy of a new class of lipid lowering drugs which are competitive inhibitors of HMG-CoA reductase. One of these inhibitors, lovastatin formerly called mevinolin has been approved as a hypocholesterolemic drug by the U.S. Food and Drug Administration (FDA). Considering the same rationale and the fact that HMG is a competitive inhibitor of HMG-CoA reductase both in vivo and in vitro (Fimognari and Rodwell, 1965; Beg and Lupien, 1972; Moorjani and Lupien, 1977), back in 1967,
the work from this laboratory for the first time indicated that HMG has hypocholesterolemic activity (Beg and Siddiqi, 1967). Although work from different laboratories has unequivocally established the hypolipidemic effect of HMG (Castaner and Paton, 1978) there is a need to reinvestigate the mode of hypolipidemic action of HMG in light of the work on lovastatin and other HMG-CoA reductase inhibitors (Cressman et al. 1988).

The data given in Table 1 show significant decrease of total lipids in plasma of normolipidemic (18.6%) as well as hyperlipidemic (20%) rats on HMG treatment. This decrease was also reflected in total cholesterol, phospholipids and triglycerides. The compound was also significantly effective in lowering plasma total cholesterol, phospholipids and triglycerides of diabetic rats. It is evident from this table that fat-rich cholesterol diet as well as alloxan treatment caused significant elevation of all lipid parameters in plasma. It appears that HMG has a more pronounced hypotriglyceridemic effect in hyperlipidemic rats but a more pronounced hypocholesterolemic effect in diabetic rats. The results are in agreement with the findings of Francesconi et al (1987) in normal as well as diabetic rats but differed in magnitude which could be due to sex difference in the animals. We used male rats whereas they used female rats. In diabetic rats the difference in magnitude of reduction
could also be attributed to the difference in experimental manipulations of producing diabetes. They used streptozotocin whereas we used alloxan. Even though we did not measure the concentration of plasma total lipids of diabetic rats, the reduction in lipid parameters confirms the hypolipidemic action of HMG in diabetic rats also.

Diabetic condition is known to be associated with hypertriglyceridemia (Bohr and Kraemer, 1988). Since HMG treatment significantly lowers triglyceride concentration in plasma of diabetic rats, it is safe to conclude that HMG appears to have a general hypolipidemic mode of action. The hypolipidemic action of a compound may involve its interference with lipoprotein synthesis or release or impaired lipid absorption. Since the defective lipoprotein metabolism or lipid transport could be a possible cause of hyperlipidemia or several kinds of hyperlipoproteinemias, the most logical mechanism of action of a hypolipidemic compound could be its interference with lipoprotein metabolism.

It is now well established that several modalities of hypolipidemic therapy, including treatment with bile acid binding resins, fibric acid derivatives and weight reductions, produce significant alterations not only in the concentrations but also in the composition, structure and metabolism of lipoproteins (Goldberg et al., 1987). It was interesting to note that in our experiments, HMG treatment
produced significant alterations in the plasma levels of lipoproteins and the concentrations of lipid components of almost all classes of lipoproteins. In all the experimental groups of rats, HMG treatment resulted in a fall of plasma levels of VLDL plus LDL accompanied by an increase of HDL in plasma (Table 2 and Table 3). VLDL and LDL are considered as atherogenic lipoproteins whereas high levels of HDL are protective against the development of atherosclerosis (Eisenberg, 1984).

Although total lipid content of VLDL in all the groups and of LDL in hyperlipidemic group was significantly lowered on HMG treatment but analysis of individual lipid components provided a better information about the effect of HMG on the lipoprotein lipids. Among HDL and its subfractions (HDL₂ and HDL₃), total lipid content of HDL₃ was significantly decreased only in normolipidemic rats (Table 4). A significant change in the protein content of all plasma lipoproteins was not found (Table 5) except that VLDL+LDL protein was decreased in hyperlipidemic (16.9%) and VLDL protein was lowered in diabetic animals (22.2%). However, HDL protein was increased in normolipidemic rats significantly (22%). In other groups also it showed a tendency to increase.

Further insight into the mechanism of plasma lipoprotein changes is provided by the analysis of lipoprotein lipids
and the key enzymes of lipoprotein metabolism. The changes caused by HMG in lipid parameters of a combined fraction of VLDL plus LDL (Table 6) are such that these lipoproteins show a tendency to decrease as is clearly evident from the decrease in the concentration of all lipid components in this lipoprotein fraction (Fig. 6).

A significant lowering effect of HMG on the concentration of triglycerides and cholesterol associated with VLDL+LDL, which are the determining factors of these lipoproteins, suggest that HMG exerts a stimulatory effect on the catabolism of these lipoproteins. In the earlier reports, on the basis of observation that HMG treatment in rats caused a decrease in blood lipids without any increase in the liver lipids, Yousufzai and Siddiqi, (1976b), ruled out the possibility of HMG inhibiting the release of VLDL and LDL from the liver. The observations were more clearly evident when effect of HMG on lipid parameters was seen in separate fractions of VLDL and LDL.

Table 8 and Table 11 show the effect of HMG on VLDL and LDL lipids respectively. Plasma triglyceride lowering activity of HMG is found to be associated with a concomitant decrease of triglycerides of both VLDL and LDL. Plasma cholesterol lowering activity also seems to be contributed by lowering of both LDL-cholesterol and VLDL-cholesterol. The observation that HMG lowers atherogenic lipoproteins in
plasma (Table 2 and Table 3) can be explained from these changes caused by HMG in the concentrations of VLDL- and LDL-lipids. Probucol also acts by lowering LDL-cholesterol but it causes more marked regression of xanthommas than would be expected from the extent of lowering of LDL-cholesterol. Probucol seems to act by increasing LDL removal by a receptor independent mechanism. Since all the lipid components of VLDL are decreased (Table 8, Fig. 7) it is likely that HMG either brings about the degradation of these lipoproteins resulting into lowering of their plasma levels or inhibits the synthesis of VLDL.

On the basis of the observation that HMG markedly enhances the activity of post-heparin plasma lipoprotein lipase (Table 22), it is most likely that the catabolism of VLDL is enhanced. Upon hydrolysis surface lipids are removed from VLDL and the remnants thus produced are taken up by receptor mediated process by the liver. Thus, enhanced hydrolysis of VLDL caused by HMG might result into less availability of LDL precursor i.e. VLDL thereby decreasing the concentrations of LDL lipids also (Table 11). Contrary to this, Arca et al. (1986) suggested that since HMG reduces hepatic cholesterol synthesis, this might inhibit the secretion of LDL precursor (VLDL) from the liver. Their data in FH and polygenic hypercholesterolemia support this hypothesis.

The effect of HMG seems to depend on the metabolic condi-
tions of animals. In induced hyperlipidemia, concentration of cholesterol in VLDL was maximally decreased (Table 8, Fig. 7). In contrast, phospholipids, triglycerides as well as total lipids of VLDL were significantly decreased in rats fed on basal diet, indicating an overall reduction in the total mass of this lipoprotein. In diabetic rats also, the concentrations of all the lipid components of plasma VLDL were lowered (Table 8, Fig. 7). Similarly the differences in the response to HMG was observed for LDL-lipids as shown in Fig. 8. Normolipidemic group showed an increase of LDL-phospholipids associated with the decrease of other lipids. The hyperlipidemic rats showed a lowering of total mass of this lipoprotein but diabetic rats did not show any change in LDL-triglycerides although both cholesterol and phospholipids were decreased (Table 11). Carlson, et al (1977) have reported that clofibrate caused an elevation of LDL-cholesterol in hypertriglyceridemic patients with LDL-cholesterol below 140 mg/dL but the reverse is true when LDL-cholesterol levels are over 140 mg/dL. As far as the percent composition of lipoproteins is concerned, the composition of LDL (Table 12) was altered more significantly than the composition of VLDL (Table 10) in normolipidemic and hyperlipidemic groups. However, phospholipid and triglyceride composition of VLDL was significantly changed in diabetic rats, without any significant change in LDL composition.
These observations do not give any indication about significant effect of HMG on the percent lipid composition of circulating lipoprotein particles. Enhanced hydrolysis of VLDL upon HMG treatment might have resulted in a substantial rise of LDL levels. Since we did not find any increase in LDL-cholesterol, which is an indicator of plasma LDL levels, it is likely that either LDL particles are increasingly removed from the circulation or there is a rapid uptake of VLDL remnants by the liver in response to HMG administration. This is in agreement with the rationale of using HMG-CoA reductase inhibitors to effectively lower plasma LDL-cholesterol levels. The inhibition of intracellular cholesterol biosynthesis would result into an increased expression of receptors on the cells surfaces, responsible for the uptake of both VLDL remnants and LDL (Fig. 3).

Diabetic condition is regarded as the model for hypertriglyceridemia as is also evident from elevated levels of plasma triglycerides in diabetic rats in comparison to normal rats (Table 1). Lipoproteins and lipids are frequently altered in diabetes. These lipoprotein alterations are of interest because of their possible role in the origin of accelerated atherosclerosis found in diabetes. It is well known that in diabetes there is an increased formation of acetyl CoA, a precursor for both cholesterol and acetoacetate synthesis.
Macrophages from insulin deficient mice have increased activity of HMG-CoA reductase and subsequently an increased rate of cellular cholesterol synthesis (Kraemer, 1986). More recently, a decreased secretion of lipoprotein lipase has been reported by insulin deficient macrophages (Behr and Kraemer, 1988). The changes in cholesterol metabolism appear to be secondary to a decrease in the number of receptors responsible for the uptake of VLDL (Kraemer, 1986). These receptors probably represent LDL receptors in that the uptake of both LDL and VLDL by macrophages is mediated by the LDL receptors (Koo et al., 1986; Ellsworth et al., 1987). In the presence of decreased LDL receptors, the low level of lipoprotein lipase secreted by macrophages would be expected to further hinder the uptake of triglyceride rich lipoproteins (Behr and Kraemer, 1988). As opposed to lipoprotein lipase secretion, in vivo insulin treatment normalizes the activity of HMG-CoA reductase, cellular cholesterol synthesis and LDL receptor expression.

High levels of triglycerides associated with high levels of atherogenic lipoproteins in plasma has consistently been reported in diabetes. Our results also confirm these changes in diabetic rats in comparison to normolipidemic rats (Table 1 and Table 2). Since VLDL are the main triglyceride carrying lipoproteins, effectiveness of HMG under diabetic condition
is of significance. HMG induced compositional changes of lipoproteins in normolipidemic rats appear to differ from those produced in rats of diabetic group.

A high cholesterol : triglyceride ratio in VLDL fraction has been reported in animals receiving diet rich in butter and cholesterol (Olivier et al., 1988). Our results also show an increased cholesterol : triglycerides ratio of VLDL in hyperlipidemic rats (fed with fat-rich cholesterol diet as described in methods) (Table 9). This ratio was not changed significantly upon HMG treatment in normolipidemic group. In contrast, hyperlipidemic and diabetic rats treated with HMG showed a low mean cholesterol : triglycerides ratio of VLDL indicating the selective depletion of cholesterol in this lipoprotein fraction in comparison to triglycerides. It may mean that HMG did not produce proportionate decrease of cholesterol and triglycerides in VLDL. The effectiveness of HMG on the composition of lipoproteins resembles to that of fenofibrate (Goldberg et al., 1987) and acipimox (Taskinen and Nikkila, 1988). In both the cases the decrease of VLDL lipids was associated with a rise of HDL-cholesterol. In our observations the magnitude of increase in HDL-cholesterol was more pronounced in hyperlipidemic and diabetic groups (Fig. 9). Thus VLDL and LDL lost cholesterol in response to HMG administration and HDL fraction became enriched in cholesterol and relatively more depleted in triglycerides.
(Table 13). The rise of HDL-cholesterol seems to be mainly due to a rise of HDL₂-cholesterol (Table 14, Fig. 10) in both hyperlipidemic and diabetic groups. Since cholesterol and phospholipids are the major components of HDL, their concentrations are indicative of the plasma levels of this lipoprotein. Although the concentration of phospholipids in HDL did not change significantly but it also exhibited an increasing trend indicating an increase of total HDL mass. Again this effect of HMG was more pronounced in HDL₂ subfraction (Table 14) which was highly significant in diabetic rats (p <0.001, 317.0%). The increase of both cholesterol and phospholipid concentration in HDL₂ suggests a rise in HDL₂ mass. In HDL₃ subfraction phospholipid concentration was decreased (Table 15, Fig. 11). These observations can be correlated with those reported in Table 2 and Table 3 and it may be suggested that the elevated plasma HDL levels, as indicated by turbidimetric analysis as well as agarose gel electrophoresis (Fig. 5), are mainly contributed by HDL₂ subfraction. Although we have not measured the level of HDL subfractions in plasma, the analysis of subfraction lipids supports the idea that action of HMG involves mainly HDL₂ subfraction. This is again in agreement with the increased ratio of HDL₂-cholesterol : HDL₃-cholesterol of treated rats (Table 16). The process of enhanced hydrolysis of VLDL, in response to HMG treatment and accompanied transfer of
surface components of VLDL to nascent HDL associated with the uptake of tissue cholesterol by HDL₃ to form HDL₂ (Fig. 2), is supported by the observation of increased lipoprotein lipase activity in HMG treated rats (Table 22). The possibility of inhibition of VLDL production and release by liver can again be ruled out since HMG does not inhibit the lipolysis as a result it may not reduce the flux of FFA to the liver and consequently VLDL production. In this regard HMG differs in its action of lowering VLDL lipids from acipimox (Taskinen and Nikkila, 1988) which lowers VLDL production by inhibiting FFA flux to the liver.

It is, therefore, logical to conclude that HMG resembles a new class of lipid lowering agents namely competitive inhibitors of HMG-CoA reductase. They exert their action by stimulating a compensatory increase in the expression of LDL receptors on cell surface (hepatocytes) of liver as shown in Fig. 3, which reclaim circulatory cholesterol (LDL-cholesterol) to meet cellular metabolic needs (Gordon and Rifkind, 1987). This process augments the removal of VLDL remnants (produced as a result of lipoprotein lipase activity on VLDL) and LDL from the circulation. In addition, production of LDL is reduced, since these cholesterol-rich lipoproteins are derived from VLDL remnants (Cressman et al., 1988). Hence the level of intracellular cholesterol synthesis is an important determinant of blood LDL-
cholesterol levels. In our investigation on the effect of HMG on LDL lipids (Table II) it is interesting that the percent reduction in total plasma cholesterol and LDL-cholesterol levels is quite similar and significant in normal as well as hyperlipidemic and diabetic rats in contrast to HDL-cholesterol and HDL₂-cholesterol which are significantly increased in hyperlipidemic and diabetic rats. Hence, it may be proposed that the augmented removal of VLDL remnants and LDL from the circulation, as a result of inhibition of intracellular cholesterol synthesis (Fig. 3) in presence of HMG, might in turn enhance the activity of lipoprotein lipase to maintain a critical intracellular cholesterol level. This proposed mechanism gets strong support from our observation that HMG treatment resulted in significant enhancement of the activity of lipoprotein lipase in post-heparin plasma of both normolipidemic and hyperlipidemic rats to the extent of 147% (p < 0.001) and 100% (p < 0.05), respectively (Table 22). These effects are similar to lovastatin and bile acid sequestrants. Both have been postulated to increase the activity of LDL receptors (Paoletti and Poli, 1987) and this common mechanism has been put forth to explain the reduction in levels of LDL-cholesterol in hypercholesterolemic patients during drug therapy. Clofibrate and bezafibrate also exert their effects on lipoproteins by increasing the catabolism of triglyceride
rich lipoproteins (Goldberg et al., 1979; Vessby et al., 1982). Similar to the action of fibrates (Sirtori and Franceschini, 1988), the increased lipoprotein lipase activity in HMG treated rats may also result from the elevation of the apoC-II content of VLDL which is the activator of lipoprotein lipase. An increase in apoC proteins of VLDL + LDL fraction is shown in Fig. 12. The rise in apoC content of VLDL in HMG treated rats seems to be due to the transfer of apoC-II and apoC-III from HDL. At the same time increased activity of hepatic lipase in post-heparin plasma seems to be a secondary effect produced as a result of increased lipoprotein lipase activity (Table 22). Since surface components of triglyceride rich lipoproteins, upon hydrolysis, are transferred to HDL₂ resulting into the production of less denser HDL₂ particles, the enhanced activity of lipoprotein lipase reflects, indirectly, the enhanced HDL₂ levels.

It has been demonstrated that lipoprotein lipase regulates firstly the catabolism of triglyceride rich particles and secondly, also the formation of HDL (HDL₂) (Patsch et al., 1978; Nikkila, 1984). Thus lipoprotein lipase activity is not only a regulator of triglyceride concentration in plasma but is also a determinant of HDL levels. In vivo lipoprotein lipase and hepatic lipase activity are regulated independently but act concomitantly (Nikkila, 1984) and, therefore,
the ratio of two lipase activities would be crucial. Triglyceride rich HDL₂ particles serve as a good substrate for hepatic lipase and are converted to HDL₃ whereas triglyceride poor HDL₂ is not degraded by hepatic lipase (Patsch et al., 1984). Hepatic lipase breaks down HDL₂ particles through its phospholipase activity and is thus the key enzyme in the removal of HDL₂ particles. It may be pointed out that average value of lipoprotein lipase activity was lower in hyperlipidemic than in the normolipidemic rats and HMG treatment resulted in the enhancement of the activity of both enzymes in post-heparin plasma (Table 22). A similar effect of HMG was observed on hepatic lipase measured in liver homogenates (Table 21). Myocardial lipase, which was measured in heart homogenate, differs from hepatic lipase in its metabolic function. This is localized on the capillary wall of the endothelial cells of rat heart (Thu et al., 1975) and has been reported to hydrolyze diglyceride monolayers as well as triglyceride monolayers (Chung and Scanu, 1977) and monoglyceride in miceller or albumin bound substrates (Thu et al., 1976). The physiological role of this lipase lies in the assimilation of triglyceride fatty acids into the tissue.

Our results indicate that the triglyceride concentration of HDL is maximally decreased (Table 13). This effect of HMG on HDL triglycerides was also clearly reflected in its
subfractions (*Table 14* and *Table 15*). Patsch *et al.* (1984) showed that the triglyceride content of HDL₂ was a crucial factor in hepatic lipase mediated conversion of HDL₂ to HDL₃. Increased triglyceride concentration in HDL has been reported in postprandial lipemia in normotriglyceridemics (Patsch *et al.*, 1984) and in hypertriglyceridemics with elevated triglyceride levels (Eisenberg *et al.*, 1984). Since triglyceride rich HDL₂ particles serve in vitro as a good substrate for hepatic lipase whereas triglyceride poor HDL₂ is not degraded by hepatic lipase (Patsch *et al.*, 1984), it is likely that HMG might enhance this process and hence, as a result, HMG treated rats showed presence of triglyceride poor HDL₂ in plasma (*Table 14*). Increased activity of hepatic lipase in treated rats (*Table 21* and *Table 22*) seems to be responsible for the clearing of triglyceride rich HDL₂ in these rats as compared to control rats showing significantly higher concentrations of triglycerides in their HDL₂ subfraction. Increased cholesterol and phospholipid concentrations of HDL and HDL₂ in HMG treated rats suggested increase in plasma concentration of HDL in comparison to saline treated rats. Same conclusion can be drawn from the results obtained after SDS-PAGE of apolipoproteins of HDL subfractions. Here again the apolipoproteins of HDL₂ and HDL₃ provided more clear information than total HDL, regarding the increase of HDL. The increase in total
mass of HDL can be explained by a visible increase of apoE and apoA-I of HDL₂ in both hyperlipidemic and diabetic rats (Fig. 14). This was also associated with an increase of apoA-IV and apoA-II in case of diabetic rats treated with HMG. However, the increase in HDL₂ was not reflected in apolipoproteins of normal rats. These results are consistent with the proposition that HDL rise is mainly reflected in its HDL₂ subfraction. Although apoE of HDL₃ was also increased in diabetic treated rats but apoA-I was considerably decreased (Fig. 15) which does not reflect a net increase in HDL₃. Although HMG and lovastatin belong to the class of HMG-CoA reductase inhibitors, their mode of hypolipidemic action appears to be different. Lovastatin action on lipoprotein profile does not seem to be mediated by lipoprotein lipase or hepatic lipase (Helve and Tikkanen, 1988) both of which are known to regulate HDL levels. Therefore, lovastatin-mediated lowering of LDL-cholesterol has been explained in terms of increase in the number of LDL receptors only (Goldstein and Brown, 1984b; Bilheimer et al, 1983). HMG significantly increases the activity of both lipases exhibiting an effect similar to that of bezafibrate (Gavish et al. 1986). Hence, it is tempting to speculate that mode of action of HMG is not only mediated through increase in LDL receptors, as suggested by Goldstein and Brown (1982) for the inhibitors of cholesterol biosynthesis,
but also increase in the activity of these lipases. Like bezafibrate the decrease of plasma triglycerides is most probably attributed to the increased lipoprotein lipase activity. It is noteworthy that an elevation of HDL-cholesterol was observed inspite of an increase in the activity of hepatic lipase. Several investigators suggested that higher hepatic lipase activity are associated with decreased HDL-cholesterol concentrations (Kuusi et al. 1980; Rao et al. 1982). It may be inferred that this mechanism either does not operate or is masked by activation of pathways that caused increased HDL-cholesterol concentration in plasma as discussed in previous pages.

The protective action of HDL against the development of CHD is believed to arise through the process of reverse cholesterol transport by which surplus cholesterol from the extrahepatic tissues, including artery walls and tendon xanthommas, is transported in association with HDL to the liver for excretion in the form of bile salts (Reichl and Miller. 1986; Eisenberg, 1984). Although a low level of HDL-cholesterol is recognized as a major cardiovascular disease risk factor, there is evidence that it is the \textit{HDL}_2 subfraction which is the principal determinant of risk. \textit{HDL}_3 does not appear to be a risk factor (Hong et al. 1988). While the majority of studies have not implicated \textit{HDL}_3 as a risk factor, its plasma levels have been shown to be
sensitive to diet and alcohol intake (Williams et al., 1985; Diehl et al., 1988). Low HDL-cholesterol levels are also a particular feature of hypertriglyceridemia. There is a well established inverse correlation between plasma triglyceride concentration and HDL-cholesterol, primarily the HDL₂ subfraction (Deckelbaum et al., 1984; Hongo et al., 1988). This strong negative relationship of HDL₂-cholesterol with plasma triglyceride found in many studies is compatible with the hypothesis that HDL₂ is formed, at least in part, from HDL₃ by the lipolysis of triglyceride rich lipoproteins, mediated by lipoprotein lipase (Taskinen and Nikkila, 1981).

The ratio of plasma cholesterol to HDL-cholesterol (Castelli, 1984) and the ratio of VLDL + LDL-cholesterol to HDL-cholesterol (Miller et al., 1982) has been reported to be indicators of CHD risk. Several reports have indicated that an elevated ratio of plasma cholesterol to HDL-cholesterol might contribute to the development of coronary atherosclerosis (Wallantin and Moberg, 1982; Jackson and Lee, 1984). From our data (Table 20) it can be seen that rats from different groups showed significantly different coronary risk indices. As expected, this was highest in hyperlipidemic rats and decreased after HMG treatment, significantly, in all the groups. The lowering of these ratios can be explained on the basis of the HDL-cholesterol increasing effect of HMG. Accordingly, the decrease was
maximum in diabetic rats. In a study involving subjects with different coronary risks indices, Griffin et al (1988) have recently reported a higher concentration of HDL-cholesterol in the lower risk group. It can be suggested that, like cholestyramine (Jones et al, 1984) and bezafibrate (Gavish et al, 1986), HMG administration is also effective in reducing these indicators of atherogenic risk. According to the current concepts of atherogenicity of plasma lipoproteins, these effects are desirable and should result in a decreased tendency to develop atherosclerotic disease.

The ability of HMG to counteract triton-induced hyperlipidemia in rats suggested that HMG might be exerting its hypolipidemic effect through inhibition of lipoprotein synthesis (Yousufzai and Siddiqi, 1976c). Since HMG was unable to overcome orotic acid-induced fatty liver changes in rats (Yousufzai and Siddiqi, 1977c), the possibility of HMG inhibiting the synthesis of VLDL and LDL rather than release was considered more plausible as the decrease in serum lipids was not accompanied by a rise of liver lipids (Yousufzai and Siddiqi, 1977a). The hypocholesterolemic and hypotriglycerideremic activity of HMG has also been demonstrated in streptozotocin-induced diabetic rats (Francesconi et al, 1987). As shown in Table 1, HMG is equally effective in alloxan-induced diabetic rats. In light of these observations and the current knowledge of role of
cAMP in regulating the lipolysis. It is tempting to suggest that like nicotinic acid, activity of HMG is mediated through its action on adenylate cyclase. It would, therefore, be expected that level of this enzyme would be increased in triton-induced hyperlipidemia. It has been recently shown that hepatic adenylate cyclase as well as guanylate cyclase increased by 234.0% and 96.0%, respectively, in triton-induced hyperlipidemia as compared to control rats. The administration of HMG (i.p.) for two weeks at the dose level of 25 mg/Kg body weight decreased the activity of adenylate cyclase and guanylate cyclase to the extent of 40.0% and 34.0%, respectively [Personal communication, Dr. A.N.K. Yusufi, Department of Biochemistry, Faculty of Life Sciences, Aligarh Muslim University, Aligarh]. The inhibitory effect of insulin, nicotinic acid and prostaglandin E₁ on adipose tissue lipolysis has been explained in terms of inhibition of the synthesis of cAMP possibly at the level of adenylate cyclase site or by stimulating phosphodiesterase. Since HMG decreased the activity of adenylate cyclase, it is quite likely that the action of HMG is mediated through the inhibition of adenylate cyclase. Francesconi et al. (1987) have observed that HMG administration to streptozotocin-induced diabetic rats produced only a slight increase of HDL-cholesterol (4.0%), the level of which was significantly lower (p < 0.01) in the
plasma of these animals than in the plasma of controls. The trend, towards an increase of HDL-cholesterol levels parallel to the tendency towards a decrease of plasma total cholesterol, was considered to indicate an effect of HMG on lipoproteins related to its hypolipidemic and hypoketonemic action. These observations led the authors to believe that HMG administration partially counteracts some of the effects produced by diabetogenic agent streptozotocin. In the present study we have shown the effect of HMG administration in normolipidemic, hyperlipidemic and alloxan-induced diabetic male rats. HMG administration to our diabetic animals produced a marked increase in plasma HDL-cholesterol 48.0% (p < 0.05) than in the plasma of control animals (Table 13). As shown in Table 1 the magnitude of increase in plasma HDL-cholesterol was approximately of the same order as that of decrease in total cholesterol (32.0%, p < 0.05). Therefore, we confirm that HMG administration has the capability to counteract the effects produced by diabetogenic agent alloxan. Our observations also confirm the findings of Francesconi et al. (1987) that the effect of HMG was more pronounced in diabetic than in normal rats. This is due to higher uptake of HMG found in the diabetic group in comparison with the controls (Francesconi et al. 1987). Considering that there is an increased HMG concentration in urine (Deana et al. 1982) as well as in plasma (Lippe et al.,
of both diabetic patients and rats, the authors have speculated that increased uptake and excretion of HMG in diabetes may be due to an alteration of the membrane permeability or to some other unknown factors. A model for the hypolipidemic action of HMG has been proposed in Fig. 16. Platelet activation has been shown to be associated with hyperlipidemia and atherosclerosis both in humans and experimental animals (Aviram and Brook, 1987). Considerable evidence suggest that high plasma LDL-cholesterol levels are also associated with increased platelet aggregation. Hence the atherogenic property of LDL and VLDL as well as antiatherogenic property of HDL have been attributed to their effects on platelet function (Aviram and Brook, 1987). Plasma lipoproteins affect platelet activity by modulation of arachidonate pathway, cAMP formation and phosphoinositide metabolites (Aviram, 1988). The overall metabolism of arachidonic acid was found to be increased in platelets from patients with type IIA hypercholesterolemia (Eynard et al., 1986) probably due to the modification of the lipid composition of platelet membranes. Kamido et al (1988) have reported a lower phosphocholine : cholesterol ratio in platelets from such patients. This ratio is an important determinant of platelet membrane fluidity. The lipid composition of platelets affects not only membrane fluidity of platelets but also prostaglandin metabolism and thus
Fig. 16  Proposed model for the hypolipidemic action of HMG (Abbreviations given in Figs. 1 & 2)
+ ...positive effect of HMG
- ...negative effect of HMG
affects aggregation of platelets. In vitro studies have also shown that cholesterol enriched platelets produced increased amount of thromboxane $B_2$ as compared to cholesterol poor platelets. The increased formation of arachidonic acid metabolic products, not only via cyclooxygenase but also via lipooxygenase pathway, may thus contribute to hyper-aggregability, associated with the hyperlipidemic state (Tremoli and Nicosia, 1983).

As also discussed earlier, it is now well established that compounds inhibiting platelet aggregation might retard atherogenesis. Witiak et al. (1988) have recently reviewed the activity of various antilipidemic and antiaggregatory agents, some of which also lower serum LDL and VLDL concentrations. Hence the effectiveness of HMG (in vivo) in decreasing the platelet aggregation (Table 23) can well be attributed to its capacity to reduce LDL-cholesterol as well as plasma levels of atherogenic lipoproteins (LDL+VLDL). The ability of HMG to inhibit ADP-induced platelet aggregation both in vivo (Table 23) and in vitro (Table 24) suggest that the compound may physiologically modulate platelet function. This modulation is also exhibited by the alterations in the speed of aggregation as well as time for maximal aggregation. Since platelets contribute to the formation of atherosclerotic lesions by enhancing foam cell formation (Takagi et al., 1988), the inhibitory action of HMG
on platelet aggregation support the usefulness of this compound as an antihyperlipidemic and antiaggregatory agent. It can be suggested that HMG treatment produces an alteration in the lipid composition of platelets as a result of the changes produced in plasma lipids and lipoproteins. Aviram and Brook (1982) have reported that increased platelet activity in patients with FH is dependent on the exposure of platelets to some plasma constituent(s). It appears, more than likely, that the cholesterol containing particles such as LDL, through interaction with the platelets, are important in this regard since platelets possess specific binding sites for LDL (Aviram and Brook, 1981a,b) and serve as cholesterol storage organs (Block et al. 1988) after the uptake of lipoprotein cholesterol. The changes in the membrane cholesterol content of platelets, as a result of alteration in plasma lipids produced by HMG treatment, are therefore expected to be responsible for the decreased aggregation. This may involve the alterations in the arachidonic acid metabolism. Theoretically, compounds inhibiting platelet aggregation could possibly do so by a variety of mechanisms (Philp, 1981). Increased platelet aggregation in type IIA hypercholesterolemic patients have been attributed to increased metabolism of arachidonic acid (Eynard et al. 1986). Hence, it is most likely that hyporesponsiveness of platelets, derived from HMG treated
rats, to aggregating agent may be consequent to the cholesterol (LDL-cholesterol) lowering effect of HMG in the plasma and the effect of HMG seems to be mediated via the arachidonic acid pathway. Eynard et al (1986) have explained the changes of arachidonic acid metabolism by at least 2 mechanisms: modifications of the cholesterol and phospholipid content of platelet membranes and or modifications in the platelet enzyme activities. The effect of HMG on platelet aggregation is of interest because of their relationship to atherosclerosis. It will be desirable to perform platelet aggregation studies in diabetic rats also.

From the foregoing discussion it appears that HMG may be a more beneficial therapeutic agent than other conventionally used compounds and drugs. However, more biochemical, pharmacological and clinical studies are needed in higher primates before it can qualify as a hypolipidemic drug for human consumption. A comparison of lipid lowering effect of HMG and other widely used compounds/drugs is summarized in Table 25 which supports the contention that HMG may be a potential drug of future in combatting hyperlipidemia.
Table 25

COMPARISON OF HMG AND OTHER HYPOLIPIDEMIC COMPOUNDS/DRUGS

<table>
<thead>
<tr>
<th>HMG</th>
<th>PARAMETERS</th>
<th>FIBRATES</th>
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<th>NICOTINIC ACID AND ANALOGUES</th>
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1 SIRTORI and FRENGESCHINI (1988)
2 CRESSMAN et al (1988); HELVE and TIKKANEN (1988)
3 TASKINEN and NIKKILA (1988); CREPALDI et al (1988)

* ↓ decrease  ↑ increase —— no change  NA data not available