Light-Microscopic and Electron-Microscopic Study on the Development of Experimental Arteriosclerosis

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Arteriosclerotic vascular changes were produced by experimental high lipid feeding and by various methods in forty eight white rabbits. These changes were more severe and frequent after prolonged feeding over three months. Histopathologically, vascular lesions consist of not only fat deposition but also elastosis, fibrosis and so on. Electron microscopic examination was also performed. From the results of the experiment, the author suggests active metabolic disturbances of vascular wall as pathogenesis of the development of arteriosclerosis. The concept of "capillarosclerosis" was also introduced in this report.

RECENTLY, in Japan also, the clinical importance of cerebrovascular and cardiovascular diseases has been recognized progressively. In most cases, these diseases have some relationship with arteriosclerotic vascular changes.

Detailed examination about generalized vascular changes from the anatomical stand point has been done. While the problems that seemed to have some relationship with the vascular lesion being studied clinically, attempts to produce experimental arteriosclerosis with various animals also has been conducted¹⁾. Based on these studies, many theories about the pathogenesis of arteriosclerosis have been introduced²⁾, especially, abnormality of lipid metabolism has been the most disputed topic.

Many detailed observations and discussions about abnormality of plasma lipid level and about atherosclerotic changes as its final result have been reported, however, what kind of intermediate process were there or what kinds of factors had influence in the development of these vascular lesions have not been discussed often. On the other hand Rinehart and others say that fat deposition of vascular wall plays only the secondary role in pathogenesis of

arteriosclerosis³⁾.

Furthermore, because of too much interest in searching for generalized factors, not a few people overlooked local difference in development of arteriosclerosis. Of course there are some investigators who put emphasis on local factors especially on effects of hemodynamics but they seldom pay attention to metabolic abnormality of vascular wall. Up to now, vascular changes such as atherosclerosis, Mönckeberg's sclerosis, and arteriolosclerosis have been clarified already but no one has reported on abnormality of smaller blood vessels including capillaries.

In consideration of above mentioned facts, the author is reporting here some interesting results of experiments about development of arteriosclerosis in rabbits by lipid feeding and by various other methods.

MATERIALS AND METHODS

1) Materials

Forty eight healthy, male albino rabbits from four to five months of age, about 2 kg in weight, were used and divided into several groups as shown in Table I. As the control groups the non-treated rabbits and infant rabbits of one month old were used.

2) Methods

TABLE I EXPERIMENTAL PROCEDURE IN RABBITS

Group	Method	Period of Experiment	No. of Cases	Instrument used
I	Intramuscular Injection of Cholesterol	1 Mon.	5	L. M.
II	Intramuscular Injection of Cholesterol + Intravenous Injection of Allylamine	1 Mon.	5	L. M.
III	Lanolin Feeding	1 Mon.	4	L. M.
		3 Mon.	6	L. M.
		6 Mon.	2	E. M.
IV	Lanolin Feeding + Intramuscular Injection of Adrenaline	1 Mon.	2	L. M.
		3 Mon.	2	L. M.
V	Lanolin Feeding + Intramuscular Injection of Noradrenaline	1 Mon.	2	L. M.
		3 Mon.	2	L. M.
VI	Lanolin Feeding + Intramuscular Injection of Adrenaline + Intravenous Injection of Allylamine	1 Mon.	3	L. M.
VII	Lanolin Feeding + Intramuscular Injection of Noradrenaline + Intravenous Injection of Allylamine	1 Mon.	2	L. M.
VIII	Lanoline Feeding + Intramuscular Injection of Thyrsin	1 Mon.	1	L. M.
		3 Mon.	1	L. M.
IX	Lanolin Feeding + oral administration of NaCl	3 Mon.	2	L. M.
X	Lanolin Feeding + Constriction of Renal Artery	3 Mon.	3	L. M.
XI	Lanolin Feeding + Constriction of Renal Artery + Intravenous Injection of Allylamine	3 Mon.	2	L. M.
XII	Lanolin Feeding + Pressoreceptor Denerve	1 Mon.	2	L. M.
		3 Mon.	2	L. M.
XIII	No. Feeding (Control)		10	L. M. E. M.

L. M. = Light Microscopy

E. M. = Electron Microscopy

a) Feeding :

The rabbits were fed in the following manner.

- I) Cholesterol group: Daily intramuscular injection of 125 mg cholesterol.
- II) Cholesterol allylamine group: Daily intramuscular injection of 125 mg cholesterol and twice a week intravenous injection of 10 mg allylamine.
- III) Lanolin group: Daily oral administration of 10g lanolin. (Lanolin was mixed with about 400g of been-curd refuse. All other oral administration was done in this manner.)
- IV) Lanolin-adrenaline group: Daily lanolin feeding 10g and every other day intramuscular injection of 0.2 mg adrenaline.
- V) Lanolin-noradrenaline group: Daily oral administration of 10g lanolin, and every other day intramuscular injection of 0.2 mg noradrenaline.
- VI) Lanolin-adrenaline-allylamine group:

Daily oral administration of 10g lanolin, every other day intramuscular injection of 0.2mg adrenaline and twice a week intramuscular injection of 10mg allylamine.

- VII) Lanolin-noradrenaline-allylamine group: Daily oral administration of 10g lanolin, every other day intramuscular injection of 0.2mg noradrenaline and twice a week intravenous injection of 10mg allylamine.
- VIII) Lanolin-thyrsin group: Daily oral administration of 10g lanolin, and every other day intramuscular injection of 1cc thyrsin.
- IX) Lanolin-NaCl group: Daily oral administration of 10g lanolin and 3g NaCl.
- X) Lanolin-unilateral constriction of renal artery group: Daily oral administration of 10g lanolin and operation of unilateral renal artery (at the early period of feeding by using silver clip at one side renal artery making its stenosis.)

XI) Lanolin-unilateral constriction of renal artery-allylamine group: Daily oral administration of 10g lanolin, unilateral renal artery operation and twice a week intravenous injection of 10mg allylamine.

XII) Lanolin pressoreceptor denervation.

b) Light microscopic examination

Experimental animals were sacrificed after the period of one month, three months and six months of experimental feeding and examined histopathologically. Specimens were obtained from brain, lung, aorta, heart, kidney, liver, spleen and pancreas. Heart was always sliced approximate vertically to its long axis and tissues from other organs were also cut in the manner to make them as large as possible. As to fixation, mainly 10% formalin was used. Absolute alcohol and cold acetone were also used in combination. In most cases the paraffine embedding was used but gelatin embedding was also used for a part of tissue specimens.

Hematoxylin-Eosin stain (hereafter this will be abbreviated as H-E stain), Mallory stain, Weigert stain, periodic acid Schiff's method, (PAS method) Hale Rinehart's colloidal iron method, etc. were used. For fat stain the Oil Red O and Nilblau were used. And as a rule the PAS method was used together with the salivary digestion method.

c) Electronmicroscopic examination

10 rabbits were examined electronmicroscopically. (6 were experimentally fed and 4 were used as the control group.) After having killed the rabbits the thorax and pericardium were opened immediately, and specimens of thoracic aorta and myocardium were examined. The specimens taken from aorta and myocardium were fixed in refrigerator for 2 hours in 1 percent osmic acid with acetate-veronal buffer (pH 7.5). They were washed in distilled water and dehydrated by ethanol in an ordinary way.

Finally, tissues were embedded in butyl methyl methacrylate with benzoyl peroxide catalyst.

Sections were made with a Porter's microtome

equipped with a glass knife. The sections were examined without removing the plastic in a Hitachi-electron microscope. Electron micrographs were taken at an original magnification of 1500 to 10,000, and enlarged photographically as desired.

RESULT

1) Development of vascular lesion

I) Feeding and vascular lesion

Incidence of development of arteriosclerotic vascular changes in each group is shown in Table II. That is, in seven out of twenty six cases with one month feeding, slight changes were recognized lightmicroscopically. In this group, arteriosclerotic findings were noticed, respectively, one out of five cases in the group I and II, one out of four in the group III, one out of two in the group V, one out of three in the group VI, one out of two in the group VII, one out of two in the group XII. No abnormality was recognized in group IV and VIII. But in the group with three months feeding, vascular lesions were observed in seventeen out of twenty cases. Namely, all the cases in groups III, IV, V, VIII, XI, one of two cases in the group IX, two of three in the group X, and one of two cases in the group XII showed arteriosclerotic vascular lesions. All cases of six months feeding showed arteriosclerosis. And findings of three months feeding group were much more severe than those of one month feeding group.

II) Localization of the vascular lesion

On examining vascular changes of all organs mentioned above, arteriosclerotic findings were demonstrated most frequently at coronary artery, at aorta, and at pulmonary artery in the decreasing order. In renal artery they were recognized only in few cases. But in other organs, such as liver, brain, spleen, pancreas, almost no abnormal finding of the artery was observed.

As to the aorta, aortic arch was mainly examined. Even in the one month feeding group, the vascular lesion was already recognized in some cases by light-microscopic examination. In the

TABLE II FREQUENCY OF POSITIVE FINDINGS

Period of Feeding	1 Month			3 Months			6 Months		
	Group	No. of Cases	Positive Findings	Group	No. of Cases	Positive Findings	Group	No. of Cases	Positive Findings
Findings	I	5	1	III	6	6	III	2	2
	II	5	1	IV	2	2			
	III	4	1	V	2	2			
	IV	2	0	VIII	1	1			
	V	2	1	IX	2	1			
	VI	3	1	X	3	2			
	VII	2	1	XI	2	2			
	VIII	1	0	XII	2	1			
	XII	2	1						
	Total	26	7 (27%)		20	17 (85%)		2	2 (100%)

TABLE III HISTOPATHOLOGICAL FINDINGS

Group	of Period Exper.	Coronary artery						Aorta						Pulmonary artery					Renal Artery	Liver & Spleen
		Intima			Media			Intima			Media			Intima			Media			Lesion
		Thickening	Hyaline change	Elastosis	Fibrosis	Fat deposition	A. M. P. S.	Thickening	Hyaline change	Elastosis	Fibrosis	Fat deposition	A. M. P. S.	Elastolysis	Thickening	Elastosis	Fibrosis	Fat deposition	Lesion	
I	1 Mon.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
III		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
V		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VI		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VII		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
XII		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
III	3 Mon.	+++	+	+++	+++	+++	±	+	+++	+	+++	+++	+++	-	+++	+++	+++	+	-	-
IV		+++	+	+++	+++	+++	+	+	+++	+	+++	+++	+++	-	+++	+++	+++	+	-	-
V		+++	+	+++	+++	+++	+	+	+++	+	+++	+++	+++	-	+++	+++	+++	+	+	-
VIII		+	+	+	+	±	+	-	-	-	-	-	-	-	-	-	-	-	-	-
IX		+	+	+	+	+	+	-	+	+	+	+	+	-	-	-	-	-	-	-
X		+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	-	-
XI		+++	+++	+++	+++	+++	+	+	+	+	+	+	+	-	+	+	+	+	-	-
XII		+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
III	6 Mon.	+++	+	+++	+++	±	±	±	+++	+++	+++	+++	±	+	+++	+	+++	+++	+	-

group of three months feeding atheromatous plaque was observed macroscopically. And in the group of six months feeding more remarkable atheromatous change was present. In many cases and groups, vascular change was most frequent in coronary artery. But in the group of one month feeding changes were recognized mostly in small branches of coronary artery in myocardium, but hardly observed in main stem of coronary artery. In the group of three months feeding more remarkably and more frequently, abnormality in the smaller arteries of myocardium were demonstrated. But in this group, main stem of coronary artery also showed the change rather frequently. In one month group some findings in pulmonary artery were observed. In the three months group, these findings were more frequent and in the six months group these were more remarkable.

Also in renal artery slight and a few abnormalities were observed in 3 and 6 months groups but there was no vascular change in other organs such as liver, brain and spleen.

By the study on the frequency of abnormality at the various part of coronary artery with different diameters in three months feeding group, distribution of vascular change was observed as shown in Fig. 1. To observe the distribution, 500 samples of coronary arteries were examined at random. As it's easily understood from the Fig. 1, vascular changes were observed most frequently in the blood vessels of 40 to 90 μ in diameter. As to same

Fig. 1. Relationship between the Frequency of Vascular Lesions and Diameter of Vessels.

diameter group, abnormalities were most frequent in vessels of left ventricular wall, then ventricular septum and right ventricular wall in decreasing order.

III) Histopathological and electronmicroscopical findings

a) Aorta

No macroscopical change was obtained from the one month feeding group, but histopathologically elastolysis, granulation and small cell infiltration in media and intimal thickening corresponding to these changes of media, were recognized in one case of the first group. The thickened intima showed hyaline change in H-E stain, and elastosis was recognized by Weigert stain. Abnormality was hardly detected at internal elastic membrane adjoining to the intimal thickening and in the superficial layer of media elastic fiber seemed to be slightly

decreased in density. Some of the elastic fibers appeared to be swollen around the foci of the medial elastolysis.

Among three months feeding groups, group III, IV and XI showed macroscopical, atheromatous plaque at aortic arch. The most remarkable example was seen in a case of group IV. In this case, a yellowish gray protuberance was observed along the entire course of ascending aorta and it covered more than a half of inner surface at the beginning of the aorta.

Ulceration was not recognized in any case. Histological study of the atheromatous plaque revealed the intimal thickening forming a hill on the internal elastic membrane which consists of elastosis, fibrosis, fat deposition, cell proliferation and degeneration of cells including karyolysis.

In some cases elastosis was remarkable around the elastolysis of internal elastic membrane, but in other cases the former develops in intimal thickening without any relationship with the latter.

No calcification was observed in all cases. There was no remarkable change in the media and adventitia.

Histochemically, acid mucopolysaccharide was observed in early atheromatous plaque. In general acid mucopolysaccharide was demonstrated in the deep layer of the atheroma especially in its margin. Amorphous substance with low density was observed electronmicroscopically as the ground substance in atheromatous plaque, and formation of collagenous fibres with periodicity was also observed in it. Intimal surface was covered with thin dense layer of presumably endothelial origin.

The findings of six months fed group did not differ essentially from those of three months. The most remarkable difference was the severity of the change of internal elastic layer. And in this group the elastosis and collagenosis of the intima were irregular in their fibre formation. Fat deposition was also more remarkable, but acid mucopolysaccharide seemed to be decreased.

b) Medium and small size arteries

All vascular lesions in one month fed groups (group II, III, V, VI, VII and XII) were found light microscopically and lesions were demonstrated mainly in branches of coronary arteries and in pulmonary arteries. No characteristic change for the group was recognized histopathologically.

Intima of small arteries in myocardium was most frequently affected. Intimal thickenings were mostly localized and there found the slight elastosis and collagenosis. Cell proliferation was scarcely accompanied and fat deposition was hardly recognized. Acid mucopolysaccharide was demonstrated histochemically in the intimal thickening. Con-

striction of the vascular lumen was not marked even in area of the thickening. Internal elastic membrane was almost intact except a few cases in which elastolysis was demonstrated. As to media, around the elastolysis of internal elastic membrane, irregularity in the course of elastic fibres was partly recognized. But in general, the media was almost intact with normal thickness. Adventitia was intact except one case with slight elastosis.

In one month fed group all the above mentioned findings were found in the small coronary arterial branches and no abnormality was found in the main stem.

In three months fed groups, lesions were observed not only in small branches but also in stem of coronary artery. Histopathological findings were not different in various groups of the experiment.

In the groups III, IV, V and XI marked intimal thickening, fat deposition, elastosis and collagenosis of the stem arteries were noticed. The abnormality of the branches of coronary artery was severe and vascular lumen was constricted or obliterated by the intimal thickening.

In most cases, fat deposition, elastosis and collagenosis in the intima were accompanied and the acid mucopolysaccharide was also proved histochemically. Internal elastic membrane was sometimes ruptured and this change accompanied the abnormal lining of the elastic fibres in media. But medial necrosis or atrophy was not demonstrated in any case. Elastosis of the adventitia was observed in some cases.

As to pulmonary artery, elastosis of the intima was the main finding and the vascular change was more severe than those of one month fed group.

c) Electronmicroscopic findings in small vessels especially in capillaries

It has been difficult to point out the abnormality of capillary by light microscopic examination and there has never been any report on the subject.

As the normal control the author examined the heart of ten rabbits of one month old with no experimental feeding. In this group, capillaries had endothelium with smooth surface and relatively uniform thickness and the density of endothelial cytoplasm had no irregularity. These capillaries were tightly surrounded with parenchyma of organ without too large pericapillary space.

In three months fed group, partial swellings of endothelial cells were observed and the cytoplasm was irregular in density. The difference of density was most evident at the junction of endothelial cells. Endoplasmic reticulum showed the abnormal distribution and deformity. Marked protuberance or processes often developed in endo-

thelial cells, and sometimes small lumens were formed secondarily with them. In subendothelial or perivascular space, amorphous substance and possible initial collagenosis appeared in three months fed group. Therefore, the connection between the vascular lumen and parenchym becomes rather loose in this case. After six months feeding, collagenosis also became evident in the pericapillary space.

DISCUSSION

Arteriosclerotic vascular changes were produced by experimental high lipid feeding with lanolin and cholesterol and by other various medications. Arteriosclerosis were demonstrated more frequently after prolonged feeding over three months with increasing severity. Atheroma consisting of marked collagenosis and elastosis was found in aorta with fat deposition. Moreover, acid mucopolysaccharide was recognized at the early stage of atheromatous change.

In three months feeding group, remarkable collagenosis and elastosis were found with some fat deposition in the intima of the aorta. After six months feeding fat deposition of the aorta was the main finding in some cases. Some atheromatous plaques were associated with marked cellular proliferations but others were not associated with it.

In medium and small size arteries, intimal thickening with collagenosis and elastosis were most frequent and severe especially with groups fed over three months. Remarkable fat deposition were also observed in some cases.

In one month feeding group, entire arteries including aorta, medium and small size arteries showed very slight fat deposition. Vascular lesions were demonstrated most frequently in the coronary vessels, especially in the small branches of coronary arteries of myocardium of left ventricle, then in the aorta and pulmonary arteries in the decreasing order, but they were scarcely seen in other organs. In the branches of coronary artery, arteriosclerotic changes were observed most frequently in the arterioles with 40 to 90 μ diameter.

For long time, many authors have been discussed about the relationship between the development of atherosclerosis and disturbances

of lipid metabolism of plasma. In early stage of the experiment adrenaline, digitalis, BaCl, toxins and other various agents were applied⁴⁾, but they were abandoned because of the effect to produce the Mönckeberg's sclerosis.

Since Ignatowski and Saltykow succeeded to produce the atherosclerosis of rabbits in 1908⁵⁾, general trend of the experiment turned to the production of atherosclerosis. Therefore, recent reports on the experimental arteriosclerosis were concerned with atherosclerosis produced by various methods, and many investigators had been paid attention only to the lipid of atheromatous plaque.

Because of these stand points, research techniques to prove the lipid have been mainly progressed. "Surface technique" also was established from this view by Duff and Ritchie⁶⁾. Indeed, Lautsch reported the deposition of lipid in intima only four hours after the start of cholesterol feeding.

In the current experiment about development of arteriosclerosis, morphological aspect has been almost neglected and in some extreme cases, studies on the blood chemistry alone have been reported as the study of arteriosclerosis itself.

The study on relationship between hormones and arteriosclerosis appears to represent one of these erroneous ideas. Katz, in his book titled "Experimental Atherosclerosis" in 1953, discussed about the pathogenesis of atherosclerosis with many references, but description on the morphological findings was very scant⁷⁾.

These facts suggest that many investigators prefer the definition of atherosclerosis by Mc Millan and Duff, namely "it is a pathological change, affecting the intima of arteries, that is characterized by focal thickenings of the intima in which stainable lipids can readily be demonstrated⁸⁾".

But as it was clearly shown in author's experiment, even by the lipid feeding experiment of rabbits, and even in the atheromatous plaque, the collagenosis, elastosis and their correlation to acid mucopolysaccharide were demonstrated. These elements such as collagen, elastin and acid mucopolysaccharide would

not be proved in serum.

According to the previous report of our laboratory, the development of arteriosclerosis has no distinct correlation with serum cholesterol level, cholesterol phospholipid ratio and lipoprotein index⁹⁾. Indeed, clinical and anatomical studies in men often fail to prove the direct correlation between serum lipid and arteriosclerosis.

Judging from these facts the author believes that it is a mistake to attach too much importance to lipid as the causative factor of arteriosclerosis.

Some investigators suggest the active metabolism of vascular wall¹⁰⁾. The author also believes the existence of metabolic disturbance in the wider sense, namely in lipid and other substances during development of arteriosclerosis in vascular wall.

Investigators who pay the attention to only the lipid in serum and in vascular wall, tacitly prefer the plasma inhibition theory as the pathogenesis of arteriosclerosis.

Duff thought that there are two factors, one to deposit lipid in and the other to remove it from intimal wall and that the fat deposition might occur by the unbalance of these factors⁸⁾.

Mackenzie states that fat in the intima is produced by the cells of intima¹¹⁾.

By the concept of "arterial wall as an organ", Russel, Holman and others say that the development of arteriosclerosis is an active metabolic process influenced by the hormones and by other factors¹²⁾. But their consideration was limited to lipid metabolism.

Some advances in the study of pathogenesis of arteriosclerosis were made by their concept of active metabolism of arterial wall. Recently, Shore and Zilversmit proved the phosphatide synthesis in the arterial wall by their experiment using radioactive isotope¹⁰⁾.

As it is evident by author's experiment, even in the atheroma produced by the lipid feeding many substances other than the lipid are detected.

These facts suggest that serum lipid is merely one of the many factors playing the causative role in the development of arterio-

sclerosis and that vascular wall undergo arteriosclerotic changes from its own metabolic abnormality not only in the lipid but also in the other substances. Namely, arteriosclerosis develops by the integration of disturbances of active metabolism of arterial wall in broad sense.

From this stand point, it is meaningless to discuss whether the fibrosis or fat deposition is the primary or secondary change in the development of arteriosclerosis¹³⁾.

Up to date, plasma inhibition, anoxemia, toxin, lipid, diet and hormones and so on have been described as the pathogenesis of arteriosclerosis, but the pathogenesis could not be explained completely by one of them alone. And those investigators who proposed their theories from these factors have been interpreted the vascular wall as only passive one.

Hemodynamic factor must be emphasized concerning the localization of the development of arteriosclerosis. The both factors of tension and elasticity of vascular wall and flow pressure ought to have a equilibrium each other, but in fact, its diameter and tension and flow pressure do not always have parallel relationship.

Therefore, the equilibrium between blood flow and tension of vascular wall is often broken¹⁴⁾. Hass and Williams recognized the development of elastic fibre by the functional demand of vascular wall¹⁵⁾. Hemodynamic influence may be most remarkable in the area where the flow pressure changes greatly.

Up to date, electronmicroscopical studies on the vascular wall has been done¹⁶⁾ but there has been no report about the findings of capillaries in the cases with arteriosclerosis in larger vessels. The author found such changes of the capillaries electronmicroscopically as marked deformation of endothelial cells, unequal density of the cytoplasm, abnormalities of endoplasmic reticulum including deformity, abnormal distribution and changes in size, deposition of amorphous substance and collagenosis in the pericapillary space.

Up to date, atheroma, Mönckeberg's sclerosis, arteriosclerosis and arteriolosclerosis have been only referred as the sclerotic vascular

change. As it is evident by author's experiment, a new concept of "capillarosclerosis" must be established.

In general, arteriosclerosis is supposed to accompany metabolic disorder of organs and decreased blood supply to the organs has been considered as the main cause of it. But measurement of cerebral hemodynamics in our laboratory revealed the fact that the decrease of blood flow and cerebral oxygen consumption do not always show the parallel relationship¹⁷⁾.

Of course, dysfunction in cells of each organ must be considered but even when the cells have normal activity, abnormality of capillary may directly cause the metabolic disorder of organs. Because of these facts, abnormality of the capillary should not be disregarded in the study of the arteriosclerosis in future.

SUMMARY

1) Arteriosclerotic vascular changes were produced by experimental high lipid feeding and by various methods.

2) Frequency and degree of vascular change increased remarkably by the prolonged experimental feeding over three months.

3) Main histopathological findings in the vascular wall were collagenosis, elastosis, increase of mucopolysaccharide and fat deposition in the intima. But the fat deposition was not considered to be the cardinal component of the vascular change.

4) The importance of the role of active metabolic disturbance of vascular wall in the development of arteriosclerosis was suggested.

5) Local-difference and organ-difference were recognized in regard to the frequency and degree of the vascular change. This suggests the influence of hemodynamics to the metabolism of vascular wall.

6) By electronmicroscopic examination, various changes were observed in capillaries. These findings may be referred as "capillarosclerosis".

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EXPLANATION OF PLATES

(1)-(12): Light-micrograph

- 1) A medial elastolysis and a slight intimal thickening from the aorta of one month fed rabbit. Weigert stain
- 2) A marked intimal thickening and hyalinoid degeneration of the aorta of three months lanolin fed rabbit. Hematoxylin-Eosin stain
- 3) Elastosis of the intima in the same artery. Weigert stain
- 4) Fibrosis of the intima in the same artery. Mallory stain
- 5) Acid-mucopolysaccharide stained in atheroma of the aorta. Rinehart method
- 6) The lipid deposition in an atherom. Oil red O stain

- 7) Thickened intima of a small coronary artery of rabbit which received three months lanolin feeding, constriction of renal artery and allylamine injection. Hematoxylin-Eosin stain
- 8) Fibrosis of the intima in the same artery. Mallory stain
- 9) Elastosis of the intima of the same artery. Weigert stain
- 10) The polysaccharide deposition in the intima. Periodic acid Schiff's method
- 11) Extensive lipid deposition of intima in small coronary artery. Oil red O stain
- 12) Marked elastosis of the intima in pulmonary artery of three months fed rabbit. Weigert stain

(13)-(24): Electron-micrograph

- 13) An endothelial cap with dense cytoplasm. This section was taken from an aorta of the three months fed rabbit.
- 14) An endothelial cell of the atheroma containing a large ovoid vacuole.
- 15) The deposition of amorphous substance and early collagenosis in the deep layer of the atheroma. Initial elastic lesions are also observed.
- 16) Collagenosis in the atheroma. The periodicity is seen in fibres.
- 17) A cross section through a normal rabbit capillary in heart muscle.
- 18) A longitudinal section of a normal rabbit capillary.
- 19) The thickening of endothelial cells in three months lanolin fed rabbit.
- 20) The illustration of the different density of cytoplasm at a conjunction of two endothelial cells.
- 21) Amorphous substance with low density and initial collagenosis in pericapillary space. The density of cytoplasm of endothelial cell is increased.
- 22) The ciliary "processus" formation of endothelium.
- 23) Plasma-like dense material immediately beneath the endothelium. The processus formations are also observed.
- 24) A view of collagenosis in pericapillary space of six months fed rabbit.