Ultrastructure of Arterial Spasms as Related to Atherosclerosis and Hyperlipidemia

Masahiro ITO

Department of Pathology, Atomic Disease Institute,
Nagasaki University School of Medicine
Nagasaki, Japan

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Morphological changes induced in contracted arteries by repeated electrical stimulation with a 3.5 volt pulse wave were examined by light and electron microscopy. The effects of mild hypercholesterolemia on contracted arteries were also investigated. Renal or femoral arteries from rabbits were stimulated over periods of time ranging from 15 minutes to 14 days. A total of 46 animals were examined. They were divided in the following three groups: (1) Electrical stimulation. (2) Electrical stimulation with a cholesterol load. (3) Control including a sham operation.

In the electrical stimulation group, scanning electron microscopic examination revealed various endothelial changes such as irregularities in cell orientation, adherence of leukocytes and platelets, swelling of the marginal fold and the appearance of microvillied cells. The incidence and degree of these changes increased as the stimulation time increased. Occasionally, characteristic cytoplasmic elongations of the endothelial cells were observed. However, obvious endothelial denudation was not observed. Transmission electron microscopy revealed junctional damages and activated pynocytosis in the endothelial cells. The numbers of vacuoles observed in the medial smooth muscle cells increased rapidly after only a short period of stimulation. Medial necrosis was observed in only two cases. Fibromuscular intimal thickening, up to 3 cell layers, had formed by the 14th day of stimulation.

In the group receiving both electrical stimulation and cholesterol administration, endothelial changes were generally more intense than in the former group. Emigration of monocytes through the endothelium was frequently observed. Endothelial denudation was not detected in this group either. The mean serum cholesterol value for this group was 288 mg/dl just before electrical stimulation was started. Foam cells, which were often accompanied by plasma insudation, appeared in the subendothelial spaces within the first 3 days of stimulation. Morphologically, these foam cells appeared to have originated from monocytes. Early stages of atherosclerosis were obtained within 7 to 14 days of treatment.

No significant intimal thickening or foam cells were detected in the control and sham operated animals.

These results suggested that endothelial changes, especially junctional damages, may be directly involved in the production of atherosclerotic lesions during angiospasm, whereas endothelial denudation might not be necessary for lesions to occur. In addition, mild hyperlipidemia enhanced the development of atherosclerosis contracted arteries, but did not appear to be an initiating factor.
Since 1957, when BERNARD\textsuperscript{2} first proposed his theory implicating angiospasms as one of the initiating factors of atherosclerosis, many experiments dealing with angiospasms have been conducted.\textsuperscript{11,13,14,19,35,37} The relationship between angiospasms and diseases, such as ischemic heart disease, RAYNAUD's disease, hypertensive encephalopathy, primary pulmonary hypertension and stress induced ulcers are clinically important. The pathogenesis of spasms has been clarified, and certain cations, such as $\text{Ca}^{2+}$, $\text{K}^+$ and $\text{Mg}^{2+}$, apparently participate in this process. These cations are important factors in the regulation of membrane stability. However, detailed mechanisms of this process on the molecular level are still unclear. Naturally, reproducing physiological spasms in experimental model in vivo may present inevitable methodological problems. SEMBA et al.\textsuperscript{29} have provided detailed descriptions of the physiology of vessel contractions including information on the vasomotor nerves and stimulation sites. In order to obtain an arterial contraction as physiologically close to the natural process as possible, we employed a low voltage electrical stimulation method.

Many reports have been published which indicate that experimentally induced spasms promote the development of atherosclerosis, and certain morphological changes appear to be specific to angiospasms. However, very few scanning ultrastructural studies\textsuperscript{19,21} of contracted arteries are presently available and to date, no studies on the effects of hyperlipidemia on contracted arteries are available even though hypercholesterolemia is the most common risk factor associated with atherosclerosis today.

Three major parameters were examined in this study mainly by scanning and transmission electron microscopy: They included severity of endothelial damage, medial change, especially vacuolar change and angionecrosis, and the effects of cholesterol administration on contracted arteries.

**MATERIALS AND METHODS**

**Animals and Diet:**

A total of 46 albino rabbits of the Japanese white race, 2 to 3 months of age and weighing about 2.5 kg, were used in these experiments. Animals were selected irrespective of sex, housed in individual cages and fed a regular commercial ration for rabbits. The renal artery and peripheral femoral artery, which are muscular type arteries, were selected as stimulation sites. The experimental animals were randomly divided into three groups as follows: Group 1 was subjected only to electrical stimulation of the renal or femoral artery. Group 2 received a 1\% cholesterol diet for 3 weeks after which the femoral artery was subjected to electrical stimulation. The cholesterol diet was fed continuously until the time of sacrifice. An additional five rabbits served as the control group.

**Experimental Methods:**
Animals were anesthetized with ether and ketamine chloride and retroperitoneal or inguinal incisions were applied to expose either the left renal or femoral arteries respectively. Stimulation was administered by planting bipolar silver electrodes, measuring 0.3 mm in diameter, in the outer wall of the above arteries while taking care not to cause mechanical stenosis. The electrodes were then connected to a NIHON KODEN Co. Electrical Stimulator MSE 20 unit via a subcutaneous, vinyl-covered lead wire. An intermittent current (30 seconds of stimulation followed by a one minute pause) was administered using a duration 5 msec, 20 Hz, and 3.5 volt pulse wave. Stimulation of the femoral arteries was performed only on the left side while a sham operation was performed on the right side of each animal. The controls for the renal artery group, which consisted of separate animal, received a sham operation. The operations were conducted as aseptically as possible, and a dose of 50 mg/kg of body weight of synthesized penicillin was injected intramuscularly into each animal. Stimulation times were 15 minutes, 1 hour, 3 hours and 6 hours in the short period group, and 2 to 14 days with 6 hours of stimulation per day in the long period group. The stimulation time for each group and the number of experiments are shown in Table 1. In a preliminary study, sufficient contraction in both the renal and femoral artery was confirmed by surgical microscopy.

**Blood Sampling:**

Blood samples were collected from the animals fed 1% cholesterol one day before cholesterol feeding began, 3 weeks after cholesterol feeding began, and at the time of sacrifice. The serum cholesterol and triglyceride levels were measured and shown in Table 2.

**Fixation and Sampling for Morphological Studies:**

After completion of the experiment, all animals were sacrificed by a rapid injection of sodium pentobarbital into the ear vein. Perfusion fixation was conducted immediately with 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) through the thoracic aorta for approximately 20 minutes. The perfusion pressure was maintained at 110-120 mmHg after cardiac arrest. Specimens were obtained 0.5 to 1.0 cm from the electrodes on the peripheral side of the arteries in order to avoid areas of granulation directly adjacent to the electrodes and the direct effects of electricity. The arterial segments were then cross-sectioned into three specimens and fixed in the 3% glutaraldehyde solution at least 24 hours. One segment was used for scanning electron microscopy. The other two were prepared for transmission electron microscopy.

**Light and Transmission Electron Microscopy (TEM):**

For TEM, samples were post fixed in 1% OsO_4 for one hour, serially dehydrated in ethanol and embedded in epoxy resin. The embedded specimens were then sectioned on an ultra-microtome. Thick sections, which were stained with alkaline toluidine blue, were observed with a light microscopy allowing the orientation of the arteries to be determined. The thick sections of the renal arteries were used for observing and counting the number of vacuoles in the medial smooth muscle cells. The number of vacuoles in each section was counted at a high power of magnification (×400) with a light micro-
Table 1. The Number of Experimental Group

<table>
<thead>
<tr>
<th>Group 1. Electrical Stimulation (n=28)</th>
<th>Group 2. Electrical Stimulation with Cholesterol Load (n=13)</th>
<th>Control (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Renal Artery (n=21)</td>
<td>Femoral Artery</td>
<td>d ; 6 hours stimulation per day</td>
</tr>
<tr>
<td>15min 1 hr 3 hr 2 d 3 d 5 d 6 d 7 d</td>
<td>15min 3 hr 1 d 3 d 7 d 9 d 14 d</td>
<td>0 1 d 3 d 7 d 10 d 14 d</td>
</tr>
<tr>
<td>2 3 4 1 3 (1) 1 1 2 (3)</td>
<td>1 1 1 1 1 1 1</td>
<td>1</td>
</tr>
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<td>( ) ; the number of sham operation</td>
<td></td>
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<tr>
<td>b. Femoral Artery (n=7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15min 3 hr 1 d 3 d 7 d 9 d 14 d</td>
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<td>1 1 1 1 1 1 1</td>
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Group 2. Electrical Stimulation with Cholesterol Load (n=13)

<table>
<thead>
<tr>
<th>Femoral Artery</th>
<th></th>
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<tbody>
<tr>
<td>0 1 d 3 d 7 d 10 d 14 d</td>
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<tr>
<td>1 3 2 3 2 2</td>
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</tbody>
</table>

Control (n=5)

d ; 6 hours stimulation per day

Table 2. Serum Lipid Levels of Group 2.

<table>
<thead>
<tr>
<th></th>
<th>unloaded</th>
<th>3 weeks</th>
<th>at sacrifice</th>
</tr>
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<tbody>
<tr>
<td>Total cholesterol</td>
<td>65±15</td>
<td>288±145*</td>
<td>406±255*</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>122±52</td>
<td>95±40</td>
<td>155±133</td>
</tr>
</tbody>
</table>

* p <0.001 n=13 mean ±SD (mg/dl)

scope. (Fig. 1) The ultra-thin sections were stained with uranyl acetate and lead citrate and then observed with TEM 7A and TEM 100B electron microscopes.

Scanning Electron Microscopy (SEM):

The vessel segments, which were opened longitudinally, were immersed in a solution of 2% tannic acid in glutaraldehyde for 4 hours and then fixed in 1% OsO₄ for 4 hours. Tissues were subsequently dehydrated in solutions of 50%, 70%, 80%, 90% and 100% ethanol in water. After dehydration, the specimens were rinsed in solutions 25%, 50%, 75% and 100% iso-amyl acetate in ethanol and then subjected to critical-point drying with carbon dioxide. The tissues were then mounted on brass stubs, coated with a layer of gold 300 Å thick and examined with a JEOL JSM 35 C scanning electron microscope at 15 KV.
Fig. 1. Vacuoles in the medial smooth muscle cells of a renal artery from Group 1. ○ indicates the mean value of the number of vacuoles per section.

Effects of Electrical Stimulation on Arteries:

Arteries that were stimulated for 30 seconds with a one minute pause produced sufficient reactions against each intermittent stimulation. The areas immediately surrounding the electrodes exhibited marked contractions upon stimulation, especially in the short period groups. The regions in which the electrodes were embedded became granulated after 3 to 5 days of stimulation. In spite of the granulation, contractions were still visible macroscopically, but the intensity of the reactions decreased. After 7 to 14 days of stimulation, the reactions became so weak that contractions could not be observed macroscopically.

RESULTS

Control Animals:

TEM examination of both the renal and femoral arteries from the control rabbits indicated that internal elastic lamina formed a gentle sine curve directly above the area in which flat endothelial cells were arranged. Myointimal cells could also be observed attimes in the subendothelial space. The tunica media comprised of compact smooth muscle cells. SEM examination revealed longitudinal folds running regularly between deep grooves on the surfaces of the vessels. The endothelial cells were arranged in neat rows parallel to the blood stream. Marginal folds with lateral stripes at their crests took on a cobblestone appearance on the surfaces of the endothelial cells. Endothelial cells
with microvilli on their surfaces were sometimes observed running vertically in the
grooves between the longitudinal folds.

Group 1, Electrica Stimulation:
a. Endothelial change

A stimulation period of 15 minutes produced no noticeable changes, but after 1-
3 hours of stimulation, SEM examination revealed a decrease in the width of the endo-
thalial folds while the grooves had become more shallow. Mild torsions and disturbances
in the arrangements of the endothelial cells were also noted. Platelet attachment and crater
formation were observed on their surfaces (Fig. 2). TEM examination revealed edematous
swelling in the subendothelial spaces, and monocytes were sometimes encountered. This
edematous swelling appeared more rapidly in the intimal which comprised of myointimal
cells. These cells stood up vertically and their cytoplasms invaginated. When the stimula-
tion time increased to 6 hours, the folds became even more irregular and at times com-
pletely disappeared. Crater formation was evident on the surface of the endothelial cells.
Numerous leukocytes and plateles became attached to the cell surfaces and degeneration
and partial disappearance of the endothelial cells were noticed (Fig. 3). But, evident
denucleation had not occurred. After 2 to 3 days of stimulation, swelling of the marginal
folds evident in addition to increased disturbances in the endothelial cell arrangements;
microvilllied cells proliferated in and around the grooves (Fig. 4). TEM examination

Fig. 2. Surface view of the endothelium of a contracted renal artery after 1 hour of
electrical stimulation.
The surface has a rippled appearance. Craters (arrows) have formed on the
top of the endothelial cell. Platelets (PL) are adhered to the endothelial cells.
Bar=10μm
Fig. 3. Femoral artery after 6 hours of electrical stimulation. Many leukocytes (L) are adhered to the endothelial cell, some of which extend into the endothelium. The longitudinal folds are irregular. Bar=10μm

Fig. 4. Renal artery after 3 days of stimulation. The longitudinal folds are often obscure. Endothelial cells with short microvilli (arrows) appeared during this period. The cell in the upper half of this micrograph is distorted. Bar=10μm
showed the arteries to be in a contracted state, with the endothelial cells rounded and protruding in such a way as to form valleys between themselves and neighboring endothelial cells. Smooth muscle cells were observed penetrating the fenestra of the internal elastic lamina from the media. After 7 to 14 days of stimulation, changes in the endothelial cells became varied and intense. Cellular integrity, however, was still maintained. The marginal folds were generally swollen and TEM examination revealed lumen formation in the cytoplasmic flaps, complications in the junctional areas and organelle growth in the dilation of the gap junctions (Fig. 5). At this point, intimal thickening of 2 to 3 cell layers occurred (Fig. 6), accompanied by expansion of the interstitial spaces and a proliferation of modified smooth muscle cells. The internal elastic lamina was occasionally interrupted (Fig. 7).

b. Medial changes

Changes were most commonly observed in the inner media. Damage was pronounced in the smooth muscle cells lying just beneath the internal elastic lamina. Ghost cells, cell debris, and degenerated cells were occasionally present and their numbers and degree of damage increased as the stimulation period increased. Careful examination revealed fibrin-like material deposits just beneath the internal elastic lamina (Fig. 8). The degenerated smooth cells displayed various organelle changes, such as swelling of the mitochondria, dilation of the rough endoplasmic reticulum, the appearance of myelin figures and destruction of the limiting membranes. Such degenerated cells were frequently observed after 2

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Fig. 5. Junctional damage is pronounced in this renal artery after 7 days of stimulation. A large lumen has formed from a cytoplasmic flap and the gap junctions are distended (arrowheads). Part of a modified smooth muscle cell displays marked dilated of the rough endoplasmic reticulum. $\times 22,500$. 
Fig. 6. Early sclerotic lesion in a renal artery stimulated for 7 days. Slight fibromuscular intimal thickening is apparent. Note the two monocytes emigrating into the endothelium and a medial smooth muscle cell penetrating the fenestra. IEL: internal elastic lamina, M: monocyte. × 3,750

Fig. 7. Interruptions in the internal elastic lamina of a renal artery after 7 days of stimulation. × 330.
to 5 days of stimulation. Medial necrosis was another unmistakable finding. Severe medial necrosis was detected in only 2 rabbits stimulated for 1 to 3 hours (Fig. 9). These necrotic cells exhibited pyknotic nuclei, rarefied cytoplasms, and a disarray of myofibrils. After 7 to 14 days of stimulation, the volume of extracellular components including collagen fibers, cell debris and ground substances had increased considerably in the inner media.

c. Medial vacuoles

Translucent vacuoles, usually oval or circular in shape, were found in the media even in the control animals (Fig. 10). These vacuoles varied in size and were usually present in the smooth muscle cells located in the inner and outer media. The contents of the vacuoles, which were usually more electron lucent than the surrounding cytoplasm, included myelin figures, mitochondria, ribosomes, and remnants of cellular organelles. The limiting membranes of all the vacuoles were 2 layers thick as observed by an electron microscope. The numbers of vacuoles increased dramatically after a short period of stimulation and then decreased again gradually to the basal level by the 7th day. There were fewer vacuoles in the femoral arteries than in the renal arteries.

Group 2, Electrical Stimulation Combined with Cholesterol Feeding

a. Endothelial change

The serum cholesterol levels of rabbits that were fed a 1% cholesterol diet for 3 weeks were slightly elevated averaging 288 mg/dl. Generally, endothelial cell changes in

![Fig. 8](image-url)
Fig. 9. Angionecrosis in a renal artery after 3 hours of electrical stimulation. Remarkable smooth muscle cell degeneration has occurred. These degenerated cells exhibit condensed or rarefied cytoplasm with condensed nuclei. Some have vacuoles. Note the aggregation and disarray of the myofilaments. $\times4,250$

Fig. 10. Medial vacuole in the renal artery. This smooth muscle cell contains an electron lucent vacuole in the cytoplasm adjacent to the nucleus. A portion of the vacuole has a double limiting membrane. $\times8,250$
the cholesterol fed group were more intense than in the group that was electrically stimulated only. SEM examination revealed swelling of the marginal folds, the presence of microvillied cells, and cells with long cytoplasmic processes projecting from their margins (Fig. 11, 12). The frequency and severity of these changes increased as the duration of the electrical stimulation increased. Although, no foam cells were found in the intimal after 6 hours of stimulation. TEM examination revealed smooth muscle cell penetration of the media in the slightly thickened intimal. Ingested materials and a lipid droplet containing macrophage were observed in the cell body after 3 days of stimulation. Insudation of the subendothelial space was occasionally noted (Fig. 13). Macrophages were identified by their characteristic nuclear chromatin and pseudopod processes (Fig. 14). After 7 to 14 days of stimulation, intimal thickening consisting of 3 to 4 cell layers, was formed (Fig. 15). These lesions comprised of lipid containing cells, ground substances, and smooth muscle cells which occasionally contained lysosomal bodies (Fig. 16). Monocytes were also observed emigrating through the endothelial cells. However, obvious endothelial denudation was not detected.

b. Medial changes

Basically, medial changes in this group were similar to those of Group 1, except for lipid deposition. These changes were also evident beneath the internal elastic lamina. Occasionally, neutrophils and foam cells, which originated from smooth muscle cells, were present. (Fig. 17).

There was very little difference between changes in the renal and femoral arteries of the experimental animals, except that the changes occurred earlier in the former.

Fig. 11. Swollen marginal folds in a femoral artery from a rabbit fed 1% cholesterol and electrically stimulated for 7 days. Swelling and distortion of the marginal folds (arrowheads) are apparent in the endothelial cells. Bar =10μm
Fig. 12. Femoral artery from a rabbit fed cholesterol and electrically stimulated for 14 days. Endothelial cells with radially extended cytoplasmic processes (arrows) appear in the upper half of this micrograph. The junctions between these cells are open. 

Bar = 10 μm

Fig. 13. This subendothelial insudate (arrow) appeared after 3 days of electrical stimulation combined with cholesterol feeding. A lipid-laden macrophage (M) is present among the insudate. 

×7,200
Fig. 14. Two lipid-laden macrophages are identifiable by the lack of a basement membrane, the presence of well developed ruffles (arrow), secondary lysosomes and a characteristic distribution of chromatin. M: macrophage. IEL: internal elastic lamina. ×12,500

Fig. 15. Early stage of an atherosclerotic lesion in a femoral artery after 7 days of stimulation combined with cholesterol administration. Lipid droplets are present in the endothelial cells, smooth muscle cells, and monocytes. M: monocyte. IEL: internal elastic lamina. ×5,000
Fig. 16. Proliferated smooth muscle cells from the femoral artery of a rabbit that received the combination electrical stimulation and cholesterol diet for 14 days. Some of the intimal smooth muscle cells contain lipid droplets. Note a smooth muscle cell containing numerous secondary lysosomes. ×5,100

Fig. 17. A foam cell, which originated from a medial smooth muscle cell, is present in the upper media of a femoral artery after 14 days of electrical stimulation combined with cholesterol administration. The presence of dense bodies and a basement membrane identify this cell as a smooth muscle cell. ×4,250
Sham operations:

In the arteries of the sham operated animals in both the electrical stimulation and the cholesterol supplemented group, no foam cells or lipid droplets were noticed. Except in a few special cases, nothing more than mild intimal thickening resulted from granulation, even after 7 to 14 days of stimulation. The endothelial cells also remained in a virtually normal state, leading us to believe that the effects of granulation can be excluded from consideration.

DISCUSSION

Angiospasms may well be responsible, at least in part, for certain vascular diseases such as ischemic heart disease, cerebrovascular attacks, and Raynaud’s disease. Angiospasms have been extensively studied, but most of these studies employed methods of drug administration or arterial ligation. When drugs are used to induce angiospasms, there is a tendency to administer excessive doses, so the effects of the drugs themselves cannot be ignored. A major criticism of these studies is that, in general, the doses of vasoactive substances administered have been shown to produce peak values higher than those which are known to occur spontaneously. Ligation methods are disadvantageous because the elasticity of the arterial wall is lost. The present study avoided such problems by using a low voltage electrical stimulation method in order to obtain arterial contractions as close to the natural form as possible.

By using the electrical stimulation method, TAKEBAYASHI and HARANO confirmed through light microscopic observation, the induction of intimal thickening and fibrinoid necrosis in the media of the gastric, renal, and cerebral arteries. KAMIO observed, by transmission electron microscopy, focal cytoplasmic necrosis and the "crush-up" effect in the medial smooth muscle cells of the gastric artery.

The present study was conducted in order to determine the types of changes that occur in endothelial cells and in the media as a result of repeated arterial contractions over a period of time, what role angiospasms play in the occurrence of atherosclerosis, and also to examine the effects of hyperlipidemia on angiospasms.

The arterial endothelium functions as a selective permeable barrier and provides a non-thrombogenic surface. Endothelial cells are vital in the production of substances like plasminogen activator, prostacycline (PGI₂), Factor VIII, platelet inhibiting substances, etc. Endothelial cells are easily altered by chemical and mechanical manipulation. Most researchers agree that endothelial cell damage is directly involved with the occurrence and development of vascular disease complicated or induced by spasms. ROSS, et al. postulated the "response to injury" hypothesis, which originally suggested that smooth muscle proliferation was promoted by a platelet-derived growth factor (PDGF) which was released by platelets when they adhered to exposed subendothelial connective tissue in regions of endothelial denudation. There is no evidence, however, that in vivo, denudation of the magnitude included by balloon catheterization, ever occurs.
It is now an accepted fact that intimal thickening can occur without endothelial denudation and that other factors, such as monocytes and lipoproteins, are also important in the formation of fibroproliferative lesions. It is also apparent that even a high turnover rate of endothelial cells is not necessarily associated with denudation. Endothelial cell turnover is generally low (10⁻³ cell/day), but a high turnover rate occurs around the bifurcations of the intercostal arteries in the aorta. These regions develop atherosclerosis more frequently and severely than other regions of the aorta, suggesting that increased endothelial cell turnover accelerates the development of atherosclerosis. However, histological examination of such areas has shown the endothelium to be intact, suggesting that turn over is not necessarily associated with desquamation. The cells with elongated cytoplasmic processes that were observed in this study might be involved in cell turnover.

Joris et al. observed that mononuclear cell adhesion occurs on endothelial cells and not on denuded areas in hyperlipidemic rats. They suggest that atherosclerotic plaques may be initiated by mononuclear cell adhesion and emigration. They also criticized the "response to injury" theory. Walker et al. showed that confined injury to the endothelium failed to induce intimal thickening in normocholesterol rabbits. Intimal proliferation was observed only when the media of the vessel was also damaged.

Researchers, in most of the previous studies concerning arteria spasms, have reported occurrence of broad and severe endothelial denudation during the spasms. However, in this study, obvious endothelial denudation was never observed. This discrepancy is apparently due to the methods used to produce spasms. It is likely that most of these previous experiments resulted in the production of spasms which could not physiologically and spontaneously occur. It is concluded therefore that naturally occurring spasms might not necessarily cause endothelial denudation and endothelial denudation is not required for the initiation of atherosclerosis.

Some of the endothelial damage observed in this study may have resulted in functional changes as well. Plasma insudation and fibrin-like material deposition in the endothelium probably increased its permeability. The swelling of the marginal folds reflected an opening or a breakdown of the junctional areas, which was confirmed by TEM. It is assumed that passive contraction and overextension caused by medial smooth muscle contraction give rise to junctional damage. In addition, the possible influence of hemodynamic stress and hypoxia should be considered in this study.

Microvilliated cells (blebbing cells) were first described by Smith et al. in the pulmonary artery, and their existence became evident later in numerous other anatomical regions in many experimental species. These cells are perceived to be highly active with accelerated vesicular transport systems that possess numerous swollen pinocytic vesicles in their small cytoplasmic processes.

The cells with the elongated cytoplasmic processes represent yet another characteristic endothelial change, possibly resulting in an active transport system. Mori and Sekimoto also observed the formation of cells with elongated cytoplasmic processes which they suspected to be free endothelial cells originating either from the blood or from mitosis.
Their relationship to spasms however, is still unclear.

The source of the vacuoles which appear in the media during spasms is still a controversial subject. They may or may not be the results of smooth muscle cell contraction. Investigators who have studied the pathogenesis of the vacuole use terms such as evagination, protrusion, intrusion, and herniation to explain their formation. One of the most important morphological findings in the investigation of arterial spasms is that these vacuoles have a double limiting membrane. Vacuoles with only a single limiting membrane are generally the results of poor fixation during the tissue preparation process. TAKEUCHI postulated that the peri-nuclear vacuoles that appear in the media smooth muscle cells of the renal artery may serve as a morphological index of arterial contraction. Some investigators suggest that these vacuoles are simply a result of cell necrosis. The number and type of vacuoles induced by spasms in the present study were similar to those produced by a single administration of nor-epinephrine in another study. Another observation of the present study was that the number of vacuoles produced was directly proportional to the magnitude of arterial contraction as confirmed by surgical microscopy. These findings suggest that vacuole formation is highly correlated with arterial contraction.

Previous studies have concluded that angionecrosis occurs during spasms at various rates, and it has been identified in several cases of the Möncheberg type atherosclerosis. In the present study, only two cases out of 46 displayed medial necrosis. This low rate of medial necrosis suggests that it does not play an important role in the initiation of atherosclerosis during spasms.

Hypercholesterolemia is the most common risk factor associated with atherosclerosis in modern society. It has long been known that high levels of LDL are associated with the acceleration of atherosclerosis, both in experimental animals and in humans. Many different animal species and experimental designs have been used to study diet-induced atherosclerosis. In this study, mild hypercholesterolemia, i.e., cholesterol levels falling well within the range of spontaneous or physiologically normal values, was induced. The effects of cholesterol on angiospasms varies across studies. MOHRI et al. reported that the neurogenic contracting mechanism in the arterial wall breaks down as cholesterol levels increase whereas TOMOIKE observed that high levels of cholesterol increased the response of ergonovine to desquamated sites in the coronary artery over time. SHIMOKAWA et al. recently reported that experimentally induced atherosclerotic lesions in the coronary arteries of swine constrict more extensively with the administration of histamines than do nonatherosclerotic lesions. They then postulated that coronary atherosclerosis is probably the primary factor involved in the pathogenesis of coronary arterial spasms. However, several cases of cardiac infarction induced by spasms without the presence of atherosclerotic lesions were reported. The present study suggests that hyperlipidemia and arterial spasms may interact additively, creating a vicious cycle in the artery.

Focal interstitial intimal serofibrinous insudation was found concomitant with both
early subendothelial accumulations, accumulations of macrophages and alterations in the surface contours of the endothelium. The insudate appeared to be a mixture of plasma components and/or their products. However, whether the insudate was due to the loss of selective permeability, to active transport of plasma components, or to a combination of these, is not clear. Monocytes\textsuperscript{7)}\textsuperscript{8)} were the major foam cell precursors in early atherosclerotic lesions in this study.

Duration of arterial spasms is another critical point to be considered. Angiospasms rarely lasted over two weeks in studies of humans and experimental animals.\textsuperscript{14)}\textsuperscript{37)} Preliminary studies concerning regression\textsuperscript{43)} are presently being investigated by the author.

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