Heart disease is one of the most common causes of natural death in civilized communities in the more temperate zones of the world. It is responsible for at least two-fifth of all such deaths. In U.S.A. atherosclerosis of the coronary arteries is almost universally present in males and in postmenopausal females (Doyle, 1959). The prevalence of manifest of coronary heart disease (CHD) among middle aged men is approximately 5.5 per cent (Epstein, 1964). In 1973, cardiovascular disease (CVD) accounted for 41 per cent of deaths in men aged 35-44 years and 52 percent in men aged 45-54 years (Royal College of Physicians, 1976). In the younger age group almost three quarters, and in the older age group more than three quarters of these deaths were due to coronary heart disease. In women aged 45-54 years CVD accounted for 28 per cent of deaths and less than half of these was due to CHD. Both the incidence and mortality curves have been rising steadily for many years.

A number of factors are regarded to be involved in the aetiology of vascular disease specially for the development of coronary atherosclerotic heart disease. The aetiologic factors may be broadly divided into three groups - (1) Genetic and environmental (2) Immunological,
(3) Dietary factors and those relating to the blood and blood vessel walls.

In the last 20 years, epidemiologic and experimental studies have provided considerable evidence linking certain risk factors to the development of atherosclerotic lesions.

Cardiovascular disease includes the lesions of coronary and cerebral arteries and also those of leg arteries and veins, whereas CHD includes three distinct but overlapping syndromes in middle age, myocardial infarction, angina pectoris, and sudden death. Virtually, all CHD results from severe atherosclerosis, but not all individuals with severe atherosclerosis do not develop CHD.

Coronary atherosclerosis is a pathologic condition of coronary arteries, characterised histologically by lipid accumulation in the intima together with a variable connective tissue reaction in the vessel wall resulting in narrowing of the lumen from the consequent thickening of the vessel wall. Possibly accompanied by other changes in the circulating blood, these result in thrombus formation.

The underlying reason in the atheroma is the structural damage to the intima and there is a deposition of fibrin or a thrombus on this damaged intima, reducing the lumen of the blood vessels and rendering them more
liable to thrombotic occlusion. The mural thrombi are incorporated into the wall of the artery and undergo fatty change as they form atheromatous plaques.

Cholesterol and particularly its ester, is the predominant lipid that accumulates during plaque formation (Smith, 1965). The proportion of ester in the plaque increases during development of the lesion (Smith, et al. 1967) and so, too, does that of phospholipids, but triglycerides are always present in only small amounts. The well-known fact that the nature of the deposited lipid substances in the atherosclerotic plaques changes steadily towards decreased amount of phospholipids led to the belief that the ratio of total sterols to phospholipids is the best index to the extent of atherosclerosis (Mead and Gouze, 1961).

The lipids that accumulate in the plaques are derived primarily from the circulation, but there is also some local synthesis within the arterial wall. Active glycolysis occurs, generating ω-glycerophosphate for the synthesis of glycerides from fatty acids (Opie, 1973). High activity of the pentose pathway in arterial tissue also provides intermediates necessary for the de novo synthesis of glycerides. There is little evidence of cholesterol synthesis in arterial tissue and the major
proportions comes from the plasma (Zilversmit, 1968; Lofland and Clarkson, 1970).

Perhaps, the most important cause of cholesterol accumulation in the arterial wall is the inability of the tissue to significantly metabolise the sterol, apart from some minor degradation and the formation of ester. All these compounds because of their hydrophobic nature, induce sclerosis. Only about 20% of the cholesterol ester in atheroma is produced by local synthesis, but the ester principally produced, cholesterol oleate (Bowyer et al., 1968), is strongly sclerogenic (Abdulla et al., 1967).

Ischaemia of the arterial wall may play a role in the deposition of lipids. Normally, the inner two-thirds of large human vessels is nourished directly from the lumen while the outer third is nourished by the vasa vasorum in the tunica adventitia (Kirk and Laursen, 1955). Thickening of the tunica intima in the aorta results in ischaemia of the tunica media and the effect of this is to impede the outflow of cholesterol from the endothelium through the tunica media and so promote accumulation of cholesterol in the tunica intima as seen in atherosclerosis.
Sociological research on cardiovascular disease is virtually scanty. Giffords' (1966) study and the Los Angeles Heart study (Chapman et al., 1966) demonstrated a trend of differences according to occupation and socioeconomic status between patients with CVD and those with CHD. In both studies the majority of patients with CVD were in the upper socioeconomic half, while CHD incidence – again in both studies shows no differences according to socioeconomic status. Chapman et al. (loc. cit) presented these discrepancies between CVD and CHD as "important differences in aetiological and environmental factors governing the occurrence of these two major vascular disorders".

Mc Gill (1968) reviewed the epidemiological evidences and clearly demonstrated that coronary artery disease is much more prevalent in affluent than in poor countries.

Similar data have been compiled by many investigators. Keys (1963) has reported that in the poor segment of the population in Italy and Spain, the prevalence and incidence of coronary heart disease and mortality from CHD is much lower than in the remainder of the population.

Strong and his colleagues (1968) analysing the extent and severity of atherosclerosis in relation to ischaemic
heart disease in different countries throughout the world confirmed the general relationship but drew attention to some important facts that the incidence of myocardial infarction is much greater in Norway than in Chile, that the mean extent of coronary atherosclerosis is greater in those with, than in those without myocardial infarction in both populations.

Considerable differences in mortality due to coronary heart disease exist between geographical areas of the United States have been reported (Acheson, 1966; Sauer, et al 1966, Stallones, 1965; Gordon, 1966). Bronte Stewart et al (1955) reported the racial differences in the incidence of coronary mortality among men of all three races in South Africa whose financial position and fat intake were approximately the same.

Several autopsies have been made in order to determine the differences of atherosclerosis in the aorta and the coronary arteries by Groom et al (1964, 1966) in two genetically similar populations. Selective involvement of coronary arteries were detected in this process. The workers further reported that atherosclerosis has its inception in childhood, and its distribution in different vascular beds of the body as well as its rate of development are conditioned by environmental factors (Groom et al, 1966).
Gore et al. (1960) compared the severity of coronary and aortic atherosclerosis in their autopsy material from Jamaica, Japan and the U.S.A. The severity of aortic atherosclerosis in Jamaica and Japan approached that observed in aortas from the U.S.A. population autopsy study. However, atherosclerotic involvement of coronary arteries of Jamaicans and Japanese subjects were markedly less than that found in the Americans.

Strong and McGill (1963) concluded from their autopsy studies: "The results of the test for correlation between aortic and coronary lesions indicate that the aorta is not adequate as an indicator of the extent of coronary lesions in the investigation of ischaemic heart disease."
GENETIC AND ENVIRONMENTAL FACTORS:

Epidemiologic research in atherosclerotic vascular disease has turned recently towards hereditary factors after the environmental influence in the natural history particularly of ischaemic heart disease has been elucidated to a large extent. Certain families with essential familial hypercholesterolaemia exist in whom many members became victims of an early and unexpected cardiac death.

Genetic factors were recognised during 1940 in the genesis of myocardial infarction. Those patients, who became victims of myocardial infarctions, were considered obviously to have the strongest genetic predisposition. In the 60's involvement of genetic factors were considered less important in the development of myocardial infarction.

Genetic factors must be considered with respect to the nutritional metabolic hypothesis of atherosclerosis. The Ceylonese appear to have a high incidence of coronary heart disease (CHD) associated with a low fat diet and low concentration of serum cholesterol. Conversely, the Masai tribe of East Africa are virtually free of coronary heart disease and have low blood cholesterol levels inspite of the fact that their normal diet of meat and blood is high in cholesterol. Fidanza (1970) explaining the Masai problem has reported they may be have a genetic trait which produces
an efficient feedback mechanism which protects them from hypercholesterolaemia.

Roth and co-workers (1967) have examined 53 patients with myocardial infarction under the age of 40. Seventeen patients gave a negative family history for myocardial infarction. Thirty six, however, gave a positive history of myocardial infarction in siblings, parents and grandparents at relatively young ages. Therefore, strong family history of myocardial infarction was considered a predisposing factor when the disease occurred in the younger patients. They found a marked clustering of infarction in a few families. Of ten patients whose siblings had myocardial infarction, one or both parents were also afflicted with the same illness.

Slack and Evans (1966) investigated the family history of 121 male and 96 female patients with myocardial infarction. The male relatives of these patients showed an excess of deaths from heart attacks only when myocardial infarctions occurred below the age of 55 years and in men and 65 years in the case of female patients. Among the older patients (men and women) with myocardial infarction, there was no familial aggregation of death for any of the relatives from myocardial infarction.
Walker and Gregoratos (1965) surveyed 100 military men who survived a transmural myocardial infarction at the age 40 years or below. At this young age group 47 per cent of the patients with myocardial infarction had a positive family history and only 20 per cent of the control persons.

Gertler et al (1959) also examined 100 patients with myocardial infarctions under the age of 40 years. They found remarkable clustering in some families "when coronary heart disease appears in the family, it is more significant if both parents have it; with appearance in father and sibling(s) or mother and sibling(s) of slightly lesser significance. Likewise, familial aggregation of coronary heart disease was shown in Rose's study (1964) in some families.

Mulcahy and co-workers (1967) in a study of CHD in 100 women less than 60 years of age, found that 66 of these patients had no coronary heart disease in their families. The authors concluded from their findings that they had only 23 patients with at least one parent under 70 years or at least one sibling under 60 years who have suffered or died from coronary heart disease.

On the evidence so far available it is reasonable to assume that family aggregation of CHD is at least partly
due to higher frequency of risk factors in some families than those in others. Indeed there is little confirming support for more profound hereditary factors.

Research workers have long been familiar with families in which several members have cutaneous or tendinous lipid deposits and very high plasma lipid levels. The actual mode of inheritance of these obviously genetically determined disorder, the nature of biochemical abnormalities and their relationship to the general range of ischaemic heart disease were obscure for a long time.

**Lipoprotein types as a genetic marker in cardiovascular diseases:**

Fredrickson et al (1967); Fredrickson and Levy (1972) described five different patterns of hyperlipidaemia, type I, IIa, IIb, IV and V mainly on the basis of paper electrophoresis of lipoproteins and introduced a simplified system for quantifying the various classes.

Familial and acquired type II hyperlipoproteinemias are important since they are closely associated with premature CHD (Goldstein et al 1973a; Slack and Nevin, 1968; Stone and Levy, 1972).

More recently, a genetic approach could be applied to the hyperlipidaemias from a large epidemiological study of survivors of myocardial infarction and their families.
This familial disorder was the most frequently observed abnormality, recognised as familial combined hyperlipidaemia (Goldstein et al., 1973a).

Stone and Dick (1973) studied lipoprotein patterns estimated by membrane filtration and nephelometry in a random sample of healthy men aged 30 to 69 years and in an age matched group of male patients with ischaemic heart disease. The frequency distribution of these patterns was not significantly different between the random sample of appreciably healthy and ischaemic heart disease groups. In the latter group either plasma cholesterol, triglyceride or both may be elevated along with a variety of lipoprotein patterns (type IIa, IIb, IV and sometimes V, Fredrickson typing) were observed in number of families. Type IV hyperlipidaemia comprised 53% of all abnormal patterns in both random samples and ischaemic heart disease groups.

Goldstein et al. (1973b) studied serum cholesterol and triglyceride concentrations in over 2500 relatives of 149 hyperlipidaemic patients and the distribution of lipid levels in these first, second, third and fourth degree relatives. Their data best fitted a genetic classification in which three autosomal dominant disorders were distinguished - hypertriglyceridaemia, hypercholesterolaemia and a familial combined hyperlipidaemia.
Two family studies by Nikkila and Aro (1973) have added considerably to the knowledge of the genetics of hyperlipidaemia. In both, the relatives of patients also had myocardial infarction. Serum lipid and lipoprotein analysis, using qualitative electrophoresis or quantitative ultracentrifugal analysis were undertaken. Surprisingly wide variations of lipoprotein between that of the index patients and those of his first degree relatives were found. In many families there was a variety of lipoprotein patterns (viz. types IIa, IIb, and IV) classified as multiple lipoprotein type hyperlipidaemia.

Goldstein et al. (1973a) and Schrott et al. (1972) have provided evidence that this familial form of hyperlipidaemia is monogenic, this has, however, been disputed by Slack (1974). Familial hypercholesterolaemia — a major risk factor for the prevalence of CHD, however, showed both monogenic and polygenic modes of inheritance (Khachadurian 1964, 1972, Carter et al., 1971). Other studies failed to show the same trend (Jensen and Blankenhorn, 1972).

Monogenic and polygenic varieties of familial hypercholesterolaemia may in fact have different pathogenic mechanisms. It is also conceivable that they may not be of equal importance in the predisposition of ischaemic heart disease.
Genetic approach may be of importance in assessing the risk patterns and has been reported in the recent study of Brunzell et al. (1976). Pure hypertriglyceridaemia may be seen both in familial hypertriglyceridaemia and familial combined hyperlipidaemia. These authors showed myocardial infarction to be far more frequent among hyperlipidaemic relatives of patients with hypertriglyceridaemia of familial combined hyperlipidaemia than those among hyperlipidaemic relatives of those with familial hypertriglyceridaemia. The frequency of myocardial infarction in the latter group was in fact similar to that of the normolipidaemic relations.

Very recently increased coronary mortality in relatives of hypercholesterolaemic school children was reported (Schrott et al., 1979).

Population studies have been made in search of aetiological factors of cardiovascular diseases. Studies were carried out mostly with serum lipids. Serum lipid concentrations in normal subjects vary widely. This holds good even within homogeneous communities, but, between communities, comparisons show that there may be even a three-fold difference among mean cholesterol concentrations. Seasonal variation in serum cholesterol concentration was also reported (Throp, 1963, Paul et al., 1963,
Doyle et al., 1965. Luyken and Jansen (1960) reported a concentration of cholesterol ranging from 109 mg/100 ml in Pacific islanders to 269 mg/100 ml in Eastern Finland (Keys, 1965b). Serum triglyceride levels also show major geographical differences (Bang et al., 1971; Micheli et al., 1973; Lewis et al., 1974). They tend to vary parallel with cholesterol levels, suggesting that some regionally differing factors influence the metabolism of both lipids in a similar manner. Several factors are known to contribute to this variability, including physical activity, endocrine function, genetic determinants, and diet.

Population studies in different countries:

A concise account of some of the population groups studied in relation to serum lipid and lipoprotein patterns from different parts of the world are summarised below:

The Framingham Study:

Kennel et al., (1971) studied the population of Framingham, Massachusetts, and Bethesda, Maryland, to examine the risk of coronary heart disease according to their antecedent cholesterol and lipoprotein status. Their study indicated an increased risk proportionate to antecedent serum cholesterol among these people.
The Scandinavian study:

Plasma lipid concentrations in men and women aged 16-35 years and living on the west coast of Sweden were studied by Svanborg and Svennerholm (1961). Triglyceride and phospholipid concentrations were found to be higher in female subjects as compared to those in males, whereas cholesterol level was higher in males than those in males with higher prevalences of CVD.

Studies on Japan, Hawaii and California population:

Study of serum lipids was undertaken by Kato et al. (1973) as part of a large and comparative study of cardiovascular disease. They observed consistent negative relationship between serum cholesterol and dietary intake of complex carbohydrate, but no association was observed with simple carbohydrate.

Study of Australian Rural Population:

Serum cholesterol levels in adult population of Busselton in West Australia were studied by Welborn et al. (1969) to establish the prevalence of coronary heart disease. The overall prevalence rate of "probable" coronary heart disease was 68/1000 for males and 58/1000 for females. Their results conform with the evidence for increased coronary heart disease incidence in
asymptomatic hyperglycaemia presented by Keen, Jarrett (1970).

Study on South African white and Bantu prisoners:

A comparative study of fasting serum triglyceride and fatty acid level was conducted on South African whites and Bantus by Antonis and Bersohn (1961). It showed that ischaemic heart disease patients of both sexes and white males over 40 years had fasting serum triglyceride levels significantly higher than those of young white males or premenopausal females and of Bantu males of all ages. Further high triglyceride levels were associated with a greater degree of saturation of the fatty acid components. In view of the widely differing incidence of ischaemic heart disease among these subjects - rare in Bantu but becoming increasingly prevalent among younger white males, it was suggested that raised serum triglyceride levels could be the result of impaired fat transport and clearance mechanism either produced or aggravated by excessive intake of dietary fats rich in saturated fatty acids, which in turn produces ischaemic heart diseases.

Study on immigrants to Israel:

In order to investigate the incidence of atherosclerosis, Toor et al., (1960) conducted a detailed study of immigrants, belonging to seven racial groups to Israel.
from Western and Eastern countries. Serum cholesterol and lipoproteins and protein fractions were studied. They reviewed 5000 hospitalized cases of myocardial infarction and showed a very low incidence for Yemenites (inhabitants of Israel), higher for immigrants from European countries. A statistical survey of the entire population for the years 1953-1959, showed that the mortality rate from atherosclerosis was extremely low for Yemenites and high for European immigrants. These results suggested the influence of socioeconomic conditions, diet and caloric balance on serum cholesterol values and atherosclerosis morbidity.

Stockholm prospective study-I.

Carlson and Lindstedt (1969) conducted this study to determine the relative serum concentration of cholesterol and triglycerides as well as other potential factors in healthy persons predicting the future development of coronary heart disease. Subjects of both sexes, aged 15-74 years, were studied. In the age group of 15-19 years, females had higher cholesterol level than males. At 20-24 years, the level was the same in both sexes and increased in parallel up to 50 years and then continued to increase in females while it levelled off in males. Women thus had
significantly higher cholesterol values than men at 50-64 years. The serum triglyceride concentration was same in men and women at 15-19 years. In men it measured to a maximum at 45 years and then decreased significantly. In women it increased to about 55 years, the male level was about 25% higher than the female.

**Study on Jamaican rural population:**

This study was undertaken by Florey et al. (1973) to investigate the correlation between serum lipid and blood glucose with cardiovascular function. The main cholesterol and triglyceride concentration in male subjects aged 25-64 years were 236 mg/100 ml and 80.1 mg/100 ml and in females of similar age group to 226.7 mg/100 ml and 79 mg/100 ml respectively.

**Study on Seattle population:**

Lipid level of survivors of myocardial infarction were compared with controls by Goldstein and his group (1973a). It was concluded that 31% had hyperlipidaemia. These lipid abnormalities were most commonly found in males under 40 years (60%) and females under 50 years of age (60%). Elevation in triglyceride level with or without associated elevation in cholesterol is 3 times more frequent in survivors than a high cholesterol level alone.
Study on Ceylonese population:

Obeyesenere (1964) studied serum cholesterol level in normal and ischaemic heart disease (IHD) patients and its correlation with consumption of dietary fat. The serum cholesterol levels of the male patients were found to be significantly higher than those of the controls in fourth and fifth decades.

Edinburgh study:

Logan et al (1978) studied risk factors for ischaemic heart disease in normal male aged 40 years. Edinburgh men were shorter and fatter having higher levels of serum total triglyceride and triglyceride in VLDL, LDL and HDL fractions. Rose et al (1976) determined serum cholesterol values in three populations of children and adolescents aged 1 month to 20 years and detected 15 cases of primary hyper beta lipoproteinaemia (overall frequency 1:251). The upper limit of normal serum cholesterol concentrations was approximately 200 mg/100 ml at all ages studied. In their study a finding of major importance was very high frequency of a case related family history of hyperlipidaemia or coronary artery disease or both (in 22 of the 27 affected families identified).
Studies in India:

At present, it is well established that occurrence of cardiovascular diseases specially the coronary heart diseases is quite high among the Indians. Naturally, it has become interesting to study the subjects suffering from myocardial infarction, hypertension and atherosclerosis, in search of the aetiologic factors.

Studies have also been carried out to establish the effects of the dietary factors, age, sex and environmental factors on serum lipid and lipoprotein variants.

Dietary serum cholesterol level was studied in rural and industrial workers of Delhi by Padmavati et al (1958). This survey was undertaken to investigate the incidence of atherosclerosis and hypertension in these groups. Average serum cholesterol levels in male industrial workers below 40 years age was 168 mg% while that in those above 40 years age 169 mg%. Rural male populations below 40 years had serum cholesterol level of 181 mg% while those above 40 years showed 188 mg%. The female rural subjects on the other hand had serum cholesterol of 175 mg% and in subjects above 40 years, this level was found to be 183 mg%.

Mathur (1960) undertook a field survey of general population and CHD patients in Agra to ascertain various
factors relating to heart disease. Average serum cholesterol in normal subjects was $182 \pm 38 \text{ mg}\%$, while in CHD patients it was found to $250; \pm 52.25 \text{ mg}\%$.

Bandyopadhyay and Banerjee (1964) studied plasma lipids in normal subjects and patients suffering from different cardiovascular disorders. In normal subjects level of total cholesterol was $157 \pm 10 \text{ mg}\%$ while triglyceride level was $125 \pm 16 \text{ mg}\%$.

Vytilingam (1966) studied the incidence and epidemiology of CHD in South India. In 22 patients the serum cholesterol level was below $42 \text{ mg}\%$; in 150 patients it ranged from $140-200 \text{ mg}\%$, while rest of the 28 patients showed raised cholesterol levels ranging from $201-500 \text{ mg}\%$. The age of the subjects ranged between 21-90 years.

Nigam et al (1973) undertook investigation of factors influencing early mortality in 342 acute myocardial infarction patients. They reported that as many as 239 had serum cholesterol level below $250 \text{ mg}\%$, 44 had concentration ranging from $251-300 \text{ mg}\%$ while 8 patients showed serum cholesterol concentration above $300 \text{ mg}\%$.

A comparative study of subjects belonging to below 40 years age group and above 55 age group was carried out by Wadia et al., (1973) to investigate precocious coronary artery disease. Serum cholesterol and triglyceride levels
were studied in subjects belonging to both the groups. The former varied from below 200 mg\% to above 300 mg\% in above both groups. They found two cases with normal cholesterol showing hypertriglyceridaemia.

Mowar et al (1976) studied serum lipids and urinary catecholamines in patients suffering from acute myocardial infarction. The study was undertaken keeping in mind that hypertriglyceridaemia and hypercholesterolaemia are considered as high risk factors of IHD. They observed that all lipid parameters and urinary catecholamines were significantly elevated in all the patients within 48 hours of the onset of chest pain. The levels decreased gradually except that of nonesterified fatty acids and triglycerides as compared to normal subjects.

Raman and co-workers (1976) worked out serum cholesterol and serum magnesium concentration to identify the incidence of ischaemic heart disease in subjects of different age groups and patients with ischaemia. They have reported the presence of comparatively high serum magnesium level in younger subjects and high physically active groups. In the groups of well fed subjects and ischaemic heart disease patients blood cholesterol was significantly high and serum magnesium was found to be reduced than the basal level.
2. IMMUNOLOGICAL FACTORS:

It is believed that at least three factors - thrombosis, lipid imbibation, and arteritis - are involved in the pathogenesis of atherosclerosis. It is suggested that increase of endothelial permeability due to inflammation may lead to increased lipid entry. A number of experimental findings suggest that immunological mechanisms could be involved in the inflammatory process (Poston and Davies, 1974; Mathews et al., 1974). Thus, in the rabbit, atherosclerosis can be inhibited by anti-inflammatory and immuno suppressive drugs. Ingestion of antigenic foreign protein exacerbates the atherogenic potential of high lipid diets (Minick et al., 1966; Levy, 1967; Van Winkle and Levy, 1970). An increased incidence of positive skin tests to tobacco leaf protein have been found in patients with CHD who smoked (Harkavy, 1963). This appeared to be an independent risk factor, for the positive groups had a lower incidence of hypertension and hypercholesterolaemia. Absorption of intact protein from the gut known to occur in normal infants of 1-13 months (Gruskay and Cooke, 1965). Increased levels of circulating milk protein antibodies have been found in patients with CHD (Davies et al., 1969; Davies et al., 1974) and there is epidemiological evidence that denatured or altered milk protein may be atherogenic (Annand 1967, 1972). Mathews et al. (1974)
have postulated an immune-complex mechanism whereby circulating immune complexes could set up a vicious cycle of damage to the vessel walls.

By means of various immunological methods it was established that the atheromatous arterial intima contained $\beta$-LP of plasmal origin (Gero et al. 1961a, Tracy et al. 1961, Watts 1963). A further contribution of the above data has been the discovery of the antigenic nature of plasma lipoprotein (LP), which are substantially involved in the pathogenesis of arteriosclerosis. The antigenicity of $\beta$-LP was examined first by Kunkel (1950), who prepared immune serum in rabbits using human $\beta$-LP as an antigen.

Based on the assumption that immunological events may result in some structural changes of the vessel wall interfering thereby with the deposition of plasma lipids, several attempts have been made to influence the development of cholesterol induced arteriosclerosis by a previous immunization with heterologous $\beta$-LP (plasma lipoproteins) (Gero et al. 1961b, 1961c).
3. DIETARY FACTORS AND THOSE RELATING TO THE BLOOD AND BLOOD VESSEL WALL:

Dietary factors especially carbohydrates and lipids are believed to be involved in the aetiology of cardiovascular diseases. Minerals, vitamins and certain other hormones also have some effect on the development of atheroma. Carbohydrate, protein and lipids are the main constituents in the diet, vitamins are required in minor amount. The role of other dietary components in the development of atheroma have also been studied.

i) Effect of Carbohydrates:

Since, diabetes constitutes a potent risk factor in the development of CHD, carbohydrate and its metabolism have been studied extensively.

Recent population studies, such as the Framingham and the Du pont company ones, have shown that there is a two to three fold increase in cardiovascular morbidity and mortality in diabetics as compared with those in the non-diabetics.

In the 16-year follow-up of the citizens of Framingham, USA, mortality from arterial disease was two to four fold greater in the diabetics than in the population as a whole (Garcia et al 1974).
In the careful 10 year follow up study of male diabetics working for the Du pont company, Pell and D'Alonzo (1970) found three times more deaths attributable to coronary artery and allied diseases among diabetics than among a matched non-diabetic population.

The Bedford survey in Great Britain (Keen et al., 1965) and the Tecumseh Community Health Study (Epstein et al., 1965; Ostrander et al., 1965) have both shown that the prevalence of clinical coronary artery disease is higher in the presence of than in the absence of hyperglycaemia, this association being independent of serum cholesterol and blood pressure levels.

In another population study in Bedford, Keen and Jarrett, (1970) examined the relationship between blood sugar and arterial disease and found that there was approximately a two fold increase of coronary heart disease in the hyperglycaemic group. This observation was subsequently extended and confirmed (Keen, 1972).

Epidemiological studies such as these show a definite relationship between glucose intolerance and arterial disease and may lead to the conclusion that glucose intolerance is a quantitative risk factor in relation to arterial disease. This does not imply, of course, that the glucose intolerance is the only factor.
Glucose metabolism and atherogenesis are related through the intermediary of a disorder of lipid metabolism. Hyperlipidaemia is a common accompaniment of diabetes. Several of the genetic types of hyperlipoproteinaemia, e.g. Fredrickson types III and IV, have an associated impairment of glucose intolerance.

The role of dietary carbohydrate in producing both hyperglycaemia and hypertriglyceridaemia are well established. Carbohydrate induced hypertriglyceridaemia (Mancini et al., 1973, Sales et al., 1975, Glueck et al., 1969) commences within 48-72 hours in man (Mancini et al., 1973) and reaches a maximum in 1-5 weeks. There is evidence that the mechanism of carbohydrate induced hypertriglyceridaemia may involve both increased VLDL secretion and impaired clearance of triglyceride from plasma.

The quantity and the type of dietary carbohydrate is important in the relationship of cardiovascular disease. Yudkin and Makenzie, (1964) have reported the relationship of sucrose to cardiovascular disease. Normal dietary intakes of young men were measured and 200 g of sucrose were exchanged isocalorically with starch. After two weeks of such feeding, significant increase in the concentration of plasma triglycerides were found in all subjects. In about 30 percent of the subjects there was an increase in

A high concentration of insulin has been shown to stimulate lipogenesis in arterial tissue where it is also known to inhibit lipoprotein lipase (Stout, 1968), thus, increasing deposition of (Szanto and Yudkin, 1969) lipid and inhibiting its removal thereby facilitating production of coronary heart disease. Similar changes may account for the occurrence of atherosclerosis in diabetes mellitus (Stout, 1968, Opie, 1973) in which there may be further aggravation by osmotic damage in the arterial wall due to conversion of glucose to sorbitol.

Animal experiments gave some support to the view that hyperinsulinism in response to sucrose makes the individual susceptible to coronary heart disease though there are species differences (Yudkin, 1967, 1971; Yudkin and Moyland, 1967; Yudkin and McKenzie, 1964; Yudkin and Roddy, 1964). For example, in rats sucrose given for 10 weeks produces a fall in serum insulin concentration, where
it produces a rise. Again, in rats a rise in plasma triglyceride, and occasionally of cholesterol concentration occurs, whereas in pigs there was only a transitory rise in these constituents and in cockerels there was a considerable rise in cholesterol only. Sucrose causes some enzyme changes; for example, in the rat an increase in fatty acid synthetase, glucose 6-P dehydrogenase and pyruvate kinase occurs in the liver at the same time as there was a decrease in fatty acid synthetase in adipose tissue. These enzyme changes may be induced quite quickly, e.g. within 18 hours after high sucrose feeding, but there was an equally rapid fall in fatty acid synthetase activity and triglyceride levels when sucrose was withdrawn. Sucrose has been shown to produce diseases in the arterial wall in rats in which it was associated with an increase in platelet aggregation. Yudkin and Roddy (1964) have shown that sucrose produces an increase in the concentration of lipid in the aortic wall of the rat. They have also shown in cockerels that sucrose can induce aortic atheroma and that the area of the atheroma is directly proportional to the level of plasma cholesterol.

ii) Effect of other dietary components:

The possible role of other dietary components in the development of atheroma have been studied. The role of
certain minerals in the drinking water and vitamins were also important in this respect. In different parts of the world hardness of drinking water had been correlated with cardiovascular mortality (Crawford and Crawford 1967; Crawford, 1972; Neri et al., 1972); the softer was the water, the higher was the death rate. There was a good correlation between mortality rates and the concentration of calcium in water. Knox (1973) established a negative correlation between calcium intake and ischaemic heart disease mortality. Large calcium supplements have been found to reduce serum cholesterol levels in the rabbit (Iacono et al., 1960) and in man (Yacowitz et al., 1965). Serum triglycerides and phospholipids were reported to decrease (Yacowitz et al., 1965).

At low levels of calcium intake, magnesium metabolism was altered and indeed, tissue concentrations of calcium and magnesium have been found to differ in hard and soft water areas.

Anderson (1975) demonstrated a significant decrease in myocardial magnesium in people who died of accidents who have lived in soft water areas as compared to those in hard water areas.

Magnesium concentration have been found to be lower in heart muscle from subjects who died suddenly from myocardial degeneration than in muscle from subjects who died from other causes (Chipperfield and Chipperfield, 1973).
Studies in various areas of Finland have shown that the incidence of cardiovascular disease is lowest in areas where the drinking water has the highest concentration of magnesium and fluoride and, conversely, the incidence of cardiovascular disease is highest among the residents of areas where the concentrations of magnesium and fluoride are low (Luoma et al., 1973).

Thus, it became evident that magnesium may play a key role in heart disease. Seelig and Heggtveit, (1974) have suggested a therapeutic role for magnesium in acute coronary heart disease.

Dalderup (1973) suggested that the intake of vitamin D may be important because of its effect on calcium metabolism. Other workers have failed to show an effect of vitamin D on serum cholesterol levels in healthy men with an average age of 35 years (Carlson et al., 1970).

There is now abundant evidence largely but not entirely from animal experiments, that ascorbic acid is one of the many factors able to control serum and organ cholesterol levels. In some studies, vitamin C has reduced serum cholesterol concentration (Spittle, 1970; Ginter et al., 1971) while other workers have been unable to demonstrate any effect (Elwood et al., 1970; Anderson et al., 1972). In man, effect of large supplements of ascorbic
acid on the serum cholesterol concentrations appears to depend on concentration. Thus, there are only few reports of a fall produced in normocholesterolemic subjects but more in which a fall was found in hypercholesterolaemic ones. In the guinea-pig, ascorbic acid deficiency causes a reduction in conversion of cholesterol to bile salts (Ginter, 1973), which is the main route of excretion of sterol.

Other dietary components are known to alter blood lipid levels. Experimental evidence derived from animal studies suggests interaction of various dietary components in changing blood lipid levels and it is essential that the total diet be examined (Kritchevsky, 1976). Kritchevsky (loc. cit) suggested from his data that the increasing death rate from coronary heart disease in the United States parallels the increasing ratio of animal to vegetable protein. Experiments in a number of different species suggest that animal protein is more cholesterolaemic than vegetable protein. In the human, Walker et al (1960) reported that a young woman ingesting a diet containing 50 g of vegetable protein had lower serum cholesterol levels than a woman eating 50 g of animal protein.

Torwell (1972) has suggested that the risk of coronary heart disease is inversely related to the amount of complex mixture of celluloses, hemicelluloses and pectins. The
mechanism of involvement of these components in the metabolic processes is not clear. However, there is undoubtedly some epidemiological support for Torwell's hypothesis.

In India, Malhotra (1967) studied railroad workers from different parts of the country in which the risk of CHD differed. In Udaipur, the area of lower risk the workers ate more fat with a higher degree of saturation than did the workers in Madras, the higher risk area. There was no difference in the concentration of cholesterol in the serum. Malhotra noted that 22 of 28 individuals from Udaipur had an abundance of vegetable fibres in their faeces, whereas only 6 of 28 individuals from Madras had fibres and these were less plentiful.

Bread can be an important source of dietary fibre, and Morris et al (1963) reported that bread consumption was related negatively to plasma cholesterol level in 99 bank employees.
iii) **Effect of Lipids:**

At the symposium on nutrition and cardiovascular diseases in Oslo (1963), Westlund discussed the cholesterol levels of 4300 men (40-59 years of age) who were free of CHD and diabetes (Westlund, 1964). There appears to be a striking correlation between the increase of the serum cholesterol and the rising incidence of myocardial infarction (Westlund, 1964). Dramatic changes in lipids and lipoproteins have been reported during and for several weeks after acute myocardial infarction (Besterman, 1957; Fredrickson, 1969). Cholesterol and LDL are often transiently decreased after infarction. Plasma triglyceride is very often elevated at the time of acute myocardial infarction, and the elevation may persist for weeks (Besterman, 1957; Fredrickson, 1969).

Dietary trials in survivors of myocardial infarction revealed that when patients were placed on low fat diet much lower risk factors were associated (Morrison, 1960; Koranyi, 1963; Hood et al., 1965).

In 1960 Morrison reported the favourable outcome after a 12 year observation period of 100 patients with myocardial infarction. 50 patients, placed on a 25 g fat (= 15% fat) 50-70 mg cholesterol, 1500 caloric diet and losing weight significantly. They showed a 38% of survival
rate. The other 50 patients who remained on their usual American diet had a 100% mortality rate. The low fat diet produced a decrease in cholesterol from 312 mg% to 220 mg%.

Koranyi (1963) in a three year study of myocardial infarction (MI) survivors found 8.6% mortality in the low fat group (35-40 g per day) as compared with 19.7% in his control group.

Hood et al. (1965) gave an account of long term follow-up studies on 112 strict dieters (cases of asymptomatic hypercholesterolaemia, angina pectoris and patients with MI). Considering myocardial infarction group separately, only 2 men died of re-infarction among 22 infarction cases— as compared with 12 reinfarction deaths among 22 controls. In 1967 Bierenbaum et al., published their 5 year observations on 100 male patients aged 20-50 with myocardial infarction under dietary management with a 28 percent fat diet. The group was identical with another group of 100 myocardial infarction patients who were not under dietary regime. The study group showed an incidence rate per 100,000 of 4450 for new MI and 2108 for deaths from MI. The control group showed an incidence rate per 100,000 of 7143 for new MI and 4911 for deaths from MI. The non-dieted group had a 160% higher MI rate and a 233% mortality rate than the study group. Mean serum cholesterol in the group fed 28% fat diet reported lowered from 259 mg% on admission to 235 mg% at the time of evaluation.
Laren (1966) reported a 5 year follow up study in Norway involving 206 male survivors of M.I. (1 or 2 years prior to the study) who were put on a low cholesterol diet with restriction of saturated fatty acids and substitution through Soybean oil. The randomly selected control group consisted of 206 former M.I. patients of the same age (30-67 years) without dietary treatment. Initially cholesterol concentration in both the groups were similar (296 mg%) which dropped in the diet group to 244 mg% and remained at 285 mg% in the control group.

Thirty four of the treated group had 43 new M.I.; 54 of the control group had 64 new M.I. There was 27 sudden deaths in each group. Laren explains this surprising congruity with the relation of sudden death to rapidly fatal arrhythmias which are probably more closely related to the site than to the extent of lesions resulting from the initial infarction.

In another dietary trial by Rose et al., (1965) the addition of approximately 64 g of corn oil to a low caloric diet for M.I. patients showed that the depression of the cholesterol values was highly significant compared with the controls and the patients given olive oil diet. But at the end of two years the proportions of patients remaining free of major cardiac events is greater for the control group (75%) than for the two oil groups (Olive oil...
The inference drawn was that corn oil cannot be recommended as a dietary treatment for ischaemic heart diseases. It is most unlikely to be beneficial, and possibly harmful. The excess calories derived from oil increases total calories from 33% to 50% in oil-fed group to render harmful effect. There are striking international and regional variation in the occurrence of severe atherosclerosis, as has been shown by the International Atherosclerosis Project (IAP) (Keys, 1970). Those communities with little atherosclerosis have virtually no coronary heart disease, and it has been shown that an increase in the extent of coronary atherosclerosis is accompanied by an increased susceptibility to the disease. Keys (1970) showed that the severity of atherosclerosis was closely related to the serum cholesterol and with the proportion of total energy derived from fat. Many studies mostly reviewed by McGill (1968) have suggested that severe atherosclerosis is closely associated with dietary patterns seen in the affluent countries of the world characterised by a high intake of energy - total fat, saturated fat and cholesterol. Prospective community studies have shown that as the blood cholesterol concentration increases there is an increased association with coronary heart diseases.

Kaufman et al., (1975) produced a very striking reduction in serum cholesterol merely by adjusting the
Keys et al., (1965) have shown that the cholesterol raising effect of the saturated fatty acids is mostly related to those containing 12-16 carbon atoms, suggesting a key role, therefore, for lauric, myristic and palmitic acids (Horlick, 1959; Hegsted et al., 1965, Grande et al., 1970; Grande et al., 1972). Conversely, stearic acid and saturated fatty acids of greater than 18 carbon atoms appear to have little effect on blood cholesterol levels (Hegsted et al., 1965; Grande et al., 1970) though they are not entirely free from them. Oleic acid may also show a slight cholesterol elevating effect (Brown, 1969).

Kinsell et al., (1952), Friskey et al., (1955) Bronte-Stewart et al., (1956), Ahrens et al., (1955) and Vergroesen (1975) in separate investigations have reported that when saturated fat is replaced isocalorically by fat rich in polyunsaturated fatty acids, serum cholesterol falls. The decrease is of the order of 15-20%, is usually maximal in 2-3 weeks and appears to be maintained indefinitely as reported by Leren (1966).

Jolliffe (1961) has suggested that dietary pattern, that defines the proportions of polyunsaturated and saturated fat (p/s) influence the serum cholesterol, the amount or
chemical characteristics are not responsible for alteration in serum cholesterol.

Vergroesen (1975, 1972) in two experimental set ups found no other additional reduction on serum cholesterol when linoleate intake were increased from 20-25% of calories to 45%. Grande et al., (1963) noted that the very highly unsaturated fatty acids of certain fish oils reduces serum diunsaturated linolenic acid. Kingsbury et al., (1961) have reported that cod liver oil produces a greater fall in serum cholesterol level than corn oil.

Kritchevsky et al., (1971) have shown, in animal studies on high cholesterol intake, that long chain saturated fatty acids such as arachidic (20:0) and behenic (22:0) may be markedly atherogenic without raising the blood cholesterol concentration.

Grande et al., (1972) have produced some evidence in man showing that stearic acid, and saturated fatty acids with fewer than 12 carbon atoms, e.g. caprylic and capric acids, can induce hypertriglyceridaemia. Thus, it may be concluded that a diet high in saturated fats and cholesterol is implicated in atherogenesis and coronary heart disease, and should be avoided by dietary means.

A number of human experiments using formula diet have shown that lowering of mono unsaturated fatty acids alone has virtually no effect on cholesterol concentration.
It would appear that the greatest effect can be obtained by replacing saturated fatty acids by polyunsaturated ones (Karvonen, 1972). The mechanism by which polyunsaturated fats decrease serum cholesterol levels is still a subject of debate. Recent evidence suggests that the main effect is to redistribute cholesterol between plasma and tissues rather than to remove it from the body (Grandy and Ahrens, 1970).

Based on certain observations clinical investigators have produced evidences that dietary fat could be one of the risk factors in the aetiology of heart diseases. Clinical trials in various countries have shown that vegetable oil results in the abnormalities of human myocardium.

Investigation relating different dietary fat habit and its effect on serum cholesterol was studied from last 3 decades. The observations were mostly reviewed by Jolliffe (1961) and Page et al., (1957).

Earliest evidence relating low serum cholesterol concentration to low fat intake was observed from investigations on vegetarian communities by Kinsell et al., (1952) and Groen et al., (1952).

Groen et al., (1952) reported a nine months study on 60 normal human volunteers in whom the total cholesterol content of the serum decreased gradually but significantly on replacement of animal fat with vegetable fat. Recently
Hankin et al., (1970) in a study with the inhabitants of South pacific and in California showed a wide range of saturated fat intake which correlated well with serum cholesterol, and triglyceride levels. Several investigators suggested that the nature of the dietary fat might have a greater significance for the lipid metabolism and possibly also for the atherogenesis, than the total amount of fat in the diet (Ahrens et al., 1955, Bronte-Stewart et al., 1956; Frisk et al., 1955).

Serum lipids are influenced by the nature of fatty acids, as well as their quantity. The degree of unsaturation, stereo-isomeric difference and chain length of all alter the response of the serum cholesterol concentration to dietary fat. The main example concerns 18:1 fatty acids of which the natural cis-isomer elaidic acid is produced during partial hydrogenation of certain natural oil.

Increase in serum cholesterol in man as a result of elaidic acid intake has been reported (Vergroesen, 1972).

Natural foods rich in saturated fats, largely of animal origin and contain cholesterol, while many sources of polyunsaturated fat are from vegetable sources, e.g., seed oils, and contain plant sterol. Plant sterols are poorly absorbed and interfere with cholesterol absorption, play some part in the reduction of serum cholesterol level induced by corn
oil (Beveridge et al., 1964), which is richer in sitosterol than most other seed oils.

Mancini et al., (1973) in a short term dietary experiment in man in which fat intake is reduced to 5 g/day and is isocalorically replaced by carbohydrate showed decrease in serum cholesterol concentration and rise in triglyceride levels in a normal subject.

Results of Animal Experiments:

The mode of action of modified fat diets in reducing serum cholesterol levels remain somewhat controversial. Cholesterol synthesis has been reported by Wiech et al., (1967) to decrease and increase by Wood and Migicovsky (1958) in the laboratory rodents fed polyunsaturated fat and to be unchanged in man (Ahrens, 1957; Grundy and Ahrens, Jr. 1970).

There is some experimental evidence for increased plasma cholesterol on diets containing unsaturated fat (Byers and Friedman, 1958, Pinter et al., 1964). Increased cholesterol absorption with polyunsaturated fat were reported in rat by Byers and Friedman (1958) and in man by Pinter et al., (1964). Other animal studies suggest that neither changes in cholesterol absorption nor its intestinal secretion, would account for the effect of unsaturated fat in reducing serum cholesterol level (McGovern and Quackenbush, 1973a).

Cholesterol has been directly measured from tissues
during dietary manipulation in animals and man. On diets rich in polyunsaturated fat, in rats, cholesterol content of the liver was reported to be increased (Avigan and Steinberg, 1958; Gerson et al., 1961, Bloomfield, 1964) or unchanged (Anderson et al., 1959) or decreased (Alfin-Slater et al., 1954). In man cholesterol in liver decreased (Frantz and Carey, 1961). In the rabbit cholesterol content in liver and heart (Moore and Williams, 1964) were lower and of the whole carcass (Malmros, 1969) when polyunsaturated fat was fed. An increased muscle cholesterol was also reported (Bieberdorf and Wilson, 1965). In monkeys, the cholesterol content of several tissues was compared during consumption of saturated and polyunsaturated fats, there was no demonstrable differences (Portman and Sinisterra, 1957).

Serum phospholipid concentrations changed likewise as those of cholesterol in response to the dietary fats (Grande et al., 1961, Mc Gandy et al., 1970, Grande et al., 1972).

Wissler et al., (1953) found lipomatous coronary artery changes in rats fed on high fat diet containing lard. This was not in the animals fed on corn oil (Shapiro and Freedman, 1955). Portman et al., (1956) found higher serum cholesterol levels on feeding hydrogenated cotton seed oil than those fed with corn oil at various levels and lowering of serum cholesterol levels on replacement of hydrogenated
by non-hydrogenated cotton seed oil.

Wigand (1959) found a distinct increase in the serum cholesterol levels after six weeks in the rabbits fed butter fat and hydrogenated cotton seed oil. On the other hand, hypercholesterolaemia was not pronounced in the group fed hydrogenated coconut oil or lauric acid although several animals in these groups showed moderate weight loss. Supplements of linolenic acid diet appeared to improve weight gains. Similar results were also reported by Thomasson (1955).

Besides investigations on serum cholesterol level in relation with atherosclerosis, Anfinsen (1955) suggested that concentration of triglyceride in the serum could possibly play an important role in atherogenesis. Allbrink and Mann (1959) found elevated serum triglyceride levels in 85-90% of patients with coronary artery disease. Attention had also been focused on certain protein lipid relationships in the serum and their importance in the pathogenesis of human atherosclerosis and its early diagnosis (Gofman et al., 1950). Increased beta lipoprotein levels have been found in patients who have survived coronary occlusion (Barr et al., 1951) and in patients with various forms of hypercholesterolaemia (Swahn, 1953). Jencks et al., (1956),
using electrophoretic techniques, analysed serum from 77 males with atherosclerosis and myocardial infarction and demonstrated a drop in the alpha and an increase in the beta-lipoprotein fraction.

The hydrogenated cocoanut oil which exerted the strongest hypercholesterolaemic and atherogenic effects together with dermal symptoms, is completely devoid of polyunsaturated essential fatty acid. On the other hand, oils such as corn oil and rapeseed oil which are rich in polyunsaturated fatty acid had hardly any enhancing effect on the serum cholesterol level.

**Effect on TG:**

Saturated fat when replaced by polyunsaturated fat in the diet, serum triglyceride concentration decreases (Nestel et al., 1970; Chait et al., 1974).

This similarity in the effect of the fat modified diet on serum levels of both cholesterol and triglyceride has recommended changes in dietary pattern in ischaemic heart disease prone communities. Representative medical bodies (official collective recommendations on diet in the Scandinavian countries, 1968; American Heart Association, 1969) have thus advocated restriction of saturated fat intake with partial replacement by polyunsaturated fat.
Postprandial serum triglycerides were somewhat higher during consumption of saturated fat than those during consumption of polyunsaturated fat. The fall in serum triglyceride concentration induced by polyunsaturated fat was due to reduction of VLDL levels (Chait et al., 1974). They also reported that when a diet with high polyunsaturated/saturated (P/S) ratio was fed, the reduction in VLDL triglyceride was greater than that in VLDL cholesterol, the composition of lipoprotein changed significantly. The reduction in serum cholesterol concentration was due largely to lower levels of LDL cholesterol but decreased VLDL cholesterol concentrations contributed to the changes in whole serum cholesterol levels.

Dietary stearic acid, although without influence on serum cholesterol concentration, increases serum triglyceride level when it replaces carbohydrate in the diet; palmitic acid has an effect similar to carbohydrate (Grande et al., 1970; 1972). Saturated fatty acids of medium chain length produce higher triglyceride levels than do diets richer in C_{12-16} fatty acids (Uzawa et al., 1964). When polyunsaturated fat was fed in rat or men, rat adipose tissue lipoprotein lipase activity (Pawar and Tidwell, 1968) and post-heparin lipolytic activity in man (Bagdade et al., 1970) were observed to increase.
In another study of polyunsaturated fat containing diets by Chait et al., (1974), post-heparin lipolytic activity in human plasma did not increase significantly and no change occurred in the fractional rate of removal of injected triglyceride emulsion in subjects in whom a large reduction of serum triglyceride level was produced.

Hypertriglyceridemia was observed to be closely associated with the development of premature CHD (Brown et al., 1965; Carlson and Bottiger, 1972; Havel, 1969; Levy and Glueck, 1969). Scharde et al. (1960) stated that, of the blood lipids in 452 cases of arteriosclerosis, the triglycerides were increased to a greater extent than cholesterol and phospholipids. Furthermore, hyperlipidaemia was discovered in 71% of cases of CHD, in 60% of cases with vascular disease and in only 46.5% of cases of cerebral atherosclerosis.

The oils most frequently consumed are those obtained from soybean, peanut, sunflower and rapeseed. In India, mustard, groundnut, cocoanut and til oil are also used widely by people of different regions. Rapeseed oil is a progressively popular dietary oil. The main producing countries are India, Canada, China, Pakistan and among European ones, Poland, France, Germany and Sweden.
Traditional rapeseed oil from *Brassica napus* and *Brassica campestris* differs from most edible oils in that it has a relatively low content of oleic and linoleic acids (both acids being the major components in the other oils), accounting for more than 80% of total fatty acids. Erucic acid is one of the fatty acid with 22 carbon atoms and one double bond (22:1) at the Δ13 position.

Marine oils have always been part of the human diet, mainly consumed as fresh fish or oils from marine animals. Commercial marine oils are generally divided into two broad classes based on their docosenoic acid (22:1) content. One variety contains 1-4 percent of docosenoic acid, while oils from herring, capelin, sand lance, and some marine mammals contain noticeably higher quantities (10-20 percent). The principal docosenoic acid is cetoleic acid (22:1, n-11). The docosenoic acid content is not greatly altered by partial hydrogenation, but positional or geometrical isomers are formed in the partially hydrogenated product. Generally, the position of the remaining unsaturated bonds are close to those in the parent structure (n-11), and about half of them are of the trans-configuration (Ackman *et al.*, 1978).

Mustard oil (*Brassica juncea*) is commonly consumed as edible oil in some parts of India. In Bengal and Eastern India, it is most widely used. It also contains erucic acid approximately in the same proportion as rapeseed oil (44-48%).
oleic acid ranges from \( \approx \) 15\% and linolenic acid from 14\%-18\%. The fatty acid composition of different edible oils is given in the table below:

<table>
<thead>
<tr>
<th>Carbon atom: double band.</th>
<th>Ground nut oil (Peanut oil)</th>
<th>Rapeseed oil</th>
<th>Mustard oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic</td>
<td>16:0 14.4</td>
<td>3.3</td>
<td>0.7-1.5</td>
</tr>
<tr>
<td>Palmitoleic</td>
<td>16:1 -</td>
<td>traces</td>
<td>-</td>
</tr>
<tr>
<td>Stearic</td>
<td>18:1 2.5</td>
<td>0.6</td>
<td>0.4-1.0</td>
</tr>
<tr>
<td>Oleic</td>
<td>18:1 42.8</td>
<td>14.0</td>
<td>20-22</td>
</tr>
<tr>
<td>Linoleic</td>
<td>18:2 34.8</td>
<td>14.3</td>
<td>14-18</td>
</tr>
<tr>
<td>Linolenic</td>
<td>18:3 1.1</td>
<td>7.8</td>
<td>-</td>
</tr>
<tr>
<td>Arachidic</td>
<td>20:0 1.0</td>
<td>0.4</td>
<td>0.5-1.0</td>
</tr>
<tr>
<td>*Gadolic/gesenoic</td>
<td>20:1 0.8</td>
<td>7.4</td>
<td>7-8</td>
</tr>
<tr>
<td>Behenic</td>
<td>22:0 2.5</td>
<td>traces</td>
<td>2-3</td>
</tr>
<tr>
<td>*Eruic Docosenoic/ctaleic (n-11)</td>
<td>22:1 -</td>
<td>51.9</td>
<td>40-44</td>
</tr>
</tbody>
</table>

*Isomers depending on the conformation and position of double bond.

Effects of Rapeseed oil on Lipids:

It has been known since 1955 that ingestion of rapeseed oil containing high concentration of erucic acid produces pathological changes in some tissues of experimental animal. (Abdellatif, 1972; Jacquot et al., 1969; Rocouelin and Cluzan, 1971). Most studies were developed to assess the alterations appearing in the rat (Abdellatif and Vles, 1970 b, c), both in histopathological and biochemical aspects. Subsequently, other species, such as ducklings (Abdellatif and Vles, 1970c), pigs, monkeys, gerbils (Beare-Rogers and Nera, 1972), rabbits, guinea pigs and dogs have been investigated for various lesions induced by rapeseed oil ingestion and important differences in the responses of these species emerged.

Naturally erucic acid is not found or occurs in traces in body fat, but when the diets contains rapeseed oil, erucic acid is found in depot fat, organ fat and milk fat. Erucic acid is metabolised in vivo to oleic acid.

Several attempts have been made to study the lipid pattern in the heart tissue and also in the blood of experimental animals fed oils of Brassica variety. Span, Oro, tower, campestris are the common oil products of Brassica. Earlier report of toxicity of the oils from Brassica, viz., rapeseed oil, are available (Roine and Uksila, 1959).

Rapeseed oil has a growth retarding effect on rats (Kramer et al., 1973), swines (Crampton et al., 1960), hamsters,
mice and ducklings (Thomasson et al., 1967). Growth was retarded in hicks receiving a diet with 25% rapeseed oil (Carroll, 1957) but not with 4% or 8% rapeseed oil (Sell and Hodgson, 1962). No retardation in growth with dogs receiving rapeseed oil were observed (Crampton, et al., 1960).

Rats eat less a diet containing 20% rapeseed oil (RSO) compared with one containing 20% peanut oil. The decreased appetite is not caused by organoleptic factor (Thomasson et al., 1967), but involves an effect on hypothalamus (Beare and Beaton, 1967). The energy content of rapeseed oil is same as that of other oil (Thomasson, 1955a; Thomasson et al., 1967).

The unfavourable growth obtained with rapeseed oil diet is caused by the erucic acid content of this oil (Thomasson and Boldingh, 1955; Carroll and Noble, 1956; Beare et al., 1959a). The imbalance in the ratio of saturated/monounsaturated fatty acids may also be of importance for growth (Hopkins et al., 1955; Beare et al., 1963a,b; Rocquelin et al., 1970).

No growth retardation have been observed with Cambra oil diet in rat experiments (Rocquelin and Cluzan, 1968; Rocquelin et al., 1970; Craig and Beare, 1968; Abdellatif and Vies, 1970a) and the growth was unaffected by adding
saturated fat to a diet containing canbra oil (Craig and Beare, 1968). Further experiments with dietary erucic acid in the form of rapeseed oil revealed the growth retardation in other animal species (Paloheimo, et al., 1959; Thomasson et al., 1967).

The digestibility of rapeseed oil (RSO) has been examined on rats (Hornstra, 1972, Rocquelin and Leclerc, 1969), chicks (Vogtmann et al., 1973, Clement and Renner, 1977), rabbits (Carroll, 1957) lambs (Walker and Stokes, 1970), swine (Crampton et al., 1960).

Erucic acid is not naturally found in animal tissue lipids. Rats fed rapeseed oil diets show less erucic acid and more oleic acid in their tissue lipids than in the diet.

Metabolic conversion of long chain fatty acids from dietary rapeseed oil to oleic acid has been suggested (Carroll, 1962, Craig et al., 1963, Craig and Beare, 1967) and experimentally proved (Carreau et al., 1968, Lapous et al., 1970).

By the use of labelled fatty acids, it has been demonstrated that erucic acid is oxidised at the same rate as oleic acid but the yield of the oxidation is lower (Bach et al., 1969).
Rapeseed oil in the diet has no effect on the blood cholesterol level in rats, mice and dogs whereas a tendency to hypercholesterolaemia is shown in guinea pigs and chicks (Carroll, 1957). A lower level of rapeseed oil in the diet did not influence the blood cholesterol in the rabbit (Kritchevsky et al., 1972; Wigand, 1959). The results on experiments on rabbits with higher amounts of rapeseed oil in the diet are contradictory (Carroll, 1957; Abdellatif & Vles, 1971a).

Bergland (1975) observed that the diet with rapeseed oil increased the heart weight of rat and the weight of heart decreased significantly after the rats had been transferred to the control diet.

Rocquelin et al., (1970) did not find any histological lesions in the liver of rats fed with rapeseed oil diet for two months, but 1-2 years intoxication caused fatty infiltration and degeneration in the central parts of the lobes (Abdellatif and Vles, 1970a, 1971a; Thomasson et al., 1967).

Abdellatif and Vles (1970a, 1970b, 1971a) noted that after 16 weeks or more on rapeseed oil diet, rats showed changes in the kidneys. These changes include increased kidney weight and nephrosis characterised by vacuolation of the tubular epithelium, tubular dilatation and focal connective tissue proliferation. The severity of changes increased with time.
Rapeseed oil diet increased the size and fat content of the adrenal gland (Carroll, 1951, 1953, 1958; Abdellatif and Vles, 1970a, 1970b, 1971a). Growth retardation and pathological changes were observed in adrenal (Jacquot et al., 1969) and skeletal muscle (Rocquelin and Cluzan, 1971). The adrenocorticotropic hormone induced synthesis of prostaglandin in vitro was substantially lowered in adrenal homogenate of rats fed on rapeseed oil diet compared with rats fed on a corn oil diet (Carney et al., 1972). Changes in prostaglandin as a result of polyunsaturated fatty acids in heart muscle was also reported (Gudbjarnason and Oskarsdottir, 1977).

In 1960, Roine et al., first reported that rats fed rapeseed oil for two or three months suffered from myocarditis. The effects of rapeseed oil on different animals were also reported by several workers (Rocquelin et al., 1970, Abdellatif and Vles, 1970a, 1971a).

Abdellatif and Vles (1970a, 1970b, 1971a) observed that the effects of rapeseed oil on the myocardium of the growing rats could be distinguished into three stages - intracellular lipidosis, histocytic infiltration and finally, fibrosis. They also observed that the intracellular myocardial lipidosis started some hours after supplying rapeseed oil and reached a maximum after 3-6 days, thereafter decreasing rapidly. Similar observation was also made by Houtsmuller et al., (1970).
Abdellatif and Vles (1973) also observed that all the rats fed with rapeseed oil for 3 to 6 days showed a dose related lipidosis of the myocardium.

Beare-Rogers et al. (1971) fed on a different dose of Canadian rapeseed oil for 1 week in rats observed that 5 percent rapeseed oil had no effect but 10 percent rapeseed oil in the diet showed some abnormal fat accumulation in the myocardium of the experimental animal. Similar changes were also observed by Engfeldt and Brunius (1975). According to them about 2 percent erucic acid in the diet did induce pathological fatty accumulation in the heart muscle cell of rats while 1 percent did not, but on electron microscopic study they, however, observed some fat droplets in some locations even at the low erucic acid level.

Kramer et al. (1973) also observed that the influence of rapeseed oil on the lipid contents of the rat hearts depended on the amount of erucic acid given in the diet. High percent of erucic acid in the diet showed a significant increase in the triglycerides of heart homogenate. They also observed that the total phospholipid decreased with increased dose of erucic acid, and the concentration of phosphatidylcholine showed a tendency to increase and
the concentration of phosphatidylethanolamine to decrease. The concentration of cardiolipin remained unchanged.

Beare-Rogers et al., (1974) showed incidence of cardiac lesions in rats following 16 weeks feeding trial with liquid rapeseed oil. Similar incidence was observed with partially hydrogenated rapeseed oil and with liquid cambra oil. The incidence of lesions appeared to be related to the level of the test oil and not its content of erucic acid.

Kramer et al., (1975) have also reported myocardial necrotic lesions as a result of feeding rapeseed oil low in erucic acid for 16 weeks to male rats.

Beare-Rogers and Gordon (1976) observed that rapeseed oil containing diets had no effect on the myocardial nucleotide levels, nor any change was observed in the ability of the mitochondria to oxidise substrates or in their capacity for oxidative phosphorylation. The characteristic lipidosis was observed in rats fed on high erucic rapeseed oil for 1 week. Incorporation of eicosenoic acid and erucic acids occurred in the cardiac mitochondrial and microsomal preparations.

Beare-Rogers et al., (1977) have reported cardiac lesions in rats fed on rapeseed oil diet containing low or high levels of erucic acid.
Lipidosis of the heart muscle caused by feeding rapeseed oil has been observed not only in rats but also in ducklings, guineapigs, gerbils, rabbits and monkeys (Kramer et al., 1973; Abdellatif and Vles, 1971a, 1970b, Beare-Rogers and Nera, 1972 and Vles and Abdellatif, 1970).

Kramer and Hulan (1978) observed increased heart weight in rats fed on diets containing high erucic acid rapeseed oil, possibly due to elevated fat levels. The triglyceride level in the cardiac lipid was high after 3 days, and still higher after 1 week on this diet as compared to rats fed diets containing soybean, olive, or tower rapeseed oil throughout the experiment. However, inclusion of about 5 percent erucic acid in the vegetable oils resulted in higher triglyceride levels at 3 days and 1 week. The fatty acid and triglyceride composition were greatly influenced by the dietary fatty acids. High percentages of long chain monoenones (20:1 and 22:1) were found in cardiac triglycerides of rats fed on diets containing either free or esterified erucic acid for 1 week. The pattern for long fatty acids (22:1) in cardiac free fatty acids was similar to that in cardiac triglycerides, a rise during the first week of feeding followed by a decline to a much lower level at 16 weeks. However, the relative
abundance of saturates (16:0 and 18:0) for the high erucic acid rapeseed group was significantly less than that among all other groups. It was thus observed from the earlier observations that the deposition of lipid in the myocardium was directly related with the erucic acid content in the diet.

Apart from the fat accumulation in the heart, other alterations was also observed after a period of two to four months, viz., myolysis, infiltration of myocardium by histocytes and, finally, scarring. Similar pathological effects on the myocardium were also reported by Abdallatif and Vles (1970a). It was reported that differed infiltration of mononuclear cells histocytes and proliferation of the fibroblasts appeared in the myocardium of rats on rapeseed oil diet after 4 to 8 weeks. These changes increased in severity and became less cellular and more fibrotic in the course of time.

Rocquelin et al., (1973a), Beare-Rogers et al., (1974), and Borg (1975) observed that some toxic components present in rapeseed oil causes myocardial necrosis. Hulan et al., (1976) found that rats fed on diets containing commercial lard added with 5.4% free erucic acid or pig fat containing 5.4% esterified erucic acid developed the same or lower incidence and severity of myocardial necrosis as those in hearts of rats fed on commercial lard alone. They
concluded that erucic acid per se was not responsible for the heart necrosis when rapeseed oils were fed to rats.

Vles et al., (1976) found that incidence and severity of heart lesions to be the same in rats fed on Canadian low erucic acid rapeseed (LEAR) oil, French LEAR oil having 0.3% erucic acid or sunflower oil. In case of very high levels of erucic acid (30% or more) were added to non-rapeseed oils (i.e., olive oil, soybean oil) did cause an increase in cardiac lesions (Beare-Rogers, 1975; McCutcheon et al., 1976).

Kramer et al., (1975), from their studies, concluded that the primary myocardiotoxic factor was the triglycerides of the oil (i.e. fatty acid composition and/or balance). They failed to remove any specific cardiotoxic factor from rapeseed oil by exhaustive molecular distillation and adsorption chromatography.

Kramer et al., (1975, 1973) have suggested that the myocardial lesions in rats fed on rapeseed oil resulted from a fatty acid imbalance not only from the presence of erucic (22:1) and eicosenoic (20:1) acids but also from altered saturated to unsaturated fatty acid ratios, low concentrations of palmitic (16:0) acid, high concentration of oleic (18:1) and linolenic (18:3) acids, and low 18:2/18:3
The result was in conformity with the hypothesis that the addition of 22:1 to a control oil (olive) naturally high in 18:1 greatly increased both the incidence and severity of myocardial necrosis. The relative amount of various polyunsaturated acids in cardiac lipids markedly influence the development of myocardial necrosis following overstimulation with catecholamines.

Williams and Oliver (1961) have reported development of myocardial lesions (necrosis, fibrosis, calcification and ceroid pigment deposits) followed by feeding diets containing 32% to 71% synthetic saturated triglycerides.

More recently, Hulan et al. (1977a, 1977b) have reported that the presence of excess oil in the diet of the young growing rats could also create a stress situation and thereby influence the incidence of myocardial necrosis and/or fibrosis.

There are many reports in the literature which indicate that the cardiovascular damage brought on by various dietary stress situation was not uncommon in the growing rodents. In one particular study, more than 50% of the mice fed on sugar, lard and casein diets showed atrial myocardial necrosis, with formation of mural thrombi, calcification and fibrosis (Ball et al., 1965). Lack of such increased incidence was observed in female rats.
(Kramer et al., 1973, Hulan et al., 1977a) and pigs (Friend et al., 1975a, Aherne et al., 1975) fed on rapeseed oil when compared to those fed on control oils.

Gudbjarnason and Oskarsdottir (1977) observed modification of fatty acid composition of rat heart neutral and phospholipids by feeding a diet containing 10% cod liver oil. The results reflect the dynamic state of esterified fatty acids in neutral and phospholipids of heart muscle. In cardiac neutral lipids there was a moderate but significant increase in exogenous fatty acids, 20:1 (n-9), 22:1 (n-11), 20:5 (n-3) and 22:6 (n-3) in animals fed cod liver oil, and a relative decrease in endogenous fatty acids, 16:0, 18:2 and 20:4. The most severe lesions consisted of lipidosis, necrosis and fibrosis which appeared in the myocardium when animals were given rapeseed oil or erucic acid in the diet. Erucic acid was suspected to be the responsible pathogenic factor.

There are little data on the pathogenicity of rapeseed oil in human beings (Tremolieres et al., 1971, 1972, Tremoliers and Carre, 1972, Jaillard et al., 1973). Del Carmine et al., (1975) studied the blood serum to assess the exposure levels of Italians to rapeseed oil containing erucic acid. They studied the methyl esters of fatty acids by gas chromatography and reported that the amounts of palmitic, oleic and linoleic acid were similar to those found in previous surveys accounting
for about 80% of the total. Forty subjects were seen to have measurable erucic acid levels ranging from 0.3 to 4.8 of total fatty acid (average 1.1 ± 0.14). These levels resembled those found in similar surveys in France and Italy. Eleven of the subjects had erucic acid (1.9±0.35) in the absence of measurable behenic acid (0.42±0.08). Twenty six additional subjects had measurable behenic acid (0.55±0.03) in the absence of erucic acid.

Friend et al. (1975a,b) and Aherne et al. (1977) have reported absence of myocardial necrosis in pigs fed on rapeseed oil containing low level of erucic acid. Swine on such diets deposits erucic acid in adipose tissue (Walker, 1972).

The rats fed on rapeseed oil showed large amounts of cholesterol esterified to erucic acid in adrenal gland. Carroll (1962) and Walker and Carmey (1971) related this situation to a reduced secretion of corticosterone in the cold. Cortical cell hypertrophy appeared to continue throughout the experimental period of up to 64 weeks (Vles, 1971).

Hulan et al., (1977c) further studied the effects of dietary fatty acid balance on myocardial lesion in male rats fed on the different vegetable oils. Heart and heart lipid weights did not differ statistically from each other. Fatty acid analysis of the cardiac lipids revealed that the fatty acid composition of heart lipids reflected that of the diet fed.
Heart lipids of rats fed on rapeseed oil contained the same or a significantly ($P < 0.01$) higher level of the saturates (16:0, 18:0) than those of the heart lipids of rats fed on other vegetable oils.

Menenguz et al., (1977) studied rapeseed oil induced modification in the fatty acid composition of lipids in myocardium, skeletal muscle, kidney and liver tissues of mice. In myocardium and liver tissues of peanut oil fed mice, the quantitatively important fatty acids like palmitic, stearic, oleic, linolenic and arachidonic acids accounted for more than 96% of the total fatty acids, and 85% in the rapeseed oil fed mice. This difference of about 11% was attributed to deposition in rapeseed oil fed mice of erucic acid (7.8%), eicosenoic (2.5%) and linolenic (0.7%) acids which were absent or present in traces in peanut oil-fed mice. Beare-Rogers and Gordon (1976) reported four times greater level of myocardial fatty acids in young rats fed on rapeseed oil than in those fed on olive oil.

Connellan and Masters (1965) separated the lipid components of rat heart kidney, skeletal muscle and liver by chromatography on silicic acid into cholesterol ester, triglyceride, free fatty acid and phospholipid fractions.
Palmitic acid was always present in greatest percentage in the triglyceride fraction. Arachidonic acid was observed in highest concentration in phospholipid and in lowest concentration is triglyceride fractions. The fatty acid compositions of the cholesterol ester fractions were broadly similar to those from all the extrahepatic tissues. Some differences were observed in fatty acid composition of the phospholipids between the hepatic and extrahepatic fractions.

Hung and Hulub (1977) suggested that the early accumulation of triglycerides in the heart of rats fed on rapeseed oil arose from a higher rate of synthesis of triglycerides via acyl-CoA 1,2 diglyceride acyl transferase as well as from a lower activity of microsomal lipase in this tissue.

Branca et al., (1977) have reported the effect of carnitine on heart steatosis induced by rapeseed oil in rats.
High concentration of rapeseed oil has been found to alter mitochondrial metabolism \cite{Houtsmuller et al.,1970; Christopherson and Bremer,1972a,b}. They have observed a decreased level of oxygen consumption with substrates of tricarboxylic acid cycle in animals on rapeseed oil diets, i.e., decreased capacity of the heart mitochondria to oxidize substrates. Diminished mitochondrial ability to oxidise various substrates was observed when animals are fed diets containing erucic acid \cite{Vodovar et al.,1973; Swarttouw,1974; Heijkenskjold and Ernster,1977; Hsu and Kammerow,1977; and Loow, et al.,1978}. This perhaps could explain the extramitochondrial accumulation of lipids.

Dow-Walsh et al., \cite{1975} found no such change in the presence of heparin, but observed a reduced rate of oxidation in its absence. Kramer et al., \cite{1973} and Cheng and Pande \cite{1975} detected no change with fatty acid substrates. Heart mitochondria obtained from rats fed on rapeseed oil (50 cal%) or corn oil diet for 3 days showed similar abilities for the coupled oxidation of various substrates and similar carnitine palmitoyltransferase activities \cite{Cheng and Pande, 1975}.

Houtsmuller et al., \cite{1970} reported that the rates of oxygen uptake and of ATP synthesis in heart mitochondria isolated from the heart of rats fed on sunflower seed oil,
were three times higher than those from hearts of rats fed on rapeseed oil.

Hsu and Kummerow (1977) observed the effects of corn oil (CO), rapeseed oil (RSO), hydrogenated fat (HF) and (HF+CO) on the respiratory activity of isolated heart mitochondria, their hormone sensitive lipase activity, and the fatty acid compositions of the phospholipids of the mitochondria from rats. The results indicated that heart mitochondria isolated from rats which were fed on corn oil (CO) had a higher rate of oxygen uptake, higher ADP/O ratio and a higher rate of ATP synthesis than the heart mitochondria isolated from those fed rapeseed oil or hydrogenated fats. The rates of oxygen uptake of the rat heart mitochondria isolated from each dietary group of rats was in the following order: oleyl carnitine > erucyl carnitine > elaidyl carnitine. The decreased capacity to oxidise substrates by heart mitochondria which were isolated from the heart of rats fed on rapeseed oil or hydrogenated soybean oil as compared with those from hearts of rats fed on corn oil as a sole source of dietary fat seemed related to the mitochondrial lipid composition. The type of dietary fat fed had a pronounced influence on the mitochondrial fatty acid compositions of phosphatidyleholine, phosphatidylethanolamine, and cardiolipin. The lipase
activity of the rapeseed oil fed group did not show any increase with either epinephrine or supplement ATP treatment. The substrate preference for lipase activity in myocardium was corn oil triglycerides $\triangleright$ trierucin $\triangleright$ trielaidin $\triangleright$ tripalmitin. However, cardiac lipid accumulation did not seem to be related to lipase activity in the myocardium.

Clandinin (1978) also observed that by dietary rapeseed oil in rats mitochondrial function and its conservation of energy were affected. Measurement of cardiac mitochondrial respiratory functions showed decline in net rate of oxygen uptake and, therefore, ATP synthesis was found to decline with prolonged feeding of 15% oil containing diets. Significantly reduced ADP/0 ratios were observed for groups fed on high or low erucic acid rapeseed oil. Only prolonged feeding of low erucic acid rapeseed oil diets resulted in significant alterations in the efficiency of oxidative phosphorylation. Similar observations were made by Renner et al., (1979) in chicks. Those workers observed that the composition of fat ingested affected the fatty acid composition of mitochondrial diphosphatidyl glycerol (cardiolipin) more than the fatty acid composition of phosphatidylcholine or phosphatidylethanolamine. Their studies indicated that a complex dynamic mechanism exists associating dietary fat with mitochondrial structural, functional changes and energetic efficiency in the growing chick.
Blomstrand and Svensson (1975) reported significant increase in triglyceride of heart homogenate and mitochondria. They also noted that changes in mitochondrial phosphatidylcholine and phosphatidylethanolamine. Cardiolipin concentration remained unchanged. They have demonstrated that monounsaturated C22 fatty acids were incorporated into cardiolipin with a corresponding decrease of linoleic acid. Long chain polyunsaturated acids were capable of replacing the shorter and more saturated linoleic acid in phospholipids (Gudbjarnason and Hallgrimsson, 1976).

In mammalian tissues, cardiolipin (diphosphatidylglycerol) occurs exclusively in mitochondria (Rouser et al., 1968). This compound is synthesised by mitochondrial enzymes (Davidson and Stancev, 1971; Hostetler et al., 1971). Cardiolipin is tightly bound to cytochrome oxidase. This suggests that this phospholipid could be an important structural component of the respiratory chain (Avasthi et al., 1970; Chuang and Crane, 1975). As a marker of mitochondrial structure, the concentration of this compound, as well as its turnover, could be followed in order to study both the biogenesis of mitochondria and the development of their inner membrane.

Linoleic acid is the precursor for arachidonic acid by a series of desaturations and elongation. Arachidonic acid,
in turn, is used for endogenous prostaglandin synthesis. Gudbjarnason and Hallgrimsson (1976) have suggested that such prostaglandins could play an important regulatory role in cardiac muscle.

Clandinin (1976) observed that high and low erucic acid rapeseed oil containing diets induced a highly significant reduction in membrane saturation and similar reduction in unsaturation index; after 7 days of feeding, lipidosis was maximum. In all rapeseed oil treatments, elevated levels of n-9 fatty acids were observed for both membrane fractions. Rapeseed oil containing diets induced fatty acid composition changes in lipids of the inner mitochondrial membrane, with change in mitochondrial function. He further observed that dietary induced change in specific mitochondrial membrane lipids could affect the flux of substrates and efficiency of oxidative phosphorylation or dehydrogenase activity.

Dewailly et al., (1977) observed that phosphatidyl ethanolamine (PE), phosphatidyl choline (PC) and cardiolipin (CL) contents of heart were not modified in rats fed on high or low erucic rapeseed oil for 20 weeks.

Dewailly et al., (1978) observed changes in fatty acid composition of cardiac mitochondrial phospholipids. Treatment
with rapeseed oil containing 46.2% erucic acid showed incorporation of 22:1 (5.6%) into isolated cardiolipin from heart mitochondria. After high or low (5.7%) erucic rapeseed oil feeding, linolenic acid was slightly incorporated into cardiolipin. Moreover, both these rapeseed oils induced a significant increase of linoleate:arachidonate ratio in phosphatidylethanolamine and phosphatidylcholine. The ratio was also significantly increased in fatty acids esterified to the $\beta$-position of these phospholipids. On the basis of such results, the role of linoleic acid, which was present at high level in different rapeseed oils used, could act as a possible inhibitor of heart microsomal enzymes involved in linoleate arachidonate conversion. Such alteration could account for mitochondrial fragility and myocardial lesions obtained in long term rapeseed oil feeding experiments.

Marine oils also contains erucic acid. Oils from herring, capelin, sand launce contain noticeably higher quantities (10-20 percent) of docosenoic acid (22:1 n-11). The docosenoic acid content was not greatly altered by partial hydrogenation. Eicosenoic acids (20:1) often presented in the oils in considerable quantity.

Effects of feeding partially hydrogenated marine oils for short and long term in experimental animals was studied. (Ackman et al., 1978). Docosenoic acid (22:1) induced cardiac
lipidosis can be demonstrated when the fat contributes 4-6 percent energy to the diet. In rat the disappearance of lipidosis on continued feeding (adaptation) was similar to that observed from feeding on Brassica oils. The appearance of long term lesions shows species differences.

Ackman et al., (1977) Ackman and Loew (1977) observed the effects of high levels of fats rich in erucic acid from rapeseed oil, or cetoleic and cetelaidic acids from partially hydrogenated fish oil in a short term study in a non-human primate. Myocardial lesions were observed in both the experiments.

Forsyth et al., (1977) observed the effects of feeding partially hydrogenated herring oil containing docosenoic acid on the heart mitochondrial oxidation of palmityl-CoA and pyruvic acid in rats and monkeys. Glycerides in herring oil contained cetoleic acid which brought about a decrease in palmityl CoA oxidation in rats initially, but significant metabolic adaptation was reported with increasing pyruvate and palmityl CoA oxidation above control levels. There was no significant adaptive change in pyruvate or palmityl CoA reported in monkeys after prolonged feeding of partially hydrogenated herring oil.

Schiefer et al., (1978) investigated the morphologic
effects of dietary plant and animal lipids rich in docosenoic acids on heart and skeletal muscle of cynomolgus monkeys. The histopathology of the myocardium showed some lipido­sis attributable to the rapeseed oil and a somewhat lesser lipidosis in the case of the partially hydrogenated marine oil, both being more pronounced than the lipidosis in the lard-corn oil control group.

Populations of Eskimos in Greenland have had a lifetime exposure to natural marine oils containing docosenoic acid (22:1). Recent surveys and epidemiological studies indicated that cardiovascular diseases were uncommon (Ackman et al., 1978).

The hearts of 54 Norwegian men aged 20–69 who had died suddenly in accidents, were screened from some 600 hearts (Ackman et al., 1978). They were selected as being free of myocardial infarction, coronary thrombosis, myocardial hypertrophy and valvular disease. Histochemical lipidosis, mostly of moderate degree, was found in 60 percent of the hearts. The fatty acids of the total lipids of the 54 hearts were examined and all were found to contain docosenoic acid (22:1) at 1 percent or less of the fatty acids.

Canadian data from a more limited study of the fatty acids from human heart triglycerides, also showed 1 percent
of docosenoic acid (22:1) (Ackman et al., 1978). These forensic data indicated that very mild cardiac lipidosis, not necessarily associated with deposits of docosenoic acid (22:1), could be widespread in humans consuming a Western high fat diet, irrespective of the type of fat consumed.

The literature indicated that diffuse myocarditis in man is not an unusual phenomenon, but there was no report associating with docosenoic acid (22:1) originating from partially hydrogenated marine oils in particular (Ackman et al., 1978).

In the examination of 54 human hearts reported above, no evidence of multifocal heart muscle lesions of the type seen in rats fed on rapeseed oil was found in normal men from a population suspected of long term exposure to partially hydrogenated marine oil. In many feeding experiments on rats a considerable time lag was found between the lipidosis phenomenon and the finding of muscle cell necrosis suggesting that moderate lipidosis was not injurious to the cells of the myocardium.

The absence of acute heart muscle cell necrosis or myocardial fibrosis, despite the concurrent presence of mild to moderate cardiac lipidosis, in a human population exposed to partially hydrogenated marine oil, supports this view.
Effects of Mustard oil on Lipids:

Gopalan et al (1974) studied myocardial changes in monkeys (Macaca radiata) fed with mustard oil. They kept adult male monkeys for a little over one year on diets containing 20% mustard oil, peanut oil or hydrogenated peanut fat respectively. Serum cholesterol in the animals of the peanut group remained unchanged throughout the study, while on hydrogenated dietary fat showed significantly higher values in 6 months. Animals on mustard oil diet, however, exhibited significantly higher levels of serum cholesterol at 6, 8 and 12 months. Lipid phosphorous in the serum of the monkeys of the peanut oil and hydrogenated peanut fat groups was significantly elevated at 4 months above initial levels and remained more or less elevated thereafter. The lipid phosphorous in the animals of the mustard oil group was significantly higher at 6 months than the basal values. Levels of either serum cholesterol or lipid phosphorous however, did not exhibit any differences between the groups at any of the intervals in the study. The significant findings of their study were the myocardial changes in monkeys maintained on a diet which provided 40% of calories from mustard oil for more than a year. This was not the manifestation of a high fat intake per se, as was shown by the absence of such changes in the hearts of animals consuming similar amount of either peanut oil or hydrogenated peanut fat.
Bhatia et al. (1978) studied the effect of feeding 15 percent mustard oil to female rats on the in vitro oxidation of $^{14}$C-oleic and $^{14}$C-erucic acid and their incorporation into lipids by liver, heart and skeletal muscles. Initially, the oxidation of $^{14}$C-erucic acid was lower than that of $^{14}$C-oleic acid, but the oxidation of $^{14}$C-erucic acid was enhanced in all tissues in rats conditioned to mustard oil diets supplying erucic acid. Oxidation of erucic acid was maximum in liver and least in heart. Incorporation of $^{14}$C-oleic acid into triglycerides and phospholipids were not affected by the type of diet or tissues. Incorporation of $^{14}$C-erucic acid was mainly into triglycerides of heart and skeletal muscle of rats not accustomed to mustard oil diet.

Bhatia et al. (1979) further studied effects of feeding mustard oil to rats on the in vivo lipid metabolism in heart and lung and reported severe lipidosis on 2nd day of the experiment in heart tissue. Significant incorporation of erucic acid in the total lipid of heart was also demonstrated on 2 and 10 days on 20% mustard oil fed group.

Sharma et al. (1979) observed the effects of 0, 5, 10 and 15 percent of mustard oil on the lipid profile of liver, kidney, adipose tissue, spleen, adrenals and ovaries in female rats on 2, 10, 30 and 120 days of feeding. Total lipid
contents were marginally increased in kidneys, adipose tissues, adrenals and ovaries. Accumulation of lipids were accounted by the increase of cholesterol ester in adrenal and ovaries, free fatty acids in spleen, triglycerides in the adipose tissue and triglyceride and cholesterol ester in the kidney respectively. Analysis of fatty acids of total lipids from these organs revealed accumulation of erucic acid which gradually declined on prolonged feeding of mustard oil. These results indicated that all the organs could have developed ability to oxidise erucic acid to a varying degree.

Ray et al. (1979) studied the effects of 15 percent mustard oil, rapeseed oil and groundnut oil feeding on the development of myocardial lipidosis and fibrosis in rats at intervals of 10, 20, 30 and 150 days. Significant increase in triglyceride contents of heart tissue with high incorporation of erucic acid was reported in rats on mustard oil and rapeseed oil diet after 10 and 20 days of feeding. Increase in cholesterol ester was reported after feeding mustard and rapeseed oil diets for 150 days. A higher collagen content in the heart of mustard and rapeseed oil fed rats was observed.

Sen and Sengupta (1979) studied the effects of feeding common edible oils to rats on the lipid profile of heart
Lipid profile of rat myocardium were studied following feeding of 10 percent groundnut or mustard oil at intervals of 5, 15, 40, 80, 120 and 160 days. Rats on mustard oil diet showed cardiac accumulation of triglycerides rich in erucic acid. Free cholesterol and free fatty acids showed similar trends on mustard oil diet at 15, 120 and 160 days. Collagen content increased in rats on mustard oil diet at 80, 120 and 160 days of observation.

Increased in triglyceride in mustard oil group could be due to increased synthesis or decreased degradation. It appears that increased free fatty acid in the heart tissue in the rats on mustard oil diet could facilitate esterification for triglyceride synthesis. Otherwise the accumulation of triglyceride could be due to increased rate of synthesis through Acyl CoA :1,2 diglyceride transferase or inhibition of microsomal lipase in hearts of rat fed oil containing erucic acid.

From the above findings it was noted that oils containing erucic acid in higher concentration could produce adverse effects on growth, organ weight, and bring about lipidosis and fibrosis in the heart of experimental animals. Mitochondrial energy metabolism with consequent production of ATP, oxidising ability of various substrates, its lipids and fatty acid profile were well documented with rapeseed oil and marine oil diets.
HEART DISEASES AND ENZYMES

The assay of the activities of certain enzymes have some practical importance in the diagnosis of myocardial infarction. First report on increased serum enzyme activity as a result of leakage from infarcted myocardium was known since 1954, LaDue et al (1954) have reported increased serum GOT activity in human acute transmural myocardial infarction. Till then measurement of the activity of specific enzymes was standard procedure in the diagnosis of myocardial infarction (Goldberg and Winfeld, 1972, Hughes, 1969, Jennings et al, 1957). The coronary syndrome includes small rises in specific enzymes in serum though within normal range, which is supposed to reflect a very small myocardial infarction (Batsakis and Briere, 1967, Herman, 1971, Konttinen, 1971).

A series of workers have reported a positive correlation between the activity of GOT and LDH to the size of acute myocardial infarction (LaDue and Wroblewski, 1955, Wroblewski, 1957, Kibe and Nilsson, 1967, Ekelund et al, 1972). Moller and Thygesen (1976) reported maximum enzyme activities according to age, sex and different sites of infarction in acute myocardial infarction. In general, the more severe and extensive was the myocardial infarction, the greater was the increase in enzyme activity in serum (Forster, 1968). But in acute myocardial infarction in man, the appearance of enzyme in serum and their maximum level did not coincide...
(Waldenstrom et al., 1977). The reason for this could be different permeability of the cell membrane, different volumes of distribution or different rates of degradation.

LDH can be elevated for several reasons. Interest has focused on the analysis of isoenzymes of LDH to indicate specific involvement of myocardium. Kraft et al. (1978) reported determination of isoenzyme of LDH in a prospective study of 201 patients with suspected acute myocardial infarction. The LDH isoenzymes are known to be LDH$_{1-5}$ after their electrophoretic mobility. LDH$_{1-2}$ were reported to be elevated in acute myocardial infarction.

Determination of glucose-6-phosphate dehydrogenase (G6PD) activity is known to have an increased enzymatic activity during myocardial infarction (Kerpolla et al., 1960). The simultaneous assay of several enzymes and isoenzyme pattern are preferable for diagnosis of heart diseases.