Atherosclerosis is a condition characterised by a variable combination of changes in the intima of the arteries consisting of focal accumulation of lipids, complex carbohydrates, blood and its products, fibrous tissue and calcium deposits with changes in tunica media. The earliest change usually detectable in arteries is the so-called fatty streak. It is described as a small area yellow to gray in colour and containing an intracellular accumulation of lipid as well as extracellular lipid and fibrin. The cells are mostly smooth muscle cells and foam cells and the lipid is mostly cholesterol ester (Smith, 1965). Besides, fatty streaks the early change in the arteries, in this condition, may also be in the form of fibrous plaques. These cause the affected area of the intima to assume a pearly white appearance and are much larger than fatty streaks. The cholesterol ester composition of fibrous plaques closely resembles that of plasma. Despite these differences many workers believe that the plaques are derived from fatty streaks. These early lesions
change with age to various types of complicated lesions. McGill et al (1963) described aortic fatty streak consisting of intima of varying thickness containing stainable lipids in various forms, as a stage intermediate between intimal thickening and fibrous plaque formation. While describing the early atherosclerotic lesion Hess & Stäubli (1969) stated that there is retention of lipid first in the subendothelial space which is subsequently metabolised by the smooth muscle cells of the intima and with increasing influx these cells assume phagocytic properties and possibly later change into myogenic foam cells. With the passage of time the above process gradually extends in the direction towards the adventitia. Sappington & Cook as early as 1936 observed that arteries with most marked intimal thickening are most prone to develop severe atherosclerosis but intimal thickening itself perhaps does not inevitably lead to atherosclerosis (Mcgill et al, 1963).

In the aorta of rats in whom atherosclerosis was induced experimentally using a diet similar to that of Hartroft in the present investigation showed the following changes:
(1) Sudanophilic deposition in the intima and subintimal layers (Fig. 2).

(2) Intimal thickening possibly due to endothelial cell proliferation and also infrequent intimal discontinuity (Fig. 3).

(3) Thickening and reduplication of elastic fibres in the elastic lamina (Fig. 4b).

No obvious change, however, could be demonstrated between the control and the atherosclerotic group of animals histochemically using OTAN technique in the aorta of rats used in the present investigation (Fig. 6b). In rats rendered atheromatous on a cholesterol-peanut oil-cholic acid-thiourea diet Hess & Staubli (1963) reported that sudanophilic neutral fat is present in the aortic intima and detected only traces of phospholipid with acid haematin method and little or no cholesterol with PAN method. The above observations are in close agreement with the findings as stated above in the present investigation. Moreover, it may be noted here that the morphological features of the lesions in rat, according to Hartroft and Thomas (1963), are more similar to those in man than those of most other experimental animals. Also
that even with currently known techniques it has not been possible to produce atheromatous lesions in rat comparable to advanced lesion seen in man.

Although, informations on atherosclerotic lesions both in the clinical and experimental fields are available with reasonable details, progress toward an understanding of the etiology of the atherosclerotic process had been very slow. Among many, the most obvious reason for this state of our knowledge is perhaps the fact that no satisfactory method was available until recently to study this early phase of the disease in-vivo. Nevertheless, a number of etiological factors have been discussed in the literature, and a multitude of hypotheses have been proposed over the last century to explain the pathophysiology of the atherosclerotic lesions. More important of them may be stated as (i) the encrustation of fibrin and subintimal haemorrhage, (ii) mechanical and haemodynamic (Rheological) and (iii) lipid infiltration hypotheses.

In 1851, Carl Von Rokitansky put forward the view that human atherosclerosis could be the result of fibrin deposition from the blood onto the arterial wall, a
suggestion subsequently supported by Virchow (1856) and Clark et al (1936). Following these earlier observation Duguid (1946, 1948 & 1952) established the hypothesis by presenting evidences to show that fine films of thrombus and fibrin are being continuously deposited or encrusted on the inner surface of arterial wall throughout life. Support for Duguid's hypothesis has also come from experimental studies (Harrison, 1948; Heard, 1952 and Hand & Chandler, 1962). Since Duguid formulated the above hypothesis information were available on the contrary which provide little or no support for the encrustation theory of atherogenesis. (Lloyd, 1959; French et al, 1965; Gresham and Howard, 1963). Moreover, the theory does not provide a satisfactory explanation for the massive deposition of lipids which occur in atherosclerotic plaques under the condition (Duff & McMillan, 1951) neither it was possible to convert experimentally organised thrombus into a typical plaque. (Wartman, 1948; Friedman & Byers, 1964 a; Constantinides, 1965).
Virchow in 1856 and Rindfleisch in 1872 proposed the haemodynamic mechanical damage to be the cause of atherosclerotic lesion. In reviewing Virchow's conclusions Aschoff (1824) stated that mechanical strain on the arterial wall stimulates proliferation of connective tissues and hence gives rise to sclerotic elements of atherosclerosis. This was subsequently supported by Duguid (1926) and Duguid Robertson (1957). Texon (1957) proposed that the haemodynamic suction pressure of blood flow exerts a lifting action on the tunica intima at bends and curves and over plaques leading to such lesions. Rodbard (1963) while supporting the above hypothesis argued that the cell free plasmatic zone in the blood at the endothelial surface present in laminar flow is reduced or disappears in areas of turbulence, at constrictions and at bends and the loss of plasmatic zone may allow chylomicrons and platelets to become adherent to the endothelium in these regions as a precursor to the occurrence of such lesions (Gordon, 1963). It is interesting to note here that the shear stress of human blood increases in chylomicron hyperlipidaemia.

On the other hand, there are several indications that lipids play a significant role in the production of
atherosclerosis although other factors are also important. The evidences that lipids play an important role in atherogenesis may be summerised as follows:

1. Plasma lipid level and atherosclerosis:

The subject has been studied extensively in the survey known as Framingham and Oslo studies (Kannel et al, 1964; Westlund & Nicolaysen, 1966) and has provided many important and interesting observations supporting the above hypothesis. In these studies, the initial plasma lipids in apparently healthy subjects have been found to correlate with the subsequent incidence of coronary heart disease. Incidence of coronary heart disease was found to vary as a function of serum cholesterol level. The high incidence of atherosclerosis and coronary heart disease in essential hypercholesterolaemia provide still other evidences in support of the above theory. It is said that a cholesterol free diet reduces the serum cholesterol level (Keys et al, 1965) and concurrently a therapeutically low fat diet are hypocholesterolaemic. Grande et al (1965) have also suggested that the serum cholesterol
level is proportional to the square root of dietary cholesterol intake. It is interesting to note here that the percentage calorific fat intake is low in Japan and Bantu community of south Africa, while the intake of fat is high in United States, Canada and Britain and that the mortality from ischaemic heart disease is much higher in these latter countries than it is in Japan or South Africa. Recently a great deal of attention has been directed on such observations, to the extent that it suggests that, as if, the only important causative agent in atherosclerosis is the lipid level of the diet. Also plasma lipids may play a second role in the production of atherosclerotic vascular lesions. This is because lipids are important not only in blood clotting but are also involved in the primary aggregation of platelets on the damaged areas of the endothelium which may influence atherogenesis and its complications by affecting thrombus formation.
2. Nature of lipid contents in atherosclerotic lesions:

Chemical analysis of atherosclerotic aorta in humans shows that it may contain cholesterol up to a value of 2-3 mg per 100 mg while individual plaques may contain up to 50% cholesterol (Adams, 1966). Cholesterol constitutes about one third of the total lipids of the aortic intima in adult subjects but this may be as high as half of the total lipids in elderly subjects (Smith, 1965). Böttcher (1964) further observed that atherosclerotic lesions from early small fatty streaks to mature calcified lesions have cholesterol content varying between 65 to 80 percent of lipid fractions. From these analyses it is also known that the proportion of esterified to unesterified cholesterol is high in both early and late forms of atherosclerotic lesions. Further, gas chromatographic analysis of the cholesterol esters contained in human atherosclerotic lesions shows a pattern of fatty acids which, in general, is similar to that in plasma (Böttcher et al, 1960a, b; Böttcher, 1963; Lawrie et al, 1964). Similar observations have also been made by Björntorp et al (1963) in chicken.

These observations strongly support the view that in atherosclerosis there is an increased transport
of cholesterol from plasma to arterial wall. It is also known that cholesterol can be synthesized endogenously by arterial tissue (Siperstein et al., 1951; Bisley and Pritham, 1955; Arzanoff, 1958; Lofland et al., 1965).

The problem of endogenous cholesterol synthesis in arteries is probably much more complicated than what it appears at the first sight. Such activity on the part of arterial tissue perhaps is possible only under normal physiological condition and that this synthetic process is disturbed under morbid condition or declines rapidly with age (Dayton, 1961). Nevertheless, it has also been observed that fatty acid synthesis is increased in the aortic intima of cholesterol-fed rabbit, but incorporation of labelled acetate or mevalonate into cholesterol (i.e., Sterol synthesis) could not, however, be demonstrated under the condition (Whereat, 1964). Further, in-vivo isotope studies on cholesterol-fed rabbit had shown that the major portion of cholesterol in aortic atheromatous lesions comes from blood while only a small fraction is synthesised within the arterial wall itself (Newan & Zilversmit, 1962; Dayton, 1959; Christensen, 1964). Gould et al. (1963), on the other hand, considers that endogenous synthesis of cholesterol is unimportant in atherosclerotic arteries.
Analytical, chromatographic and histochemical studies in man and rabbits on atherosclerotic plaques have further revealed that sphingomyelin is the characteristic phospholipid (Adams and Bayliss, 1963; Adams, 1964 a, b; Adams et al, 1963 a, b; Adams et al, 1964 a, b; Höttcher, 1964) in these lesions. Weigensberg and McMillan, (1964), however reported the predominance of cephalin instead of sphingomyelin in such lesions produced in cholester fed rabbit. Since sphingomyelin is not the characteristic phospholipid of either plasma(Philips, 1958; Nye et al, 1961) or red cells in various species, Westerman et al (1963) suggested that atheroma phospholipids are not derived from plasma or from the red cells of encrusted thrombus but are actively synthesized in the arterial wall. The idea has been further reinforced by the works of Bjorntorp et al (1963) and Young et al (1964). Many experimental evidences have also been advanced in support of the above view and these are: 1) Phospholipid is labelled more rapidly with P-32 in rabbit and human aortic wall than the corresponding blood plasma (Zilversmit et al, 1961; Newman et al, 1966). ii) C-14 acetate labels phospholipid hundred times more than cholesterol in aortic intima (Newman et al, 1961). iii) The rat aorta and the normal
human arterial intima incorporate most lipid precursors into cephalins and lecithins rather than sphingomyelin (Chobanian Hollander, 1966; Billimoria, 1967). iv) Certain enzymes and metabolic pathways concerned with phospholipid metabolism could be detected in homogenates of aorta. (Stein et al, 1963).

3) Experimental induction of atherosclerosis by feeding high cholesterol diet.

It has been possible to induce atherosclerosis in several species by feeding high cholesterol diet. Experimental atherosclerosis produced in this way has been under study since the time of Ignatowsky in 1909 when he first produced experimental aortic atherosclerosis by feeding rabbits on a diet of meat, milk and egg. Since then atherosclerosis has been induced in chicken (Katz and Pick, 1961), dog (Steiner and Kendall, 1946), rat (Wissler et al, 1954; Fillios et al, 1956), mouse (Cuthbertson et al, 1960), guineapig (Altschul, 1950), hamster (Goldman and Pollak,
1949), domestic pig (Reiser et al., 1959) and monkey (Mann et al., 1953; Cox et al., 1958; Taylor et al., 1959, 1962) using suitable atherogenic diets of which cholesterol forms one of the essential ingredients. Such diet helps to produce gross hyperlipaemia and hypercholesterolaemia and as the plasma cholesterol levels rise, pathological changes develop in the arteries with development of subsequent processes producing a lesion homologous to the fatty streak in man. In the present investigation a successful early lesion could be developed in rats fed with a modified Hartroft diet.

4. Factors affecting plasma lipid level and atherosclerosis:

It is generally accepted that one of the factors by which hormones influence the development of atherosclerotic vascular disease is by altering plasma lipid levels. Of the several hormones that have been implicated in atherogenesis, oestrogens and thyroid hormones have received special attention. The influence of oestrogen on atherosclerosis received attention from the fact that more atherosclerosis exists in males under forty years of age
than in female and that at middle age coronary heart
disease in males is greater than in females. The possible
mechanism by which oestrogens are related to atherosclero-
tic lesions is its effects on plasma lipid metabolism,
producing an increased plasma level of phospholipid. It
has further been observed by Katz (1952) that oestrogen
reverses coronary lesions induced in cockerels by
cholesterol feeding.

Interest in the thyroid hormones in relation
to atherosclerosis stems largely from the relation
between myxoedema and plasma lipid levels. Moreover, it
has also been observed that thyroid hormones can minimise
the atherosclerotic lesions in cholesterol fed rabbits
and that the rats can be made to develop atherosclerosis,
if made hypothyroid. Katz & Stamler (1953) and Kritchevsky
et al (1961) also reported that the administration of
thyroxin to the cholesterol fed chick and rabbit lowers
the serum cholesterol level whereas thyroidectomy or
administration of thiouracil increases serum cholesterol
in the rabbit (Fisher, 1964). It has further been
observed that thyroxine and dextro-thyroxine increase:
excretion, synthesis and absorption of cholesterol
(Friedman et al, 1952; Duncan, 1961; VanItallie and
Hasim, 1965) but since the first predominates there is
a fall in the serum cholesterol level. Thiouracil, on the
other hand, depresses biliary excretions of bile acids and
cholesterol and perhaps also the conversion of cholesterol
to bile acids (Friedman et al, 1952; Abel et al, 1956).
However, Kritchevsky (1965) found no evidence to indicate
that thyroid hormone increases oxidation of cholesterol
side chain instead he observed that it may control a
steroid oxidising enzyme which converts cholesterol to a
hydroxylated coprostanol derivative (Kritchevsky & Tepper,
1965).

Administration of cortisone increases the serum
cholesterol, triglycerides and phospholipid levels in the
rabbit, rat dog and man (Adlersberg et al, 1951; Rich et al,
1951; Adlersberg, 1959; Moran et al, 1966). Likewise,
atherosclerosis is found to be more severe and in Cushing's
syndrome (Katz & Stamler, 1953) and in cortisone treated
cholesterol fed rabbit. Conversely, it is well known that
patients suffering from Addison's disease have relatively
little atherosclerosis.

5. Hypocholesterolaemic agents
and atherosclerosis:

The effect of a number of hypercholesterolaemic agents on plasma cholesterol level is well known. It is possible to reduce the plasma cholesterol level by reduction in absorption on endogenous synthesis of cholesterol by agents like \( \beta \)-sitosterol, oestrogen and Atromid 'S'. The same can also be effected by increasing the excretion of cholesterol by unsaturated fatty acids and hormones. Nevertheless, the therapeutic use of these agents has not proved beneficial in patients with established ischaemic heart disease which might be due to a relatively late administration of these drugs when the lesion has already reached an inert metabolic state.

Mechanism of lipid transport and its deposition in tissues.

Although, there are valuable evidences, as enumerated above, about the intimate relationship
between blood cholesterol level and formation of atherosclerotic lesions, the mechanism by which plasma cholesterol level affects the development of atherosclerosis is still debatable. In man, cholesterol is mainly carried in low density lipoprotein and in rat it is concentrated in \( \text{L}_1 \) and \( \text{L}_2 \) lipoproteins (Adams et al., 1964). Page (1954a, b) suggested that lipoprotein enters the arterial wall, as it is carried in the blood, where it becomes dissociated into its constituent lipids and apolipoprotein. Watts (1961, 1963) produced immunohistochemical evidence to show that a number of plasma proteins normally filter through the arterial wall. Simultaneously, Adams et al. (1962) and subsequently Adams and Morgan (1966) put forward autoradiographic evidences using varied kinds of tracer substances viz., tritiated cholesterol (Biggs & Kritchevsky, 1951), C-14 labelled triglyceride (Milch et al., 1965) and 4C-14 cholesterol (Dayton, 1959). Electron microscopic studies also suggest that lipoprotein and chylomicron are transported across the endothelium within pinocytic vesicles or through the intercellular gap between the endothelial cells (French, 1963; Florey, 1967). However,
there is still much uncertainty about the routes by which these macromolecules pass through the arterial endothelium. It appears from above that there is a considerable body of evidence which indicates that lipoproteins normally filter through the arterial wall. There are still other investigators who are of the opinion that lipid may also be deposited by blood monocytes (Macrophages, Lipophages) produced by active proliferation of atherosclerotic plaque. Such entry of lipids is perhaps facilitated by increased endothelial permeability by histamine like substances (Harman, 1962) or by the increased permeability resulting from depolymerisation of mucopolysaccharide in the ground substance (Schallock, 1962; Böttcher, 1964).

Whatever might be the mechanism of entry of lipids into the arterial intima they enter the arterial wall in the lipoprotein form and it is the instability of the lipoprotein that perhaps results in lipid deposition within the intima. So the physical nature of macromolecules that transport lipids may thus be a determining factor in atherogenesis. The above physical nature is perhaps determined to a large extent by phospholipid cholesterol
(P/C) ratio. Histochemical studies show a considerable increase in phospholipids in the early stages of atherosclerotic process (Adams, 1959a; Adams and Tuqan, 1961; Day, 1962; Adams et al., 1963a). Biochemical estimation, on the other hand, shows that P/C ratio in the aortic wall remains above 1.0 in the early stages of the diseased process but later in a fully developed atheroma the increase in cholesterol greatly outweighs that in phospholipid resulting in a fall in the above ratio to a value as low as 0.23 (Adams et al., 1963a, 1964a). It has also been observed that aortic atheroma in the cholesterol fed rabbit becomes severe when P/C ratio in plasma falls below 1.0 (Moore and Williams, 1964).

It follows, therefore, that the endogenous synthesis of phospholipid, occurring in the arterial wall in early phase of atherosclerosis, may be looked upon as a homeostatic mechanism. Phospholipids by their dispersing action on non-polar hydrophobic lipids can correct the inherent instability of lipoproteins (Maardi et al., 1960; Schön, 1960; Rossiter & Strickland, 1960). A number of evidences may be advanced in support of the above hypothesis and these are: 1) In patients with severe
atherosclerosis the serum phospholipid to cholesterol ratio (P/C) is lower at the same level of serum cholesterol than it is in control subjects (Gertler et al, 1950; Morrison, 1952; Steiner et al, 1952; Moore et al, 1963).

ii) Administration of oestrogen affords arterial protection to the cholesterol fed cockerels and oestrogen is known to increase the serum phospholipid level and also the phospholipid, cholesterol ratio (Stamler, 1963; Oliver & Boyd, 1966; Cramer, 1961; Blomstrand and Christensen, 1963).

iii) Hypercholesterolaemia associated with biliary cirrhosis is not accompanied by increase in the severity of atherosclerosis. Conversely, there is often an increase in the severity of atherosclerosis in nephrotic syndrome with hypercholesterolaemia. There is a decreased serum phospholipid level in the latter whereas an increase in the former.

iv) There is formation of atheromatous plaques in the tunica intima (Hartroft et al, 1952; Best, 1956) in rats fed with diet deficient in choline, required for the synthesis of lecithin (Kennedy & Weiss, 1956).

To summarise, it may be stated that intimal thickening in atherosclerosis may be due to all the three factors viz., encrustation, lipid infiltration and
mechanical stress. Thickening of intima then leads to ischaemia of the outer zones of the arterial wall. The ischaemia so produced would then interfere with the synthesis of lipotropic factors locally *viz.*, phospholipid and protein required for the lipid transport across the arterial wall and may also interfere with the arterial lipase required to hydrolyze infiltrated triglycerides and thereby set up a positive feedback cycle. It is likely that in this lipid accumulation process in atherosclerosis, metabolic impairment of the normal outward flow of lipid or lipoprotein plays a more important role than increased entry of lipid through the endothelium. The outward flow of lipid or lipoprotein through the arterial wall is affected by a number of factors. Perhaps the most important of them is the local phospholipid concentration. The phospholipids disperse the hydrophobic lipids such as cholesterol and triglyceride by their surface active property and thus help the transport of cholesterol and triglyceride out of the arterial wall. To do this the optimum phospholipid to cholesterol ratio
should be above unity. The human body tries to maintain this homeostasis even during early phases of atherosclerosis by increased local synthesis of phospholipid. To be more precise, it may be said that phospholipids perhaps increase the rate of entry of cholesterol into the arterial wall when the serum level of the sterol is high and helps to clear the same from the arteries when the serum level is low. This means that phospholipid increases the equilibration speed of cholesterol and other hydrophobic lipids between tissues and blood. Still another important mechanism by which lipid is transported out of the arterial wall is by active esterification of cholesterol within the arterial wall itself. This esterification is probably mediated by a transacylating enzyme that transfers fatty acids from unsaturated phospholipid to cholesterol.

Chemical estimation of brain lipids and its fractions, as observed in the present investigation, gave a mean concentration of total cholesterol per gram as $18.94 \pm 0.59$ mg whereas those for ester cholesterol and free cholesterol as $2.46 \pm 0.41$ and $16.47 \pm 0.53$ mg.
(Table II) respectively. This indicates that in normal brain concentration of free cholesterol is greater than that of ester cholesterol, an observation also made by others (Johnson et al, 1949). Mcllwain (1966), however, is of opinion that in normal adult brain, cholesterol exist entirely in the free state. This, of course, is in contrast to its occurrence in many other organs of the body, where cholesterol ester predominates. The ester cholesterol concentration for normal brain as estimated in the present investigation in rats was higher than that observed by others, the possible reasons for which have already been discussed. But whatever might be the extent of error involved in estimation it is true that most workers have reported the presence of a low concentration of ester cholesterol in adult brain. This perhaps signifies that a slow esterification of cholesterol does take place also in normal adult brain.

The concentration of lipid phosphorus hence phospholipid per gram of normal brain tissue, on the other hand, as
estimated in the present investigation gave a mean value of 1.87 ± 0.09 mg (Table II).

The lipid phosphorus when correlated with total cholesterol (Fig. 28) in normal brain, the two were found to have a highly significant positive correlation \((r = +0.96, p < 0.001)\) the regression equation being \(y = 0.1396x - 0.7700\). This means that with the increase in cholesterol concentration in the brain there is a corresponding increase in that of phospholipid. This perhaps represents a kind of homeostatic mechanism that normally operates in the brain which prevents deposition of cholesterol in excess of what is present normally. This is achieved, as stated before, by the surface active phospholipids present in the brain which corrects the instability of hydrophobic lipids and thus accelerates the equilibrium of such lipids between plasma and the brain tissue. Increase in phospholipid may possibly be due to an increased synthesis proportional to the increase in the hydrophobic component. The details of the exact mechanism is, however, still lacking. Another possible homeostatic mechanism which might prevent excess
cholesterol deposition in normal brain is perhaps an increased esterification of cholesterol. The rate of such esterification is, however, slow as is evident from the relatively low concentration of esterified cholesterol estimated in the present study. Such esterification of cholesterol, of course, requires the presence of transacylating enzyme which helps to transfer fatty acid from unsaturated phospholipid to cholesterol. Whether such homeostatic mechanism is present at all may perhaps be verified by the demonstration of the presence of such enzyme in the brain.

On induction of atherosclerosis the concentration of total cholesterol and free cholesterol per gram of brain tissue were found to increase significantly \((p < 0.001, p < 0.001)\) excepting ester cholesterol in which there was a significant decrease \((p < 0.02,)\) Table-IV. This suggests that there is a positive increase in the total cholesterol concentration in atherosclerotic brain and that this increase is mainly due to an increase in the free cholesterol component. An increase in total cholesterol in atherosclerotic brain
has also been corroborated by histochemical study (Figs. 9a, b - 13a, b). The significant positive correlation between free and total cholesterol ($r = 0.97$, $p < 0.001$ - Fig. 29) further supports the above view. Whether a part of free cholesterol is contributed by active deesterification of ester cholesterol simultaneously is a matter of further verification. In this connection it may be noted that the ester cholesterol which had a highly significant positive correlation with total cholesterol in normal brain ($r = 0.81$, $p < 0.001$ - Fig. 30) reduced to low insignificant correlation ($r = 0.2$, $p < 0.5$ - Fig. 31) in atherosclerotic animals (Table - XIV). This is perhaps due to a derangement of the slow esterification process normally present in face of a general derangement of the homeostasis of lipid metabolism in the brain in atherosclerosis which is discussed as under.

It has been observed that in atherosclerotic brain there is a significant increase ($p < .01$) in the mean concentration of phospholipid compared to that in normal brain (Table - IV). In addition to this the most
significant change in lipid concentration of brain in atherosclerotic animals observed in contrast to control was a reversal in the interrelationship between phospholipid and total cholesterol concentrations. While there was a significant negative correlation (r = -0.8, p < 0.001) between lipid phosphorus and total cholesterol in atherosclerotics (Fig. 32) the same was significantly positive in normal controls (Fig. 28). This means that in atherosclerosis with the increasing cholesterol concentration in brain there is a fall in the concentration of phospholipid and hence in the phospholipid cholesterol (P/C) ratio in contrast to normal brain, a situation closely simulating that in aorta or plasma under atherosclerotic condition. (It may be mentioned here that the mean P/C ratio in normal brain is greater than that observed in aorta (1.0) and in our estimate it was 2.5. This decreased to 2.3 in atherosclerosis).

From what has been stated above it may be postulated that the lipid deposition in the atherosclerotic brain may be due to one or a combination of the following factors:
1) Negative correlation between the phospholipid and cholesterol concentration in the brain resulting in reduced P/C ratio.

ii) Reduced rate of cholesterol esterification producing a decrease in mobilization of cholesterol.

iii) Increased rate of entry of cholesterol in presence of high plasma sterol level.

It may be argued here that the increased concentration of cholesterol, as observed in the atherosclerotic brain, may be due to increased local synthesis under the condition. The facts that exogenous cholesterol depresses cholesterol synthesis (Manalo-Estrella et al, 1963; Bhattathiry and Siperstein, 1963) and that the thyroid hormone, which is known to increase cholesterol synthesis, blocked by methyl thiouracil, local synthesis is unlikely to occur. For all probability perhaps there is a breakdown of the blood-brain-barrier due firstly, to a high plasma cholesterol level and secondly, to a direct increase in the membrane permeability under thyroid deficient state (Lange, 1944). This facilitated the
transport of cholesterol from the plasma into the brain where it is deposited subsequently under the action of one or a combination of the factors discussed above.

Further concentration of cholesterol in the brain of both control and atherosclerotic group of animals when plotted against phospholipid showed a continuous distribution pattern (Fig. 33). A critical analysis of the distribution curve indicates that initially as the cholesterol concentration increases there is a corresponding increase in the concentration of phospholipid. This is, perhaps, what is necessary to maintain the homeostasis of lipid stability, such that there is no excess deposition of lipid than what is necessary for normal brain function. The above, however, holds good up to a certain critical concentration of cholesterol. As soon as that critical level is crossed the correlation between cholesterol and phospholipid is reversed i.e., with increasing cholesterol concentration there is a gradual decrease instead of increase in the phospholipid concentration when there is a complete derangement in the homeostasis of lipid stability causing an excess deposition of cholesterol in brain. However, even under the condition of deranged homeostasis, in its
Fig. 28. Shows the correlation between total cholesterol and lipid phosphorus of brain in normal rats.

Fig. 29. Shows the correlation between total cholesterol and free cholesterol in brain of atherosclerotic rats.
Fig. 30. Shows the correlation between total cholesterol and ester cholesterol in brain of normal rats.

Fig. 31. Shows the correlation between total cholesterol and ester cholesterol in brain of atherosclerotic rats.
Fig. 32. Shows the correlation between total cholesterol and lipid phosphorus in brain of atherosclerotic rats.
Fig. 33. Shows the correlation between total cholesterol and lipid phosphorus in brain of normal (*), atherosclerotic (X) and TSH treated (Θ) rats. The plot between total cholesterol and lipid phosphorus when taken together shows a continuous distribution pattern. The correlation with their significance are given in Table-XIV. For details see text.
early phase, at least, there is an attempt on the part of brain to correct it. Increase in the mean concentration of phospholipid in brain (Table-III), particularly in its lecithin fraction, in the atherosclerotic group of animals as compared with that of normal controls bear testimony to the above fact.

Chemical analysis of pituitary lipids and its fractions revealed a mean total cholesterol concentration per gram of fresh pituitary to be of the order of $11.1 \pm 0.16$ mg and the concentrations of ester and free cholesterol being $9.34 \pm 0.33$ and $1.76 \pm 0.05$ mg respectively. In the pituitary ester cholesterol was present in much greater concentration than free cholesterol in contrast to brain (Table II & X). This may be due to the basic difference in function of the two organs. While brain subserves primarily the function of neural transmission, the function of pituitary is mainly secretory in nature. So far, the relative concentrations of ester and free cholesterol are concerned pituitary resembles more closely to other secretory organs viz., adrenals etc., than brain.
The concentration of phospholipid per gram of pituitary, as determined by lipid phosphorus estimation in the present study, had a mean value of $1.43 \pm 0.11$ mg. Lipid phosphorus and total cholesterol in normal pituitary showed a low insignificant correlation in contrast to brain where the two had a highly significant positive correlation (Table XIV).

The total lipid and the concentration of its various fractions were found to increase significantly in pituitary of atherosclerotic animals excepting the free cholesterol where the increase had no significance (Table VII). On the other hand, it has been observed that there was a significant positive correlation between lipid phosphorus and total cholesterol in pituitary under atherosclerotic condition. Also whatever increase in the cholesterol concentration has taken place here was all due to an increase in the ester cholesterol fraction (Table VII). The increase in ester cholesterol fraction perhaps has taken place in any of the following two ways or their combinations: 1) direct infiltration
of ester cholesterol from plasma and ii) infiltration of free cholesterol from the plasma which is subsequently esterified in the pituitary itself. A significant increase in phospholipid under atherosclerotic condition with a significant positive correlation with total cholesterol supports the possibility of the later process to be effective under the condition.

In 1954 Seifter & Baeder reported the presence of a lipid mobilizing substance (LM) in the plasma of various animals and man. They later isolated the substance from posterior pituitary of hogs and found it to be an octapeptide (Seifter and Baeder, 1957). Subsequent works indicate that LM has a significant role in increasing blood lipid level particularly under conditions of surgical stress (Zarafonitis et al, 1957, 1959), pregnancy (Zarafonitis et al, 1958), nephrosis (Seifter & Baeder, 1954) and certain hyperlipidaemic states (Zarafonitis et al, 1957). In man, it has also been observed that injection of crystalline LM (1.2\,\mu g) produced a prompt rise in plasma total fatty acids, cholesterol and phospholipids (Zarafonitis et al, 1958).
A review of literature on the subject indicates that liver is perhaps the source of excess cholesterol and phospholipid appearing after LM injection (Baeder and Seifter, 1968). It has also been suggested that LM possibly acts on omental and mesentric fat beds discharging neutral fat into the portal circulation (Zarafonetis, 1962).

The role of pituitary gland in the regulation of lipid metabolism and transport can be found in literatures published during the last thirty years. A search of these literatures provides ample evidence that injection of various substances of pituitary origin decreases the fat content of carcass and adipose tissue, increases the liver lipid (Best and Campbell, 1936; Campbell and Lucas, 1951) increases the ketone body in blood and urine (Campbell and Lucas, 1951; Burn & Ling, 1929; Anselmino & Hoffman, 1931), depresses the respiratory quotient (Fisher et al, 1936) and increases plasma FFA (Raben, 1958). Increases in liver lipid has also been reported following injection of anterior pituitary extracts and its various fractions viz., adrenotrophic, somatotrophic
and thyretrophic preparations (Payne, 1949; Rosenberg, 1953). Other investigators (Evans, 1933; Munoz, 1933; Baumann & Marine, 1932; Campbell et al., 1953) also reported occurrence of lipaemia in dogs and rabbits following repeated injections of anterior pituitary extracts or purified growth hormones. Sturm (1959) enumerated the four factors of TSH and identified one of these as a lipid mobilizing factor. It has also been reported that adipokinetic pituitary hormones like ACTH; TSH, etc., exert important effects on other target organs (Rudman, 1963).

It is interesting to note here that in spite of the presence of powerful adipokinetic substances of the anterior pituitary and lipid mobilizing factor of posterior pituitary cholesterol is deposited in a number of organs viz., liver, spleen, adrenals, kidneys, bone marrow, subcutaneous tissue (Anitschknow, 1913, 1933; Weinhouse & Hirsch, 1940) and brain and pituitary as observed in the present study when the animal is maintained on atherosclerotic diet. It is possible that
under the circumstances the functions of these organs may be deranged due to failure on their part to cope with the lipid load. Evidences of such functional derangements are perhaps well known in a fatty liver resulting from impairment of hepatic apolipoprotein synthesis, in the myocardium following excessive fat deposition, in brain in conditions like, Tay-Sachs, Niemann-Pick, Gaucher's disease and Hand-Schuller-Christian syndrome where there an excess deposition of lipid material. In the pituitary as observed in the present investigation there was nearly a 9% increase in the total lipid load in the rat fed with atherosclerotic diet (Table X & XI). It may be postulated that the extra lipid so accumulated might have deranged the function of the pituitary so far its lipid mobilizing action is concerned.

TSH being one of the adipokinetie substances of anterior pituitary was employed in the present investigation to assess its role in lipid mobilization, if any, from brain of atherosclerotic animals. With this end in view TSH in doses of 0.2 I.U. was administered
in a group of rats maintained on atherosclerotic diet for 20 weeks. The atherosclerotic diet was continued during the period of TSH therapy in order to simulate clinical condition. Methyl thiouracil as one of the constituents of the diet was also continued in order to assess the effect of TSH in absence of thyroid hormone.

Following TSH therapy no significant alteration in the concentrations of total cholesterol, free cholesterol or total phospholipid was observed in brain tissue. Two significant changes were, however, observed and these are:

1) Increased esterification of free cholesterol-the mean concentration of ester cholesterol increased from a value of 2.16 ± 0.18, observed in atherosclerotic group to 2.44 ± 0.29 mg following TSH therapy (Table VI). The above indicates a significant rise in the concentration of ester cholesterol in TSH treated rats from that of atherosclerotic controls (p < 0.02).

ii) A decrease in the negative correlation between phospholipid and total cholesterol from that
obtained in atherosclerotic group. In atherosclerotic group this was of the order of $r = -0.3, p < .001$ which changed over to $r = -0.197$ on TSH therapy (Figs. 33 & 34 - Table XIV). The significance of the changed correlation was ($p < 0.5$). This indicates, that there was an attempt on the part of the hormone to revert the correlation between phospholipid and total cholesterol back to its normal relationship ($r = + 0.94, p < 0.001$ - Fig. 33).

Further, it may be noted that TSH had practically no effect on the lipid content of the pituitary in atherosclerotics excepting on its phospholipid constituents. There was a slight increase in the phospholipid content in pituitary following TSH therapy as the mean value differed significantly in all the three groups - control, atherosclerotic and TSH treated (Table VII, VIII and IX), the reasons for which have already been discussed (Chapter VI).

The effect of TSH on brain lipid in animals under atherosclerotic condition is difficult to assess.
Fig. 34. Shows the correlation between total cholesterol and lipid phosphorus in brain of TSH treated rats.
on the basis of the present data alone. The information obtained in support of the above in the present investigation was on a very small sample and to what extent the small sample error is influencing the interpretation is difficult to assess at this stage. In this connection it may be recalled that Rudman and Seidman (1958) reported that purified preparation of adrenotrophic, somatotrophic, thyrotrophic hormones etc., had very little effect on serum lipids even at a dose five times greater than the crude extract. These findings suggest that although the hormones individually has a lipaemia producing effect the possibility remains that two or more anterior pituitary hormones might act in a synergistic manner. On the light of what has been enumerated above it seems that the elucidation of the effects of TSH on brain lipids in atherosclerotic animals requires further elaborate investigation. In continuation it may be stated that Ghoshal (1964) could demonstrate the fact that it is possible to ameliorate some of the initial symptoms in atherosclerotic subjects by TSH therapy for a reasonably long period.
The initial symptoms of so-called cerebral atherosclerosis are psychic in nature. The somatic and neurological symptoms show up at a comparatively later period. The initial psychic symptoms often cannot be distinguished from those occurring with age and may confuse the whole picture. However, the symptoms which are variable initially later gives rise to an uniform pattern. Lassitude, physical and psychial debilitation and neurasthenia are some of the typical initial symptoms. Forgetfulness and loss of memory are still other important features of cerebral sclerosis. While eliciting the preliminary history it often happens that although the patient had subjective complaints during the initial phase they are either forgotten later or are submerged in the somatic and neurological symptoms of defects developed subsequently. Depressive mood is another initial symptom which appear with the further progress of the disease. According to Schulte and Harlfinger (1969) "depressions of this kind on the threshold of arterial sclerosis cannot be accounted for
entirely - if, at all, on psychological grounds. Being for the most exogenously symptomatic, they are largely the direct expression of cerebral degeneration itself, hence fundamentally a physical psychosis. They are, however, part and parcel of the initial phase. Moreover, it may be pointed out here that atherosclerotic process although could affect any and every organ, it is perhaps the renal vessels which are affect earliest and cerebral vessels last. Moses (1963) observed that cerebral atherosclerosis is minimal even at the later decades of life and tends to be focal rather than generalised.

From what has been stated until now, it is doubtful whether cerebral atherosclerosis could be held responsible for giving rise to initial cerebral symptoms in atherosclerotic subjects. It is true that if cerebral atherosclerosis is to produce these initial symptoms it will do so as a result of regional or generalised cerebral ischaemia. Brain with its most powerful homeostatic mechanism will certainly not allow such derangement in homeostasis to occur within such
short periods. For all probability, therefore, it is unlikely on the part of the atherosclerotic process to give rise to these early symptoms.

A change in the lipid metabolism in the brain as enumerated in present thesis work could perhaps explain such early derangements in the functions of brain. The extra lipid deposited in the brain perhaps interferes with the neural transmission in some unknown way giving rise to the above symptoms. Whether the change is in the total cell impedance or in the manner of saltatory conduction or in the synaptic transmission under the condition is a matter of further interesting investigation. The study of brain, lipids, therefore, poses an important problem in all conditions where there is a derangement in lipid metabolism associated with cerebral symptoms. The subject requires an intensive study.