قسم الباثولوجيا الاكلينيكية
كلية الطب - جامعة أسيوط
رئيس القسم : أ٠د/ سعد عطا ٠

تصلب الشرايين التجريبي

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أجريت دراسات على تصلب الشرايين التجريبي الناتج عن اعطاء مادة الكولستيرول يوميا عن طريق الفم لمجموعة من الذكور الأرانب ٠ وقد استمرت التجارب لمدة أربعة شهور ٠ ولقد قيست المتغيرات الحيوية الآتية شهريا : الفحص الباثولوجي للشرايين والقلب ، نسبة كولسترول الدم الى الدهون الفوسفورية ، ضغط الدم الشرياني وقورنت النتائج بعينة ضابطة ٠

ومن خلال متابعة التجارب لوحظ الآتي :

أحدث أعطاء الكولسترول يوميا عن طريق الفم لمدة أربعة شهور تصلبات بالشرايين خصوصا في الأورطي النازل وتضخم في البطين الأيسر للقلب وذلك بعد مدة شهرين من بدء التجربة ، وقد لوحظ أيضا زيادة كبيرة في نسبة الكولسترول الى الدهون الفوسفورية في الدم وزيادة في ضغط الدم الشرياني ٠

ان المتغيرات السالف ذكرها لوحظ بعد شهر واحد من بدء اعطاء الكولستيرول وباستمرار التجربة ازدادت التغيرات وضوحا ٠

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AN EXPERIMENTAL ATHEROGENESIS

(With 3 Tables & 4 Figs.)

By
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SUMMARY

Experimental atherogenesis was induced in male rabbits. The experimental animals were fed with cholesterol in order to induce atherogenesis. The experiments were lasted for four months. During the procedure, the following biological variations were measured monthly. These include, the pathological changes in the arteries and heart, plasma cholesterol/phospholipid ratio, and blood pressure.

After two months from the start of induction of experimental atherosclerosis, pathological changes were noticed in the gross appearance of blood vessels (mainly arteries). There was also concentric hypertrophy of the left ventricle. The following changes, were produced after one month of cholesterol feading and with continuous administration the changes became more manifested. There were a significant increase in the plasma cholesterol/phospholipid ratio, elevation of the arterial blood pressure.

From our data, we can conclude that, atheromatous plaques in the arterial walls induced by cholesterol feeding is mainly due to hypercholesterolaemia, an increase of plasma cholesterol/ phospholipid ratio.

INTRODUCTION

High levels of serum cholesterol are associated with a high incidence of ischaemic heart disease (IHD); this association has emerged in virtually all epidemiological studies. However, although the early studies appeared to show a linear relation between serum cholesterol level and risk of (IHD) KANNEL, et al. (1971). It now appears that the relationship is more complex. In some of the populations studied, significant increase in (IHD) risk has only been found in the top 25-30 per cent of the cholesterol distribution, and in others there seems to be a J-shaped relationship in which (IHD) risk may actually be higher in the lowest quintile (20%) of the distribution than in the second and median quintiles. BLACKBURN, et al. (1983). Arteriosclerosis is one of the most important factors causing (IHD).

The first pathological change associated with atherosclerosis, is not found in the endothelium, but in the intima. DAOUD; JARMOLYCH; ZUMBS; FANI and FLORENTIN (1964) have pointed out that it varies from one human to another. The inner coat of the vessel thickens owing to local increase of tissue fluid in the intimal ground substance. At the same time there is

an influx along the paths mentioned of protein which may also be coupled with lipid. The latter do not become visible until they are uncoupled. They also claimed that, this initial process is a borderline form of normal interchange and is certainly in many cases reversible.

Cholesterol-induced atherosclerosis in the rabbits has been by far the most commonly used animal model, hence it was proved that rabbits are much susceptible due to certain enzymatic activity (KRITCHEVSKY; MAYER; TESSAR; LOGAN; BROWN; DAVIES and COX in 1964). In 1964, KRITCHEVSKY found that, the rabbits respond directly to cholesterol feeding, within 2 to 3 weeks of feeding diets containing: 1% cholesterol and a triglyceride vehicle such as 10% vegetable oil as sunflower seed (corn or peanut oil and butter also can be used). For induction of atherosclerosis 12 weeks are needed. Therefore he concluded that, the administration of cholesterol alone, produce a severe atherosclerosis.

ZEMPLENYE (1964) described the pathohistological findings in rabbits, which occurred during experimental atherosclerosis induced by cholesterol-fat feeding. Cholesterol was given in a dose of 1gm and 10 gms of fat (margarin) was added to the normal food of the rabbits/ rabbit daily. He noticed that grossly visible lesions were seldom seen before the end of the first month, of cholesterol feeding. In the aorta, the lesion appeared in the form of small slightly raised round or oval spots with yellowish colouration. The lesion was observed first in the aortic ring and then in the aortic arch. With more prolonged period of feeding, dilatation of the aorta and calcification may also be found. Similar changes to those described in the aorta can also be seen in other arteries. This was especially noticed in the coronary, pulmonary and in ether arteries elsewhere.

In addition to the level of serum cholesterol, various serum lipid levels have been embloyed as indices of atherosclerosis, including: 1). The plasma cholesterol/phospholipid ratio. 2) the concentration of plasma alpha and beta lipoprotein or the percentage of cholesterol in alpha and beta lipoproteins. 3 (The concentration of lipoprotein molecules of specific size in the plasma, as disclosed by the analytical ultracentrifuge, and 4). Serum triglycerides.

AHRENS and KUNKEL (1949); suggested that the ratio of cholesterol to phospholipid concentration is an index of the stability of lipid solutions in the blood and that this cholesterol/ phospholipid ratio and not the cholesterol concentration alone, was a determining factor in the development of atherosclerosis. Cholesterol is insoluble in aqueous solution but phospholipids tend to keep it in solution in blood. Elevation of the cholesterol/phospholipid ratio may predispose to the precipitation of cholesterol in the arterial intima. PAGE and BERNHARD (1935); found that, the cholesterol/phospholipid ratio is less than 1 in normal rabbits. In rabbits with atherosclerosis, induced by cholesterol feeding, the ratio is greater than 1.

The plasma cholesterol/phospholipid ratio had also been correlated with human atherosclerosis. GERTLER, GARN and LERMAN (1950), found the average serum cholesterol/phospholipid ratio sugnicantly higher in patients, who had survived a coronary artery occlusion than in normal people of similar age. BILLIMORIA; IRANI and MACLAGAN (1965); found that, total cholesterol was increased in ischemic heart diseases. STEINER and his associates, (1952), found that the serum cholesterol, serum phospholipid, and also the cholesterol/phospholipid ratio, were increased in most of patients with clinical coronary atherosclerosis. However, in many persons with coronary occlusion the cholesterol/phospholipid ratio is with in the normal range.

The hypothesis that atherosclerosis is ralted to the physical state of the blood lipids or lipoproteins has led to the study of another agents which, like the phospholipids, may influence lipid solubility. KELLNER; CORRELL and LADD (1951) found that, the injection of surface-active agents, such as the detergents, tween 80 and triton A 20, into cholesterol fed rabbits

not only caused a marked, sustained elevation in serum phospholipid but also greatly inhibited the development of atherosclerosis. Also SCHROEDER and PERRY (1955), showed that, the metal binding agents, such as the chelator EDTA and hydralazine, were found to cause a prompt fall in plasma cholesterol in man.

DAWBER (1961), reported the relation between the elevated blood pressure, serum cholesterol and the development of coronary artery disease. Serum cholesterol would appear to be a stimulus acting uniformly throughout the vasculature. However, many observations indicate that not all arterial beds are equally affected.

The studies of DEMING and his associates (1958) revealed, in an experimental work, the relationship between hypertension, atherosclerosis and the metabolism of cholesterol. These data, of experimental atherosclerosis (on rats), indicated that, hypertension intensifies dietary atherogenesis and that pharmacological control of the blood pressure reverses this effect. The author showed that hypertension increases the amount of cholesterol in the arterial wall prior to the development of visible atherosclerosis, that the amount of cholesterol in the arterila wall is more closely dependent on blood pressure than on diet or serum cholesterol, and finally, that, hypertension increases the rate of synthesis of cholesterol in the arteries and the liver.

We investigated the model of arteriosclerotic rabbits in order to get some data on under laying pathophysiological condition. (BARAKA, et al. 1978).

MATERIAL and METHODS

Boskat male rabbits weighing 200 to 300 gm. of the same age and from the same mother were divided into 2 groups.

The First group	: Untreated (a): 7 Rabbits.
The Second group	: Treated (b): 28 Rabbits.

The first group is the control group was used to measure the normal plasma phospholipid, cholesterol level, blood pressure and area of cross section of the aorta, coronary and femoral arteries.

The second group received a daily dosage of 0.5 gm/kg. orally for each animal for 28 days per month for four months. Plasma phospholipid level, cholesterol level and blood prossure were measured.

Pathological examination

7 rabbits, were slaughered monthly, during the feeding of cholesterol. The changes in aorta,coronary and cerebral arteries were examined by the aid of a lens and microscopically. Similarly, wall of the heart was studied to indicate the severity of cases. The histopathological examinations, were performed by staining of the sections with haematoxylin stain according to LILLIE (1948).

RESULTS

The changes produced by cholesterol feeding appeared after 2 months from the starting of administration in 30% of animals. These pathological changes can be summarized as follows:

By macroscopical examination of heart, by taken transverse section, passing through the ventricles, there was concentric hypertrophy of the left verticle, as well as yellowish colouration

of the musculature (Fig. 1). By opening of the aorta longitudinally,revealed yellowish oval mass, raised above the surface, in the descending aorta were observed.

Histopathological examination, revieled deposition of fat between intimal fibres of the aorta (Fig. 2), coronary (Fig. 3) arteries.

After three months of cholesterol feeding, the pathological changes were seen in 50% of the animals. The changes were more manifested than those noticed by the second month, e.g. multiple small atheromatous plaques appeared in the intima of the descending and aortic arch.

By the fourth month of cholesterol feeding, large yellowish white plaques, were noticed in 80% of rabbits. These were due to calcification in the arch and descending aorts, causing narrowing of the opening of the branches arises from the aorta (Fig. No. 4-2). There was also an increase in the fat deposits around the pericardium.

For measurement of the arterial blood pressure fo rabbits, the medial ear artery was compressed between a glass disc and a special air cuff (1). The latter is connected to a spring-type manometer (2) with a scale graduated in mm Hg. At the same time the colour of the artery was noticed with the eye through the glass disc. The cuff was inflated for some time after the visible colour disappeared. The air was gradually let out of the cuff. The point at which the colour reappeared corresponds to the mean arterial blood pressure. This method is used for the comparison of the mean value of the arterial blood pressure of normal and treated rabbits, i.e. the data is used for comparison only. And for accuracy, each measurement was repeated several times and the arithmatic mean value was taken.

During the experiments, the normal values of cholesterol and phospholipid plasma levels, were recorded monthly.

It is seen from table No. (1) that, the mean value of the plasma cholesterol level varied from 104.8 to 108.1 mg%, with a mean of 106.7 mg% ($\pm$ 1.8). It is evident that animals not receiving cholesterol or drugs have got a moreless constant cholesterol level all-over the period of observation, which continued for four months.

Similarly, the phospholipid plasma content varied all over this period of observation from 84.4 to 91.6 mg%, with a mean value of 88.0 mg% ($\pm$ 2.7). This also, indicates the phospholipid concentration in plasma is a constant one in the experimental animals used (rabbits) all-over the period of the studies (four months).

Relating the cholesterol to phosphelipid content of the plasma in the form of ratio (cholesterol/phospholipid ratio), it is seen that such ratio did not change significantly during the four months of investigations. The individual values of cholesterol/phospholipid ratios, varied, during this period, form 1.18 to 1.245, with a mean value of 1.214 ($\pm$ 0.03). So, it is evident that, neither plasma cholesterol and phospholipid contents nor their ratio significantly changed from month to month during the fulfilling of the experiments (P 0.05). Also the standard errors are small and closely near to each other. Therefore we can consider the arithmatic mean values of plasma cholesterol, phospholipid and their ratio as the normal stable values.

When the experimental animals (rabbits), were subjected to oral administration of a daily dose of 0.5 gm/kg of cholesterol for 28 days (with rest for two days before the sampling of the blood) monthly, there was a significant elevation of plasma cholesterol level. The latter elevated from 106.7 ($\pm$ 1.8) to 289.7 ($\pm$ 4) mg%. By the end of the second, third and fourth months of cholesterol feeding this value was elevated to 327.2 ($\pm$ 4.8), 747.3 ($\pm$ 10.8) and

967 (± 19.3) mg% correspondingly. So, it is seen that the elevation of the plasma cholesterol content was significantly elevated (P 0.05) during the procedure of inducation of atherosclerosis. The plasma phospholipid level was also significantly increased from 88.0 (± 2.7) to 107.5 (± 1.7), 113.53 (± 5.6), 123.4 (± 2.9) and 122.5 (± 3) mg/100 ml., by the end of the first, lecond, third and fourth months correspondingly, of the procedure. (Table No. 2).

In our experiments, the measurement of arterial blood pressure, by using middle ear artery technique described in the methods. The mean arterial blood pressure was recorded in normal rabbits and during the inducation of experimental atherosclerosis, using feeding in a dose of 0.5 gm/kg, day for 28 days monthly.

The mean value of the arterial blood pressure in the untreated group of animals (normal) was 71 (± 1.8) Mg Hg. The standard error is seen to be small, which emphacise the validity of the method.

Cholesterol feeding of rabbits for one month, in the above mentioned doses, caused an elevation of the arterial blood pressure from 71 (± 1.8) to 77 (± 1.2) Mm Hg. The increase in blood pressure was significant (P 0.05) and the standard error was less than that of the normal group of animals. By continuation of feeding for another month, i.e. after two months of feeding, the blood pressure was elevated to 77 (± 2.2) Mm Hg. This value was much more than in the previous month, and the rise in arterial blood pressure was significantly increased (P 0.05) in comparison with the normal value. After three and four months of cholesterol feeding, the mean values of the arterial blood pressure were correspondingly, 84.5 (± 1.2) and 85 (± 1.5) Mg Hg. Table No. 3. It is noticed that the increase in the arterial blood pressure reached a level, after the third month of the procedure, beyond which the continuation of cholesterol feeding did not produced a correlating parallel rise in blood pressure. This indicates that, by the end of third and fourth months of induction of atherosclerosis the maximum rise in arterial blood pressure is nearly reached. These data go parallel to the previously described pathological lesion in the arteries in consideration to the time of occurrence of both biologicl variations.

DISCUSSION

In atherosclerotic conditions, the biological functions of the body are affected, on account of the narrowing and rigidity of the arteries. As a result ischemia of the corresponding organs occurs. These changes are always accompanied by biochemical changes in the composition of blood. The heart is the most sensitive vital organ to these changes.

The fine details about the aetiology of atherosclerosis is uptill now uncertain. But it is quite accepted that a great role in the aetiology of atherosclerosis is played by metabolic disorders. Detection of the disease in its preclinical period is very important since prophylactic measures can be effective especially in this period.

In this work, experimental atherosclerosis was induced in rabbits, represent the most sensitive experimental animals to the induction of atherosclerosis (KRITCHEVSKY and his COWORKERS, 1954). The female rabbits were excluded, hence they are less susceptible to the disease. This is attributed to an increase in the function of thyroid gland, and to the presence of oestrogens (MALINOW, et al. 1962).

During administration of cholesterol, the following biological variations were measured monthly. These include, the pathological changes in the arteries and heart, plasma cholesterol/ phospholipid ratio, and blood pressure.

After two months from the start of induction of experimental atherosclerosis, pathological changes were noticed in the gross appearance of blood vessels (mainly arteries). These were seen in 30% of the animals and consisted of yellowish oval masses, raised above the surface of the descending aorta. There was also concentric hypertrophy of the left ventricle of the heart. This indicates that, the myocardial muscles of the left ventricel hypertrophied to compensate the narrowing of the aorta. The hypertrophy of the left ventricle, in experimental atherosclerosis is in agreement with data of CABRERA and MONROY (1952), who reported similar gross changes of the left ventricle.

By the continuation of cholesterol feeding, the percentage of the affected animals was increased till it reached 80%, at the end of the 4th. month of the procedure. Histopathological examination revieled deposition of fat between the intimal fibres of the descending aorta, cerebral, and coronary arteries. These histopathological lesions were accompanying the gross appearance changes which started two months of cholesterol feeding. The question; why the pathological lesions appeared after two months, only in 30% of the animals although the experimental conditions are the same to all animals and why this percentage increase by continuation of cholesterol feeding, remains obscure. Perhaps individual susceptibility and obscure metabolic pathways, of individual character, play role in this consideration.

MEYER, WALTZ and ZAK (1959) proved that, elevation of the plasma cholesterol level alone in man is not indicative for induction of atherosclerotic lesions in arteries. They indicated that the plasma cholesterol/phospholipid ratio gives a good indication for diagnosis of atherosclerosis. They attributed such suggestion to the dissolving power of phospholipid to cholesterol to keep it in aqueous solution. PAGE and BERNHARD (1935) found that the cholesterol/phosphilipid ratio in the plasma of normal rabbits is less than that in rabbits with experimentally induced atherosclerosis (by cholesterol feeding). Our results indicate a significant increase in the plasma level of total cholesterol and cholesterol/phospholipid ratio during the induction of experimental atherosclerosis in rabbits. These changes, were produced after one month of cholesterol feeding and with continuous administration of cholesterol the changes became more manifested. So, it is evident that the elevation of the total plasma cholesterol and the cholesterol/phospholipid ratio occurred prior to the formation of the pathological lesions. Therefore the tendency of atheromatous plaques formation is accelerated when there is no sufficient phospholipid to emulsify the insoluble cholesterol in the blood. Such decrease in the phospholipid leads also to decrease in the power of mobilization of cholesterol from the sites, where by it forms plaques. Clinically it was proved (GERTLER, CARN and LERMAN, 1950). That the average serum cholesterol/phospholipid ratio is significantly higher in patients with coronary artery occlusion than in normal young men of the same age. STEINER (1952) attributed coronary atherosclerosis not only to increase in cholesterol/phospholipid ratio but also to increase serum cholesterol and serum phospholipid.

During the process of induction of experimental atherosclerosis the rise in the arterial blood pressure started to be observed after one month. The rise in the arterial blood pressure gradually increased till reached the maximum readings after four months. The change in the arterial blood pressure is mostly secondary to the pathological changes, induced by the biochemical in blood. It is evident that changes in the cardiac activity and hypertroph of the myocardium (left ventricle) are secondary to hypertension than to biochemical changes. This can be explained that hypertrophy has occurred in the left ventricle and not in other chambers of the heart. DEMING and his colloegues (1958) have indicated that hypertension intensifies dietary atherogenesis and the pharmacological control of blood pressure reverses this effect. They demonstrated that hypertension can cause an increase in the amount of cholesterol precipitated

in the arterial wall. This can occur prior to the development of any visible atherosclerotic lesion.

The plasma cholesterol/phospholipid ratio was significantly elevated during the induction of experimental atherosclerosis and the atheromatous plaques were visible macroscopically at the end of the second month.

From the previous data, and the observations of the pathological changes, we can conclude that, atheromatous plaques in the arterial walls induced by cholesterol feeding is mainly due to hypercholesterolaemia, increase in the permeability of the arterial wall and also depending on the plasma cholesterol/phospholipid ratio. The increase in the blood pressure does not significantly affect the induction of the atheromatous plaques formation. This indicates that, there are serial changes in the blood constituents. The elevated blood pressure shares in the ischemia of the different organs, especially of heart. This causing hypertrophy of the left ventricle, with constriction of the coronary vessels leading to coronary ischemia.

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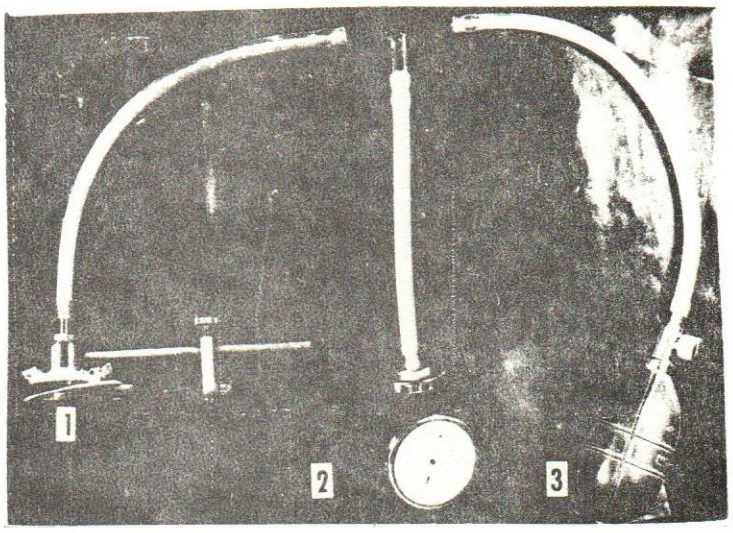
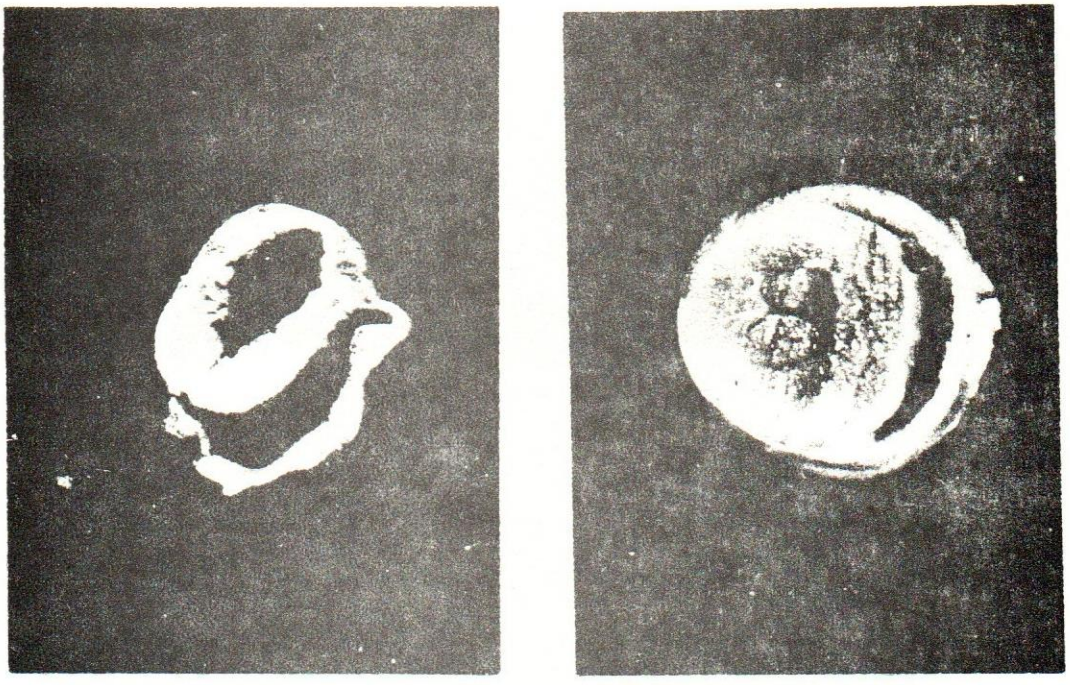


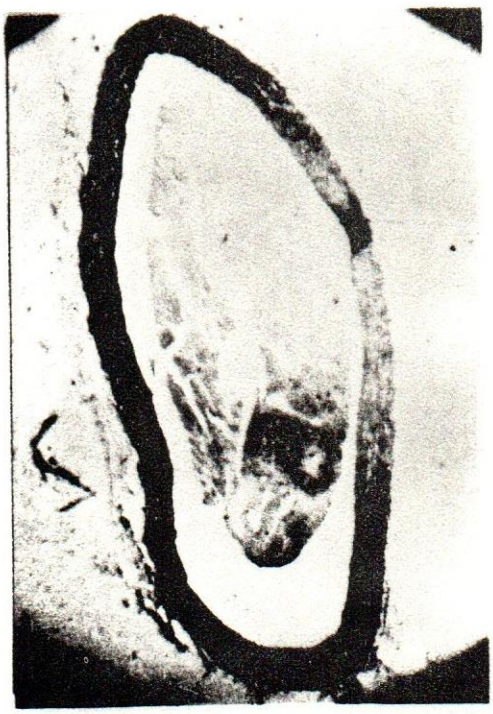
(Measurement of blood pressure of rabbits)



Normal

(After 2 months of cholesterol feeding)

Fig. (1): Concentric hypertrophy of the left ventricle after 2 months of cholesterol feeding.
(0.5 gm/kg/day for the 1st 28 days monthly)



Normal (4x8)
descending aorta

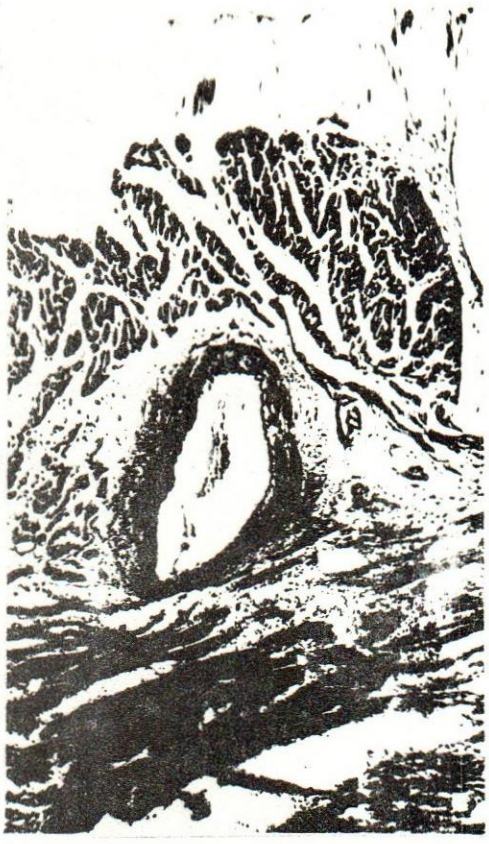
(haematoxylin stain)

(4x8)
Descending aorte after 2 months of cholesterol feeding.
(haematoxylin stain)

Fig. (2): Deposition of fat between the intimal fibres of the descending aorta, after 2 months from the beginning of cholesterol feeding.
(0.5 gm/kg/day for 28 days monthly)

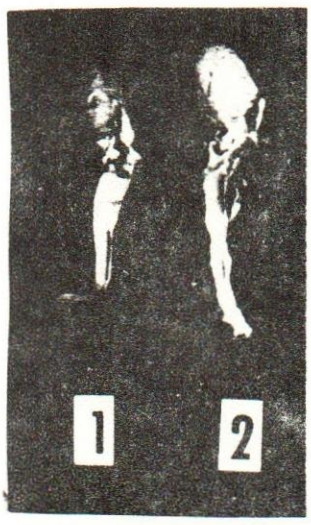
Normal (40x8)
Coronary artery

(haematoxylin stain)



(40x8)
Coronary artery two months after cholesterol feeding.
(haematoxylin stain)

Fig. (3): Deposition of fat between the intimal fibres of the coronary artery after 2 months from the beginning of cholesterol feeding.



1- Normal rabbit 2- Rabbit treated with cholesterol
Note atheromatous plaques in No. 2.

Fig. (4): Longitudinal opening of the aorta after 4 months of cholesterol orally in a dose of 0.5 gm/kg/day for 28 days monthly.