CHAPTER II
REVIEW OF LITERATURE
Chapter II: Review of existing literature on the subject used as a guide to the evaluation of the present status of the problem

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Chapter II

(i) REVIEW OF LITERATURE

The information collected for this review has been classified under three main sections, namely those which deal with aspects concerned with (i) ascorbic acid (AA), (ii) cholesterol, and (iii) AA-cholesterol relationship. In addition, some aspects of propylene glycol (PG 1,2-propanediol) and of gum guggul have also been reviewed to complete the information required for the present study. The literature has been surveyed up to the end of 1980.

1) Ascorbic acid.

The subject of ascorbic acid (AA) has already been extensively reviewed (3, 18-23-a to d), and a voluminous literature is available concerning the different aspects of its activity. Both the vitamin C as well as the non-vitamin C properties of AA have been documented. The present resume of literature has therefore been restricted only to those aspects which were considered relevant to the present investigation.

Certain physicochemical and chemical characteristics of AA. The ionization characteristics, the optical absorbance, the hydrophilic and the hydrophobic characters, the capacity to lower interfacial tension, the capability of participating in H... bonding reactions, and the significance of these characteristics have been discussed (3). Likewise, the dual
function of AA as anti- and pro-oxidant, and its functions as a potent reductant, a hydroxylant, and a metal chelator have also been reviewed (20). Recently, certain biochemical characteristics of AA have attracted particular attention; these are: de-lactonization of AA by phosphodiesterase (PDE), the role of AA in electron transport, in microsomal drug metabolism, and as a detoxificant, the action of AA on various enzymes, and the significance of AA-2-sulphate. These are being reviewed briefly.

Delactonisation of AA by PDE. It became known in early sixties (24,25) that L-gulono-γ-lactone hydrolases (aldonolactonases) delactonized DAA to give DKGA. In 1976 (26) a similar reaction in respect of AA became known. The facts that both AA and c-AMP are only intramolecular (cyclic) esters which contain a negatively charged oxygen atom attached to the ring and thus have a structural similarity, and that c-AMP is hydrolyzed by PDE led Lewin (1976) (3) to consider that it could be feasible that PDE might also be capable of hydrolysing AA, which would actually amount to its declactonization. Moffat et al. (1972) (27) had shown that AA- was one of those substances which inhibited the hydrolysis of c-AMP by PDE. From these facts Lewin (1976) (3) concluded that the inhibitory action of AA- on this hydrolysis was due to a competition for the active sites in PDE. His experiments proved that this in fact was the case. As PDE is activated by divalent cations Mg²⁺, Co²⁺, and Mn²⁺ and without these it remains inactive, stabilization of AA by
chelating agents like EDTA would render the enzyme (PDE) inactive with respect to AA. Further metabolism of delactonized AA, whether or not it is re-lactonized in tissues, or whether mega doses of AA could compensate this loss, all remain unknown so far.

Goldberg (1977) (28) reported that guanylate cyclase (GMP-cyclase) activity of guinea pig splenic cells was activated by oxidation, and by AA, DAA, fatty acid hydroperoxides and prostaglandin endoperoxides.

AA and electron transport. It was about the year 1931 that Szent Gyorgyi suggested that AA participates in electron transport reactions. Since then several reviews have appeared; recently Sebrell and Harris (1967) (18) and Weis (1975) (29) have given excellent account of the subject. This electron transport chain involving AA has been considered as operative in conjunction with the liver microsomal systems; GSH, several cytochromes, particularly cyt b₅, and NAD⁺/NADH have been implicated as the components of the chain. However, the complete knowledge in respect of this redox chain, and the role of AFR and DAA, if any, still remain to be established. The role of AA in -hydroxylation of dopamine to noradrenaline and that in the bioperteine and THF systems has been discussed (20).

AA and microsomal drug metabolism. This aspect has been reviewed recently (Zannoni et al., 1972, 1973, 1975, 1977; Zannoni et al., 1978) (30-35).
Drug metabolism is markedly influenced by factors such as age, sex, stress, presence of hormones or other drugs, environmental chemicals, and nutritional status of the animals; it is species-specific as well. The mechanism involved in hepatic drug metabolism is quite complex. It involves the hepatic microsomal electron transport system, which contains the hemeprotein cytochrome P-450, which is reduced by NADPH through a flavoprotein, usually referred to as NADPH-cytochrome P-450 reductase. Many metabolic reductive reactions utilize this system. For oxidative metabolic reactions, the reduced state of cytochrome P-450 (i.e., the Fe\textsuperscript{2+} form) incorporates one atom of oxygen into the drug substrate and the other oxygen atom into water, i.e., mixed function oxygenase-type drug hydroxylation reactions. In addition, there is a lipid component (phosphatidylcholine), which is associated with the electron transport and is an obligatory requirement for drug metabolism.

AA deficiency is known to result within 10-21 days in decreased metabolism of a variety of drugs. The decrease occurs in enzymes involved in O- and N-demethylation, nitro reduction, and hydroxylation, including that of cholesterol and other steroids, as well as in the individual components of the liver microsomal electron transport system, such as cytochrome P-450 and NADPH-cytochrome P-450 reductase. The decreased activities of the enzymes occurred only when the microsomal AA had reached 30% of its normal value. Recent studies by Sikie et al. (1976) (36) indicate
that both cytochrome P-450 and aminopyrine N-demethylase decreased to 60% of control values in liver microsomes at 21 and 25 days of deficiency. Repletion with AA resulted in complete recovery within 7 days; this proved beyond doubt that these enzyme activities were markedly dependent on the liver concentration of AA. The lipophilic ascorbyl palmitate was almost equally effective on a molar basis; D-iso-AA was much less active, though not totally inactive. In the AA-deficiency state, both the quality as well as the quantity of cytochrome P-450 are altered in liver microsomes. Both type-I and type-II substrate binding spectra are atypical, indicating an alteration in the alteration in the essential phospholipid moiety (phosphatidylcholine) needed for functioning cytochrome P-450.

Lipid peroxidation is known to be detrimental to drug metabolising activity; anti-oxidants, which inhibit lipid peroxidation, protect drug enzyme activities (Carpenter, 1972) (37). AA might be acting as antioxidant in these cases, as shown by three independent methods of assay, namely oxygen consumption, NADPH disappearance and MDA formation (Sato and Zannoni, 1976) (34). Furthermore, AA-deficient microsomes showed 18% decrease in the amount of phosphatidylcholine compared to the normal microsomes.

AA-deficient microsomes are also less stable to sonication, dialysis, and to treatment with Fe$^{2+}$ chelators ($\mu$-dipyridyl and o-phenanthroline). The decrease in the activities of cytochrome P-450 and O-demethylase with dialysis could be prevented by AA. AA could also protect
cytochrome P-450 from inhibition by Fe$^{2+}$ chelators. These studies suggest that there is an interaction between AA and cytochrome P-450 involving the heme iron.

Thus the requirement of AA in the metabolism of drugs, steroids and cholesterol in man may be important. It would be of clinical interest to determine if increased intake of AA in man would decrease the plasma half-life of the commonly used drugs and cholesterol.

**AA as a detoxificant.** AA is capable of detoxifying both the endogenous and the exogenous toxic substances. The reduction of aminochromes (which interfere with many enzymatic reactions, and the elimination of compounds which interfere with deactivation of neurotransmitters, (such as the action of AChE on ACh) are examples of the former substances. The latter type includes increased resistance by rats to lethal doses of barbiturates when given AA (Einhauser, 1939) (38), and similarly by man to the toxic effects of Pb$^{2+}$ (Marin, 1941) (39), and Hg$^{2+}$ (Marchmont-Robinson, 1941) (40).

Protection afforded by AA against several environmental chemicals and other cytotoxic agents like heavy metals (41), organochlorine pesticides (42), cytotoxicants like alloxan and 6-hydroxydopamine (43), carcinogens like chloretone (3) and N-nitrosamines (44,45), and cigarette smoke (46) have been reviewed.

Seregi (1978) (47) reported protective effect of AA against lipid peroxidation. Panigrahy (1977) (48) observed
absence of lathyrogenic effect of B-aminopropionitrile (BAPN, an osteo-lathyrogen) in male garden lizard tissue as revealed by collagen characteristics and AA content. Yunice (1977) (49) observed that AA and ZnSO₄ could afford protection against ethanol toxicity.

**Action of AA on enzymes.** The role of AA redox reactions and hydroxylation reactions has already been explained above. It has also been seen that, apart from these activities, AA affects the activities of several other enzymes, either activating them or inhibiting their action. The mechanism of these effects seems via the chelating effect of AA on the metal ions associated with, or necessary for, the enzymes for their action. Further, AA is also capable of influencing the de novo synthesis of certain enzymes, such as those involved in hydroxylations. These latter activities involve either the quaternary structure of enzymes, i.e. enzymatic aggregation/de-aggregation, or the contribution of AA towards the biosynthesis of enzyme components, as in the case of the incorporation of iron into heme molecule. To cite a few examples: AA has been found to adversely affect the activities of aminotransferases, as those involved in glutamate-glyoxylate, glutamate-oxaloacetate and alanine-glyoxylate systems (Srivastava and Sirohi, 1969) (50). Bossa (1974) (51) reported the effect of AA on aspartate aminotransferase. AA reduces the activity of catalase (H₂O₂ → H₂O+O₂) even at as low concentration as 2 x 10⁻⁶.
Likewise, the effect of AA on lipoprotein lipase (53), on hyaluronidase inhibition (54), and on enzymes of carbohydrate metabolism (55) has been reported.

**Mode of operation of AA in biological systems.** AA⁻ operates to exert its significant influence on biological activities in several ways (3):

- **Directly**, via its characteristic physicochemical characteristics, like its ionization, redox behaviour, free radical formation, chelation and hydroxylation reactions, H... bond formation, and lowering of interfacial tension. These characteristics, in their own respective ways, are of significance in bringing out its actions like its anti-histamine activity, and tissue repair action in wound healing, etc., by helping in the synthesis of new collagen.

- **Indirectly**, via its action on enzymes, cyclic nucleotides and hormone production.

**Enzymes:** AA can affect the level of enzyme activity. It can also influence enzyme biosynthesis. The observation that AA⁻ is present in calf-thymus and liver nuclei (56), and enhances RNA biosynthesis in wheat nuclei (57) point to its possible involvement in biosynthetic activities at the genetic level.

**Cyclic nucleotides:** It can affect the levels of c-AMP and c-GMP, which are mutually antagonistic, and thus balancing their concentrations and hence their activities in the cellular reactions.
Hormone production: It is involved in the production of adrenaline, noradrenaline and serotonin.

Recently, Hograbin et al. (1979)\(^{(58)}\) have proposed that another fundamental mechanism involved in the action of AA could be the regulation of prostaglandin \(E_1\)-formation; AA is known to stimulate its synthesis in human platelets.

Mode of action of DAA: DAA is the transport form of AA for entry into lipid membranes. High concentration of DAA have deleterious effect, because DAA is capable of binding GSH, thus lessening the availability of both the SH compounds as well as of DAA itself for the required redox reaction DAA to AA. Further degradation of DAA to DKGA reduces irreversibly the amount of AA which is considered lost to the body, along with the production of toxic \(C_2\)-acids like oxalic acid and glyoxylic acid, both of which are physiologically deleterious. DAA is diabetogenic \(^{(59)}\) and can induce cataracts \(^{(60)}\). It is a potent pharmacologic substance and cannot be given safely \(^{(61)}\). Banerjee (1977) \(^{(62)}\) has discussed the physiological role of DAA. Recently, Yano (1976) \(^{(63)}\) reported the formation of free radical products by the reaction of DAA with amino acids.

Action of AA on blood, various blood cells and lymphocytes.

Leucocytes: The concentration of ascorbate in leucocytes is one of the highest known, and is next only to the adrenals. The functions of leucocytes which involve AA\(^{-}\) include ascorbate...
transport, protein synthesis (synthesis of antibodies), phagocytosis, and formation of c-AMP.

**AA transport**: Leucocytes are known to converge on infected areas and on wound areas (3); recently it has also been observed (64) that they move towards infarcted coronary sites and deposit their AA- there. The major function of leucocytes, which in fact is more than that of blood or lymph, thus seems to be to transport large amounts of AA to damaged tissues, to assist in the biosynthesis of protein which is to form part of the new tissue. AA is needed for the synthesis of collagen as well, which is required for the repair of the damaged tissue. AA enhances chemotactic response and microtubule assembly in human leucocytes (65).

**Synthesis of Y-globulins**: Leucocytes are involved in the synthesis of antibodies (γ-globulins). These antibodies are known to contain a large number of -S-S-bonds in comparison with most other proteins; the function of these disulphide bonds is to bridge the light and the heavy chains. The primary polypeptide chains actually contain only cysteine residues, which subsequently are oxidized by DAA to -S-S-bonds. Thus high concentration of leucocytes helps to attain the redox activity needed for the synthesis of the γ-globulins.

**Phagocytosis**: Phagocytes (also called polymorphonuclear leucocytes, PMN) have been known since long to be one of the chemical mechanisms of the body defence against bacterial attack. They use their lysosomes for the ingestion and
disintegration (destruction) of foreign bodies (bacteria), and make available their antibodies for attacking the bacterial invaders. This activity has been shown to be associated with adequate presence of AA in the white blood cells. Persons with low leucocytal AA show low phagocyte activity (66, 67), accompanied by lowered fragility (68). This aspect has been reviewed recently (69).

AA in relation to neutrophils, lymphocytes, granulocytes & erythrocytes: In order to determine whether tissue differences in DAA-reducing activities correlate with differences in sensitivity to ionizing radiation and oxidant stresses, Stankova et al. (1975) (70) measured DAA uptake and reduction during incubation of neutrophils, lymphocytes and erythrocytes with DAA. They found that DAA reduction is only indirectly coupled to NADPH oxidation in PMN and lymphocytes, and is closely coupled in RBC; RBC is known to contain GSH-dependent DAA reducing activity (71). They concluded that DAA reduction differs qualitatively among mature human RBC, PMN and lymphocytes, and further, that the DAA reducing capacity might be an important part of cellular mechanisms for inactivating oxidants, and free radicals, especially lipid peroxides, and therefore may be one determinant of radiosensitivity.

Mengel and Greene (1976) (75) reported the effect of AA (5 gm/day for 3-5 days) on erythrocytes obtained from healthy human volunteers; they observed a significant increase in the lytic sensitivity of the erythrocytes
to H2O2, caused probably by the oxidant stress. In contrast, Winterbourn (1979) (76) and Williamson and Winterbourn (1980) (77) have reported a significant protection afforded by AA against acetylphenylhydrazine-induced Heinz body formation in normal and G-6-PD-deficient erythrocytes. Germano et al. (1978) (78) observed a synergism between AA and -mercapto-propionylglycine in protecting erythrocytes against poisoning by acetyl-hydrazine.

Smith et al. (1975)(72) reported that exposure of human granulocytes to AA in vitro resulted in stimulation of the HMP shunt pathway (73), increase in random migration and chemotaxis, and inhibition of aldehyde formation and iodination of protein; the bactericidal and the phagocytic capacity of these cells remains unaltered (74). The ingestion of massive doses of AA by man may influence the function of human granulocytes, and could possibly prevent viral infection in man.

In summary (79), it is emphasized that the size of the body pool of AA is related to plasma AA− values. In contrast, leucocytal AA concentrations are representative of tissue AA saturation. Aspirin, tetracycline, narcotics and most other drugs desaturate leucocytal AA; they also increase urinary excretion of AA in normal man (79).

**c-AMP formation:** The adenyl cyclase of leucocytes is stimulated by catecholamines to potentiate c-AMP formation (80). AA also inhibits the hydrolytic break down of c-AMP by PDE. It is thus feasible that one of the functions of
high AA level of leucocytes is the upkeep of sufficiently high level of c-AMP and its transport to the required site.

Membrane transport of AA. This aspect has recently been reviewed (81). It is clear that tissue concentration of AA\(^-\) (some 5 to 40 mg/100 gm wet tissue) is several times more than that present in the blood, which is of the order of 0.8 to 2.0 mg/100 ml plasma. In view of the fact that, after absorption, the dietary AA\(^-\) finds its way into the blood from where it is transported to other tissues, where its concentration is much higher than that in the blood, it is concluded that active transport is responsible for the higher tissue concentration. AA\(^-\) is transported from the plasma to the nonmotile erythrocytes, and by the motile leucocytes to other tissues. The transport of AA is partly as unattached (free) and partly as attached (bound). Being ionic, AA penetrates the erythrocyte membrane very slowly in comparison with DAA, which is non-ionic and more lipid-like; DAA is thus the transport form of AA. Hughes and Maton (1968) (82) explained the greater penetration of DAA, than AA, because of this phenomenon (i.e. more lipid-like character); it could also be explained on the basis that DAA possesses a much greater power to reduce the interfacial tension, and therefore can correspondingly increase membrane permeability (3). The free- AA\(^-\) in the blood is propelled into the lymph via the pores in the membrane of the walls of the blood vessels (3). Free AA\(^-\) being a relatively small molecule, can pass readily with the fluid through the pores into the lymphatic space; it
can also be carried by the leucocytes as they penetrate into the inter-cellular spaces of the blood vessel walls. In this manner \( \text{AA}^- \) moves out from the blood into the lymph spaces at the arterial end and re-enters the vascular system at the venous end (3).

The bound form of \( \text{AA} \) combined with blood serum proteins has recently been discussed (83). Sharma (1978) (84) has reported on the binding of \( \text{AA} \) to ovarian tissue proteins.

**Effect of insulin and certain sugars on the membrane transport of \( \text{AA} \):** Ralli and Sherry (1939) (85,86) observed a decrease in the level of plasma \( \text{AA}^- \) in dogs after insulin administration. This effect was confirmed in diabetic animals (87) and in human beings (88). Since neither the urinary \( \text{AA}^- \) nor metabolic products were increased by the insulin treatment, it was assumed that this effect was the result of an accelerated tissue uptake (81). Sherry and Ralli (1948) (86) reported that both *in vitro* as well as *in vivo* insulin caused a transfer of \( \text{AA}^- \) into leucocytes, but not into erythrocytes. Hughes and Maton (1968) (82) reported that the uptake of \( \text{AA}^- \) by erythrocytes was not influenced by enzyme inhibition. McIlwain *et al.* (1956) (89) suggested that \( \text{AA}^- \) uptake in brain was energy assisted. Sharma *et al.* (1963) (90) showed that the uptake of \( \text{AA}^- \) in brain cortex and adrenal cortex of guinea pigs was an energy dependant process, and that ouabain and 2:4-dinitrophenol suppress \( \text{AA}^- \) uptake into brain cortex; further that ACTH inhibited \( \text{AA}^- \) uptake by brain cortex. Mann and Newton (1975) (81) reported that D-glucose inhibited the transport of DAA into human erythrocytes, a non-insulin-dependent tissue.
They further found that several other sugars (D-mannose, D-xylose, D-galactose, 3-C-methyl ether of D-glucose) do the same, thereby suggesting that DAA, and D-glucose share a common transport mechanism. Some other sugars (L-xylose, L-arabinose, L-sorbose, D-DAA and 2-deoxyribose) had only a weak effect. D-fructose, D-ribose and D-arabinose, on the other hand, increased slightly the transport of DAA into erythrocyte membrane. Likewise, Cu\(^{2+}\) facilitated this transport (ref. 3, p. 120). The molecular mechanisms of active transport of AA\(^{-}\) into leucocytes and other tissues, and those of the release of the AA\(^{-}\) when required, are not yet known. The energy required for the active transport of DAA across the erythrocyte membrane seems to be financed by the ability of the oxidation of AA\(^{-}\) (ref. 3, p. 121). It thus seems that the method of transport of AA\(^{-}\) into erythrocytes has been selected by evolution not only to enable passage through permeable lipid membranes but also to provide the required energy for the transport by the AA - DAA oxidation (ref. 3, p. 121). That the intracellular availability of DAA is impaired in certain tissues by either hyperglycemia or lack of insulin suggests that diabetic microangiopathy, the main complication of human diabetes, may be a consequence of local AA\(^{-}\) deficiency (81). Oxidized isoascorbic acid lacks vitamin C activity, but it has a definite, though weak, effect on the transport of AA through erythrocyte membrane; it is of significance as isoascorbic acid is widely used as a food additive. It could become a competitor of AA\(^{-}\) for transport (81).
AA in relation to cyclic nucleotides. AA is known to increase the levels of cyclic nucleotides, c-AMP and c-GMP; it helps both in the formation of these nucleotides, as well as in the inhibition of their hydrolytic breakdown to 5'-AMP and 5'-GMP. c-AMP and c-GMP are mutually antagonistic (3).

Several hormonal activities utilize these nucleotides as mediators (second messengers), such as those of adrenaline, glucagon, ACTH, LH, TSH, vasopressin, and oxytocin. Thus it (c-AMP) has a wide extent of influence, though it is tissue-specific. The type of influence which it exerts, however, is determined by the enzyme profile of the cell in which the action takes place. For instance, in the liver, c-AMP inhibits the conversion of acetate to cholesterol (Berthet, 1960) (91), but in several other tissues such as adrenal cortex, corpus leuteum, and ovaries and testes, it stimulates the conversion of cholesterol to pregnenolone. Thus, increased levels of AA should favour lower cholesterol levels. Several investigators have, indeed, found reduced levels of cholesterol after giving mega doses of AA (ref.3, p.93). In fact, the AA-induced increase in the levels of these nucleotides reflects that AA indirectly influences all such physiological actions.

AA and insulin action: The pancreas is known to have a remarkably high level of AA⁻. The distribution between the α- and the β-cells, however, is not yet known (3). The rat pancreas also contains a high level of c-AMP (Turtle and
Kipins, 1967) (92); c-AMP is known to enhance insulin release from pancreatic islets (ref. 3, p. 94). AA also enhances the synthesis of pro-insulin in the pancreatic B-cells, and thus, indirectly, of insulin.

High intake of AA parallels insulin activity in tending to result in lowering blood sugar levels (ref. 3, p. 94). It has been shown that i/v administration of AA (0.3 to 1.2 gm) results in a significant lowering of blood sugar curves in normal and in diabetic patients (ref. 3, p. 94). Pfleger and Scholl (1937) (93) and Bartelheimer (1939) (94) observed that high intake of AA decreased the required insulin dose in several diabetic patients. Stone (1972) (95) reported that mega doses of AA reduced the amount of insulin required to control diabetes. Diee and Daniel (1973) (96) reported that each gm of ingested AA decreased the insulin dose by 2 I.U. in a juvenile onset diabetic. Scarlet et al. (1976) (97 a,b) showed that AA had no effect on glucose tolerance in non-diabetic subjects. Vijay Kumar and Vasudevan (1980) (98) and Kumari et al. (1980) (99) reported that AA (3 gms/day for 7 days) decreased glucose tolerance in diabetic patients, though it had no such effect in the normal controls. The effect of high doses of AA on the carbohydrate and lipid metabolism in diabetics using insulin (100), and the role of AA in relation to diabetes mellitus (101) have been reported.

AA and protein and carbohydrate metabolism: AA enhances protein synthesis, especially of proteins which contain
-S-S- bonds (3). In AA deficiency, as in scurvy, there is reduced protein metabolism, enhanced protein catabolism and an imbalance of serum proteins. As regards carbohydrates, AA plays an important role; this fact has been known since 1942 (102). Later work in that era concentrated attention on carbohydrate metabolism in AA deficiency state (scurvy) (Banerjee et al., 1947-1962) (103-105), using scorbutic guinea pigs. The abnormal glucose tolerance in scorbutic animals could be corrected by injection of insulin (104). Non-diabetic obese subjects showed decreased glucose tolerance curve (106).

DAA and blood sugar levels: Patterson (1950, 1951) (59,60) had observed that injection of large doses of DAA in rats led to degranulation of B-cells of pancreatic islets, accompanied by hyperglycemia. Nandi et al. (1973) (107) reported that large doses of DAA (30-60 mg/100 gm body weight/day) were toxic to guinea pigs fed high cereal diet; the DAA level in these animals increased markedly in blood, urine and liver, along with a concomitant increase in the blood sugar level. Similar results (108) were observed when guinea pigs were given AA in place of DAA. With 100 mg AA/100 gm body weight/day for 15 days, the DAA level increased from 0.01 mg% to 1.2 mg%, and the 2-hr post-prandial sugar levels increased to 0.3 mg% and 130 mg%. The increase in blood sugar was found dependent on the increase in the blood DAA level. When AA administration was discontinued, DAA decreased progressively, and along with it there was a
corresponding decrease in the blood sugar level. When the experiment was repeated with guinea pigs fed on wheat diet fortified with casein (c. 15%) the blood AA levels (1.2 mg%) were found similar to those obtained using high cereal diet, but without any increase in DAA and the sugar levels. The same dose of AA, when given to rats for 30 days, irrespective of the high cereal diet or casein-fortified wheat diet, caused no increase in DAA, nor in the sugar level. Feeding of large doses of AA to normal human volunteers fed high cereal diet led to high blood DAA levels. The increase with 2 and 4 gm of AA daily was: AA from 0.55-0.9 mg% to 1.0-1.1 mg%, DAA from 0.1-0.3 mg% to 1.2-2.1 mg%, and the 2 hr postprandial blood sugar from 80-100 mg% to 100-140 mg%.

These facts clearly bring out the differences between the species which can and those which cannot biosynthesize their own requirement of AA.

Blood DAA levels in diabetes mellitus: The facts ascertained above, that accumulation of DAA in blood leads to hyperglycemia, led Chatterjee et al. (1975) (108) to conclude that human diabetes was possibly caused by some metabolic disorder leading to the accumulation of DAA in blood. They found that, in contrast to normal individuals, all the established diabetic patients examined by them had markedly high blood DAA levels. In the guinea pig they had found a correlation between the blood DAA level and the blood sugar level; such a correlation was not found by them in diabetic patients, possibly because the latter were under treatment.
with antidiabetic drugs (insulin and sulphonylureas). In most patients the blood sugar levels had come down after the drug treatment, but the blood DAA levels had still remained high. In these cases, discontinuation of the drug treatment for a considerable time (4-6 months) brought back the hyperglycemic state. It was worthy of note that in none of the established diabetic patients there was a significant fall in the DAA level, irrespective of the intake of the antidiabetic drugs, even for long periods up to 18 months. Thus the persistent high blood DAA level appeared to be specific to diabetes mellitus. Although some moderately high blood DAA levels were found associated with several other disease conditions, these higher levels returned to normal during convalescence (Stone, 1974; ref. 95). These workers further found that the high DAA levels were possibly due to the inhibition of 2,3-DKGA-decarboxylase in diabetes. According to Lewin (ref. 3, p. 115), the high levels of DAA in blood and other tissues resulting from feeding of guinea pigs with cereal diet + AA suggests blockage of the mechanism by which AA is regenerated, resulting in DAA breaking down to DKGA and its further fragmentation. It seemed that DAA did not possibly act as an antagonist to the circulating insulin, because in the human volunteers who received 2-4 gm of AA/day there was marked increase in the blood DAA level without significant increase in the blood sugar level, commensurate with the high DAA levels. Histological studies of pancreas of guinea pigs fed high cereal diet with large doses of AA (100 mg/100 gm body weight for 16 days), indicated...
that with a blood DAA level of 1.6 ± 0.2 mg% there was indication of degranulation of B-cells (108), similar to the findings of Patterson cited above. Further, DAA is known to react with sulphur amino acids, and in general also DAA degraded amino acids (cf. alloxan). Since sulphur amino acids were essential for insulin synthesis, DAA could be considered as inhibiting insulin synthesis. Both these findings would ultimately lead to inhibition of protein synthesis. Lack of protein metabolism is in fact one of the features of diabetes mellitus. Exact mechanism of action of DAA, however, still remains to be worked out. The fact that the clinical manifestations of diabetes appear long after the onset of the disease, is supported by the fact that in some patients the blood DAA level is found to be high without high blood sugar level, and in most of such cases blood sugar levels too become high after some 6 months. In view of such observations, they (108) concluded that diabetes mellitus could possibly be controlled by preventing formation of DAA and dissipation of the accumulated DAA in the blood. The role of casein type diet could possibly afford some clue in this respect.

Certain other roles of AA. Several other roles have been ascribed to AA; these are being summed up because they do not come under the direct perview of the present investigation. Some of the more important of these are: anti-stress function of AA, and its role(s) in wound healing, skin disorders, ageing, anti-viral defence mechanism, common cold, cancer, cardiovascular
defects, calcification and fatty acid deposition, and cholesterol deposition, etc. Besides, possible toxic effects and abuse of AA have also been discussed. Ample references have been given in references No. 1 and 3. Some relevant additional references in these respects have been appended (109-133).

ii) Cholesterol.

Cholesterol is present in all tissues, and every cell can synthesize it; its major function is in membranes. The normal metabolism of cholesterol and its regulation have been extensively reviewed (1, 134-137). The turnover of cholesterol and its levels in blood and tissues have been shown to be the result of several processes, often mutually bound by feedback mechanisms. Among these steps, the absorption of exogenous cholesterol, its secretion into bile and gastrointestinal tract, and its catabolism to bile acids (by hydroxylation at C$_7$ and C$_{12}$, and oxidation at C$_{24}^{-}-$C$_{26}$) and to other steroids (by oxidation at C$_{17}^{-}-$C$_{20}$ and C$_{20}^{-}-$C$_{22}$) have been dealt with comprehensively (138-148a,b).

Because of relevance to our present study, the review given below has been restricted to blood cholesterol only. The whole of this material (p.31-39), including Tables 1-8, has been compiled from the monograph "Cholesterol" by Sabine (ref.1).

**Blood cholesterol**: An average adult human body (70 kg) contains c. 145 gm (0.2%) cholesterol, of which 10.8 gm (7.5%) is in whole blood, or 7.5 gm (5.5%) in blood plasma.
Blood vs. tissue cholesterol levels are:

Table -I. Blood vs. tissue cholesterol levels in man

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>gm</th>
<th>% of total body cholesterol</th>
<th>% concentration in the tissue</th>
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<td>Blood (whole)</td>
<td>10.8</td>
<td>7.5</td>
<td>0.2</td>
</tr>
<tr>
<td>(plasma)</td>
<td>7.5</td>
<td>5.5</td>
<td>0.14</td>
</tr>
<tr>
<td>Brain</td>
<td>32.0</td>
<td>22.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Connective and adipose tissues + body fluids</td>
<td>31.3</td>
<td>22.0</td>
<td>0.25</td>
</tr>
<tr>
<td>Muscle</td>
<td>30.0</td>
<td>21.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Skin</td>
<td>16.0</td>
<td>11.0</td>
<td>0.3-0.7</td>
</tr>
<tr>
<td>Liver</td>
<td>5.1</td>
<td>4.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Alimentary tract</td>
<td>3.8</td>
<td>3.0</td>
<td>0.15</td>
</tr>
<tr>
<td>Lungs</td>
<td>1.9</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Adrenals</td>
<td>1.2</td>
<td>1</td>
<td>2.6-15.0</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0.9</td>
<td>0.4</td>
<td>0.25-0.34</td>
</tr>
<tr>
<td>Heart</td>
<td>0.6</td>
<td>0.4</td>
<td>0.10-0.13</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.5</td>
<td>0.35</td>
<td>0.16-0.34</td>
</tr>
</tbody>
</table>

Thus, amongst the tissues, the highest amount is present in adrenals, and then in brain and skin.

Plasma cholesterol: There is a significant amount of cholesterol in the blood, more than that of glucose.
Table 2. Blood cholesterol content vs. other blood components in man.

<table>
<thead>
<tr>
<th>Component</th>
<th>mg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total lipids</td>
<td>500 - 600</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>125 - 280</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>165</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>145 - 200</td>
</tr>
<tr>
<td>Free fatty acids (FFA)</td>
<td>5 - 15</td>
</tr>
<tr>
<td>Glucose</td>
<td>60 - 100</td>
</tr>
<tr>
<td>Protein (total)</td>
<td>6000 - 8000</td>
</tr>
</tbody>
</table>

The amount of cholesterol in human blood in comparison with that in other extracellular fluids, the distribution of cholesterol in human whole blood and plasma, and some characteristics of the major classes of human plasma lipoproteins have been compiled below (Table 3-5):

Jackson et al. (1976) (149) have reviewed the structure and metabolism of lipoproteins. Bates et al. (1977) (150) have discussed the relationship between HDL-cholesterol and AA; the significance of LDL- and HDL-cholesterol in the etiology of atherosclerosis has been detailed by several investigators (5, 10, 11-13).
Table 3. Cholesterol content of human blood vs. other extracellular fluids.

<table>
<thead>
<tr>
<th>Fluid</th>
<th>Cholesterol (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>150-200</td>
</tr>
<tr>
<td>Bile</td>
<td>390</td>
</tr>
<tr>
<td>Seminal plasma</td>
<td>80</td>
</tr>
<tr>
<td>Lymph</td>
<td>25</td>
</tr>
<tr>
<td>Prostate fluid</td>
<td>20</td>
</tr>
<tr>
<td>Milk</td>
<td>20</td>
</tr>
<tr>
<td>Synovial fluid</td>
<td>7</td>
</tr>
<tr>
<td>CSF</td>
<td>0.44</td>
</tr>
<tr>
<td>Saliva</td>
<td>2.9</td>
</tr>
<tr>
<td>Urine</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Table 4. Distribution of cholesterol in human whole blood and plasma.

<table>
<thead>
<tr>
<th>Plasma lipoproteins</th>
<th>Whole blood</th>
<th>Blood plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/100 ml</td>
<td>% of blood</td>
</tr>
<tr>
<td>Total</td>
<td>120.9</td>
<td>68.0</td>
</tr>
<tr>
<td>VLDL</td>
<td>9.4</td>
<td>5.0</td>
</tr>
<tr>
<td>LDL</td>
<td>81.3</td>
<td>46.0</td>
</tr>
<tr>
<td>HDL</td>
<td>30.2</td>
<td>17.0</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>56.3</td>
<td>32.0</td>
</tr>
</tbody>
</table>
Table- 5. Some characteristics of the major classes of human plasma lipoproteins.

<table>
<thead>
<tr>
<th></th>
<th>Chylomicrons</th>
<th>VLDL</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (gm/ml,hydrated)</td>
<td>0.95</td>
<td>0.98-1.03</td>
<td>1.03-1.09</td>
<td>1.1-1.2</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>$10^9-10^{10}$</td>
<td>$5-10^6$</td>
<td>$2 \times 10^6$</td>
<td>$1.4 \times 10^6$</td>
</tr>
<tr>
<td>Lipids (per cent)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>96-100</td>
<td>91</td>
<td>79</td>
<td>42</td>
</tr>
<tr>
<td>Cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>10.5</td>
<td>17</td>
<td>41</td>
<td>11</td>
</tr>
<tr>
<td>Free</td>
<td>5</td>
<td>6</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Ester</td>
<td>5.5</td>
<td>11</td>
<td>33</td>
<td>8</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>78</td>
<td>55</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>7.5</td>
<td>19</td>
<td>28</td>
<td>25</td>
</tr>
<tr>
<td>FFA</td>
<td>0-4</td>
<td>traces</td>
<td>traces</td>
<td>traces</td>
</tr>
<tr>
<td>Proteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Content (per cent)</td>
<td>4</td>
<td>8</td>
<td>21</td>
<td>58</td>
</tr>
<tr>
<td>Mobility</td>
<td>pre-β</td>
<td>pre-β</td>
<td>β</td>
<td>α</td>
</tr>
</tbody>
</table>

In the blood, cholesterol is present in all the three forms, namely, as free-, and ester-cholesterol, and as cholesterol-3-sulphate. The proportion of free- and esterified-cholesterol in human plasma vs. that in other tissues is given in table 6. Cholesterol in the blood is present wholly associated with protein and membrane components.
Relevant details of the cholesterol content of different compartments is given below:

**Leucocytes.** Leucocytes, in contrast with erythrocytes, actively synthesize lipids, including cholesterol. The total lipid content of normal human leucocytes is c. 5% (cf. only 0.5% of the erythrocytes). Of this lipid, 10-12% is cholesterol, which is almost wholly unesterified. Polymorphonuclear leucocytes contain more cholesterol than lymphocytes.
**Erythrocytes.** Erythrocytes, unlike leucocytes, cannot synthesize their own cholesterol, at least not from acetate. Erythrocytes contain some 32% of the total blood cholesterol; of the total lipids present in erythrocytes, cholesterol accounts for about 25%; virtually the whole of this cholesterol is unesterified, and is localized in the erythrocyte membrane, with possibly a higher concentration around its periphery. For man, the average total erythrocyte cholesterol is c. 1.31 mg/ml of packed cells, or $1.23 \times 10^{-13}$ gm/cell (on the basis of 43 ml packed cells/100 ml blood); this amount does not vary much between species (man 1.31: rat 1.43, rabbit 1.32, guinea pig 1.45, goat 1.02, sheep 1.24, pig 1.15, cow 1.23, cat 1.62, dog 1.43).

**Erythrocyte membrane.** Jamieson and Greenwalt (1969) (151) have reviewed the structure and function of the red cell membrane. The cholesterol content of rat erythrocyte membrane is given here in comparison with that in the other rat cellular membranes in order to emphasize the variability of their lipid components (Table 7).

Cholesterol is known to be essential component of the erythrocyte membrane with respect to its functional activity, particularly for its permeability, the activity of the membrane-bound enzymes, protein synthesis, and the membrane receptors, etc. These aspects have been reviewed in detail (1, 152, 153); only a very brief summary would be included here, just to emphasize their importance and relevance to the present study. For instance, the cholesterol content of
erythrocyte membrane can be altered, both *in vivo* as well as *in vitro*. A significant fact about red cell cholesterol

Table-7. Cholesterol content of various cellular membranes in the rat (ref 1).

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Cholesterol (mg/100 g protein)</th>
<th>Phospholipid (mg/g protein)</th>
<th>Chol : P/L (molar ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte ghosts</td>
<td>7</td>
<td>16</td>
<td>0.89</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>plasma membrane</td>
<td>6</td>
<td>42</td>
<td>0.26</td>
</tr>
<tr>
<td>mitochondria</td>
<td>2</td>
<td>28</td>
<td>0.11</td>
</tr>
<tr>
<td>microsomes</td>
<td>9</td>
<td>48</td>
<td>0.17</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>myelin</td>
<td>62</td>
<td>92</td>
<td>1.32</td>
</tr>
<tr>
<td>mitochondria</td>
<td>26</td>
<td>46</td>
<td>0.51</td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mitochondria</td>
<td>1</td>
<td>11</td>
<td>0.15</td>
</tr>
<tr>
<td>sarcolemma</td>
<td>3</td>
<td>28</td>
<td>0.24</td>
</tr>
<tr>
<td>Intestine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mitochondria</td>
<td>4</td>
<td>14</td>
<td>0.60</td>
</tr>
<tr>
<td>microvilli</td>
<td>3</td>
<td>11</td>
<td>0.46</td>
</tr>
</tbody>
</table>

is that virtually the whole of it can exchange between the erythrocyte membrane and the plasma (both *in vivo* and *in vitro*), and *in vitro* between the membrane and the isolated lipoproteins (1, 154). This phenomenon is intimately related to the architecture of the membrane and the activity of LCAT; where the activity of this enzyme is low, there is
an increase red cell free cholesterol; incubation of normal blood in vitro results in depletion of erythrocyte cholesterol and increased osmotic fragility. Equally important is the presence of the erythrocyte membrane cholesterol ester hydrolase which keeps the whole of the cholesterol unesterified.

Fluidity of the membrane, which is intimately linked with the membrane phospholipids (PL), and their acyl chains, depends primarily upon the nature of the constituent PL-fatty acids, and the amount of the membrane cholesterol. Increase in the amount of the latter decreases the membrane permeability. The physicochemical state of the membrane lipid influences the activity of the membrane bound enzymes and receptors. Cholesterol also plays an important role in protein synthesis (and thus enzyme synthesis), at the level of the DNA action. A number of lipids, including cholesterol, have a destabilizing effect at high concentration and a stabilizing effect at low concentration on the DNA helix. Free and esterified cholesterol play specific roles in several stages of protein synthesis, for instance, at the levels of aminoacyl-transfer RNA synthetase, the binding of aminoacyl-t-RNA to ribosomes, and peptide chain elongation. Cholesterol sulphate also stabilizes the erythrocyte membrane.

Cholesterol turnover and excretion. The role of LCAT, which is the major source of cholesterol esters in plasma, has been reviewed (ref. 1, chapter 9). Cholesterol turnover in man, rat and rabbit is 10-20, 95-165, and 100-150 mg/kg body weight/
The main route of excretion of cholesterol is via oxidation to bile acids and fecal excretion; only a small part is excreted into milk and urine. The excretion of cholesterol in bile is: man 390, pig 170, and rabbit 6 mg/100 ml. Cholesterol is excreted in urine only to a very small extent—about 1 mg/24 hr; it increases during pregnancy, more so in the post-partum period in which even 70 mg/day have been recorded. The excretion is usually as sulphate and glucuronide. The fecal excretion is as coprostanol and coprostenone + bile acids. The significance of AA-2-sulphate in this context has been discussed (155-158).

The mammary gland is capable of synthesizing cholesterol; the amount of cholesterol in secreted milk varies with species (Table 8). Milk cholesterol is predominantly associated with FGM.

Table 8: Human milk cholesterol in comparison with that in milk from other species.

<table>
<thead>
<tr>
<th>Species</th>
<th>mg/100 ml</th>
<th>Species</th>
<th>mg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>20</td>
<td>Buffalo</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>25-65</td>
<td>Goat</td>
<td>13</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>42</td>
<td>Sheep</td>
<td>23</td>
</tr>
<tr>
<td>Rabbit</td>
<td>86</td>
<td>Pig</td>
<td>145</td>
</tr>
<tr>
<td>Cow</td>
<td>15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
iii) AA-cholesterol relationship.

The AA-cholesterol relationship has been investigated by several investigating teams during the last over three decades. Amongst this work, that of Ginter and his group carried out during 1965-upto-date stands out in particular. Some of these aspects, which were relevant to the present study, have been reviewed below briefly; greater details have been given in the comprehensive review of Turley et al. (1976) (159), which forms the basis of the following discussion.

A definite relationship between cholesterol metabolism and the amount of AA ingested by animals and man has been sought for almost about half a century. Most of the experiments have been done on guinea pigs, which like man, is unable to synthesize its requirement of AA; these animals were either deficient or marginally deficient in AA. Although studies with rabbits have generally supported the evidence for a role of AA in cholesterol metabolism and atherosclerosis, its usefulness as a model for future work is limited; the same may apply to rat and hen, which, along with rabbit, are capable of synthesizing their own requirements of AA. The evidence accumulated from these studies indicates that AA is definitely involved in the regulation of cholesterol metabolism, to some extent at the synthesis, but more so at the level of its conversion to bile acids, and to other steroids. Hence AA deficiency at the sub-clinical level could initiate a number of abnormalities associated with cholesterol
metabolism, such as hypercholesterolemia, and athero-
sclerotic, cardiac, and thrombotic lesions. The seemingly
beneficial effects of high AA consumption on the course of
these lesions could be due to its hypocholesterolemic effect
mediated through an increased rate of conversion to bile acids,
as well as possibly increased excretion of cholesterol in the
form of neutral steroids + bile acids + cholesterol-3-SO₄
(formed through AA-2-SO₄, ref. 156, 157).

AA vs. plasma and tissue cholesterol and the pathogenesis of
atherosclerosis. The significance of dietary inadequacy
of AA in the etiology of hypercholesterolemia and athero-
sclerosis first became apparent from the clinical studies
in Russia; Myasnikova (160) showed in 1947 that serum
cholesterol concentration could be lowered in hypercholes-
terolemic patients by the administration of AA. Myasnikov
and coworkers, during the period 1950-65, showed that AA
could inhibit the development of experimental hypercholes-
terolemia and atherosclerosis in rabbits (161-165). These
findings were confirmed in clinical trials (166,167); Mendoza
(1955) (168) observed that i.v. injections of AA produced
a temporary increase in serum cholesterol in 1-2 hr which
was followed by a decrease in its level. Sedov (1956) (166)
and Fedorova (1960) (167), from their clinical investigations
of atherosclerotic patients, observed a significant decrease,
up to 30%, in blood cholesterol level on daily i.v. adminis-
tration of 0.5 to 1.0 gm AA for 10-30 days; Gandzha et al.
(1961) (168a) observed similar results with 0.5 gm AA
administered orally 3 times a day, the decrease in the cholesterol level being 35-40%, is further supported by recent work (208-10).

Lowery et al. (1952) (169), Becher et al. (1953), (170), Booker et al. (1957) (171), Anderson (1958) (144), Bavina (1958) (171), Beher (1963) (138), Bronte-Stewart et al. (1963) (147), Samuel and Shalchi (1964) (172), Sokoloff et al. (1967) (173), Elwood et al. (1970) (174), Spittle (1971, 1975) (175), Nandi et al. (1973) (107), Krumdieck and Butterworth (1974) (176), Weight et al. (1974) (145), Kotze (1975) (177), and Peterson et al. (1975) (178) obtained similar results. Klevay (1976) (179) reported that AA could cause hypercholesterolemia in baboons due to its inhibition of absorption of copper from the intestinal tract; this would result in a higher ratio of ingested Zn to Cu ratio, which caused hypercholesterolemia.

Since 1965 up to date, Ginter's group (from Bratislava, Czechoslovakia) has been communicating a large number of papers from their comprehensive studies of these aspects (184-207); they too have supported these results. Their studies have procured evidence of a definite involvement of AA in cholesterol metabolism from the changes that occur in plasma and tissue cholesterol levels during dietary AA deficiency, and by the hypocholesterolemic action of AA in hypercholesterolemic animals and human subjects; and from changes in tissue AA levels that occur in cholesterol fed animals. Cholesterol feeding thus increases AA requirements of guinea pigs, and, when prolonged, reduces the tissue AA levels; there is a significant negative correlation between
the concentration of cholesterol and AA in liver, adrenal and small intestines, but not in plasma. Cholesterol feeding in rabbits and rats, in contrast, was found to result in increased accumulation of AA in tissues, and increased excretion in the urine. This indicates an increased AA biosynthesis.

Turley et al. (1976) (159) have discussed in detail the effect of AA on plasma cholesterol and triglycerides, and on the integrity of the vascular wall; the latter effect is because of the effect of AA on collagen formation, via hydroxylation of proline. Likewise, 7-α-hydroxylation to bile acids, and other similar reactions become important.

Recently Vijay Kumar and Vasudevan (1980) (98) and Kumari et al. (1980) (99) reported the effect of AA on glucose tolerance and serum cholesterol in normal and diabetic patients.

iv. Propane-1,2-diol (Propylene glycol, PG).

Propane-1,2-diol (PG) has been known since the middle of the last century; from around 1932, it has been widely used as a solvent and cosolvent, stabilizer, preservative, and for many other diverse purposes. Although PG had hitherto been regarded as fairly safe and inert solubilizer, present investigations have cast doubt on this assumption, as several adverse biological effects have recently come to light. These aspects have been briefly reviewed below.
Chemical aspects of PG. PG (C₃H₆O₂, mol. wt. 76.1) is a colourless, odourless, tasteless, viscous liquid, b.p. 187.3°, sp. gravity 1.0363 and viscosity (cps, 20°) 60.5. It is completely miscible with water, alcohols, acetone, CHCl₃ and many other solvents; it is a better solvent than glycerol for many drugs, essential oils, vitamins, resins and dyes etc. Primarily because of its low order of inherent toxicity, PG had become an important solvent for pharmaceuticals, stabilizer for vitamins, additive for foods, beverages, syrups, and an ingredient of cosmetics, tobacco, flavours and colours. It has been used as a preservative as it retards the growth of many molds and fungi. It has also been employed as emulsifier, softening agent, emollient, as a coolant in refrigerators and in antifreeze solutions, as a lubricant, as a solvent for antioxidants, and as a humectant to improve the shelf life of packaged foods, etc.

These aspects have been reviewed (210-213).

Biochemical aspects of PG. PG and its 1-phosphate occur, in small amounts, in mammalian tissue; Linberg (1946) (quoted in ref. 214) extracted this glycol from sea urchin eggs and beef brain, and Rodney (1954) (215,216) isolated the naturally occurring phosphorylated glycol from rat liver. Rodney (1954a) (215) determined, using isotope dilution technique, that the level of PG-1-phosphate represented 1-2% of the total acid-soluble phosphorus in the rat liver.

PG is, apparently, almost non-toxic in animal tests;
the single oral dose LD₅₀ for rats is more than 25 mg/kg body weight (ref. 219). It is non-irritant to the skin. It is one of the few examples of xenobiotics (foreign compounds) which are metabolized by the normal metabolic pathways. Biochemical aspects of PG have been reviewed; the earlier literature has been reviewed by Lehman and Flury (1943) (217) and Rae (1951) (211); relatively recent literature has been provided by Opitz (1958) (218), William (19) (219), and by Auerbach Association (1978) (220).

Metabolism of PG:

Catabolism. PG, being lactyl alcohol or α-deoxyglycerol, is biotransformed entirely by normal pathways to lactate and pyruvate (214-216, 221-223). The oxidation of PG to lactate depends on whether the substrate is the free glycol or the phosphorylated glycol (214-216, 223). As free glycol, it undergoes a process of sequential biological oxidation, passing through lactaldehyde, methylglyoxal, pyruvate and lactate. The conversion of PG into lactaldehyde is catalysed by alcohol dehydrogenase obtained from horse or rabbit liver (224).

\[
\begin{align*}
    \text{H}_3\text{C} & \cdot \text{CHOH} \cdot \text{CH}_2\text{OH} & \rightarrow & \text{H}_3\text{C} & \cdot \text{CHOH} & \cdot \text{CHO} & \rightarrow & \text{H}_3\text{C} & \cdot \text{CO} & \cdot \text{CH}_2\text{OH} & \rightarrow & \text{H}_3\text{C} & \cdot \text{CO} & \cdot \text{COOH} & \rightarrow & \text{H}_3\text{C} & \cdot \text{CHOH} & \cdot \text{COOH}.
\end{align*}
\]

The phosphorylated glycol is oxidized via acetol phosphate, lactaldehyde phosphate and lactyl phosphate to lactic acid.

\[
\begin{align*}
    \text{H}_3\text{C} & \cdot \text{CHOH} & \cdot \text{CH}_2\text{OP} & \rightarrow & \text{H}_3\text{C} & \cdot \text{CO} & \cdot \text{CH}_2\text{OP} & \rightarrow & \text{H}_3\text{C} & \cdot \text{CHOH} & \cdot \text{CH(OH)} & \cdot \text{OP} & \rightarrow & \text{H}_3\text{C} & \cdot \text{CHOH} & \cdot \text{COOP} & \rightarrow & \text{H}_3\text{C} & \cdot \text{CHOH} & \cdot \text{COOH}.
\end{align*}
\]
Goepfert (1940) (225) observed that PG was dehydrogenated to acetol by the mold *Fusarium lini* Bolley. Sellinger and Miller (1959) (226) have shown that rat liver and kidney homogenates, which contain acetol kinase (distinct from glycerokinase), convert acetol to acetol phosphate, using ATP. They also showed that PG-1-phosphate dehydrogenase (distinct from α-glycerophosphate dehydrogenase present in rabbit muscle extract) converts acetol phosphate to PG-1-phosphate, the naturally occurring phosphorylated glycol. Acetol phosphate is converted to lactaldehyde phosphate by triose isomerase, and the latter is oxidized further to lactylphosphate by D-glyceraldehyde-3-phosphate dehydrogenase (227, 228).

The conversion of PG to propionaldehyde through a cobamide coenzyme has also been reported (229-232). PG and PG-1-phosphate have been suggested as intermediates in the metabolism of acetone via acetol (221-233).

Baer *et al.* (1968) (234) have demonstrated by *in vitro* studies that phospholipids analogous to α-lecithins and α-cephalins can be synthesized from PG: the D-PG-α-phosphorylcholine is very hemolytic, whereas the corresponding phosphorylethanolamine derivative is not hemolytic.

PG can possibly split also to $C_1$- and $C_2$-fragments, which enter the formate and the acetate pools of the body; as a $C_2$-unit, PG may be further oxidized via the citric acid cycle, thus contributing to energy pool (223). The $C_3$-units (pyruvate and lactate) too can provide energy via TCA cycle.
Anabolism. PG also participates in anabolic reactions, becoming incorporated into hepatic glycogen via the glycolytic pathway (235-237). PG has therefore been used as a substitute for carbohydrate as an energy source for chicks (238-240), ruminants (241-245) and for rats (246). Johnson (1954) (247) suggested PG in the treatment of ketosis. Wittman et al. (1975) (248) reported the inhibition of PG-stimulated gluconeogenesis by quinolinic acid (QA) in the fasting rat. PG administration resulted in increased rate of hepatic glycogen synthesis, increased amount of hepatic glycogen, and an increased concentration of serum glucose. Administration (i.p.) of QA results in weak inhibition of these gluconeogenesis parameters in the control animals, but it results in potent inhibition of the PG-mediated gluconeogenesis, including rate of synthesis of serum glucose. This inhibition is dose dependent. Nephrectomized rats have an increased sensitivity to QA. It is concluded that the difficulties in demonstrating inhibition of gluconeogenesis in vitro by QA results from its rapid renal excretion.

Propane-1,3-diol is much more toxic than the 1,2-diol, because of the intermediate formation of malonic acid, which is an inhibitor of succinate dehydrogenase, and also causes calcium deposits. For comparison, the metabolic fate and toxicity of several other C₃- and C₄-diols and their ethers (like cellosolves) have been discussed (213,219,222).
Effect of PG on blood cells and lymphocytes.

Amongst the blood cells, both the erythrocytes as well as the leucocytes have been included for discussion.

Hemolytic effect of PG was first reported by Braun and Cartland (1936) (249) as they observed that PG in sub-lethal doses produced hematuria in rats. While employing PG in equal proportions with distilled water to eliminate erythrocytes for leucocyte counts, Randolph and Mallery (1944) (250) observed that erythrocytes passed through three stages; first, the test tubes possessed a turbid phase, followed by a transparent phase, and, finally, there was a re-development of turbidity due to precipitate formation. Microscopic observations revealed that the final turbidity was due to agglutination of the red blood cells. The erythrocytes eventually hemolysed. The hemolysis occurred in concentrations greater than 30% PG in water. Mallery and Randolph (1944) (251) reported on the in vitro effect of PG on leucocytes. Husa and Adams (1944) (252) (cited from reference 25) showed that glycerol and PG did not prevent hemolysis at isotonic concentrations; 0.3-0.5% sodium chloride was found to be effective in preventing hemolysis of rabbit and human erythrocytes when both these polyhydric alcohols were in hypo-osmotic concentrations. Hemolysis by 10% solution of both these solvents was prevented by 0.6% sodium chloride (Zanowiak and Husa, 1959) (253); cited from ref. 254). This was further confirmed by Cadwallader (1963) (254) as in his in vitro studies presence of sodium chloride prevented
hemolysis of rabbit and human erythrocytes in aqueous PG solution up to 40-45% concentration. But 10-15% concentration of PG in water could not be made isotonic for these erythrocytes even on addition of sodium chloride, which indicated more hemolytic activity of PG than of glycerol. These results were in agreement with those of Brittain and D'Arcy (1962) (255), as they also had reported that addition of sodium chloride up to 30-40% PG solution prevented hemolysis in rabbit and human erythrocytes, but did not completely prevent the hemolysis at a concentration of 50% PG. They further demonstrated that in vivo hemolytic effect of PG could be eliminated by preparation of not greater than 30% of the glycol with 0.9% sodium chloride or water. These workers cautioned that, in spite of the fact that in rabbit i.v. injections of 12.5, 25.0 or 50% of PG did not affect red cell fragility, or hemoglobin content, or total white cell count, there was a marked decrease in clotting time and increase in platelet and polymorphonuclear leucocytes as well as in lymphocytes. Weill and Renoux (1976) (256) reported that mice lymphocyte cultivated with sheep erythrocytes in the presence of PG developed in 2 to 4 days of incubation more plaque and rosettel forming cells than those in the control culture (i.e. without the glycol), suggesting that PG probably affected early stages of sensitization of lymphocytes.

Recently, Ahluwalia et al. (1980) (257) have reported their studies on the in vivo and the in vitro changes induced by PG in erythrocytes. Feeding of PG at a dose level of 0.284 ml/100 gm body weight to rats decreased their ESR,
total leucocyte count and blood glutathione. Activities of acid Pase, ATPase, and 5'-nucleotidase and the total protein content of erythrocyte were significantly increased, whereas the activity of AChE was decreased. Treatment of erythrocyte with Triton X-100 (0.1%), although it caused an increase in the protein content followed by an elevation of the activities of all the enzymes, yet the effect of PG persisted with high statistical significance.

Bekeris et al. (1979) (258) reported that PG could be a cause of elevated serum osmolality.

**Effect of PG on liver.** This includes the effect of PG on liver itself, on the hepatic redox state, and on liver enzymes.

Kennedy and Morton (1940) (259) reported that PG decreased the oxygen consumption and CO₂ output of the isolated cat liver perfused with cat blood, but increased hepatic glycogen and blood lactic acid, and decreased the utilization of glucose by the liver. Addition of insulin to the perfusion with PG caused a further decrease in the oxygen consumption, prevented increase in blood lactic acid, and increased utilization of the glycol. Ethylene- and diethylene-, and dipropylene glycols were not utilized by the perfused liver, but these too depressed oxygen consumption and increased lactic acid production. Dipropylene glycol was least toxic out of the three. Ethyl alcohol was metabolized by the perfused liver, but it depressed its functional activity as indicated by decrease in oxygen consumption,
increase in lactic acid formation and decrease in glycogen synthesis.

Newman et al. (1940) (237) reported the comparative effects of PG, other glycols and alcohols on the liver directly.

Kulesar (1970) (260) and Zaroslinski et al. (181) (261) reported the limitations in the use of PG as drug solvent in pharmacological studies. Vitalinia et al. (1973) (262) reported the various aspects of 1,2-propanediol (PG) and 1,3-butanediol metabolism following parenteral administration combined with glucose and sorbitol. Jursons (1973) (263) reported that substitution of PG for 10-20% sucrose in diet for 15 days caused a decrease in the number and the volume of nucleoli in hepatocytes, and a decrease in the number of binuclear and mega nucleoated hepatocytes in rat liver.

Effect of PG on the hepatic redox state. Velikil et al. (1975) (264) reported that gluconeogenesis was increased in liver of rats given a low carbohydrate diet as compared with a high carbohydrate diet. When PG replaced 20% of the fat in low carbohydrate diet, liver glycogen increased and lipid decreased. There was a decrease in the reducing capacity of liver cytoplasm as shown by increased NAD : NADH and ATP : ADP ratios. Phosphoenol-pyruvate carboxykinase and malic acid decreased, and oxaloacetic acid and alcohol dehydrogenase increased in liver. PG addition in the high carbohydrate diet group increased the NAD : NADH and ATP : P_i ratio and lipid content of liver.
Effect of PG on liver enzymes. Rae (1948) (265) reported the action of PG on enzymes. Conney et al. (1960) (266) reported the adaptive increases in drug metabolizing enzymes induced by phenobarbital and other drugs. Cucinell et al. (1963) (267) reported the stimulatory effects of phenobarbital on the metabolism of Dilantin.

Dean et al. (1974) (268) reported effect of PG on various hepatic microsomal enzymes. PG administrated ip to rats at a dose of 4 ml/kg twice a day for 3 days caused significant elevation of the in vitro hepatic microsomal metabolism of aniline and p-nitro-anisole, but at the same time caused a significant change in p-nitro-benzoic acid metabolism. There was no change in cytochrome P-450 level with this treatment, but the response which was dose dependent could not be repeated by the in vitro addition of PG to incubating systems. This combination of stimulation and depression, together with the apparent lack of alteration of cytochrome P-450 concentration, suggests that this is not the usual increase in hepatic enzyme activity associated with the induction process mediated by phenobarbital or 3,4 benzpyrene. Since the in vitro response was not similar to the in vivo response, it would eliminate the proposal that this was similar to responses by in vitro addition of acetone (Anders, 1968) (269). It is suggested that possibly the PG response resembles most closely to that of dimethylsulphoxide, as both the solvents may produce some physical distortion in the endoplasmic reticulum of liver which contains these enzymes. The co-administration of phenobarbital
with PG abolishes the depression of aminopyrine metabolism by the latter, possibly modifying such distortion by endoplasmic reticulum. *In vivo* inhibition of drug metabolism was demonstrated by increased hexobarbital sleeping time and zoxazolamine paralysis time by PG administration. Kinetic studies with microsomes from treated rats showed a reduced $K_{m}$ and $V_{max}$ for aminopyrine demethylation, while for aniline hydroxylation there was increase in $V_{max}$ but no change in $K_{m}$ value.

Dean and Stock (1978) (270) reported the PG-mediated stimulation of hepatic drug metabolism in the perinatal period.

Yamamoto and Adachi (1978) (271) investigated the effect of oral administration of PG on the induction of enzymes and proteins in the rat hepatic microsomes and cytosol. The rats were divided into four groups, receiving water only (the control group), and those receiving PG, phenobarbital sodium (PB), and PG + PB; PG was given orally as 1 ml of 90% PG/100 gm body weight for 7 days. Various enzymes in the hepatic microsomes, and the binding capacity of the Y- and the Z-fractions in the cytosol were assayed. As compared to the control group, there was observed an increase in the aniline hydroxylase (AH) activity, the cytochrome $b_{5}$ content, and the amount of the Z-fraction in the PG-fed group. As compared with PB group, in the PB + PG fed group the microsomal protein increased but the activities of p-nitrophenol-UDP-glucuronyl transferase (pNP-GT) and aminopyrine demethylase (AD) decreased. When the PB + PG group and the PG group
were compared with the PG group and the control group, respectively, no parallel change was observed. When PG was added to hepatic microsomes of untreated rats in vitro, there was observed no change in the difference spectrum produced by binding of cytochrome P-450 and aniline and also the activities of AH and AP-NT-GT. Based on the above observation, they concluded that PG affected the in vitro assay data of drug metabolizing enzymes without changing cytochrome P-450 by its certain in vivo action, rather than by its direct interaction with microsomes, and modified the effect of PG on the microsomal enzymes and the cytosol proteins. It seems that effects of PG varied depending on the items of examination. Even if one takes the experiment administering PG as a control to the experiment using PG as a solvent for certain chemicals, it seems impossible to rule out possible effects of PG on hepatic cellular metabolism. So it is not appropriate to investigate the effects of drugs on hepatic enzymes and proteins using PG as a solvent.

Effect of PG on lipid metabolism. This aspect includes the effect of PG on triglycerides, phospholipids and cholesterol.

Kuzin and Nevraeva (1941) (272), Anker (1948) (273) and Price and Rittenberg (1950) (274) have reported the effect of PG on lipids, particularly on cholesterol; their work supported the suggestion of an alternate pathway for the metabolism of PG. For details, see ref. 275: for comparison between PG and ethanol in this respect, see ref. 276.

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were compared with the PB group and the control group, respectively, no parallel change was observed. When PG was added to hepatic microsomes of untreated rats in vitro, there was observed no change in the difference spectrum produced by binding of cytochrome P-450 and aniline and also the activities of AH and pNT-GT. Based on the above observation, they concluded that PG affected the in vitro assay data of drug metabolizing enzymes without changing cytochrome P-450 by its certain in vivo action, rather than by its direct interaction with microsomes, and modified the effect of PB on the microsomal enzymes and the cytosol proteins. It seems that effects of PG varied depending on the items of examination. Even if one takes the experiment administering PG as a control to the experiment using PG as a solvent for certain chemicals, it seems impossible to rule out possible effects of PG on hepatic cellular metabolism. So it is not appropriate to investigate the effects of drugs on hepatic enzymes and proteins using PG as a solvent.

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induced by strophanthin, barium chloride, aconitine and adrenaline; further, PG was found to be antiarrhythmic and antifibrillatory in dogs and rats at a dose level of 0.2-0.3 ml/kg of a 70% solution. In their further work communicated in 1979 (280,281) they reported that despite the fact that PG showed a low affinity and low responsiveness in depressing atrial contractility, it enhanced the length of the effective period and produced a negative inotropic effect in isolated rabbit atria. Although PG had a lower antiarrhythmic potency, its therapeutic index was found to be 50-fold that of quinidine and procainamide. In experiments with isolated rabbit heart, PG acted as a direct coronary vasodilator, which differed from catecholamines since their effect was blocked by propanolol; propanolol, however, had no effect on vasodilator action of PG. PG demonstrated a positive inotropic effect on isolated heart, which might be due to the fact that it was a short chain alcohol, and so it might be able to interact with aqueous phase of cell membrane; why PG had a negative inotropic effect on atria and a positive one on heart is not yet clear.

Kirkman (1976) (282) observed that at 40% concentration PG caused a depression of SA node and multifocal ventricular rhythm, and in very large doses asystole. PG-induced alterations in the ECG included an increased amplification of the QRS- and the T-waves. The two mechanisms proposed for these effects were the vagal and the direct effect on myocardium.

Majewski et al. (1978) (283) reported the lethal
and sub-lethal effects of PG on the cardiovascular and respiratory system of rainbow trout. Gross et al. (1979) (284) reported the cardiovascular effects of i.v. administration of PG and of oxytetracycline in PG in calves.

Effect of PG on certain CNS parameters. Conney et al. (1960) (266) reported no change in the hexobarbital sleeping time with PG dose up to 4.16 g/kg i.p. given to mice for 4 days, suggesting no effect on microsomal activity, and no effect on enzymes of hexobarbital metabolism.

Cucinell et al. (1963) (267) observed that diphenylhydantoin metabolism was accelerated by phenobarbital, but pretreatment of mice with PG for 4 days diminished protection provided by diphenylhydantoin against MES. This observation contrasts with lack of an effect on hexobarbital sleeping time following subacute PG administration. The apparent divergence may be due to a different metabolic pathway, diphenylhydantoin being metabolized partly by ring fission and partly by hydroxylation of one of its phenyl rings, while hexobarbital is metabolized by side chain hydroxylation.

Zaroslinski et al. (1971) (261) did not recommend PG as an inert co-solvent in pharmacologic studies by i.p. route, since they observed that acute pre-treatment of mice with 2.08 g/kg of PG (equivalent to administration of 0.01 ml/g of 20% solution) produced a significant elevation in initial clonic seizure threshold to i.v. infusion of pentylenetetrazol, not apparent with this dose when a single CD99 dose of the convulsant was used. Parallel results were obtained at a
higher (5.2 g/kg) dose of PG vs. strychnine convulsions. Potentiation of hexobarbital sleeping time was observed at doses of PG below the TD50 of 5.77 gm/kg. Spontaneous motor activity (SMA) was significantly decreased (P 0.05). Doses of 6.76 and 7.80 g/kg reduced activity from 196 ± 41 counts/hr to 72 ± 16 and 70 ± 16 counts/hr respectively. Sub-chronic administration of PG did not alter hexobarbital sleeping time subsequently determined, but did diminish diphenylhydantoin anti-MES (maximal electroshock seizure) activity. Thus, at co-solvent concentrations PG exhibits a weak but significant CNS depressent activity, as well as strychnine-, pentylenetetrazol-, tremorine- and oxotremorine-antagonist action.

Toxic effects of PG. Although the synthesis of PG was described more than 100 years ago (Wurtz, 1859) (285), it attracted little attention prior to 1932 when Seidenfeld and Hanzlik (1932) (286) suggested its use as an inert and safe solvent for pharmaceuticals. These investigators reported their observations following both acute and chronic administration of PG in rabbits and rats. They found that PG even in large quantities was innocuous, since cumulative effects, both functional and morphological, were not demonstrable in the organism as a whole. Braun and Cartland (1936) (249) reported the LD50 values for PG in different animals (223). Britton and D’Arcy (1962) (255) mentioned that PG had a relatively low order of acute toxicity, and, therefore, had been used for many years as a drug solvent in subchronic and chronic studies.
PG has been found to be a compound of a very low order of toxicity as measured by the various routes of administration to experimental animals. The feeding of PG to rats over substantially their entire life time has indicated a very low order of chronic toxicity. The single oral dose of LD₅₀ for rats is greater than 25 ml/kg (Ref. 219, pp 316-17). Consequently, the glycol is considered practically nontoxic in single dose by mouth. PG is not appreciably irritating to rabbit skin or rabbit eyes, and does not penetrate their skin in any extent to be harmful. Breathing concentrated vapours for 8 hr at room temperature did not kill animals, and their eyes were not harmed when flooded with an excess of the glycol. As PG has a low vapour pressure, consequently there is no significant hazard from vapour inhalation under any usual circumstances of handling or use.

Chronic toxicity data showed that PG produced no deleterious effects when given in small amounts. Rats that drank 10% PG solution in lieu of water demonstrated pathological changes in kidneys, heart, spleen or liver after 24 weeks as reported by Seidenfeld and Hanzlik (1932) (286), and the same is true with rabbit as reported by Newman (1940) (237). Weatherby and Haag (1938) (287) observed that at this concentration the rate of growth was slower for the first 10 days, but returned to normal thereafter during their 100 days study.

Laug et al. (1939) (288) noted that shortly after administration of large doses of PG, animals showed sign of loss of equilibrium, marked depression, analgesia, coma and,
finally, death. Microscopic observations revealed nuclear pycnosis, vascular degeneration of the cytoplasm and protein debris or loose casts in the cortical tubulin of kidneys. The liver showed slight congestion and hypoxemia with no fatty change.

Perfusion experiments with cat liver have shown that PG administration alters the glycogen level (Newman et al., 1940) (237). During the 2 hr-infusion period, a toxic concentration (500-600 mg/100 ml) increased glycogen content, and a nontoxic concentration (100-200 mg/100 ml) maintained a constant level of glycogen in the liver, while levels in the controls decreased. Lehman and Flury (1943) (217), Thomas et al. (1949) (289), and Lampe and Easterday (1953) (290) also reported about the adverse effects of PG. Davis and Jenner (1959) (291) reported that PG was least toxic in comparison with N,N-dimethyl-formamide and N,N-dimethyl-acetamide (H.CO.NMe₂ and CH₃.CO.NMe₂). Waldo and Schulz (1960) (241) demonstrated an increase in blood sugar in steers fed PG. Selenka (1963) (292) attributed toxicity of PG in bacteria to inhibition of specific enzyme; the enzyme was not specified. The teratogenic action of PG and its 1,3-analogue (propane-1,3-diol) were reported by Gebhardt (1968) (293). Judith (1970) (294) reported that PG decreased motility of both male and female mice, and also inhibited the motility-increasing effect of amphetamine. Gorban et al. (1972) (295) reported that PG would acidify waste water after 14 days under anaerobic atmosphere. Gaunt et al. (1972) (296) could not detect any carcinogenic potential of PG in rats fed 2.5 g/kg/day of PG.
Thienes and Haley (1972) (297) and Rudick and La Ham (1972) (298) reported the deleterious effects of PG. Chino (1974) (299) studied the pathology caused by the perineural and intramuscular injection of PG in rats. Bartsch et al. (1976) (300) investigated the acute toxicity of various solvents in mouse and rat, and reported the LD$_{50}$ of ethanol, dimethylformamide, dimethylacetamide, dimethylsulphoxide, glycidol, N-methyl-pyrrolidine, polyethylene glycol 400, PG and Tween 20. They recommended that 1/4 LD$_{50}$ value of any solvent should be used for any preparation or investigation to avoid interaction between solvents and compounds under investigation. Martin and Finberg (1976) (301) described unconsciousness, tachycardia, tachypnea and diaphoresis in a 15 months old child eight days after daily ingestion of 7.5 mg of PG; a case of stupor was also reported by ingestion of 6.0 ml of PG.

Arulanantham and Genal (1978) (302) reported that a patient developed seizures due to long term ingestion of a medication having PG as co-solvent, while some other patients developed reversible central nervous system effects on ingestion of PG. The reversible symptoms may be related to concentration of PG, or one of its metabolite in blood and tissues.

PG at a concentration of 90% produced conductive middle ear problems in guinea pigs, but there was no loss of hair cells. Brumett et al. (1978) (303) contraindicated the use of PG in high concentrations in ear drops, especially in cases with the perforation of tympanic membrane.
Effect of PG on skin (contact dermatitis and eczema). By virtue of its superior solubilizing and humectant properties, PG has since long largely replaced glycerol both in pharmaceuticals and cosmetics. But it is being increasingly reported as a cause of allergic contact dermatitis and eczema. Seidenfeld and Hanzlik (1932) (286) reported that its application to mucous membranes or injured skin could produce local irritation, while subcutaneous and intramuscular injections had produced considerable inflammatory reaction. Warshaw and Herrmann (1972) (304) studied skin reactions to PG; they could not decide whether cutaneous reactions to PG were due to primary irritation or to allergic sensitization.

Braun (1969) (305) reported 4% patients (3 out of 78) to be sensitive to PG at 10% concentration. Shore and Shelly (1974) (306) and Fisher et al. (1974) (307) have suggested that an eczematous response to 10% PG may be interpreted as evidence of allergic sensitization. Hannuscela et al. (1975) (308) and Hannuscela and Furstrom (1979) (309) have shown experimentally that ingestion of PG in sensitized individual produced flares of PG dermatitis as well as flares at sites of positive patch test reactions. Goldsmith (1978) (310), Fisher et al. (1979) (311) and Moran et al. (1979) (312) also reported the allergic response to PG. Recently, Bekeris et al. (1979) (258) reported a reversible hyper osmolality by PG in two burned patients during treatment with a cream containing PG. PG was also detectable in sera of these patients.
Other biological effects of PG. Danham and McNeal (1946) (313) reported the inactivation of influenza virus by PG. Rae (1948) (265) reported that PG retards decomposition of ascorbic acid solutions in vitro studies using 100% concentration of PG. Further, he reported that PG at a concentration of 40% prevents fermentation of yeast, while at 21% concentration it destroys or inactivates pancreatic diastase and the oxidase of raw potato, and also rapidly destroyed penicillin at 20% concentration (Rae, 1948)

Olitzky (1965) (314) and Faregemenmann and Fredrikson (1979, 1980) (315, 316) reported PG to have an antimicrobial and antifungal activity. Further latter group (1980) (316) reported PG in concentration of 35-90 g/lit inhibited growth of certain fungi on in vivo addition e.g. pityrosporam orbiculare, Candida albicans, Trichcopyton rubruc, T. mentagrophytes ver. interdigitale and Epidermo phyton floccosum. So, it is possible to use PG in the treatment of mycoses. Miyamoto and Ishibashi (1978) (317) reported the protective effect of PG against freezing damage of mouse and rat embryos. Baden (1978) (318) reported the possible use of PG in the management of hyperkeratosits.

Inflammatory response of PG. PG was found to produce well defined inflammatory edema in mice hind paw, which was concentration dependent, and was optimum at 50% concentration of PG by subcutaneous administration. Orally administered cortisone, aspirin, phenylbutazone, indomethacin were effective against edema. There was also an increase in the capillary
permeability with this PG edema. Lack of effects of chlorpheniramine, cyproheptadine and polymyxin B on edema suggested that histamine and serotonin were not involved in the mechanism of PG-induced edema. This may be due to prostaglandin-like activity of PG (Mogre et al., 1976, 1977)(319,320). In contrast to the inflammatory response, Pandse et al.(1980) (321) have reported that PG at a dose level of 1 ml/kg, 50% ip, elicits a potent antiinflammatory response in rats against carrageenin- and 50% PG-induced inflammations (subcutaneous injection). PG was found to be inactive by oral route, as well as in 5-HT- and formaldehyde-induced inflammations.

v. Gum guggul.

For this review, see Chapter 7, page- 275- 294
ii) Lacunae in our current knowledge about these aspects.

The overall view of these lacunae as ascertained from the review of literature presented above have been summarized below:

1. AA-cholesterol relationship, if any, as well as the mechanism of the mild cholesterol-lowering effect of AA, have not been well understood; these aspects require further detailed investigations from as many different angles as possible. Further, most of these studies had been conducted either in guinea pigs or in man (i.e. these were clinical studies), whereas it was desirable that this type of data could have been made available from other species as well, for instance from studies in rat, a species known to be able to synthesize its own requirement of AA; in particular the effect of mega doses of AA in this species was warranted.

The effect of AA on the fecal excretion of cholesterol via bile acids had not been reported, not at all in rat. This aspect seemed quite pertinent in our studies.

2. In view of the fact that PG had been found not to be an inert solvent, its role in cholesterol metabolism (when used as a solvent) had not been reported. Likewise, most of the work reported on OG had also been carried out using its aqueous solution or as an emulsion in gum; the effect of PG as a solvent for OG seemed a new line of attack on this aspect.
3. There were hardly any report on the effect of AA, or even of cholesterol or OG on RBC and its membrane. This aspect seemed pertinent to the understanding of the biochemistry of PG and OG.

iii) Specific objectives to be achieved in the present study

All such aspects enumerated above needed detailed investigation. The specific areas chosen for the present study have been explained on page-4 in the "scheme of studies".