CHAPTER VII
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SUMMARY

A summary of the work done on the investigation entitled "Studies on Some Aspects of the Role of Ascorbic acid in Cholesterol Metabolism" has been presented below in order to give a brief outline of the work. The experimental work and its compilation was carried out from November 1976 to October 1981 in the Department of Biochemistry, Panjab University, Chandigarh, during the tenure of a Teaching Assistantship in Biochemistry to the present author.

The summary has been presented in the following sequence:

I. INTRODUCTION
   i) Genesis, rationale and scope of the proposed project
   ii) Scheme of studies to be pursued
   iii) Experimental design of the project and the mode of its execution

II. Results obtained in the experimental investigation

III. Discussion

IV. Conclusions and suggestions regarding lines of future work in the field.

I. i) Genesis, rationale and scope of the proposed investigation

Genesis: The proposed project originated from the fact that, in contrast to deficiency diseases, several diseases of plenty have also become known. One such disease is
an excessive amount of body cholesterol, which has been considered as quite widespread; it is encountered more often in our socio-economically more affluent communities. In fact, it has become a worldwide problem.

This factor has in recent years been implicated in a number of serious and dreaded biochemical disorders of the cardiovascular and the central nervous systems, in gallstone formation, and also in the etiology of certain forms of cancer (1). Recent researches have identified this excessive cholesterol to be mostly of exogenous influx, from the cholesterol rich diet, and arising, to a fair degree, from a deficiency of vitamin C (L-ascorbic acid, AA); the latter might not be apparent to the extent of its severe form usually known as scurvy, but might be present to the extent of only a lower order, which at present is referred to as latent marginal AA deficiency. In fact, hypercholesterolemia, atherosclerosis, and even coronary heart disease have currently been claimed to be vitamin C deficiency diseases. Current investigations on AA-cholesterol relationship tend to support the view point that mega doses of AA could prevent/suppress the above disorders of excessive cholesterol, and many other allied defects. Szent Gyorgyi-Stone-Pauling school advocates daily multi-gram doses of AA for modern man for full expression of his maximum potential for health, and for ability to resist the present day stresses, the biochemical stresses so common in our modern society-, and for bodily malfunctions.

This situation led the present author to undertake a
detailed study of certain selected areas of this very wide and multifaceted problem of AA-cholesterol relationship, specify a scheme of studies, and chalk out an experimental design to pursue investigations to secure clues which might prove useful in preventing this malady. The present project aims at achieving this end.

Rationale: The rationale of the proposed study is based on the following facts. Every cell of the human system contains cholesterol, as it is an essential requisite for the normal structural integrity and the physiological functions of the cellular membranes; every cell is also capable of synthesizing it with the possible exception of matured mammalian erythrocyte, as well as exercising a control over the activities of some 26 enzymes responsible to elaborate it from active acetate. The average daily metabolic requirement of cholesterol of an adult is known to be around 350 mg; as against this, the daily consumption from the animal-based diet (like that of the Western man) is around 500 mg plus an assorted amount of other sterols ("phytosterols"); this input has to be considered alongwith an average daily synthesis of cholesterol (endogenous cholesterol, c 1.0 gm). The total daily influx thus comes to c. 1.5 gm cholesterol. This excess cholesterol accumulates in his average 70 years of life, and ultimately turns out to be a slow and silent killer, mostly affecting his cardiovascular system. It is heartening to find that phytosterols not only remain un-
exogenous cholesterol.

Scope: Considerable evidence has now accumulated which suggests that the incidence of arteriosclerotic coronary heart disease is positively related to plasma concentration of total cholesterol, particularly LDL-cholesterol, but inversely related to HDL-cholesterol. Likewise, hyperlipidemia is known to play an important role in the induction and development of atherosclerosis, and therefore the serum lipid level must be normalized to prevent or treat atherosclerosis. The role of different lipoprotein fractions in the pathogenesis of atherosclerosis has also been elucidated: VLDL and LDL fractions are considered to be atherogenic, while the HDL fraction is thought to be anti-atherogenic. In patients with coronary heart disease, LDL-cholesterol has been reported to be significantly higher, and HDL-cholesterol significantly lower than found in the control groups.

The above information established the scope of the proposed study.

ii) Scheme of studies to be pursued

The scheme of studies was planned to be based on the following three main aspects:

1. The main study was designed to limit itself to the isolated and specific central problem of investigating the effect of ingestion of AA on blood cholesterol in hypercholesterolemic rats, both in the absence as well as in the presence of
a potent hypcholesterolemic agent, focusing attention on whole blood and plasma on the one hand, and on erythrocytes and erythrocyte membrane on the other.

2. For this purpose, the experimental hypercholesterolemia was to be induced by feeding cholesterol dissolved in a somewhat unconventional solvent, propylene glycol (FG), in place of the more conventional oils/fats, or aqueous emulsions stabilized with gums. Oils/fats are known to exercise their own sharp and significant effect, in that they increase the intestinal absorption of cholesterol, and hence its levels in the blood, thus vitiating the final results obtained by their use. In contrast, FG, being a composite lipophilic-hydrophilic non-lipid solvent/additive, could possibly give different results.

3. The hypocholesterolemic agent selected for use in the proposed study was the well known and proven ancient Indian indigenous drug gum guggul as the hypocholesterolemic agent, in place of the usual synthetic ones in current use; this would afford additional newer data.

iii) Experimental design of the project and the mode of its execution:

Experimental design: The following experimental
design was followed:

**Part I** : Studies on AA-cholesterol relationship in the absence of any hypocholesterolemic agent.

**Part II** : Studies on AA-cholesterol relationship in the presence of a hypocholesterolemic agent (oleoresin of gum guggul, OG).

**Part III** : Studies on the above two parts in respect of:

(a) Hypotonic hemolytic behaviour and red cell deformability;

(b) $^{14}$C-acetate incorporation into tissue lipids.

The above three parts were investigated test substance-wise in order to bring out clearly the effect of each test substance on the parameters studied.

Part I and Part II aimed at studying the effect of ingestion by rats the following test substances:

1. Mega doses of AA, the vehicle (PG) used for feeding cholesterol and OG; cholesterol in PG; AA in PG; and AA alongwith the hyper-cholesterolemic diet (i.e. cholesterol) in PG.

2. OG in PG; cholesterol+OG in PG; AA+OG in PG; and AA + cholesterol + OG in PG.

Part III aimed at studying the effect of each of the above test substance on red cell fragility and deformability; in addition, $^{14}$C-acetate incorporation into tissue lipids was also examined.

**Mode of execution** : Each test substance in Part I and II was studied for its effect on the following
parameters:

i) In blood:

a) In whole blood:
   1. Hematocrit, Hb, ESR, CT, TLC and DLC.
   2. Sugar, and cholesterol (total, free and esterified).

b) In blood plasma:
   Lipids and certain lipid enzymes
   1. Lipidogram: total lipids, cholesterol (total, free and esterified), lipoproteins (α- and B-), phospholipids, triglycerides, free fatty acids and TBA value.

ii) In erythrocytes:

a) In erythrocytes:
   The in vivo as well as the in vitro effects of AA on acid and alkaline Pases, 5'-nucleotidase, ATPase, and AChE, as representatives of peripheral and integral enzymes.

b) In isolated erythrocyte membrane:
   The in vivo and the in vitro effects of AA on the membrane components as well as on the enzymes.

The studies in erythrocytes were extended to include the in vitro effect of the non-ionic detergent Triton X-100 treatment on the specific activities of the erythrocyte enzymes studied.
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a) In whole blood -
1. Hematocrit, Hb, ESR, CT, TLC and DLC.
2. Sugar, and cholesterol (total, free and esterified).

b) In blood plasma - Lipids and certain lipid enzymes

1. Lipidogram: total lipids, cholesterol (total, free and esterified), lipoproteins (y, and B -), phospholipids, triglycerides, free fatty acids and TBA value.

ii) In erythrocytes:

a) In erythrocytes - The in vivo as well as the in vitro effects of AA on acid and alkaline Pases, 5'-nucleotidase, ATPase, and AChE, as representatives of peripheral and integral enzymes.

b) In isolated erythrocyte membrane - The in vivo and the in vitro effects of AA on the membrane components as well as on the enzymes.

The studies in erythrocytes were extended to include the in vitro effect of the non-ionic detergent Triton X-100 treatment on the specific activities of the erythrocyte enzymes studied.
iii) Fecal excretion

In addition to the above studies on blood, the effect of ingestion of AA and OG on the fecal excretion of cholesterol and bile acids was also studied to afford a more precise information about the type of response these test substances (AA & OG) elicit in this species.

iv) (a) Hypotonic hemolytic behaviour (red cell fragility)

(b) Red cell deformability was studied by using microscopy.

v) $^{14}$C-acetate incorporation into tissue lipids (labelled studies).

II. RESULTS OBTAINED IN THE EXPERIMENTAL INVESTIGATION

Experimental - Part I

Studies on AA-cholesterol relationship

AA (Gp II): The data presented above clearly indicated that ingestion of mega dose of ascorbic acid (AA) over a period of 30 days to healthy, normal adult male albino rats, a species which does not depend on exogenous source of AA, could bring certain alterations at the cellular level. A significant reduction in total leukocyte count alongwith a marked fall in whole blood and plasma total lipids, (total) cholesterol, ester cholesterol and glucose, and an elevation of plasma free fatty acids were observed. In addition, the specific activity of certain erythrocyte enzymes, namely, acid and alkaline phosphohydrolases and ATPase were found to be elevated significantly. At the erythrocyte membrane level, marked changes were encountered. There was a reduction in
total membrane lipids followed by an elevation of phospholipid. Changes were also observed in the activities of the membrane bound enzymes. Thus the specific activities of ATPase and AChE were increased significantly, whereas that of 5'-nucleotidase at pH 9.2 was decreased in the sodium chloride-soluble fraction. On the contrary, in the Triton X-100 soluble fraction the activities of the nonspecific phosphohydrolases were elevated markedly. However, the soluble proteins in both the fractions were reduced. Lastly, the fecal cholesterol content was markedly increased.

As AA is known to have mild surface active (detergent) property, it could most probably interact with the lipoprotein complex of the externally localized enzymes. This could be the possible explanation tentatively put forward for the lowered activity of AChE.

PG (Gp III): The data presented in the present thesis clearly indicates that the intake of propylene glycol (PG), used as solvent for dissolving cholesterol, by rats at a dose level of 0.284 ml in water (28.4%) daily for 30 days brings about certain prominent biochemical changes. The gross changes observed at the cellular level were a significant reduction (50%) in ESR and in total leucocyte count (28%). Amongst the various blood and plasma constituents analysed, the plasma lipids were uniformly lowered on feeding PG. There was also a marked decrease in the normal blood level of GSH and the TBA value. PG-fed rats showed a significant increase in their blood AA content; this increase in AA exceeded even
that of animals of group II, which had received 10 mg of AA/100 g body weight daily as described above. This action of PG suggests that it could either induce the de novo synthesis of AA as in the case of hepatic microsomal hydroxylation system, or it could deplete the AA from tissues. However, the mode of action of PG in producing hyper-AA state in blood awaits further study. It could be further postulated that GSH redox system might be working in co-operation with AA redox system as a compensatory mechanism. Both the ester and the free cholesterol are also reduced significantly in PG-fed rats, with the ratio of FC:EC.

The latest approach to develop hypolipidemic drugs has been aimed at obtaining an increased level of HDL in serum or plasma. The hypolipidemic and hypocholesterolemic effects of PG in normal rats in comparison with some of the most potent hypolipidemic agents in current use, such as Clofibrate and its derivatives, is noteworthy. Moreover, in the present work PG increased \( \beta \)-lipoprotein fraction by 70% than that of the normal rats. The finding has a great potentiality for using this solvent as hypolipidemic agent alone, or in combination with hypocholesterolemic drugs which are usually hypophilic in nature.

The lowered levels of serum lipids resulting from PG ingestion are accompanied by a marked increase in the activity of plasma lipid enzymes, namely TG-lipase, lipoprotein lipase and cholesterol esterase, whereas the activity of LCAT was markedly lowered. PG in some way accelerates the lipoprotein
lipase activity, which in turn promotes the catabolism in providing precursors for the constitution of HDL, thus increasing the \( \alpha \)-fraction of lipoproteins. For encouraging the use of PG as a hypolipidemic diet or as drug vehicle, more work at tissue level remains to be undertaken, to assess its effect on liver damage or dysfunction. Chronic PG ingestion has been found to lower LCAT. PG itself has been found to have a very strong stimulatory effect on almost all the erythrocyte enzymes studied, except for AChE, with a concomitant increase in the protein content of the cell as a whole. Similarly, in vitro treatment with Triton X-100 brought more protein in solution, followed by another elevation in the specific activities of these enzymes. The most conspicuous observation was that acid Pase activity was not changed by the detergent, which suggests this protein is readily released from the membrane.

These findings are confirmed from studies on erythrocyte membrane of PG-treated rats. All the stromal enzyme activities paralleled those of the hemolysate in the sodium chloride-soluble and the insoluble fractions. It could be visualised that the specific activities of both 5'-nucleotidase (pH 7.2) and ATPase were decreased in sodium chloride-soluble fraction, in contrast to high stimulation observed in the hemolysate. This could be explained on the basis that because they could be easily released, these proteins might be lost during washing or were partially inactivated. In the case of AChE, the activity in both the stromal fractions are elevated (34.8-fold increase over hemolysate), in contrast to the inhibition
observed in hemolysate. However, in the control group the Triton X-100 treatment did not alter the specific activity significantly in the hemolysate, whereas, in the stromal fraction there was about 16.4-fold increase. Thus, this discrepancy could not be due either to the alteration in the solubility of protein or to the presence of some hitherto unknown endogenous inhibitor being formed during PG feeding, as PG is known to affect some drug metabolizing enzymes and proteins in liver. The indirect manner in which PG exerts its effect on the biological system is further confirmed by the in vitro effect of this solvent on the erythrocyte enzymes, as none of the enzyme activities was found to be affected markedly by addition of PG from 0.28 to 28%. Thus ingestion of PG, although it brings depletion of lipids in plasma and blood, and reduction in the GSH content of blood, results in a high degree of increase in the activity of blood enzymes. The mechanism(s) by which PG exerts its effects on these parameters awaits further work.

\textbf{Ch-PG (Gp IV)} : Feeding of 10 mg of cholesterol dissolved in 0.284 ml of PG per 100 gm body weight to male albino rats for 30 consecutives days brought about a significant change in PCV, while plasma cell counts were not affected. Hence, the lowered count of total leucocytes which resulted on PG feeding could not be reversed. A 3.57% increase in blood sugar level was also observed.

Feeding of cholesterol at 1% level even in the presence of hypocholesterolemic solvent PG could elevate blood and plasma
cholesterol by 134%, as well as the total lipids. The ratio FC:EC (0.96) and the ratio of β- to α-lipoprotein (1.1) were also raised (TG-lipase and lipoprotein lipase were decreased and cholesterol esterase and LCAT were increased in Ch + PG fed rats.

As discussed above, PG itself had a stimulating effect on almost all the erythrocyte enzymes, except AChE, in erythrocytes. Thus, a close study of data on the cholesterol fed group of animals reveals that cholesterol elicits a response either intermediate between PG and the control levels, or exceeding them in the opposite direction with respect to PG action; in other words, the biological response of cholesterol feeding as found in the activities of RBC enzymes is antagonistic to that of PG. The only enzyme which exceeds in its specific activity from those of Group IV was acid Pase, while alkaline Pase was totally inhibited and was not revived even upon in vitro addition of Triton X-100. Cholesterol+PG had an inhibitory effect on the rest of the enzymes studied, as well as on protein content. These findings are further confirmed from the activities of erythrocyte ghosts. Nevertheless, alkaline Pase which could not be detected at all in the hemolysate, showed some activity though it was less than that of the control group (Gp I).

All these changes in enzymes of red cells might be due to increased lipid and cholesterol, of the membrane, as the fluidity of the membrane lipids can be controlled by cholesterol.
fatty acyl chain length and unsaturation; these factors in membrane, in turn, could be capable of controlling the activity of membrane-bound enzymes. The change in the phospholipid content of membranes with or without cholesterol feeding appears to be one of the most important contributing factors for the change in the enzyme activity observed in the present work.

**AA+PG (Gp V):** Feeding of AA in PG tended to reverse the lowering effect of PG on ESR and TLC, and augmented the hypoglycemic and the hypolipidemic responses in rats. Both the FC:EC ratio as well as the β:α lipoprotein ratio were lowered to the level of the control rats (Gp I). It also decreased the activity of TG-lipase and lipoprotein lipase, whereas it increased the activity of cholesterol esterase and LCAT. This suggests that AA in PG did not possess any synergistic action on these enzymes; rather only an additive effect was observed.

The activity profile of the RBC enzymes of rats fed AA+PG showed a significant decrease in activities of all the enzymes, except that of 5'-nucleotidase (pH 9.2) and AChE with respect to the PG-fed rats. AA had no effect on 5'-nucleotidase (pH 7.2), whereas it activated AChE. In the presence of PG, AA could only partially reverse the inhibitory effect of PG on these two enzymes. Triton X-100 treatment could elevate only the ATPase appreciably. The synergistic action of AA has been observed in the activities of 5'-nucleotidase and AChE. Thus AA had an antagonistic effect toward PG in its action on these
enzymes in vivo. Also, it resembled cholesterol to some extent in its action on 5'-nucleotidase, ATPase and AChE without adding detergent, but it differed upon addition of the detergent. The enzyme activities in group V were nearer to the normal control group (Gp I), showing that AA had some beneficial effect over PG+cholesterol fed groups. The activities of various enzymes in the sodium chloride-fraction of erythrocyte ghosts were similar to that of the hemolysate, whereas that of Triton X-100 soluble fraction resembled the activities in group IV. The changes in enzyme activities observed in this group of rats might be due to the lowered C/P molar ratio in the erythrocyte membrane.

AA+Ch+PG (Gp VI) : AA could not protect against the undesirable effects of either PG or cholesterol when they were administered together, in contrast to its own beneficial effect when it was given alone in PG. In other words, cholesterol action was found to be antagonistic to that of AA in this respect, since addition of AA to Ch+PG did not influence the lowering effect of PG and cholesterol on ESR and TLC, and could only partially reverse the increase in PCV.

Similarly, feeding of AA with Ch+PG showed an intermediate response between groups IV and V in its hypoglycemic and hypolipidemic actions. As the lipid lowering effect of AA in the presence of Ch+PG could not maintain the normal levels in any of the lipid components, it is suggested that AA at a dose level of 10 mg could afford only a partial protection against cholesterol-induced hyperlipidemia.
The addition of AA to Ch+PG potentiated the effect of PG on the lowering of activity of TG-lipase and LPL, while cholesterol-induced elevation in the activities of cholesterol esterase and LCAT could not be reversed by AA.

So, in spite of the hypolipidemic action of PG in rats, with cholesterol it could produce a markedly significant hyperlipidemia, with 3-fold increase in blood cholesterol. Similarly, with AA, PG exerts its own effect, especially in the hypolipidemic action. The combination of AA and cholesterol with PG, also definitely showed a significant protective effect against cholesterol. On feeding AA with Ch+PG, none of the enzyme activities could be restored to the control level both in the erythrocyte and the erythrocyte membrane. As compared to PG-fed group, all the activities were significantly lowered, whereas comparison with the respective cholesterol- or AA-fed groups revealed that activities of the membrane bound enzymes were modified differently.

Experimental- Part II

Studies on AA-cholesterol relationship in the presence of OG (Gp VII to Gp X)

Effect on hematocrit, etc.: The feeding of OG at a dose level of 33.3 mg per 100 gm body weight for 30 days afforded a significant protection against quite a number of adverse effects caused by PG. Although in most cases, as in studies on ESR, TLC, etc., OG tended to maintain the normal
Effect on sugar and lipids: The present study revealed that OG+PG brought about a statistically significant decrease in the content of (whole) blood sugar, and plasma total lipids and cholesterol, and increased the ratio of α- to β- lipoproteins from that of the PG-fed rats. The lipid lowering effect of OG could be attributed mainly to the esterified cholesterol, since the decrease observed in free cholesterol and free fatty acids was not significant statistically, while there was a significant increase in TG and PL to that of the PG-fed group. So, in the presence of PG, which itself has a powerful hypolipidemic effect on all these plasma lipid parameters, OG could exercise its hypolipidemic effect through cholesterol ester. However, the efficiency of OG as a hypolipidemic agent at this dose level was less than that of AA, but it caused a greater decrease in the free cholesterol level. The combination of OG+AA in PG (Gp IX) afforded no added beneficial effect in respect of lowering the lipid level than that by the individual substances. In comparison with normal rats, Ch+OG group showed lower levels of all the fractions of lipids. So it could be hypothesized that the potent lipid lowering effect of the combination of AA and OG with cholesterol also elicits a more pronounced response in hyperlipidemia, just as some of the hypoglycemic agents are effective only in diabetics.

The OG, AA and OG+AA fed rats had lower activities of TG-lipase, LPL and cholesterol esterase in comparison with the
FG-fed rats. The cholesterol-fed groups (Gp VIII and X) had lowered activities of cholesterol esterase and LCAT, which was responsible for the significant increase in free cholesterol. This observation suggested that hypocholesterolemic effect of OG in blood was mediated via transport of cholesterol in the hypercholesterolemic rats. The action of OG and AA were found to be additive in nature and might be responsible for the potent protection afforded by these two agents in maintaining the lower lipid levels in cholesterol-fed animals. Hence combination of AA+OG, as in the case of sugar, are found to be highly effective in restricting hyper-lipidemia produced by cholesterol.

**Effect on redox state**: OG-fed rats showed a significant reduction in AA, and increased GSH and TBA value than the PG-fed rats. These findings suggested that OG, unlike PG, did not increase blood AA, but still could maintain the levels of reduced GSH, and appeared to be a pro-oxidant when given alone, but could be free from this undesirable effect when AA was given along with it. So OG might be inducing the hepatic enzymes differently from those induced by PG and AA.

**Effect on erythrocyte enzymes**: A perusal of the literature cited in this context revealed that, in spite of the fact that many exhaustive investigations have been made on the hypolipidemic activity of OG, no attempt has been made to study its effect at the subcellular level.

The data on the effect of OG on erythrocyte enzymes
studied showed that OG, in general, resembled AA in its actions; AA and OG opposed the action of PG to increase the activities of all the erythrocyte enzymes studied, and tended to maintain the normal level of enzymes in erythrocyte. Significant changes were observed on OG or AA+OG feeding to rats in a direction opposite to that of cholesterol-fed rats, suggesting a beneficial effect of these two drugs in hypercholesterolemia even at subcellular level. Attempts were made to confirm the above observations by extending these studies on the erythrocyte membrane. It was observed that OG treatment alone or in combination resulted in a decrease in the specific activities of all the enzymes in consonance with the observation on blood cells, with minor exceptions in the specific activities of certain enzymes in the sodium chloride-soluble fraction. The combination of OG+AA tended to keep the normal level of enzymes against perturbations caused by cholesterol and PG, individually or in combination. So all the enzyme activities in group X were nearer to those of group I. Hence, the combination proved to be of greater benefit to the organism at subcellular level than the concerned individual substances.

Effect on fecal excretion: OG was found to enhance the fecal excretion of bile acids in normal rats, whereas in cholesterol-fed rats, both cholesterol and bile acids were excreted; addition of AA to OG proved more beneficial in this respect.
Experimental- Part III

Studies on Parts I and II in respect of hypotonic hemolytic behaviour and red cell deformability, and $^{14}$C-acetate incorporation into tissue lipids.

Concerning AA-cholesterol relationship (i.e. Part I):

Osmotic fragility: The hemolytic behaviour of the erythrocytes derived from the different groups of rats receiving AA or cholesterol, or both, in water or PG, in hypotonic solution of sodium chloride at room temperature, was found to be quite in agreement with the changes in the erythrocyte lipids.

The osmotic fragility and cholesterol content of the red cells were in inverse relationship. AA in water (Gp II) did not alter the osmotic fragility of the cells to any significant extent, whereas with PG (Gp V) it did reduce the osmotic fragility, with a reduction in C/P ratio and a lowered level of lipids, from those of the PG-fed group of animals. Therefore, it appears that the mechanism by which AA decreases the osmotic fragility was different from that of the in vitro depletion and repletion of cholesterol.

Electron microscopy: The present data clearly showed that changes in erythrocyte lipids and C/P molar ratio are accompanied by changes in erythrocyte morphology and the status of the plasma membrane of red cells.

$^{14}$C-incorporation: AA in water (Gp II) tended to
lower the incorporation of labelled acetate into the various tissues, except that in the lower intestine. In liver and the lower intestine, all lipid components had greater radioactivity than that in the control group, whereas all other compartments had a reduced level of $^{14}$C-acetate incorporation. Thus, AA stimulated the synthesis of lipids in liver and in the distal part of the intestine, while it had inhibitory effect on the proximal intestine, and, thus might be maintaining a low level of lipids in plasma (blood).

PG feeding to rats, in general, showed enhancement in the incorporation of $^{14}$C-acetate in various tissues. PG promoted the synthesis of cholesterol in liver and proximal (upper) intestine, and of cholesterol and phospholipids in the distal (lower) intestine. This was not followed by increase in lipids in the plasma, which suggests that PG exerted an inhibitory effect on the release or the transport mechanism rather than on the synthesis of lipids. The feeding of cholesterol depressed the synthesis of lipids even when given in PG, while AA tended to reverse this situation. In all these studies the effect of PG predominated over those of both cholesterol and AA. The above data clearly showed that both liver and intestine are important organs for the regulation of lipids, especially cholesterol in rats.

Concerning AA-cholesterol relationship in presence of OG (i.e. Part II):

Osmotic fragility: The addition of AA or
cholesterol to OG+PG showed an inverse relation of osmotic fragility to cholesterol level of red cells; this was not seen in the OG+PG (Gp VII) fed group, in which no change in osmotic fragility was observed with respect to the normal group (Gp I), despite the fact that it had a lower level of lipids and the same C/P molar ratio, to that of Group I. This data indicated that OG, an anti-inflammatory substance, might be controlling the osmotic fragility through the protein content of these cells and by maintaining the C/P molar ratio, in spite of its lipid lowering effect.

**Electron microscopy**: Erythrocyte morphology and status of its surface membrane in the presence of OG were found to depend on both the cholesterol content and the C/P molar ratio in the erythrocyte.

**$^{14}$C-acetate incorporation**: The administration of OG in PG had a suppressive effect on the biosynthesis of total lipids and cholesterol in the liver and the upper part of the intestine; the same effect was apparent in presence of cholesterol and AA, or both. This is suggestive of the fact that hypolipidemia brought about by feeding OG was due to its inhibitory effect mainly on hepatic cholesterol synthesis, by modification of the lipoprotein component by altering the cholesterol and phospholipid concentration of liver, and by accelerating the synthesis of bile acids. Another noteworthy observation was that AA could promote the metabolism of PG,
cholesterol and OG in liver, and thus serve as a powerful detoxifying agent. This property of AA was also desirable, in addition to its action in maintaining the plasma lipid level in the normal range, without any additive effect.
Chapter VIIb.

CONCLUSIONS

An overall analysis of the entire data obtained in the present study leads us to the following conclusions in respect of AA, OG, AA+OG, and PG:

1. **AA**: AA (in water), at the dose level studied, was found to have a fairly potent hypocholesterolemic effect in normal rats, which was more pronounced in the hypercholesterolemic animals; it was also found to possess a mild hypoglycemic effect in rat. AA elicited these responses with hardly any effect on the hematocrit, the blood redox state, or the viability of the erythrocyte membrane (as assessed from osmotic fragility and the electron microscopic studies). However, there was a slight decrease in the total leucocyte count (TLC), without any corresponding change in the differential leucocyte count (DLC), and inhibition of erythrocyte membrane bound enzymes other than alkaline Pase and ATPase. These aspects need further investigation.

   When given in PG, AA caused abnormally low erythrocyte membrane lipids leading to red cell deformability, and inhibition of the activities of the membrane bound enzymes (except that of AChE); these aspects and further detailed studies in spite of the observed beneficial effects produced
by AA.

2. **OG** : The potent hypolipidemic and hypocholesterolemic property of OG when given in oils or as aqueous emulsion has been confirmed when the latter were substituted by PG as the vehicle, and rat as the experimental animal.

It was further observed that OG resembled AA in several of its reactions, despite the fact that mechanisms of their actions differed widely; for instance, their effects on the biosynthesis of lipids in liver and intestine, the activation they caused in plasma lipid enzymes and in certain erythrocyte membrane bound enzymes (except that in the activity of the neutral nucleotidase and ATPase), the extent of protection afforded by them against red cell deformability in hypercholesterolemic rats, the pattern of the fecal excretion of neutral sterols and bile acids, and their efficacy at the dose levels used in the present study, all differed.

3. **AA+OG** : Administration of a combination of the above two substances, i.e. AA+OG (in PG as vehicle), was found to afford a significant protection against several of the undesirable effects caused by the ingestion of cholesterol (in PG). The fact that OG, in comparison to AA, did not afford the same degree of protection against the effects of cholesterol, might possibly be related to the detoxifying and the surface active properties of AA. AA might be accelerating the metabolism of OG & PG, as it does in the case of cholesterol. In fact, this could be one of
the reasons for the observed reduced efficacy of the combination (AA+OG), irrespective of the fact that individually both AA & OG were fairly active in maintaining the normal state of the system in the presence of an hypercholesterolemic agent.

It is emphasized that, inspite of the above data in respect of AA+OG combination, it is still desirable to be cautious about their possible side effects at the subclinical level when used continuously over prolonged periods; this aspect needs further detailed investigation.

4 PG: PG can no longer be considered as an inert and totally non-toxic solvent/additive; it was found to elicit, besides its hypolipidemic action, a host of significant biological activities at cellular level, such as lowering of ESR, TLC, and blood GSH level, a remarkable increase in plasma HDL and AA levels, and in the activities of several erythrocyte enzymes (except in that of AChE); PG also modifies some of the biological actions of AA & OG. These findings suggest that PG ( & even AA, & OG as well) might be affecting protein biosynthesis/cell differentiation in the liver and the bone marrow levels.

These facts warrant vigilance when using PG as a vehicle for biologically active agents.

The biochemical significance of the above findings has been discussed in detail.
Suggestions for future work:

In the light of the above findings, a few lines of approach for future work in this area are suggested.

In order to properly understand, define and delineate the actions of AA, OG and PG, more detailed information using far more refined techniques would be necessary to obtain specific answers to various questions which have arisen from the present study, but more immediately with respect to the following:

A.  
   i) **Blood**: More detailed work is warranted on blood cells (particularly on platelets with special reference to the biochemical changes involved in respect of coagulation, fibrinogen etc.), on cell membranes and plasma lipoproteins, and on the redox state of the blood.
   
   ii) **Fecal excretion**: More precise information is needed, from tracer studies more refined than used in the present preliminary work (in this respect), on the fecal excretion of cholesterol via neutral sterols (cholesterol, coprosterols, etc.) and bile acids induced by AA & OG.
   
   iii) **Toxicity studies**: In view of the fact that all these three substances (AA, OG & PG) are very widely used substances (OG is one of the most versatile and promising of the Indian indigenous drugs used in the Ayurvedic, Tibbi,
Siddha, and allied local systems of medicine), detailed studies on their possible chronic toxicity would be required, particularly at the level of liver and also of kidney, brain and the reproductive system, with respect to both their enzymic as well as the redox states.

In view of the promising results obtained with OG (the oleoresin of gum guggul), its separated individual components would have to be investigated to trace the active moieties responsible for the beneficial effects of OG. These could then lead to clinical trials.

These aspects are expected to afford a clearer picture of the actions of AA, OG and PG reported in the present work. Further work on these lines is in progress in our research team. It is hoped it would lead to newer (better) remedies against this malady of excessive body cholesterol.