169. The Endothelial Cell Damages of Pre-atheromatous and Atheromatous Lesions Observed by Scanning Electron Microscope

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The injury of endothelial cells of arteries had been traditionally and erroneously believed to be rapidly cured until Efskind (1941) and Poole, Sanders and Florey (1958) showed the unexpectedly poor repair process of endothelial damages given mechanically in rabbit aorta. The authors found the acute changes of endothelial cells of arteries occurring immediately after giving atherogenic substances to animals and the injury of the endothelial cells under the hyperlipemia has been described by a number of investigators by transmission electron microscope. However, using transmission electron microscopic method it is technically almost impossible to see the detail of the surface of each endothelial cells. In this experiment with scanning electron microscope, the changes of the endothelial surface of rabbit aorta induced by the long term treatment of animals with cholesterol diet have been observed and the results have been subjected to the present publication.

Materials and methods. Two male rabbits weighing 2.1 and 2.3 kg were used. They had been kept on cholesterol diet (1% cholesterol pellets, Oriental Co., Ltd.) for two weeks. The serum cholesterol level were 200 mg/100 ml and 240 mg/100 ml respectively. The other 4 male rabbits weighing 2.8, 2.9, 3.1, and 3.4 kg were used. They had been kept on the cholesterol diet for 15 weeks. The serum cholesterol level were 1600, 1400, 1700, and 1820 mg/100 ml respectively. Three animals of the latter group were killed by stunning blow, but the remaining animals were killed by intravenous injection of 1% EDTA-solution for the purpose to avoid the deposition of fibrin during the sampling of aortic specimens. The specimens of the aortas were obtained immediately and they were carefully washed by physiologic saline added EDTA in 0.1% and cut into small blocks 3 mm×10 mm in size. The blocks were fixed by 1% osmium tetroxide and dehydrated and then they were studied with a scanning electron microscope (JSM-II) as detailed in elsewhere.

Results. The gross observation of aortas of animals received 2 weeks of cholesterol feeding remained almost normal.
However, the scanning electron microscopic observation revealed the swelling and some irregularity of the endothelial folds\(^2\)-\(^4\) accompanied by moderately swollen intercellular bridges.\(^2\)-\(^4\) The most important finding is the appearance of spotty areas showing a remarkable change of the endothelial surface in the well-known predisposed parts to atheromatous changes of the aorta. The change is characterized by a strong irregularity of endothelial folds and sometimes with deposition of fibrin-like material, platelets, and leukocytes on some of the surface of swollen or deformed endothelial cells as shown in photos. 1 and 2. There were many remarkably swollen, splitted and deformed intercellular long bridges and short bridges and often the almost all intercellular long bridges\(^4\) and short bridges\(^4\) were broken and destroyed as shown in photo. 3.

Four animals, received 15 weeks of cholesterol feeding, exhibited fatty streaks in 65 to 80 percent surface of the whole aortas.

In scanning electron microscopic observation of elevated fatty streaks the endothelial cells show a balloon shape without recognizable intercellular bridges as shown in photo. 4. Even in the relatively unelevated lesions the destruction and disappearance of endothelial folds and the presence of extremely swollen or splitted or highly deformed or destructed intercellular bridges were characteristic. There were often found old clots, fibrin-like material as well as formed elements sticking to the rough surface of affected endothelial surface as shown in photo. 5. In the relatively unaffected area the lineal endothelial folds are well preserved, but they were irregular and the intercellular bridges exhibited various changes. In the grossly normal-looking surface there was often found a spotty or streaky area with highly degenerated endothelial cells and almost perfectly destroyed intercellular bridges. Such lesions are surrounded by relatively unaffected areas and the boundary is relatively sharp as shown in photo. 6.

Discussion. The most important finding is the early and steadily progressing damages of focal endothelial cells under cholesterol feeding seen in this experiment. The appearance of grossly visible fatty spots or streaks starts at least 6 to 10 weeks of cholesterol feeding in our condition and no animal received 2 weeks of cholesterol feeding has ever shown grossly visible changes, so that the spotty appearance of areas with severely damaged endothelial cells accompanied by irreversibly destroyed intercellular bridges is the most important fact and such areas were found in the predisposed parts to atheromatous changes in the aorta of animals received 2 weeks of cholesterol feeding. Such damages of intercellular bridges are accelerated and produced by the mechanical stress of the blood stream and are con-
Photo. 1. A spotty lesion of severely affected endothelial cells is shown in the middle part of photograph. The endothelial folds tend to disappear and fibrin-like material and clots are seen on the surface of irregularly swollen endothelial folds. The relatively unaffected surface are seen surrounding the spotty lesion, however the swelling and some irregularity of endothelial folds are noted. ×300

Photo. 2. Markedly swollen intercellular long bridges ($) and deposition of fibrin-like material are seen on deformed endothelial cells. ×1,000
Photo. 3. The destroyed intercellular long (↓) and short bridges (↓) are seen on the irregularly swollen endothelial folds. ×3,000

Photo. 4. Many balloon-shaped deformed endothelial cells without intercellular bridges are seen. Fibrin, platelets and erythrocytes are sticking to the endothelial cells. ×300
considered to lose the protecting function of the bridges against the excessive opening of intercellular cleft during vasodilative stage and such excessive opening of intercellular clefts is undoubtedly thought to increase the transendothelial transport according to Bernoulli's
law through intercellular junctions resulting into the production of arterial edema and finally of atheromatous lesions when the plasma concentration of cholesterol is at or over 100 mg/100 ml of human level, especially at high cholesterol concentration. The irregularity of endothelial folds are considered to disturb the smooth peripheral blood stream contributing to the deformation and destruction of platelets and other formed elements and to the thrombotic tendency. The further damages of endothelial cells may weaken the unwettable property of endothelial surface resulting into the activation of Hageman factor of local plasma and deposition of fibrin-like material as well as sticking of leucocytes and other formed elements as shown in photos. 1 and 3. Such changes may possibly induce the local release of permeability increasing factors.

These facts suggest the importance of the preceding chemical injury of endothelial cells and of intercellular bridges followed by further damages in the localized areas by mechanical stress of the arterial flow in the initiation and progression of atherosclerosis.

Summary. In animals received 2 weeks of cholesterol feeding there was no grossly visible lesion, but by scanning electron microscope there was found a swelling of endothelial cells and swollen, splitted, and degenerated intercellular bridges and in the well-known predisposed parts of the aorta to atherosclerosis, there was found spotty areas in which almost all intercellular bridges of endothelial surface were destroyed and the endothelial surfaces were highly deformed.

In animals received 15 weeks of cholesterol feeding the endothelial cells showed further changes and the endothelial surface covering atheroma showed a balloon-like expansion and their intercellular bridges were almost disappeared.

Such injuries of endothelial cells may undoubtedly lose its physiologically controlled transendothelial transport leading to formation and progression of atheromatous lesions.

References