CHAPTER II

REVIEW OF LITERATURE
Even in the first half of the eighteenth century Cholesterol was unknown to the Biochemists. It was Poulletier de-la-Salle (1769) who first prepared it from gall stones. Cholesterol was the initial compound of the sterol group to be separated and identified.

In 1815 Chevreul had shown it to be a component of the non-saponifiable residue. The same author coined the name Cholesterine (Chole-bile, Steros-solid) for a white waxy material isolated from the alcohol soluble part of the gall stones.

Since the introduction of the term 'Sterol' in the year 1911 this substance is now known as Cholesterol.

The milestone in understanding the chemical nature of cholesterol was the discovery by Berthelot (1859) that it was an alcohol.

On the basis of an analysis of cholesterol acetate, dibromide, Reinitzer (1888) first proposed the empirical formula for cholesterol.
However, it was Wieland (1929) who first proposed a structural formula for cholesterol. This Wieland formula was soon shown to require modification.

In 1932 Wieland and Dane, and Rosenheim and King constructed the new structural formula for cholesterol. This formula has been widely accepted.

Cholesterol has the structure as follows:

Cholesterol has a carbon ring structure of the 17-carbon cyclopentanoperhydrophenanthrene. In addition there is a hydroxyl group at C_3 and an unsaturated bond at C_5 - C_6, two methyl groups at C_{10} and C_{13} and an 8 carbon paraffin side chain attached to C_{17}. Cholesterol occurs both free and combined with fatty acids by
ester linkage at the hydroxyl group. Cholesterol in ester form is often referred to as 'bound' cholesterol esters are formally rich in linolic acid.

The biosynthesis of cholesterol is perhaps the most complex of all biosynthetic processes yet unravelled. That animal body could synthesize cholesterol was known since a long time. Among the earlier experimental work that of Beumer and Lehmann (1923) on dogs and the findings of Randeles and Kundson (1928) who studied on rats were particularly convincing. Evidence of cholesterol synthesis in man can be obtained from the work of Gamble and Blackfan (1920), Gardner and Fox (1921), Imhanser (1930) and Frazer (1953). The knowledge about the biosynthetic process of cholesterol started from the work of Bloch and Rittenberg (1942). They showed that 'Deutero-acetate' could be converted to cholesterol by rats and mice. Through the work of Bloch in U.S.A., Lynen in Germany and Popjak in England much of the biosynthetic process of cholesterol came into light. A number of established facts regarding cholesterol synthesis have been summarised below.

1. Essentially all tissues form cholesterol, liver, skin, adrenal glands and gonads being the most active. Adipose tissue, muscle, aorta and adult brain have a low order of synthesis.

2. Acetate is the principal precursor of cholesterol.
3. Of the 27 carbons of cholesterol, 15 arise from the methyl and 12 from the carboxyl of acetate.

4. The first phases of synthesis involve activation of intermediates through union with CoASH.

5. Later stages of synthesis involve participation of phosphates rather than co-enzyme A.

6. Some of the early stages of synthesis appear to be common to the extramitochondrial synthesis of fatty acids.

7. Refind yeast and liver preparations of microsomal plus a soluble protein fraction, require ATP, TPNH, Mg^{++} and Mn^{++}.

8. Mevalonic acid is a prime intermediate in the pathway.

9. Cholesterol synthesis is one example of a basic biosynthetic process in nature that involves condensation of isoprenoid (C_5) units and the formation of the active intermediate isopentenyl pyrophosphate.

10. Several mechanisms are available for the formation of the active C_5 units and multiple pathways are detectable for interconversion of biosynthesized sterols.

In human body cholesterol is derived from two sources, one, is endogenous and the other is exogenous, i.e., diet. Many tissues of the body are concerned with the formation of
cholesterol. Of them, skin, adrenal glands, kidney, small intestine and gonads are the most active in the biosynthesis of cholesterol (Srere et al, 1948, 1950, Gould et al, 1950, Robinowitz et al, 1952, and West and Todd, 1967). From the point of view the origin of plasma cholesterol, liver is the most important one (Deuel, 1957). However, Harper, Neal and Hlavacek (1953) confirmed the fact that only the liver has the capacity to form cholesterol esters. Ester cholesterol was not synthesised in the tissues of the dog following hepatectomy. By using isotopic tracers, various investigators unanimously agreed that approximately 1.0 gm to 1.5 gm of cholesterol is manufactured daily in adult liver (Popjak, 1960). Extrahepatic synthesis of cholesterol in the body amounts to about 0.5 gm per day.

Thus, the total daily synthesis of cholesterol amounted to about 1.5 gm to 2.0 gm which was considerably more than was present in the average diet (Harper, 1965). But there seemed to be a relationship between dietary intake and extent of synthesis of cholesterol within the body which resulted in suppression of synthesis when dietary intake of cholesterol was increased (Morris, 1959, and Siperstein, 1960). None the less, the endogenous contribution of cholesterol in the liver, small intestine and adrenal was not completely suppressed even by the prolonged feeding of very large amount of cholesterol. In human subject alteration of the dietary intake of cholesterol as low as 200.00 mg per day to as high as 1000.00 mg
per day did not affect the levels of cholesterol in the serum (Harper, 1965). It was apparent that endogenous synthesis rather than dietary intake of cholesterol primarily determines the concentrations of cholesterol in the blood.

Acetate is the principal precursor of the cholesterol (Bloch and Rittenberg, 1942, 1945).

According to Popjak (1964) following are the principal reactions in the biosynthesis of cholesterol.

1. Activation of acetate to acetyl CoA.
2. Condensation of two acetyl CoA to aceto-acetyl CoA.
3. Formation of beta-hydroxy beta methyl glutaryl CoA.
4. Formation of mevalonic acid.
5. Mevalonic acid is phosphorylated by ATP to form 5-phosphomevalonic acid which is converted to 5-diphosphomevalonic acid.
6. Diphosphomevalonate loses CO₂ and water to form isopentenyl pyrophosphate. This reaction is helped by ATP.
7. Isopentenyl pyrophosphate is isomerised to 3-3 dimethylallyl pyrophosphate by the enzymes present in the liver.
8. A molecule of 3,3 dimethylallyl pyrophosphate reacts with a molecule of isopentenyl pyrophosphate to form geranyl pyrophosphate.
9. A molecule of isopentenyl pyrophosphate reacts with geranyl pyrophosphate to farnesyl pyrophosphate.

10. Two molecules of farnesyl pyrophosphates condense to form squalene. The enzyme in liver that carries out this reaction is firmly bound to microsomes and the system requires reduced pyridine nucleotide and Mg\(^{++}\), Mn\(^{++}\), or Co\(^{++}\).

11. Squalene undergoes cyclization to the primary animal sterol, lanosterol by squalene oxidocyclase I. T.P.N. is required as co-enzyme as in molecular oxygen.

12. The primary animal sterol, lanosterol, is converted to cholesterol through a number of intermediates.

Lymphatic transport of cholesterol from the gastrointestinal tract to the blood was first discovered by Mueller (1916). It was later confirmed by Biggs et al (1951) and Chaikoff et al (1952). The chylomicrons had the lowest density of all the plasma lipid components. They contained cholesterol, phospholipid and protein contributed in part by alpha and beta lipoprotein molecule. The chylomicrons had a mean density of 0.94 with sf value 10,000 ± 5,000 and molecular diameter of 5,000 Å. According to Scann, (1965), and Fredrickson, (1967) it contained protein 2 percent, phospholipid 7 percent, cholesterol 2 percent, cholesterol ester 8 percent, and glyceride 81 percent.

The lipoprotein molecules were commonly graded to their
floatation rate. Those that float to the surface after high speed centrifugation were in the low density range - the beta lipoproteins. The high density molecules were alpha lipoproteins. The low density protein might further be subdivided into two classes - those at higher density end of the spectrum contained a greater proportion of cholesterol and those at the other were the very low density lipoprotein which were heavily laden with triglycerides.

In man, there was relatively high proportion of cholesterol in the low density beta lipoproteins in plasma as a whole. The total cholesterol in beta lipoproteins in man was about 70.00 mg per 100 ml (Barr, 1953). From the plasma one part of chylomicrons circulated to the liver and the other part was deposited in the various tissues of the body (Bergstrom, 1962).

The chief catabolic fate of cholesterol was its oxidation of cholic acid (cholic acid) and the chief route of excretion was into the gastrointestinal tract via the bile or through mucosal cells. Some 80 - 90 percent of the body cholesterol was ultimately metabolised to bile acids. The two primary bile acids formed from cholesterol in most mammalian species were cholic and chenodeoxycholic acids (Danielsson, 1963). This bile acids conjugated with glycine and taurine in variable ratios (Bloch et al, 1943, and Zabin et al, 1953). These conjugates were present in sodium salts - the bile salts - due to nearly complete
ionisation of the acids at physiological pH. Glycocholic acid and taurocholic acids were the conjugation products of cholic acids (West et al, 1967).

From the intestine a major portion of bile salts are reabsorbed to the liver via portal vein. A part of the cholesterol as cholic acid is excreted along with faeces. Another part of the cholesterol in the intestine is degraded by intestinal micro-organism to other sterols (Snog-Kjaer et al, 1955). The faeces in man contain about 0.3 gm to 1.0 gm of sterols daily, chiefly in the form of cholesterol, coprostanol and cholestanol (Schoenheimer et al, 1935, and Anchel et al, 1938) and very little is excreted in urine. Some is lost by way of the skin secretion. This loss is highly variable, but may amount to 100 mg per day. Squalene is lost in the sebum (Kandutsch, 1964).

The gonads and the adrenals utilise cholesterol in the synthesis of hormones. Cholesterol is the precursor of many hormones like aldosterone, corticosterone, deoxycorticosterone, androgen and oestrogen. The formation of adrenal cortical hormones from cholesterol was demonstrated by Zaffaroni et al (1951). The conversion of cholesterol to pregnandiol and proges- terone was reported by Bloch (1945). But this loss of cholesterol in hormone synthesis was not a quantitatively significant fate and
did not account for the loss of much cholesterol from the body. The principal pathway of cholesterol metabolism is shown in Fig. A.

Bile salts are important factors which stimulate the absorption of cholesterol from the gastrointestinal tract. Schoenheimer (1924) demonstrated that an optically visible lipoemia could be produced in rabbits when they were fed on a single dose of cholesterol along with the bile salts. Member et al (1944) were able to induce the deposition of cholesterol in the aortas of rabbits only when it was fed with bile salts. More recently Söderstein et al (1952) also concluded that bile salts play an obligatory role in the passage of cholesterol from the intestinal tract to the lymph. Swell and Coworkers (1953) ascribed the beneficial effects of bile salts on the absorption of cholesterol to their activation of cholesterol esterase present in pancreatic juice. Tennent et al (1962) demonstrated in human subjects and in experimental animals that feeding of resins that bind bile acids prevent their reabsorption causing lowering of blood cholesterol. The action of resins might be due to the stimulant effect on the liver to increase the rate of oxidation of cholesterol, with resulting decrease in circulating cholesterol.

One of the most potent factors which influence the metabolism of cholesterol are the secretions of the endocrine glands. Of these hormones those produced by the thyroid gland and by the islet
Pathways of cholesterol metabolism Fig. A
tissue of the pancreas share the lime light as the most important in relation to the blood lipids. However, the pituitary gland, adrenal cortex and the gonads also have an influence.

A number of workers (Heckscher, 1925, Fleischmann et al, 1940, and Handler, 1948) reported a high cholesterol level following removal of thyroid gland. High cholesterol value in myxoedema and other forms of hypothyroidism in man had been reported by a number of investigators (Hurxthal, 1933, 1934, and Gildea et al, 1939). On the other hand an increased activity of the thyroid gland resulted in reduction of cholesterol level in blood. Low blood cholesterol level was a characteristic of hyperthyroidism (Bing et al, 1923, Man et al, 1940). The addition of desicated thyroid to the diet of cholesterol fed rabbits or chicks resulted in a significant depression of the hypercholesterolema as well as slowing down of atherosclerotic process (Harper, 1965).

Hyperlipaemia and hypercholesterolemia were known symptoms of Diabetes Mellitus in man. The most convincing information about the relationship of pancreas with blood cholesterol level was available from the study of the depancreatized animals (Allen, 1922, and Gibbs, 1941). When depancreatized dogs were maintained over long periods by treatment with insulin Chaikoff and Kaplan (1935) demonstrated a fall in hyperlipaemia.
Since the adrenal cortex plays an important role in the manufacture of steroid hormones, it is natural to suppose that it would exert some control over the cholesterol content of the blood. A number of investigators (Beumer, 1923, and Reid, 1932) failed to demonstrate any change in the serum cholesterol level after adrenalectomy. However, Medvei (1935) observed that blood cholesterol is somewhat elevated in Addison's disease which primarily affects the adrenal cortex. In man, cortisone therapy resulted in an increased concentration of cholesterol in the serum (Adlersberg et al, 1951 and Conn et al, 1950). Diluzio et al, (1953) reported that plasma cholesterol was reduced in adrenalectomised dogs but deoxycorticosterone acetate (D.C.A.) maintained these animals.

Chaikoff et al, (1936) found that hypercholesterolemia occurred occasionally following hypophysectomy in experimental animals. In acromegaly hyperlipoemia was a frequent finding. It was believed that hyperlipoemia in such cases were not related to the pituitary hormones but rather to the Diabetes or other disorders usually associated with this disease. The normal values for serum cholesterol in pituitary basophilism had been reported (Man, 1946). After administration of moderate dose of adrenocorticotrophic hormone decrease of esterified cholesterol in the serum was noted (Diluzio et al, 1953).
Coleman et al (1956) found that in the case of gonadectomised male rats after sexual maturity and placed on a diet containing 15 percent cotton seed oil and 1 percent cholesterol for 6 weeks, there was a marked decrease in the cholesterol concentration in the liver as compared with values obtained for the livers of nonoperated animals on the same diet. In the case of gonadectomised female rats the cholesterol concentration in the liver increased as compared with that in nonoperated female rats.

This sex difference in absorption was also noted by Aftergood et al (1956).

In experiments on chicks, the administration of oestrogens along with cholesterol markedly inhibited atherogenesis in the coronary arteries although the atherosclerotic changes in the aorta continued to occur. Furthermore, oestrogen administration reversed coronary lesions, which are previously induced by cholesterol feeding without affecting similar lesions of the aorta (Harper, 1965). Oestrogens were also known to bring about reduction in blood cholesterol. Merola (1964) have studied cholesterol synthesis in cell free homogenates of liver from rats which had been treated with oestrone. Doses of oestrone which were associated with a reduction in blood cholesterol were found also to inhibit biosynthesis of cholesterol.
The effect of diet on serum cholesterol has been a subject of controversy. The diet may alter the serum cholesterol level in two ways, it may either alter the cholesterol absorption from the intestine or may alter its biosynthesis.

It had been shown that the presence of fat in the diet increased the efficiency of absorption of cholesterol from the gut, although the effect might vary with species. Some animal, e.g., rabbits absorbed cholesterol rapidly in the absence of fat while chickens, rats and guineapigs carried out this function less readily on fat free diet (Deuel, 1957).

Doen et al (1955) fed young chicks a commercial diet to which cholesterol dissolved in 10.00 percent peanut oil was added. After 14 days of this feeding no significant increase in cholesterol was noted in the liver, spleen, aorta and biles. But when the cholesterol content of the diet was from 0 to 1.00 percent of the ration a marked increase occurred in the range between 0.10 and 0.33 percent and at higher levels. When the cholesterol was given without fat the increase in these organs was much less, except in the liver. In this organ the cholesterol values corresponding to 0 and 0.10 percent of dietary cholesterol were significantly higher on the fat free diet than on that containing 10.00 percent peanut oil. Alfin-Slater and coworkers (1952) found higher
values in the livers of rats on fat free diet than in those of animals fed on diet containing 15 percent cotton seed oil.
Peterson et al (1951, 1952) were the first to discover that when soyabeen sterols were fed on chicks together with cholesterol high - cholesterolemia was prevented.

Beta-sitosterol, a plant sterol found most abundantly in soyabeen has a structure very similar to that of cholesterol except that it carries an ethyl substituent on the side chain in position 24. This sterol is hardly absorbed at all from the intestine, nevertheless it inhibits very much the absorption of cholesterol. The cholesterol lowering action of sitosterol may be attributed to the inhibition of the absorption not only on dietary cholesterol, but also of the absorption of cholesterol secreted into the lumen of the intestine with the bile (Popjak, 1960).

In addition to soyasterols, mixed sitosterol, beta-sitosterol and ergosterol when fed singly effected a similar inhibition of cholesterol absorption (Peterson et al, 1953). Since esterification of soyasterols inhibited their ability to prevent cholesterol absorption it was postulated that unesterified soyasterols inhibit the enzyme system concerned with cholesterol absorption which may involve the esterification of cholesterol (Beher et al, 1954).
The level of blood cholesterol also raised when choline or inositol was fed to chicks receiving cholesterol (Stamler et al, 1950).

Low fat diet had been accompanying the lowering of blood cholesterol, it was found that substituting vegetable fat for animal fat in the diet was effective in the lowering of cholesterol level. Diets containing appreciable quantities of animal fats led to higher levels than diets containing the same amounts of more highly polyunsaturated fats (oils) such as corn, cotton or safflower oil (West and Todd, 1967).

Keys (1957) observed that the cholesterol elevating effect of 1.00 gm of saturated fat might be overcome by 2.00 gms, to 3.00 gms of dietary fats (oils) high in linoleic acid, such as corn, cotton or safflower oil.

Schoenheimer and Brensch (1933) were unable to demonstrate any increase of cholesterol synthesis in mice which were fed with considerable amounts of lard and other fats. Similar results were observed by Alfin-Slater et al, (1952). However, Hildreth et al, (1951) considered that total fat content of the diet was more important in determining the serum cholesterol level than was the amount of cholesterol itself ingested.
The world wide surveys showed very clearly that there was a very strong correlation between the nature of dietary fat and plasma cholesterol levels. In countries or social groups within a country where the consumption of fat was high there was a high incidence of severe atheroma and also high blood cholesterol levels. Conversely, the consumption of liquid vegetable seed fats favoured the lower plasma cholesterol level and of lower incidence of atheroma (Brunte-Stewart, 1958).

Furthermore, it had been shown by various workers that fats which contained high percentage of saturated fatty acids raised plasma cholesterol and fats which were rich in fatty acids containing more than one double bond lowered the plasma cholesterol (Popjak, 1960).

Although the evidence on the question of the mode of action of the highly unsaturated fat was far from complete. The data reported from several laboratories showed that the rate of elimination of cholesterol and its breakdown products was substantially increased during ingestion of poly unsaturated fat.

Lewis (cited by Popjak, 1960) found that cholesterol esters of the beta lipoproteins which were prepared from the blood of animals fed on a diet of highly unsaturated vegetable fat were eliminated much faster than that of the cholesterol esters obtained
from animals kept on a butter fat diet. The evidence suggested that cholesterol esterified with polyunsaturated fatty acids was metabolised faster than cholesterol esterified with saturated fatty acids.

The administration of exogenous cholesterol had been shown to reduce markedly the proportion of cholesterol which was synthesized.

Tomkins and Chaikoff (1952) reported that when diet containing 5 percent of cholesterol was fed to rat for 8 days prior to death, the rate of cholesterol synthesis in the liver was practically nil. Likewise when the animals were fed on diets containing only 0.5 percent of cholesterol for 7 days a marked depression in cholesterol production was noted. These authors postulated that the rate of cholesterol synthesis in the liver was a subject to homeostatic regulation by dietary cholesterol. Langdon and Bloch (1953) also confirmed the inhibitory effect of dietary cholesterol on the rate of cholesterol synthesis. Cook et al (1956) consumed 6.9 gm of cholesterol in the form of dish of 20 scramble eggs failed to deserve an increase in his plasma cholesterol in spite of the fact that 5.0 gm of the dose were absorbed.

Although the experimental evidence that cholesterol feeding reduced the synthesis of cholesterol in the liver was quite convincing, Gould et al (1953) found that a similar inhibition in the rate of synthesis of cholesterol did not occur in the skin or intestinal mucosa as a result of cholesterol administration.
Aftergood et al (1956) found that total liver cholesterol content of rats fed on a diet containing 15 percent of lard with 1 percent cholesterol was significantly decreased by the addition of high amounts of Vitamin E.

The relation of diet and cholesterol level of blood had also been observed by many Indian investigators. Gopalan and Ramnathan (1958) found that serum cholesterol level was raised by butter and hydrogenated vegetable fats, both in human subjects and in monkeys. They noted that sesame oil which was an unsaturated vegetable fat caused a marked reduction in the serum cholesterol.

Reddy (1959) observed that milk, eggs, other animal fats and coconut oil (unsaturated fat) accounted for high blood cholesterol level. According to them inclusion of olive oil, corn oil and other vegetable oils into the diet in some countries accounted for low incidence of atherosclerosis.

Mathur and Wahi et al (1959) observed highest cholesterol level in the high socio-economic group of persons consuming the high amount of dietary fat. But these workers did not fully agree that dietary fat was mainly responsible for high cholesterol level.

Beside dietary fat protein also had some effect on cholesterol synthesis as well as blood cholesterol level. Several earlier
workers like, Newburgh and Squier (1920), and Newburgh and Clerkson (1923) reported atherosclerotic lesions of aorta and other arteries in rabbits fed an excessive amount of protein for a prolonged period. Olson et al (1958) and Furman et al (1959) studied the effect of protein on the blood cholesterol level. They substituted carbohydrate for protein in the diet and noted marked decrease in the concentration of blood cholesterol.

In a nutritional survey Olson (1960) found marked hypocholesterolemia in a population substituting on a diet deficient in protein but moderate in fat.

Ramnathan (1955), Frenk et al (1958) and Bagchi et al (1963) reported hypocholesterolemia on paediatric patients of severe protein malnutrition.

Nath and Saik (1959) noted hypercholesterolemia in rats on high protein diet.

From the extensive studies on the plasma lipid composition in man as well as in animals had shown that difference occured which depend more on the composition of the diet than that on other factors. In less developed countries the low level of plasma lipids including cholesterol might be due to low intake of total fat as well as protein in the diet. It had been recognised that the quality of fat was probably more important than the total fat content of diet. It had also been established in several
studies that the feeding of equipment amount of animal fat such as butter, eggs or beef maintained the plasma cholesterol at a higher level that do fish or vegetable oils. This difference could be related to the total mean unsaturation of the fatty acids reported in biotin deficient rat, cholesterol synthesis remained normal. The role of other vitamins in cholesterol genesis was not clear. Riboflavin was apparently without activity (Guehring, 1952).

Lecoq et al (1948) suggested that Vitamin A and choline were concerned with the metabolism of cholesterol, whereas nicotinamide, pantothenic acid, pyridoxine, inositol and methionine were concerned with the metabolism of both cholesterol and fats.

Padmavati et al (1958) were unable to demonstrate any significant rise in the serum cholesterol level with increasing cholesterol intake. They had not seen any significant rise in the serum cholesterol levels with increasing fat intake in any of the groups studied. However, most of the subjects in their study groups were on a diet of fat content below 50 mg per day and the number of high fat intake was small. Even in these small groups with high fat intake they noted higher cholesterol level than the other groups.

Nath et al (1957) observed that nonvegetarian persons consuming proportionately larger amounts of total calories and animal
fat had higher serum cholesterol values than the vegetarians.

Age is one of the factors which causes an alteration of blood cholesterol level. A number of workers have noted a low blood cholesterol value in early infancy (Rosenbloom, 1935, Sperry, 1936, and Boyd, 1936).

According to Page et al (1935) the blood cholesterol level did not vary with age. Their study was made in 66 male subjects, of different age groups.

Boyd (1936) reported the blood cholesterol values in born infants as only 34.15 mg per 100 ml. Whereas the reported value for total cholesterol for young and aged persons was in average of 177.00 mg per 100 ml.

Sperry (1936) observed the blood cholesterol values in babies of 4-25 days of age as 71.00 mg to 190.00 mg per 100 ml with an average of 135.25 mg per 100 ml. But, he stated that the serum cholesterol level was maintained in each healthy persons at a constant level as he had failed in his subsequent works to demonstrate any significant difference in blood cholesterol level in the sera of children, varying in age from 2 months to 13 years from that of adults.

Offenkrantz and Karshan (1936) noted a gradual rise in
the cholesterol level in the plasma between the age of 2 months and 7 years, by which time it had reached the adult level.

Eck and Desbordes (1935) showed that the blood cholesterol of children of 6 - 15 years of age was somewhat lower than that of adults.

Page et al (1935), and Sperry and Webb (1950) were of the opinion that there was no direct relationship between age and level of blood cholesterol. On the other hand Keys et al (1950) concluded that a profound curvilinear relation existed between age and serum cholesterol level in man, a maximum concentration being obtained during the sixth decade. The average normal blood cholesterol level found was 173.00 mg per 100 ml for the 19 years old subjects and 252.00 mg per 100 ml for those who had reached the age of 52 years. The average rise of serum cholesterol in their series was found to be 2.20 mg of total serum cholesterol per year.

Sperry and Webb (1950) concluded as a result of studies of blood cholesterol on the same 14 men and 8 women over a period of 13 - 15 years that the serum cholesterol concentration might be increased with age in same persons but that the increase was not an obligatory concomitant of aging.
Kornerup (1950) found no significant change in blood cholesterol level from the age of 1 year up to puberty.

Adlersberg et al (1956) found no significant relationship between blood cholesterol level and age up to puberty. But they noted a significant increase of total plasma cholesterol level between the age of 20 years and 23 years. Thereafter until the age of 60 years there was no further change.

Nath et al (1957) concluded that there was a definite relationship between age and serum cholesterol level. In their study on healthy subjects between the age of 20 years and 60 years they found the average serum cholesterol level of subjects in 20 - 30 years of age group as 177.28 mg per 100 ml, in 30 - 40 years of age as 195.00 mg per 100 ml, in 41 - 50 years and 51 - 60 years age groups as 208.00 mg and 215.00 mg per 100 ml respectively.

Gopalan and Ramnathan (1957) observed no progressive increase of blood cholesterol level with age in the subjects of low socio-economic groups. However, the subjects of high socio-economic groups of their series showed a progressive rise.

Padmavati et al (1955) noted only a slight increase in the value of total cholesterol between the ages of 20 years and 50 years. The increase per year of life in their series never exceeded 1.00 mg.
When their results were statistically analysed they noted that the difference between the mean serum cholesterol level of men aged 20 - 39 years and 40 - 59 years was not highly significant. They observed this rise among the industrial workers. But, in the rural population there were no significant increase with age either in males or females.

Mathur et al (1959) investigated 816 men of different age groups. They observed that serum cholesterol level rose with age till 50th year after which there was slight fall.

Stuart et al (1962) observed a gradual rise of serum cholesterol level from the age of 20 - 49 years.

There was very little difference in blood cholesterol level between males and females. In both sexes it rose in parallel from birth to puberty. But the level in females were influenced thereafter by menstrual cycle, pregnancy and menopause.

A decrease in the level of blood cholesterol in women prior to the onset of menstruation had been noted by several workers (Okey and Boyden, 1927, and Kaufmann et al, 1929).

Offenkrantz (1936) noted a rise in plasma cholesterol level before menstruation followed by a fall during menstruation.
Oliver and Boyd (1955) observed definite cyclical changes in plasma cholesterol level in female subjects. They noted a regular fall in total blood cholesterol level in the mid cycle with premenstrual rise.

Hypercholesterolemia during pregnancy had been observed by several workers. Gardner et al, (1929) observed that the increase in cholesterol began after second month and continued up to the 30th week after which it decreased again up to the time of delivery. According to them during pregnancy only free cholesterol was increased, while the ester fraction might even show a decrease.

Boyd (1937) failed to detect any significant elevation of total plasma cholesterol level in pregnancy.

Peters et al (1951) observed the progressive rise of total and free cholesterol, phospholipids and neutral fat from the 12th week of pregnancy to delivery after which the value returned to normal.

Oliver and Boyd (1955) established the presence of hypercholesterolemia in pregnancy and they noted maximum plasma cholesterol level in the last trimester of pregnancy.

At the time of delivery the level of total cholesterol in
maternal blood was high while that in the foetal blood had low value (Rosenbloom, 1935).

As lactation proceeded the cholesterol level in mothers' blood gradually decreased. The chlostrum of women contained considerably more cholesterol than did the milk secreted later (Fox et al, 1924).

Oliver and Boyd in their earlier work found significantly high plasma cholesterol during 52 - 59 years of age. Such postmenopausal hypercholesterolemia was also noted by Adlersberg et al (1956).

Dave et al (1960) reported a relatively sharp rise in the total cholesterol in females as compared to that of males with advancing age.

Boyd and Roy (1928) found lower blood cholesterol level among Indians than that reported from Western countries.

Gertler et al (1951) on their study on the Eskimos living in Alentian Islands did not find any significant difference of the mean adult serum cholesterol level from that of adult Americans.

According to most of the workers there was no direct

Idiopathic familial hyperlipaemia is a exceedingly rare congenital disorder occurring in both children and adults. In this condition there is frequently hepato splenomegaly; Xanthomegaly; lipemia retinalis and a high level of blood lipids specially, neutral fats and also phospholipids and cholesterol. It was believed that this condition was due to defective mechanism for the removal of blood fat by the liver. A humoral factor was also suspected to involve in this defective mechanism (Holt et al, 1939).

Reports on effects of hard work on blood cholesterol level were conflicting. Robinson et al (1927) reported a marked drop of cholesterol level in both the plasma and the corpuscles following exercise. On the other hand, Patterson (1927) noted no change of blood cholesterol after strenuous exercise.

Keys et al (1956) noted that if exercise preceded food there was no change of serum cholesterol. When food was taken before exercise he observed that mild exercise had no effect on serum cholesterol.

Keys et al (1956) observed that blood cholesterol was
increased if rest followed food. They noted no change of blood cholesterol with mild activities. But there was a fall in the level of serum cholesterol if food intake was followed by moderately severe exercise.

It was also noted that if exercise preceded food there was no change of serum cholesterol. When food was taken before exercise it was observed that mild exercise had no effect on serum cholesterol level. But, after moderately severe exercise intake of food lowered serum cholesterol level in majority of cases although there was no material change. Rest after food raised cholesterol level.

Several workers had emphasized the influence of body weight on serum cholesterol level (Nath et al, 1957). They also observed that lean and thin persons had low serum cholesterol level and obese persons no matter to which socio-economic group they belong had high serum cholesterol level. A notable feature in this study was that the obese people had blood cholesterol level as 150.00 mg. per 100 ml which was more than that of persons with thinner built.

Stress is an important factor in the production of hypercholesterolemia. This was probably mediated through the secretion of both adrenal medullary and cortical hormones (Kurland and Freedberg, 1960) as well as sympathetic nervous system. The effect
might be primary or the result of secondary release brought about by a separate pituitary mobilizing factor.

Circulatory level of free fatty acids, serum cholesterol, and phospholipids had been shown to be raised in dogs and in man after prolonged injections of epinephrine and norepinephrine. In adrenalectomised rats and dogs these actions had been markedly reduced and normal response was restored by cortisone (Havel et al, 1959, and Shafrir et al, 1959).

Serum cholesterol level is dependent on the rate of synthesis within the body as well as absorption from intestine and its catabolism specially as bile acids by the liver. Moreover, it was influenced by various factors like age, sex, nutritional status, dietary habits, functional capacity of various organs of the body and many other factors. Therefore, normal plasma cholesterol level varies widely. There is widely diversity in the normal serum cholesterol level quoted by different workers. This is partly due to different methods of estimation used by different workers and also due to influence of various factors as already mentioned. A brief survey of literature as given in this chapter will show the divergence of opinion regarding the normal serum cholesterol level reported by different workers.
Bloor (1916) using a special method for extraction gave values for normal cholesterol in whole blood as 120.00 mg to 160.00 mg per 100 ml.

Some other workers using an extracting method from dried blood found an average value as 150.00 mg per 100 ml with a range of 130.00 mg to 190.00 mg per 100 ml. While, others estimated blood cholesterol level using Salkowski reaction found an average serum cholesterol level as 180.00 mg per 100 ml with a range of 117.00 mg to 297.00 mg per 100 ml.

Boyd and Roy (1928) were probably the first to report about the normal blood cholesterol values for Indians. They worked in Calcutta and found a normal total serum cholesterol value as 82.00 mg to 184.00 mg per 100 ml. They used Mayers and Wardell method for blood cholesterol estimation.

Ghosh (1933) from Lucknow observed the serum cholesterol level of normal persons as 110.00 mg to 180.00 mg per 100 ml. Their method of cholesterol estimation was that of Bloor's method.

Boyd (1933) using oxydimetric method found an average value of 162.00 mg per 100 ml of total plasma cholesterol in normal women.

Sperry (1936) in New York using digitonin method found the
normal total plasma cholesterol level as 130.00 mg to 292.00 mg per 100 ml, with an average of 209.80 mg per 100 ml. While other workers in New York gave the value of total serum cholesterol as 168.00 mg to 260.00 mg per 100 ml, and percentage of ester cholesterol was 73.75 mg to 75.36 mg per 100 ml.

Keys et al (1950) in their studies of blood cholesterol level among the middle income group persons of Minnesota found the range of serum cholesterol level as 176.00 mg to 248.00 mg per 100 ml. Brunte-Stewart et al (1955) observed the serum cholesterol level among the people of South Africa and found a value of it as 148.00 mg to 180.00 mg per 100 ml.

According to Varley (1969) normal range of total serum cholesterol in young adults was 150.00 mg to 240.00 mg per 100 ml. Serum cholesterol was raised with age and was more marked under 50 years in men than in women and might give values over 300.00 mg per 100 ml in middle age. Gupta (1970) estimated the serum cholesterol in normal individual of different socio-economic status. Total cholesterol was done by the method stated by E.J. King (1956). The total serum cholesterol values in their series varied from 110.00 mg to 296.00 mg per 100 ml although in majority of the cases, their observed range was from 130.00 mg to 240.00 mg per 100 ml. They noted a progressive rise of serum cholesterol with advancing age in both
high and low socio-economic groups. But it was greater in high socio-economic group. They did not find any effect of sex on total plasma cholesterol level, while it was noticed that total cholesterol level showed a rise with increase in the body weight specially when obesity index rose beyond 2.5.

$$\text{Obesity Index} = \frac{\text{Weight in lbs.}}{\text{Height in inches}}$$

The atheromas had been shown to contain considerable amount of cholesterol. The cholesterol could readily be detected microscopically in atheromatous lesion, because of its characteristic property of birefringence. Moreover, from chemical analysis of atheromatous patches, it was seen that the main component of the atheromatous lesion was cholesterol (Hardegger, 1943).

The large number of workers producing hypercholesterolemia in animals and birds after feeding large amount of cholesterol in the diet had demonstrated atherosclerotic lesion (Anitschkow, 1913, Kuntz et al, 1949, and Wolffe et al, 1952).

Although hypercholesterolemia following cholesterol feeding was associated with production of an atherosclerosis in animals. Cook et al (1956) had failed to produce hypercholesterolemia after oral administration of cholesterol. Moreover, the atherosclerotic-
lesions in experimental animals differed from the diseased condition in man by the rate and development and the distribution of the lesion in the vessels (Peters and Van Slyke, 1946, Schoenheimer, 1924).

Though there was a considerable indications that cholesterol deposition was the primary cause for the development of atheromas, Peters and Van Slyke (1946) did not agree that hypercholesterolemia or any disturbance in cholesterol metabolism had been the cause of the deposition of cholesterol in the atheromatous patches.

Charnic et al (1949), and Werthessen et al (1954) had shown that cholesterol could be synthesised by the arterial wall itself.

Morrison and Johnson (1950) observed that the average cholesterol content of the coronary arteries of patients who had died of acute coronary thrombosis was four times the average for a group of control patients. Because of the predominance of the cholesterol in the atheromatous patches, it was believed that the deposition of cholesterol in the arterial wall was probably attributable to a hypercholesterolemia.

Gertler and Gern (1950) were of opinion that serum cholesterol was considerably higher in males who had experienced myocardial infarction than it was in healthy active males.
In the coronary disease group although both cholesterol and phospholipids were increased in the serum, the rise in phospholipid had not kept pace with that of cholesterol. They suggested that the factors favouring the deposition of cholesterol in the intima were enhanced because of the lack of sufficient phospholipid to act as colloid stabilizer.

Gertler et al (1950) in their studies of serum cholesterol in their cases with coronary disease observed average serum cholesterol level as 286.00 mg per 100 ml whereas in control group 140 males it was 224.30 mg per 100 ml.

Steiner (1950) estimated total serum cholesterol in normal persons and patients with coronary atherosclerosis. Average serum cholesterol level was 254.00 mg per 100 ml whereas in the coronary atherosclerotic group it was 355.00 mg per 100 ml with a range of 308.00 mg to 499.00 mg per 100 ml.

Page et al (1952) and Katz (1952) concluded that there was constant relationship between serum cholesterol level and atherosclerosis.

Nikkila et al (1953) reported that mean blood cholesterol level of their patients of coronary disease was 281.00 mg per 100 ml whereas in normal subjects it was 265.00 mg per 100 ml. The age of
their patients ranged from 40 - 62 years.

Doyle et al (1957) in their investigation of 48 patients with coronary heart disease and 40 normal subjects observed mean total blood cholesterol value to be 273.00 mg per 100 ml and 244.00 mg per 100 ml respectively.

Most of the investigators had reported high level of blood cholesterol in patients with coronary artery disease (Gertler et al, 1954, Steiner, 1952, Oliver and Boyd, 1953).

Mathur (1960) in his epidemiology of coronary heart disease in Agra found that the mean total serum cholesterol value was higher in coronary group than in the control group.

The mean total serum cholesterol values obtained by Ametuzio et al (1962) in their studies of patients with coronary diseases was 288.00 mg per 100 ml which was found to be much higher than that in a normal control group they studied.

Nikkila et al (1963) estimated plasma cholesterol in male survivors of myocardial infarction and normal subjects and found an average total plasma cholesterol value as 324.00 mg per 100 ml in myocardial infarction cases, whereas in control group it was 231.00 mg per 100 ml.
Gerald et al (1965) observed a mean total blood cholesterol level as 252.00 mg per 100 ml in patients with coronary heart disease. The age of their patients ranged from 35-70 years.

According to Verley (1970) total serum cholesterol value between 300.00 mg and 400.00 mg per 100 ml, were rather a frequent finding in coronary thrombosis and in angina pectoris.

Dayton et al (1970) in the UOLA interdepartmental conference held at Losangelos concluded from the prospective epidemiological studies that serum cholesterol level was predictive of increased risk of myocardial infarction. Lowering of serum cholesterol concentration might retard atherogenesis and delay in some of its complications.

Hypercholesterolemia in hypothyroidism and hypcholesterolemia in hyperthyroidism were reported by many workers. Peters et al, (1943) and Peters and Van-Slyke (1946) reported that hypothyroidism blood cholesterol level was elevated while hyperthyroidism it was usually below normal.

The value for total blood cholesterol obtained by Foldes et al (1946) in hypothyroidism was 414.10 ± 78.90 mg per 100 ml while in hyperthyroidism it was 156.20 ± 78.00 mg per 100 ml. The figures quoted by Peters et al (1950) in hyperthyroidism was 911.00 mg per 100 ml.
Although the hepatic synthesis of cholesterol was depressed following thyroidectomy and was increased in thyrotoxicosis the concentration of cholesterol in the plasma was frequently increased in hypothyroidism and decreased in hyperthyroidism. The explanation given by Cantarow and Schepartz (1967) was that although thyroid hormones increased the rate of biosynthesis of cholesterol they also increased the rate of its degradation, transformation (e.g., to bile acids) and excretion (biliary) to an even greater extent accounting for the lowered blood concentration.

According to Varley (1969) the estimation of blood cholesterol in myxoedema might give valuable help in diagnosis. He obtained the values of total blood cholesterol level from 500.00 mg to 700.00 mg per 100 ml in these cases. He noted that the rise roughly paralleled the fall in basal metabolic rate. Administration of thyroxine leads to a marked fall in the serum cholesterol level. In hyperthyroidism cases he found the values of serum cholesterol as low as 80.00 mg to 100.00 mg per 100 ml. However, in quite a number of cases he found the serum cholesterol within the normal range. Therefore, he concluded that the estimation in this condition must be regarded as being very limited value.

Hypercholesterolemia had been observed by many workers in renal disease. Bloor (1917) noted raised blood cholesterol in
patients of nephrotic syndrome.

Other workers like, Page et al (1936), Peters et al (1950), Barr et al (1951), and Rosenman et al (1953) studied serum cholesterol level in the patients suffering from nephrotic syndrome. They observed high blood cholesterol level in those patients and found that the range varied from 300.00 mg to 1060.00 mg per 100ml.

Rosenman et al (1953, 1954, 1956) had done experimental work on rats by injecting anti-rat kidney serum which produced chronic renal disease stimulating nephrotic syndrome and observed rise in blood cholesterol level within 6 to 8 hours after injection.


In those forms of kidney disease in which oedema is not a prominent symptom, hyperlipemia may be absent. Thus, in acute nephritis, blood lipids remained at a normal in the early stages, but a hyperlipemia developed in the later stages coincident with the oedema.
In forms of nephritis in which little or no oedema existed the blood lipids usually remained at a normal level (Page et al, 1936, and Peters et al, 1943).

Varley (1970) noted that serum cholesterol was much increased in the conditions in which serum albumin was diminished, that is, in the earlier stages of Type-II nephritis and in those cases of acute nephritis which pass into a subacute stage. Otherwise serum cholesterol was normal or nearly so.

The basic reason for the hyperlipemia in kidney disorder was not clear. According to the data of Hiller et al (1924) there would not appear to be any inability to form fat. According to Peters and Van-Slyke (1946) as impairment in the processes concerned with mobilization of lipids would seem to be the main cause of the abnormality.

Liver being the chief site of cholesterol synthesis and its catabolism a derangement in the function in this organ could lead to an alteration in the serum cholesterol level.

In infective hepatitis, higher serum cholesterol level had been reported by various workers (Mancke, 1931, and Man et al, 1945).

Oser and Karr (1925) observed the cell cholesterol increased in the jaundice or infectious diseases, the plasma cholesterol was
usually diminished. As a result whole blood cholesterol estimation gave usually a normal value.

Sperry (1936) and Ahren et al (1949) also observed hypercholesterolemia and increase in the ratio of free to total cholesterol in liver diseases.

Jones (1963) was of the opinion that marked reduction of blood cholesterol ester was suggestive of severe liver damage.

Man et al (1945) observed that in the terminal stages of cirrhosis reached subnormal level of serum cholesterol and it showed decreased amounts of functioning of liver tissue.

Philips (1960) studied the serum cholesterol in patients of cirrhosis of liver, acute hepatitis and obstructive jaundice and noted that the esterification was decreased as the bilirubin contents of plasma was increased. He also observed that there was increase in the free fraction of cholesterol in the ratio of free to total cholesterol was in the range of 51.10mg to 94.40mg per 100 ml in primary biliary cirrhosis, 31.70mg to 93.60mg per 100 ml in acute hepatitis, 34.60mg to 51.30mg per 100 ml in obstructive jaundice. In obstruction of the biliary tract, Albrink et al (1950) reported that in infective hepatitis and obstructive jaundice total serum cholesterol as well as ratio of free to esterified cholesterol...
cholesterol increased. Ratio of free to total cholesterol rose markedly in obstructive jaundice. He concluded that the total cholesterol was lowest where the element of parenchymal damage was greatest and the cholesterol level was highest when the element of obstruction was highest.

Epstein (1936) however observed that in obstructive jaundice if the obstruction was not relieved the blood cholesterol level fell prior to death.

Byers et al (1950) reported that after simple ligation of the bile ducts of the rats the free cholesterol of the plasma was rapidly increased, while a much slower and less rapid rise in the esterified cholesterol level occurred when cholesterol was injected into such rats and increase in free cholesterol was noted in the plasma. They suggested that the regulation of the free cholesterol level in the plasma of the rat might be dependent upon its destruction or excretion by the hepatobiliary system.

Brunte-Stewart et al (1962) attempted to differentiate the role of genetic environmental factors, diet, stress, exercise etc. in the etiology of Ischemic heart disease, and also the blood group distribution in those patients. They found a highly significant association between the ABO blood group and Ischemic heart disease. They showed that in coloured population of South Africa the risk of
developing myocardial infarction in A and B group individuals was more than in O group individuals. The high incidence of myocardial infarction in persons belonging to Group A and Group B had also been shown by Gertlers and White (1954). Oliver and Cumming (1962) had shown in a study of patients of Ischaemic heart disease and controls that there was no significant predominance of group A and group B over group O in those patients and there was no deviation from the distribution in control group.

It had been observed that hypercholesterolemia was associated with high incidence of atherosclerosis and coronary heart disease. The observation regarding high incidence of Ischaemic heart disease in persons belonging to group A and group B than those belonging to group O, led Oliver et al (1969) to study the association between ABO blood group and serum cholesterol level in survey carried out in healthy blood donors aged between 30 years and 59 years.

They found that men belonging to blood group A had higher mean serum cholesterol level than those belonging to group O and group B. Their findings emphasised the genetic aspect of the pathogenesis of the Ischaemic heart disease. The relationship between ABO blood group and serum cholesterol level had also been reported by Langman et al (1969). They had also shown that serum cholesterol level in blood samples obtained in healthy men and women tended to be slightly higher in people of blood group A than in those of blood group O, B and AB. On the other hand Shreevastava
and Sinha (1966) did not find any significant correlation between serum cholesterol level and ABO blood group.

Adult population groups were liable to vary in their dietary habits, mental stress and strain, physical activity etc., which might affect their serum cholesterol level. The ideal population group was available only in new born babies. If it could be shown that a relationship exists between serum cholesterol level and ABO blood group in the cord blood of new born babies it would be a very valuable evidence showing the effect of ABO blood group on serum cholesterol level.

Age is an important physiological condition which causes alteration in blood cholesterol level. According to Gordon and Cohn (1928) the level of cholesterol was only 89.00 mg per 100 ml at birth in human infants, it was increased to an average of 136.00 mg per 100 ml in 5 to 12 months and reached the adult value by the age of 5 to 6 years. Keys et al (1950) and Padminavati et al (1958) observed that serum cholesterol level rose with age.

Researches ranging from laboratory experiments to epidemiology or world wide basis had led to the hypothesis that the fat content of the habitual diet of population had an important effect on blood cholesterol level. A large number of workers all over the world had shown that high intake of saturated fat in the diet...
raised the serum cholesterol level whereas increased intake of unsaturated oils lowered serum cholesterol level. The above hypothesis had been nicely summarised by Ancell Keys (1957) in the following words: 'Though many factors of nature and nurture may involve in the multiple etiology of coronary heart disease, the development of majority of cases in a population that suffers more from it is dominated by the long time effects of rich fatty diet and innumerable fat loading meals. The result of high fatty diets are hypercholesterolemia which produces atherosclerosis and changes in the coagulability and other characteristics of the blood that favours thrombosis and fibrinolysis. All food fats are not identical in promoting hypercholesterolemia, but those most favoured and abundant in almost all fatty diets are more powerful in this direction.'

It had been shown by Gopalan and Ramnathan (1957) that if other factors remained the same, physical activity if intense and continuous lowered serum cholesterol. Gsell and Mayer (1962) were of the view that such dietary factors as the fat contained and proportion of saturated and unsaturated fats were probably important in determining the cholesterol level, but they were less important than the activity factors for they manifest their importance only in physically inactive population.