CHAPTER II

INDUCTION OF ATHEROSCLEROSIS

Marchand in 1904 while discussing various types of arterial conditions proposed to use the term atherosclerosis for the degenerative process associated with the vascular tree. The pleomorphic nature and ill understood pathologic basis of the lesion associated with it prevented any attempt to reach an unanimously accepted definition of the condition.

In an attempt to standardise the definition, World Health Organisation (1968) defined atherosclerosis as "A variable combination of changes of the intima of the arteries (as distinct from arterioles) consisting of focal accumulation of lipids, complex carbohydrate, blood and blood products, fibrous tissues and calcium deposits and associated with medial changes."

Adams (1964a) gave a more comprehensive account of the definition of atherosclerosis. He defined atherosclerosis as "a multifocal, proliferative and degenerative condition that affects the tunica intima and inner part of the tunica media of both large elastic arteries and muscular arteries in the senescent individual". Later, he explained that this proliferative nature of disease is essentially an organising
or sclerotic reaction of the connective tissues in the tunica intima while the degenerative element is manifested by lipid accumulation, fragmentation and hyalinisation of connective tissue with or without calcification and ischaemic necrosis of the centre of the lesion.

Investigations into the atherosclerotic process in human subjects, however, could only provide with some clinical information but the possibility of differentiating the influence of various factors on the process concerned is seriously limited under the condition. Moreover, the time required for such investigations is unusually long to bring out a fruitful observation and as such one has to resort to experimental induction of atherosclerosis in suitable animals.

Early attempts to produce experimental atherosclerosis proved to be a universal failure except recording few sporadic reports of success (Josue, 1903; Koltz, 1906 and Saltykow, 1908). Initial success in this direction was achieved by Ignatowski (1909) after feeding rabbits with meat extract, milk and egg yolk over a considerable length of time. This was subsequently confirmed by Starokadomsky and Sobolew (1909), Stukkey (1910); Fahr (1912) and
Chalatow (1912). These authors stressed the importance of cholesterol as an ingredient in atherogenic diet. This monumentous observation in the field of experimental atherosclerosis had been the guideline for a long time. Later observations have also revealed various other factors influencing the atherogenic process.

Principles of induction of experimental atherosclerosis.

In short most of the procedures utilised for developing atherosclerotic process in animals are based on - (i) altering the level of circulating serum lipids by artificial diets containing cholesterol and other lipids (ii) changing serum lipid concentration in normal or artificially fed animals by hormones producing altered lipid metabolism (iii) changing lipid concentration in blood and precipitating atherogenesis through altered tissue metabolism under deficiency states.
Diet induced atherosclerosis

Based on the above principle atherosclerosis has been induced in a number of experimental animals. Anitschkow (1913) and Chakravarti (1959), produced atherosclerosis in rabbit feeding artificially diet rich in cholesterol. Chakravarti and Zaidi (1961) could also achieve similar results in rabbits using certain cooking fats viz., Dalda. They also demonstrated that Dalda produces an action similar to other saturated vegetable fats in producing lipid deposition in organs. The process was also induced in chicken (Dauber & Katz, 1942, 1943; Katz & Pick, 1961; Katz, 1965), Leghorn cockerels (Nichols et al., 1960), turkeys (Gresham et al., 1963), budgerigars (Finlayson & Hirschinson, 1961) and pigeons (Lofland and Clarkson, 1963) using cholesterol, cholesterol derivatives and beef fat in their diet. In pigs and minipigs, however, the process was induced by simple addition of butter or fats, saturated or unsaturated, with or without cholesterol (Rowsell et al., 1958; Bragdon et al., 1957 and Moreland et al., 1963). While Altschul in 1950 demonstrated that atherosclerotic lesion
could also be produced in guinea pig and golden hamsters by feeding a diet consisting of milk and egg-yolk.

Steiner and Kendall (1946), on the other hand, induced atherosclerosis in dogs with cholesterol rich diet and thyroid blocking. In this connection a chronic hypercholesterolaemia was found to be more atherogenic (Schiller et al, 1964) and addition of butter and bile salt was found to facilitate atherogenic process in these animals (Diluzio O'Neal, 1962; Hartroft et al, 1962).

Atherosclerosis could also be induced in monkeys as was demonstrated by Mann et al (1953) and subsequently by Cox et al (1958), Taylor et al (1959, 1962) and Portman and Andrus (1965). They found that when monkeys were fed with a 5% cholesterol diet, developed lesions of proximal part of the aorta which were rich in lipid and partly sclerotic (Mann et al, 1953). Subsequently they could also induce such atherosclerotic lesions in different species of primates using vegetable oils with cholesterol (Portman & Andrus, 1965). Cuthbertson et al (1960) demonstrated that atherosclerosis could be induced in mice also when fed with a diet consisting of cholesterol, arachis oil,
starch, cholic acid and choline. Wissler et al (1954) and Fillios et al (1956) induced atherosclerosis in rats and they found addition of bile salts, thiouracil and cholesterol to an otherwise semi-synthetic balanced diet useful in the induction of atherosclerosis in these animals. Thomas et al (1960, 1963), Naimi et al (1965) and Kim et al (1965) also observed that incorporation of butter into the above diet could produce thrombotic lesions whereas corn and peanut oil produce more proliferative type of lesions.

In this connection it is worthwhile to note that on the contrary there are certain dietary constituents which have inhibitory effect on experimental hypercholesterolaemia (Kritchevsky et al, 1954; Shapiro and Freedman, 1955 and Sinclair, 1956). To mention some of these are safflower, corn oils, unsaturated fats etc. It follows that omission of these factors from atherosclerotic diet perhaps has a synergistic effect in producing hypercholesterolaemia.

Hormones & Atherosclerosis.

It has been observed that there are certain endocrine secretions which have a marked effect on serum lipid level.
and on atherogenic process. In support of this it may be stated that Hartroft and Thomas (1963) while discussing their collective experience on experimental atherosclerosis noted the effectiveness in the development and control of atherosclerosis of endocrine secretions. Adlersberg et al (1951), Rich et al (1951), Moran et al (1966), Hill et al (1965), Malinow et al (1965) and Bailey and Butler (1966) reported the effect of cortisone in producing hypertriglyceridaemia, hypercholesterolaemia and hyperphospholipidaemia in rat, chick, rabbit, dog and man, although views contrary to this (Adlersberg, 1959) have also been reported in the literature. In support of the previous observation, adrenocorticotropic hormone has been observed to produce severe atherosclerosis in nephrectomised breeder rats (Wexler & Miller, 1957, 1958; Kittinger et al, 1959; Wexler et al, 1960). Adrenal exhaustion and depressed dehydrogenase system of enzyme were held responsible for the above observation.

Hormones like adrenaline, noradrenaline, insulin, oestrogen and androgens have also been found to have
significant effect on atherogenic process. Adrenaline and noradrenaline have been found to cause medial sclerosis characterised by focal necrosis, calcification etc., in rabbit (Anitschkow, 1933; Bertelsen, 1961) and plaque formation in monkey (Jagannathan et al., 1964). A delay in the regression of atherosclerotic lesion (Duff and McMillan, 1949) and inhibitory effect on oestrogen (Stamler et al., 1959) were some of the effects observed with insulin in this connection. Position of insulin in the production of atherosclerotic process is still debatable (Still et al., 1964; Christensen & Jenson, 1965). The cholesterol lowering effect of oestrogen has been reported by many (Katz & Stamler, 1953; Jordan et al., 1961). As such the hormone was found to inhibit atherosclerotic lesions as demonstrated in chicks by Katz (1952), Katz & Pick (1961) and Stamler (1963) but views contrary to this as observed in cockerels have also been expressed by Malinow et al. (1965). In addition, Clarke et al. (1966) demonstrated a dose dependent biphasic effect of estrogen on atherosclerotic process induced in chicken fed with cholesterol. Helman and his associates (1959) opined that androsterone in contrast has a thyromimetic action and that a part of thyroid hormone
action in lipid metabolism is mediated through this hormone.

Thyroid hormone, on the other hand, has a serum cholesterol reducing property. Katz and Stamler (1953) and Kritchevsky et al (1961) reported this effect of the hormone in chick and rabbits whereas Walton et al (1965) observed a close association of degree of atherosclerosis with serum lipoprotein level in myxoedema. Cholesterol reducing property of thyroid hormone has also been demonstrated in dogs by experimental ablation (Marmorston et al, 1959) or blocking thyroid gland (Steiner and Kendall, 1946; Steiner et al, 1949; Abell et al, 1956; Sabiston et al, 1961; Westlake et al, 1963; Geer, 1965; Creech et al, 1955; Stephenson et al, 1962). The mechanism by which the hormone produces a drop in the blood level of cholesterol is perhaps by excessive excretion in face of increased absorption and synthesis (Friedman et al, 1952; Duncan, 1961; Chiu, 1961; Myasnikov et al, 1963; Van Itallie and Hashim, 1965). It follows therefore, that if thyroid is blocked by drugs like thiouracil, it is possible to produce high level of cholesterol in blood. The effect of thiouracil on serum cholesterol level have been examined by Friedman et al
(1952) and Abell et al (1956). According to them the mechanism by which thiouracil increases serum cholesterol level is that it depresses the conversion of cholesterol to bile acid and reduces its excretion through bile. A different viewpoint has been expressed by Boyd (1959). The definite role of thyroid hormone deficiency in the development of atherosclerotic lesions have been demonstrated by Wissler et al (1954) and later by Fillios et al (1956) in rats. They have observed that simple feeding with cholesterol failed to develop such lesions in this species but could succeed only when the thyroid gland was blocked with thiouracil. Addition of butter in presence of cholesterol, cholate and thiouracil to the diet can also produce a thrombotic lesion in rats (Hartroft & Thomas, 1957; O'Neal et al, 1959; Thomas & Hartroft, 1959; Thomas and O'Neal, 1959; Thomas et al, 1960, 1963; Gresham and Howard, 1961; Priest et al, 1962; Davidson et al, 1962; Bizzi et al, 1963; Howard & Gresham, 1964; and Kim et al, 1965). Concurrently it has also been observed that removal of pituitary can also produce atherosclerosis in rats.
Deficiency states and atherosclerosis

Certain factors in the diet viz., vitamins, minerals etc., have got significant influence on the course of development of atherosclerotic lesions. In this connection the effect of copper deficiency in the production of atherosclerotic lesion (Weissman et al., 1963; Carnes et al., 1965; Simpson and Harms, 1964), the protective action of magnesium (Neal & Neal, 1962; Bhattacharya & Mullick, 1963) and the effects of its deficiency on the process of atherosclerosis (Gottlieb et al., 1959; Hellerstein et al., 1957; Vitale et al., 1963 and Nakamura et al., 1965) require special mention. The part played by pyridoxin in the development of atherosclerosis have been discussed by Rinehart & Greenberg (1951), and Lindsay & Chaikoff (1966). Role of vitamin D in the degenerative process (Wilgram, 1958, 1959; Gillman et al.)
1960; Grant et al., 1960) and deficiency of choline in the accumulation of triglycerides and other lipids in liver with a concomitant fall in plasma phospholipid, cholesterol and triglycerides has been reported by Best (1956); Forbes et al. (1965); Haines and Mookerjea (1965); Mookerjea (1965) and Radomski & Wood (1965). The mechanism of action (Best, 1956), metabolism (Kennedy & Weiss, 1956 and Kennedy, 1956) and the nature of the atherosclerotic lesion produced by choline deficiency have been reported by Hartroft et al. (1952); Best (1956); Wilgram & Hartroft (1955). The role of choline in the production of atherosclerosis has also been discussed by Wissler et al. (1954). The paradoxical similar effect of high dose of choline and choline deficiency as reported in the literature is still not fully understood (Wissler et al., 1954; Hartroft et al., 1952).

Deficiency of essential fatty acid (Kahn, 1965; Sandler & Bourne, 1962, 1965 and Smith et al., 1966) and protein (Jain et al., 1965; Chalkoff et al., 1961) in the development of atherosclerotic lesions have also been observed. A protecting action of protein deficiency (Clarkson et al., 1962) methionine supplementation (Renaud, 1966) and lysin deficiency has also been reported.
The results of investigations on experimental atherosclerosis as enumerated above may now be summarised as observed by Hartroft & Thomas (1963).

(1) Lesions resembling human atherosclerosis can be produced in animals by artificial diet containing large amounts of cholesterol. In some species, however, adjuncts such as thiouracil have been found to be necessary in order to produce impressive lesions at least in short term experiments.

(2) The rapidity with which atherosclerotic lesions develop with cholesterol feeding is closely related to the levels in the blood. The development is slow with blood levels similar to those of man and more rapid with extreme levels.

(3) Species differ widely in their ability to handle cholesterol in the diet.

(4) Hormones and or drugs can influence considerably the nature of lesions produced in experimental atherosclerosis.

(5) Diets deficient in specific nutrients such as pyridoxine and choline can influence the development of atherosclerotic lesions.
(6) The part played by saturated and unsaturated fats appear to be different in the development of atherosclerotic disease as a whole.

Through a series of investigations over the period 1957-1959 Hartroft and his associates (Hartroft & Thomas 1957; O'Neal et al, 1959; Thomas & Hartroft, 1959; Thomas & O'Neal, 1959) presented a practical dietary method for producing atherosclerotic lesions. The diet is popularly known as "Hartroft Diet" and contains 40% butter with supplements of cholesterol, bile acid and thiouracil. Rats kept on Hartroft diet develop myocardial and renal infarction but replacement of butter with arachis oil was found to produce severe atherosclerosis alone. Butter or arachis oil alone without other supplements cause obesity only without vascular lesions.

While inducing atherosclerosis in rats in the present investigation the diet was chosen after carefully considering the influence of all these factors. The different component of Hartroft diet as it appears have a potentiating effect on each other to raise the cholesterol concentration to a high level. Use of such a diet for
producing atherosclerosis in rats is fully supported by Hegsted and his associates (1957). These workers believe that the level of serum cholesterol determines the degree of atherosclerosis produced in adult rats. In the present investigation a diet similar to that of Hartroft was used, the details of which will be discussed presently.

Choice of animal in induction of experimental atherosclerosis

Nearly two decades ago the rabbit and chick were the only common laboratory animals in which atherosclerosis could be produced readily (Katz & Stamler, 1953). The rat was then generally regarded as quite resistant to the development of such lesions and hence unsuitable for the purpose. Subsequently, remarkable changes have been witnessed in these concepts to the extent that literatures concerning the production of atheroma in the rats published during that time now number in the hundred. The investigator who wishes to produce atheroma experimentally now has the choice of a wide range of species. The ultimate choice of an animal for experimental purpose, however,
depends on a number of factors viz., the ease of handling, dietary habit, similarity of morphological features of the lesion to those of man etc. Considering these Duff (1935) criticised the choice of rabbit as an experimental animal as it is herbivorous and does not naturally ingest so much cholesterol. Disadvantages of chicken or pigeon for consideration as an experimental animal include the non-mammalian nature of the species and that the lesion does not quite resemble that of man. Dog, pig, and monkey although suitable for serial study have the disadvantages of handling complexity in diet requirements etc. The rat as a laboratory animal has certain obvious advantages. These include ease of handling large numbers simultaneously, the availability of information about its nutritional behaviours and the similarity of the experimental lesions produced to those of man (at least in its early stage). Rats were chosen as experimental animal in the present study.

Technique of inducing experimental atherosclerosis

Animals:

Experimental studies in the present investigation
were performed on rats. The animals were collected from a closely bred colony of Wistar strain of rats. The reasons for choosing rat as experimental animal have been discussed earlier. Both male and female rats were utilized in the experiments after a period of ten days acclimatization to altered environmental conditions. Animals becoming sick during this period of acclimatization were discarded at the beginning and those found to become sick during experimental study were also rejected.

Eight week old rats were used. Animals having initial weights varying from 110 to 120 gms. were isolated in separate cages built with strong wire mesh and kept in a well ventilated clean animal house.

The animals so chosen were divided in two groups. The first group served as a control and the other as experimental group in which atherogenic lesions were induced. The latter group was again divided into two sub-groups. The first sub-group remained untreated after induction of atherosclerotic lesions and the other received injection of thyrotrophic hormone "Thytropar" (Armour) for fifteen days at the end of a period stipulated for development of atherosclerosis. Atherosclerosis was induced by using a modified Hartroft diet.
Dietary regime:

A. Control group

Animals were fed (10 gms. approx) twice daily.

Composition of the food (expressed in percentage of different ingredients) used has as mentioned below:

- Casein: 20%
- Wheat flour: 40%
- Sucrose: 21%
- Coconut oil: 7%
- Cellulose: 6%
- Vitaminised Casein: 2%
- Salt mixture: 4%

Vitaminised casein was prepared by soaking 500 gms of defatted, devitaminised casein in an emulsion of vitamins mentioned below:

- Thiamin Hydrochloride: 400 mg
- Riboflavin: 400 mg
- Pyridoxin hydrochloride: 400 mg
- Calcium pantothenate: 1.2 gm
- Choline chloride: 10 gm
- Inositol: 5 gm
- Para-aminobenzoic acid: 2 gm
- Folic acid: 80 mg
- Vitamin B - 12: 300 μg
- Vitamin A acetate: 125 mg
- Vitamin D: 545 mg
- Vitamin E acetate: 4 mg
- Vitamin K: 125 mg

Last four components were dissolved in 25 ml. absolute alcohol.
Salt mixture consisted of the following ingredients:

(Expressed in percentage of components)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium lactate</td>
<td>35.15%</td>
</tr>
<tr>
<td>Calcium dihydrogen phosphate</td>
<td>14.60%</td>
</tr>
<tr>
<td>Potassium hydrogen phosphate</td>
<td>25.78%</td>
</tr>
<tr>
<td>Sodium dihydrogen phosphate</td>
<td>9.40%</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>4.69%</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>7.19%</td>
</tr>
<tr>
<td>Iron citrate</td>
<td>3.19%</td>
</tr>
</tbody>
</table>

100.00%

Arrangements were also made so that the animals could not waste the food unnecessarily or spoil it by contaminating it with urine or faeces. Water was provided in sufficient quantities with the help of a long glass tube, connected to a reservoir of water, attached to the cage.
B. Atherosclerotic group:

The atherosclerotic diet used in the present study was similar to that by Roy (1966) and is a modification of that suggested by Hartroft (1961). Dalda (a cooking fat medium produced by hydrogenating vegetable oils) was used in place of Margarine. The potency of Dalda as an atherogenic agent has been referred earlier (Chakravarty & Zaidi, 1961).

The diet consisted of the following ingredients (expressed in percentage of composition):

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>15%</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>15%</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10.5%</td>
</tr>
<tr>
<td>Cellulose</td>
<td>6%</td>
</tr>
<tr>
<td>Vitaminised casein</td>
<td>2%</td>
</tr>
<tr>
<td>Salt mixture</td>
<td>4%</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.2%</td>
</tr>
<tr>
<td>Sodium tauroglycocholate</td>
<td>2%</td>
</tr>
<tr>
<td>Methyl thiouracil</td>
<td>0.3%</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>5%</td>
</tr>
<tr>
<td>Dalda</td>
<td>40%</td>
</tr>
</tbody>
</table>

100%
Both the groups of animals were kept in their respective diets for twenty weeks.

The group of animals treated with T.S.H. received 0.2 IU of T.S.H. daily in the morning for fifteen consecutive days after the 18-week period.

The animals were sacrificed by neck fracture after twenty weeks in both groups-control and atherosclerotic. The animals treated with T.S.H. were similarly sacrificed after twenty weeks including the period of induction of atherosclerosis.

Collection of Material:

After the animals were sacrificed an incision was made encircling the neck. The trachea along with the thyroid gland was exposed and removed in block. The head of the animal was then severed from the body by cutting cautiously through bones, muscles, vessels and nerves. The portions of bones forming the vault of the skull was gradually removed with the help of a suitable nibbler. A careful dissection was carried out for exposing the olfactory lobe of the brain. Meninges covering the exposed brain were
then removed with the aid of a pair of fine dissecting forceps. The brain tissue was then retracted to one side and cranial nerve attachments were severed. It was then lifted up from the base of the skull starting from the anterior end with the help of blunt lever. On removing the brain it was placed on a clean watch glass and kept covered in a petri dish containing a piece of moist filter paper to prevent undue dehydration. Excess of blood or blood clots were removed using a piece of filter paper. A macroscopic examination was then made to note any gross abnormality. The pituitary gland, which got separated from the brain when removed, then became visible near the centre of base of the skull. The gland was separated afterwards, tearing through the thecal membranes with the help of a pointed needle. The gland so dissected was next lifted up carefully with the help of the needle. It was then collected in a clean watch glass and kept covered in a petri dish containing moistened filter paper in order to protect it from rapid dehydration and drying up.
The weights of the organs removed were recorded in a Sartorius single pan semi-micro balance. This was done as soon as possible to prevent unnecessary drying up of the tissues. The thorax and abdomen of the animal was then slit open to note the condition of heart, lungs, liver, kidney etc., and to note any macroscopic abnormality. The lungs, stomach, intestines and liver were removed from the carcass and the aorta was traced and dissected out. The diaphragm was then slit open and dissection of aorta was carried out upto the level of bifurcation of common iliac arteries. Branches to different organs were cut to lift up the vessel from its bed. The aorta was divided into two and placed in fixative solutions for histochemical studies to detect any deposition of lipid material, change in elastic tissue and presence of acid mucopolysaccharide in the arterial wall. Lipid material was stained with Oil Red O technique after Lillie (1944) and OTAN technique as described by Adams (1959a). Cholesterol was localised by perchloric acid-naphthoquinone method as described by Adams (1961). These methods have been described later (vide chapter IV, section A & B) in detail. Elastic tissues were stained after
Verhoeffs, (Culling, 1963) and acid mucopolysaccharide distribution was studied by Alcian Blue stain as described by Steedman (1950).

Observation & Results

1. Mortality:

(a) Induction of atherosclerosis with modified Hartroft diet was undertaken in a total of eighty rats taken in batches. A total of forty five animals died during the course of experiment. Twenty seven of them died within six weeks of dietary treatment and autopsy did not reveal any gross change to explain the death. Eight out of the rest eighteen animals, who died later in the course of experiment developed middle ear disease and labrynthites. Pulmonary infection was observed in ten.

(b) Five animals out of twenty five in the control group died during the experiment. Three of them
developed middle ear infection and other two dying of pulmonary infection.

2. Body weight and other changes:

(a) The average gain in weight in the control group was 50 gms. approximately at the end of a 20 weeks period. The increase in weights of animals in treated group was relatively less marked. The average gain being 5 gms. approximately.

(b) Hairs were found sparse on the body of the animals receiving atherogenic diet and the normal lustre lost compared with the control group.

3. Behavioural changes:

Lack of response to environmental changes, absence of normal micturition and defecation reflex on handling, general lethargy, lack of normal response when presented with food are some of the behavioural changes observed in the treated group as compared to controls.
4. Morbid changes:

A. Gross:

1) Macroscopic study of the internal organs, after sacrificing the animal, revealed excess of fat deposition in the liver in the treated group. The livers were relatively enlarged in size, muddy white in colour and greasy to touch while the consistency, size and colour remained within normal limits in control series.

The lungs were comparatively pale in the treated group. No macroscopic difference could be observed in the heart, kidney, spleen, and brain of the animals between the treated and the control groups. The thyroid gland was found to be little enlarged in treated animals.

ii) Aorta:

No gross difference in appearance was noted in the aortae of animals in the treated and control groups. Atherosclerotic lesions like fatty streaks, fibrous plaques or ulcerations over the intimal surface was not observed in the present series when the vessels were slit open longitudinally.
B. Microscopic (Histochemical):

1) Aorta:
(a) Sudanophilic deposition in the intima and subintimal layers (Figs. 1a, b & 2).
(b) Intimal thickening at places due to endothelial cell proliferations (Fig. 3) and also infrequent intimal discontinuity (Fig. 3).
(c) Thickening and reduplication of elastic fibres in the elastic lamina (Fig. 4a, b).

However, no obvious difference in the concentrations of cholesterol and its esters, phospholipids and acid mucopolysaccharide in the aortic wall could be demonstrated histochemically using PAN, OTAN and Alcian Blue techniques respectively (Figs. 5a, b; 6a, b and 7a, b).

ii) Thyroid:
The lining epithelium is more flattened and acini distended with colloid material as compared with that of normals (Figs. 8a, b).
Fig. 1 a. Shows transverse section of aorta in a normal rat stained with Oil Red O X 300.

Fig. 1 b. Shows the transverse section of aorta in an atherosclerotic rat stained with Oil Red O X 300. Sudanophilic depositions could be seen in intimal and subintimal layers.
Fig. 2. Shows a coloured picture of the same transverse section of aorta stained with Oil Red O X 300. The red colour indicates the sites of sudanophilic deposition in the aorta.

Fig. 3. Shows a coloured representation of the transverse section of aorta stained with Oil Red O X 300. Picture shows in addition to sudanophilic deposition, intimal thickening and discontinuation at places.
Fig. 4 a, b. Show transverse sections of a normal (a) and an atherosclerotic (b) aorta stained with Verhoeff's stain X 300. Slides show thickening and reduplication of elastic fibres in the elastic laminae of atherosclerotic aorta.
Fig. 5 a, b. Show transverse section of normal (a) and atherosclerotic (b) aorta stained with PAN X 300. Show very little change in cholesterol and its ester.
Fig. 8a, b. Show the transverse sections of normal (a) and atherosclerotic (b) aorta stained with OTAN X 300. Show very little change in the phospholipid content.
Fig. 7 a, b. Show transverse section of normal (a) and atherosclerotic (b) aorta stained with Alcian Blue X 300. Very little change in acid mucopolysaccharide content could be seen.
Fig. 8 a, b & c.

Show sections of thyroid glands from normal (a), atherosclerotic (b) and TSH treated rats stained with E&H X 300. Acini are seen distended with colloid material lines by flattened epithelium in atherosclerosis (b) as compared with normal (a). On treatment with TSH (a) very little change from that in atherosclerosis is observed. For details see text.
The suitability of Hartroft type of diet may be considered from three different view points. The high cholesterol content of the diet fulfils the first principle for experimental induction of atherosclerosis. An increase in the cholesterol level is further effect by the presence of bile salts and choline in the diet. The process is indirectly supported by blocking of thyroid by thiouracil and presence of saturated fatty material (Dalda) in excess in the diet. Thyroid gland blocked by thiouracil fulfils the second condition for augmenting the process of atherosclerosis. Replacement of fat in the diet totally by a hydrogenated oil (Dalda) indirectly produces a deficiency of essential fatty acids and thereby fulfils third criteria for atherogenesis. In short, the diet produces its effects in all possible ways usually utilized for development of experimental atherogenesis. The balanced nature of the diet and presence of all the proximate principles of food make it very suitable for routine use.
Gresham and Howard (1960) noticed a positive shortening of the life span of the animals with the diet used by them, although it was essentially of the same nature as used in the present study. None of the animals receiving atherogenic and thrombogenic diet survived in their series more than 20 weeks. The death of the animals noted during the early part of the experiment in the present series, may be attributed to refusal to accept an unacquainted diet leading to nutritional deficiency. Gresham and Howard (1960) noted a fall in the body weight in their animals during experimental atherogenesis. In contrast a positive but retarded growth has been observed in the animals in the present series.

Macroscopic observations made on different viscera agrees well with those made by the above mentioned investigators. However, these investigators noticed severe degree of atherosclerosis in their experimental animals in the form of fibrous plaques and fragmentation of elastic lamina in contrast to the low degree of atherosclerosis lesions found in the present series.
Hartroft and Thomas (1963), on the other hand, observed lesions similar to those of man in their early stage. Hess and Staëbli (1963) observed sudanophilic deposits in intima and muscle fibres and only traces of cholesterol and phospholipid in the arterial wall of rats rendered atheromatous with cholesterol peanut oil, cholic acid and thiouracil diet. These are in agreement with the present observations.

A search of literature indicates that atherosclerosis, both naturally occurring and experimentally induced has been observed particularly in rats. However, naturally occurring atherosclerotic process was not observed by the present author in any of the animals used in the control series. In this connection it may be stated that Wilens and Sproul (1938a,b) observed spontaneous medial muscular hyperphesia and calcification in the coronary and pulmonary arteries in a large proportion of their rats but no lipid deposition or intimal thickening was seen in the affected arteries.