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THE SURFACE MORPHOLOGY OF NORMAL AND ATHEROSCLEROTIC CORONARY ARTERIES IN MALE MACACA FASCICULARIS AND THE EFFECT OF CORONARY ANGIOGRAPHY

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Abstract

Selective coronary angiography is one of the procedures used frequently in the diagnosis and management of coronary artery disease. Macaca fascicularis monkeys were used to study the effects of coronary angiography on coronary artery surface morphology. Fourteen M. fascicularis were fed either an atherogenic diet (0.34 mg of cholesterol/kcal and 40 to 43% of the calories as fat) for six to nine months or a control diet. For six of these animals the Judkin method of selective left coronary angiography was done 24 h prior to necropsy. The ascending aorta, right coronary artery, left circumflex (LCX), left anterior descending (LAD) and left main (LM) coronary arteries were examined using scanning electron microscopy (SEM). The animals fed an atherogenic diet had 27% of the ascending aorta and 7% of the coronary arteries covered with raised lesions. The surface of these coronary arteries differed from those of animals fed a control diet in that the surface appeared smoother and often had numerous adherent leukocytes. The animals undergoing coronary angiography had 25% of the ascending aorta and 10% of the LM surface injured by the catheter. These areas were denuded of endothelium and covered with adherent platelets. There were no morphologic changes observed by SEM following angiography within the LCX or LAD arteries. Thus even in a setting of hypercholesterolemia exposure to contrast media during the coronary angiography procedure did not lead to surface alterations.

Key Words: Coronary Arteries, Endothelium, Coronary Angiography, Atherosclerosis, Macaca fascicularis, Monkey, Artery, Angiography, Vessel surface, Endothelial injury.

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Introduction

Heart diseases are the leading causes of death in the United States, and mortality is due primarily to coronary artery atherosclerosis. Since it was first reported in 1962, selective coronary angiography has become an established tool for the diagnosis and management of coronary artery atherosclerosis. There are currently more than 125,000 selective coronary angiographic procedures done per year in this country. Although this procedure is used widely, it is not without occasional serious complications such as myocardial infarction and cardiac arrhythmias. Besides the recognized clinical complications that are seen at the time of coronary angiography, there may be subclinical effects that have not been determined. Some effects that have been shown to occur experimentally following the injection of contrast material include damage to the endothelium and increased permeability of vessels [3, 7, 37, 46]. These effects have been attributed to jet forces generated during injection, mechanical trauma caused by the catheter, and toxic effects of the contrast media [3, 7, 37, 46].

Jet forces generated during the injection of contrast media have been recognized clinically as a cause of intramural deposition of contrast agent [4], cardiac perforation [34] and subintimal dissection [26]. Fortunately these are infrequent. A model developed in dogs has shown that the flow rates generated by a hand-held syringe, as used in selective coronary artery angiography, have the potential of generating traumatic jet forces which can cause histological damage at the injection site but would be undetected clinically [37]. The selective injection of fluid (contrast media, heparinized blood or saline) in the coronary arteries or mesenteric arteries has been associated with increased pressure and flow through these vessels [35]. Both unusually high pressure and flow have experimentally resulted in histologically identifiable alterations in endothelium [1, 14, 40]. Altered endothelium has also been demonstrated in the form of increased permeability of the vessel [3, 7, 46]. Extravasation of horseradish peroxidase and Evans blue around arterioles can occur within minutes following angiography [7].

The damage to the endothelium can vary with the concentration and type of contrast agent used as well as the duration of exposure [20, 40, 41, 50].

With this background of known effects it was important to determine if, under conditions mimicking those in the clinical setting, coronary angiography produced alterations to the endothelial surface. Scanning electron microscopy was chosen to look for these alterations since it has been used to accurately assess endothelial damage and is especially useful for evaluating large surface areas. Because selective coronary angiography is done mostly in people over thirty who have some coronary atherosclerosis, it was necessary to use a model with both atherosclerotic and normal vessels. This was especially true since the endothelium overlying atherosclerotic lesions has been shown to differ functionally and morphologically from normal endothelium[19, 30, 36, 47].

Several SEM studies have compared and characterized the normal and atherosclerotic surface morphology in a variety of species, including nonhuman primates. Some of the earliest detectable changes that have been reported in the endothelial surface following the feeding of an atherogenic diet were small cells and pores at cell borders [19]. These changes occurred prior to the formation of raised lesions and were related to regenerating cells and increased permeability. Monocytes have been reported adherent to the endothelial surface with the concomitant formation of raised fatty streaks [11, 12]. The endothelial cells over raised lesions have been reported to be rounder and thinner than normal cells [19, 30, 36, 47]. These changes suggested the possibility that the cells were more fragile and thus more susceptible to damage following coronary angiography.

This study was designed to examine the surface of normal and atherosclerotic coronary arteries in M. fascicularis by SEM and determine what effect, if any, coronary angiography has on the surface morphology. The study demonstrated that in addition to the formation of raised lesions the endothelial surface of vessels from animals fed an atherogenic diet had a smoother flatter appearance and often had numerous adherent leukocytes. In the animals that had coronary artery angiography there was disruption of endothelium with adherent platelets in the ascending aorta and left main coronary artery due to catheter damage, but no changes in the left circumflex or left anterior descending coronary arteries. The altered surface of the vessels seen in the animals fed an atherogenic diet did not appear to make these vessels more susceptible to damage from or alter their response to coronary angiography.

Materials and Methods

The fourteen male M. fascicularis monkeys used in this study weighed between 3.5 and 6.7 kg and their mean age based on dentition was 4.5 years. Four groups were studied. Group I consisted of four animals that were fed a control diet, and did not have selective coronary angiography. Group II consisted of two animals fed a control diet, and had selective coronary angiography. Groups III and IV consisted of eight animals (four in each group) that had been fed an atherogenic diet for six to nine months. The animals in group III did not have coronary angiography and those in group IV did. The control diet fed to the animals in groups I and II was either a commercial monkey chow or a diet which was modeled on the American Heart Association's dietary recommendations. They contained very little cholesterol (0.05 mg cholesterol/kcal) and were low in saturated fats. The atherogenic diet contained 0.34 mg cholesterol/kcal and 40-43% of the calories as fat. All animals were treated in accordance to the standards set in the "Guide for the Care and Use of Laboratory Animals" [25].

Total serum cholesterol and high density lipoprotein (HDL) cholesterol concentrations were determined monthly. Serum cholesterol analyses were done using the AutoAnalyzer II methodology of Rush et al. [44]. Serum HDL cholesterol concentrations were determined using the heparin-manganese precipitation procedure described in the," Manual of Laboratory Operations" of the Lipid Research Clinics Program [39].

The Judkins method of selective coronary arteriography [32] was followed as closely as possible. The animals were anesthetized with ketamine hydrochloride (15 mg/kg) and acepromazine maleate (1.0 mg/kg) intramuscularly. A 20-gauge catheter was placed in the saphenous vein and a continuous drip of lactated Ringer's solution with heparin (1,000 units/250 ml) was established. During the course of the procedure, approximately 100 ml were administered to each animal. The right femoral artery was exposed, an arteriotomy done, and a # 4.1 French radiopaque polyethylene catheter introduced into the artery. The catheter had been preformed to a shape for left coronary artery catheterization. The catheter was flushed periodically with heparinized lactated Ringer's solution. The tip of the catheter was positioned at the left coronary ostium using fluoroscopy and never penetrated beyond the left main coronary artery. Eight injections of 1-2 ml of Renografin-76 (diatrizoate meglumine and diatrizoate sodium) were delivered by a hand-held 10 ml syringe. Each injection was monitored using fluoroscopy and a sufficient amount was injected to clearly delineate the coronary arteries for several seconds. The injections were spaced several minutes apart. If there was insufficient clearing of contrast medium following the injection (an indication of obstruction of the coronary artery by the catheter), the catheter was withdrawn several millimeters and repositioned. If severe bradycardia developed, 1 ml of epinephrine 1:10,000 was given. After the angiographic procedure the catheter was removed and the arteriotomy incision was closed. Angiography was not done on the right coronary artery so it could serve as an additional control.

Twenty four hours after the coronary

angiography procedure each animal was anesthetized with ketamine (15mg/kg) and transported to the necropsy room. The animal was then euthanatized with pentobarbitol and immediately perfused in situ at 100 mm pressure with 740 ml of 0.1 M cacodylate buffer (pH 7.3) containing 0.1 M sucrose followed by 1.5 liters of 4% glutaraldehyde in the same buffer and at the same pressure. Both buffer and fixative were at room temperature at the time of perfusion. The animals were perfused through an 18-gauge needle in the left ventricle. The posterior vena cava was incised for runoff. When the outflow appeared to be free of red blood cells and contained fixative the posterior vena cava was clamped to reduce flow. Perfusion fixation lasted approximately twenty minutes. The hearts and ascending aortas (approximately the first 2 cm of the aorta) were then removed and immersion-fixed for at least 24 h in glutaraldehyde before being dissected and processed for scanning electron microscopy.

Longitudinal incisions were made between the aortic valves, dividing the ascending aorta into three sections. The left main (LM) and right coronary (RCA) arteries were transected at their ostia leaving the coronary orifices with the aorta. The three aortic sections were dissected free and usually divided transversely, producing six aortic sections. The LM coronary artery, was dissected free by transecting the left anterior decending (LAD) and left circumflex (LCX) arteries at their bifurcations from the LM. The LM was then cut longitudinally to expose the endothelial surface. The right, left circumflex and left anterior descending coronary arteries were dissected free as far along their lengths as possible (approximately 2 cm). They were cut longitudinally to expose the endothelial surface and usually divided transversely into two sections. All sections were pinned to balsa wood which had been coded with notches to identify the section of artery and the direction of blood flow.

The specimens were washed in three changes of buffer (cacodylate and sucrose or phosphate) for 30 minutes each. They were then dehydrated through graded solutions of ethanol for 10 minutes each (50, 70, 85, 95, and 100% ethanol), and dried from CO_2 by the critical point method in a Denton CPD-1 critical point drying apparatus. The specimens were mounted on aluminum stubs and coated with 200A gold:palladium (60:40) in a Denton Desk-1 sputter coater.

The sections were viewed and photographed using a Philips 501 scanning electron microscope. For each animal, a detailed map was made of the ascending aorta and coronary arteries showing the areas of atherosclerosis and catheter damage and giving a description of the endothelial cells. The map was made by tracing photographs of the sections and labeling areas while scanning the entire surface of each section. The length, width, and total area of the sections of ascending aorta, LM, LAD, LCX and RCA were measured from these maps (or the original photographs) using a Zeiss MOP III image analyzer. The area covered with raised lesions, the area of denuded endothelium covered with platelets (catheter damage) and the approximate area covered with scattered adherent leukocytes were measured also. The raised lesions often had adherent leukocytes on their surface. These areas were only measured as raised lesions and not included in the measurement of area covered by adherent leukocytes. The percentage of artery surface for each of these categories was calculated.

The length, width, and area of 519 endothelial cells in the ascending aorta and 267 cells from the straight portions of the coronary arteries were measured using a Ziess Videoplan. The axial ratio for each cell was determined by dividing the length by the width. The cells measured in the ascending aorta were not located near either of the two coronary ostia nor were they from areas with atherosclerotic lesions. The cells measured in the coronary arteries were from the straight portions of the arteries not associated with branches and not from areas with atherosclerosis. The cells were measured from photographs taken at 1250 X magnification and were selected for the clarity of cell outlines. The cells were not from predetermined locations but were taken from approximately 10 fields (photographs) in the ascending aorta and 7 fields in the coronary arteries of each animal.

In addition to the parameters measured, the endothelial surface of each section was evaluated for the presence of cells with numerous microvilli, cells with few microvilli and "holes" located at cell junctions (smooth endothelium), elongated or round cells, cells with raised edges (curled endothelium), denuded endothelium without platelets adherent, and smooth endothelium with a fibrous appearance and indistinct cell junctions (very flat endothelium). The approximate area of each of these categories was estimated. Statistical analysis was done using the Students' t test. All values are given as mean $\pm$ standard deviation.

Results

The mean serum cholesterol concentration at the time of necropsy for the animals in groups I and II (control diet) was 139 mg/dl $\pm$ 32 mg/dl and the mean HDL cholesterol concentration was 64 $\pm$ 27 mg/dl. The mean cholesterol concentration for the animals in groups III and IV (atherogenic diet) was 434 $\pm$ 210 mg/dl and the mean HDL cholesterol concentration was 30 $\pm$ 8 mg/dl. The atherogenic diet groups had significantly higher cholesterol levels and lower HDL cholesterol levels than the control diet groups ($P < 0.05$).

A summary of the qualitative and quantitative results is given by groups for the ascending aorta in Table 1 and for the coronary arteries in Table 2.

SEM observations of control diet fed animals

The animals in group I (control diet, no angiography) generally had polygonally shaped endothelial cells in the ascending aorta (Figure 1). Cell boundaries were almost always detectable and there was usually a centrally located raised nucleus. Numerous microvilli were on the

TABLE 1
QUANTITATIVE AND QUALITATIVE RESULTS
ASCENDING AORTA

Group		N	Raised Lesions (% of surface)	Platelets (% of surface)	Adherent Leukocytes[a]	Shape of Endothelial Cells	Smooth Endothelium	Very Flat Endothelium	Curled Endothelium
I.	Control diet No angiography	4	0%	0%	0%	Mostly round	None	None	None
II.	Control diet Angiography	2	0%	43.0%[b] ± 11%	0%	Mostly round	None	None	None
III.	Test diet No angiography	4	34.1% ± 7%	0%	0%	Mostly round	None	None	None
IV.	Test diet Angiography	4	19.9% ± 10%	16.3% ± 2.4%	0%	Mostly round	None	None	None

[a] Excluding plaque
[b] Mean ± standard deviation

surface. No consistent orientation of endothelial cell long axes was noted. The more distal portion of the ascending aorta occasionally had some longitudinal relief of the surface (Figure 2).

The endothelial cells of the left main coronary artery were also polygonal but had less microvilli. As the LM divided into the LCX and LAD the cells became more elongate. The cells of the LCX, LAD and RCA were generally elongated with the major axes in the direction of blood flow (Figure 3). Microvilli were only occasionally present and there were often numerous "holes" located at the cell borders although cell junctions were not always distinct. At arterial branches the cells' long axes followed the expected direction of flow with the cells at the flow divider having a rounder shape. Among the animals fed the control diet, only two areas less than 1 mm^2 were seen that had leukocytes adherent to the arterial surface. A small amount of very flat endothelium was seen in one animal. Raised lesions, areas of denuded endothelium with or without adherent platelets, or areas of curled endothelium were not observed.

The ascending aorta of the animals in group II (control diet, coronary angiography) had a mean of 43 ± 11% of the surface denuded of endothelial cells with adherent platelets covering the subendothelial surface (Figure 4). Infrequently, leukocytes were associated with the platelets (Figure 5) but, for the most part, the disrupted endothelium was covered by platelets without a leukocyte response. Thrombus formation was rarely seen and then only consisted of microthrombi associated with the more severely injured endothelium. The undamaged surface of the ascending aorta had endothelial cells which were polygonal, with microvilli and raised nuclei similar to those of group I.

Within the coronary arteries, changes in the endothelium due to the coronary angiographic procedure were confined to the LM coronary artery. The LM had a mean of 12.5 ± 17.7% of the endothelial surface denuded of endothelial cells with adherent platelets. The unaffected areas had polygonally shaped endothelial cells similar to those of group I. The LCX, LAD and RCA had elongated endothelial cells and no morphological distinctions could be made between the endothelial surfaces within the coronary arteries of the animals fed the control diet (group I) and

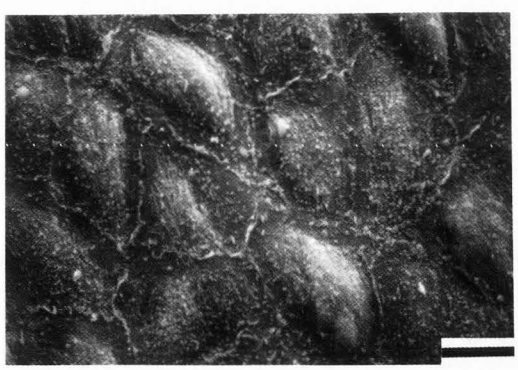

Figure 1: Scanning electron micrograph of the ascending aorta, group I. Polygonally-shaped endothelial cells. Flow was from bottom to top. Bar =10 μm

TABLE 2
QUANTITATIVE AND QUALITATIVE RESULTS
CORONARY ARTERIES

Group	N	Raised Lesions (% of surface)	Platelets (% of surface)	Adherent Leukocytes[a]	Shape of Endothelial Cells	Smooth Endothelium	Very Flat Endothelium	Curled Endothelium
I. Control diet No angiography	4	0%	0%	<1% in 1 animal only	Mostly elongate	Most of surface	<1%	None
II. Control diet Angiography	2	0%	LM only 12.5%[b] ±17.7%	0%	Mostly elongate	Most of surface	None	None
III. Test diet No angiography	4	6.8%[c] ±6.9%	0%	49% in 2 animals	Mostly elongate	Approx. 75%	Approx. 20%	Approx. 5%
IV. Test diet Angiography	4	7.1%[c] ±9.0%	LM only 8.6% ±9.5%	11% in 1 animal only	Mostly elongate	Approx. 75%	Approx. 15%	Approx. 10%

[a]Excluding plaque
[b]Mean ± standard deviation
[c]Mean of LM,LCX,LAD and RCA

the animals fed the control diet which also had coronary angiography (group II).

SEM observations of animals fed an atherogenic diet

The ascending aorta of the group III animals (atherogenic diet, no angiography) had a mean of 34.1 ± 7% of the surface covered by raised lesions (Figure 6) with a range of 24 to 40%. The distribution of raised lesions in the ascending aorta formed a consistent pattern shown in Figure 7 with most of the raised lesions forming above the left coronary cusp and non-coronary cusp. The lesions were raised with an uneven surface, giving it a "cobblestone" appearance. The endothelial cells covering these raised lesions had fewer microvilli than those in uninvolved areas and often had adherent leukocytes (Figure 8). There were areas from which the endothelium had been lost, revealing a sponge-like subendothelium which most likely represent foam cells (Figure 9). The aortic endothelial surfaces without lesions were similar to those described for the control diet fed animals. The endothelial cells were polygonal and had microvilli. Areas of denuded endothelium with adherent platelets were not observed.

In the group III animals there was less surface covered with atherosclerosis in the coronary arteries than in the ascending aorta. The LM had a mean of 9.2 ± 6.9% of the surface involved; the RCA had 8.0 ± 10.2%, the LAD had 7.4 ± 8.3%, and the LCX had the least with 2.6 ± 2.4%. The mean surface covered by raised lesions for all 4 coronary arteries was 6.8 ± 6.9%. The atherosclerotic lesions differed from those of the ascending aorta. They were not as raised and had a less "cobblestone" appearance (Figure 10). As in the ascending aorta, leukocytes were associated with the surface of raised lesions and areas in which the overlying endothelium was lost revealed cells in the subendothelial space (Figure 11).

Leukocytes were also seen on the endothelial surface of the coronary arteries of the group III animals in areas not having raised lesions. One animal which had 39% of the aortic surface covered with raised lesions had virtually no raised lesions within the coronary arteries. However, this animal had adherent leukocytes scattered over most of the endothelial surface of the coronary arteries (57% of the LAD, 89% of the RCA, 94% of the LM, and 100% of the LCX). This is shown in Figures 12 and 13.

The endothelial surface of the coronary arteries which was not affected by atherosclerosis often appeared smoother and flatter than that of groups I and II (Figure 14). This flat endothelium was present in the coronary arteries of most animals fed an atherogenic diet (groups III and IV), but was only observed in one portion of the LCX of one control diet fed animal. The nuclei of these flat endothelial cells were often seen as slightly raised oval structures with one or two prominent smaller elevations (Figure 15). In these flat areas fibrous structures in the subendothelium or perhaps within the endocytoplasm appeared to be running parallel to as well as across the long axis of the vessel. Another observation not made in the control diet fed animals but made in the group III animals was the presence of some coronary endothelial cells with what appeared to be curled edges (Figure 16). Areas denuded of endothelium with adherent platelets were not observed. Smooth endothelial surfaces with few microvilli similar to those of the control diet

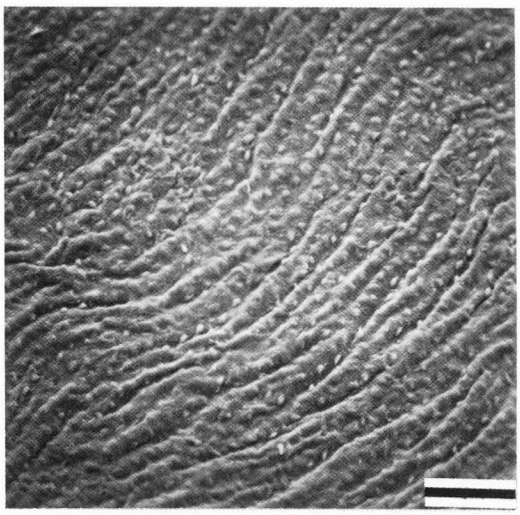

Figure 2: Scanning electron micrograph of the ascending aorta, group I. Longitudinal relief. Flow was from lower left to upper right. Bar = 100 μm

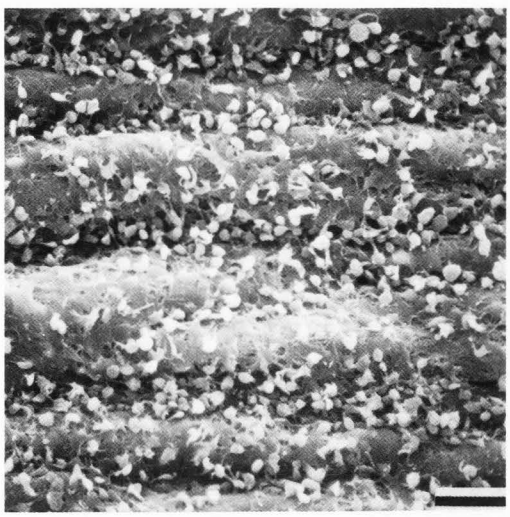

Figure 4: Scanning electron micrograph of the ascending aorta, group II. The endothelial cells have been lost and the subendothelial surface is covered with adherent platelets. Flow was from left to right. Bar = 10 μm

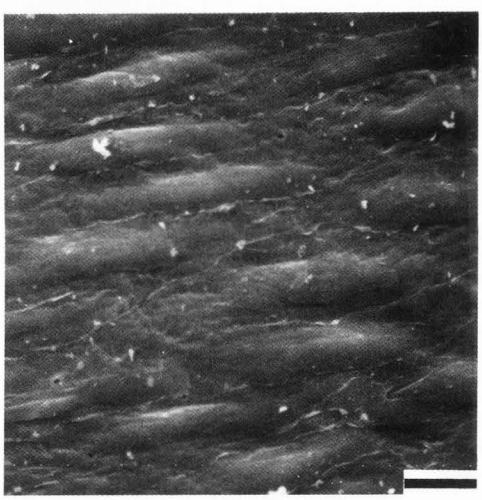

Figure 3: Scanning electron micrograph of the left anterior descending coronary artery, group I. Elongated endothelial cells, with few microvilli and the major axes in the direction of blood flow. Flow was from left to right. Bar = 10 μm

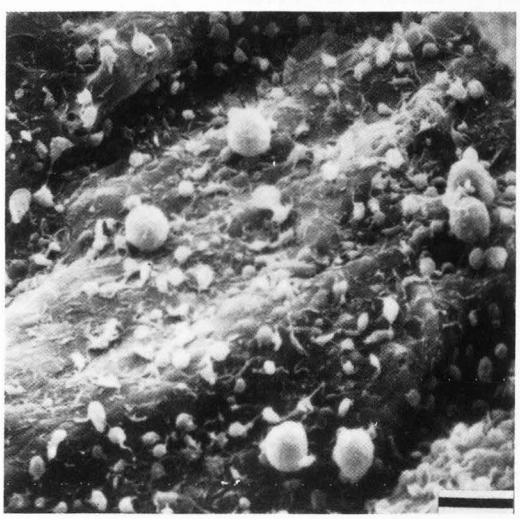

Figure 5: Scanning electron micrograph of the ascending aorta, group II. Leukocytes associated with the endothelial damage and platelet adhesion. Flow was from lower left to upper right. Bar = 10 μm

fed animals were also present in the coronary arteries of the group III animals.

The animals in group IV (atherogenic diet, coronary angiography) had the same distribution pattern of atherosclerosis in the ascending aorta as group III. Raised lesions covered 19.9 ± 10% of the surface of the ascending aorta. There was 16.3 ± 2.4% of the surface which had been denuded of endothelium and had adherent platelets covering the exposed subendothelium, as in group II (control diet, coronary angiography). The area of damaged endothelium also had a consistent pattern of distribution which overlapped that of the atherosclerosis (Figure 17). This pattern of damaged endothelium was similar to that seen in the group II animals. Once an area had been damaged and covered with platelets it was not possible to know if the area had originally been raised lesion or normal vessel. From the patterns of raised lesions and catheter damage it

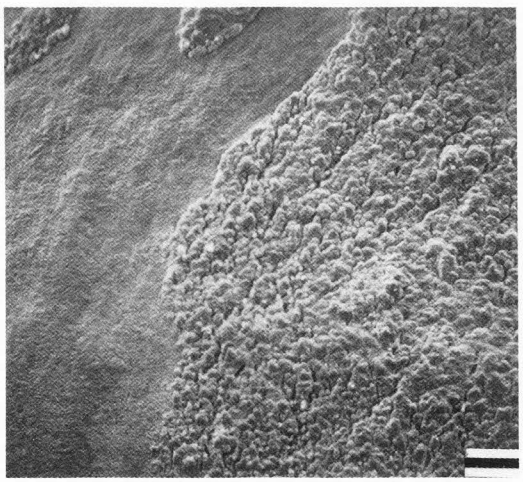

Figure 6: Scanning electron micrograph of the ascending aorta, group III. Raised atherosclerotic lesion. Flow was from bottom to top. Bar = 250 μm

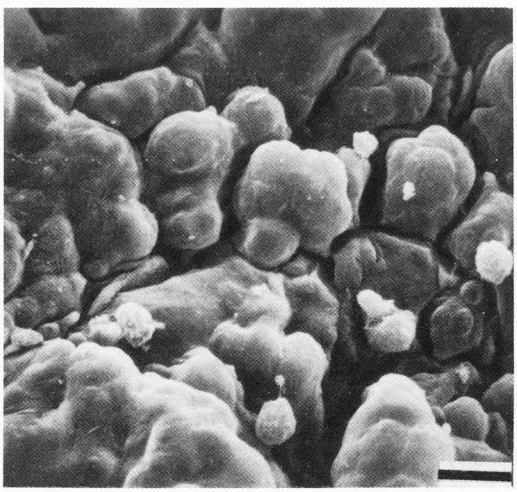

Figure 8: Scanning electron micrograph of the ascending aorta, group III. Endothelial cells with few microvilli and adherent leukocytes. Bar= 20 μm

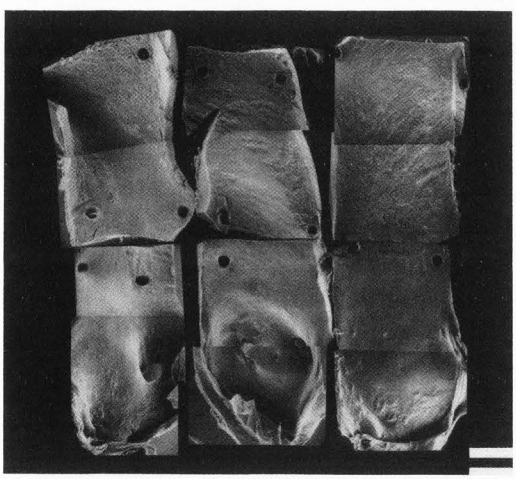

Figure 7: Composite of scanning electron micrographs of ascending aorta, group III. The left coronary cusp is located at the left of the photograph. Most of the plaque is located above the non-coronary cusp and left coronary cusp. Flow was from bottom to top. Bar = 2 mm

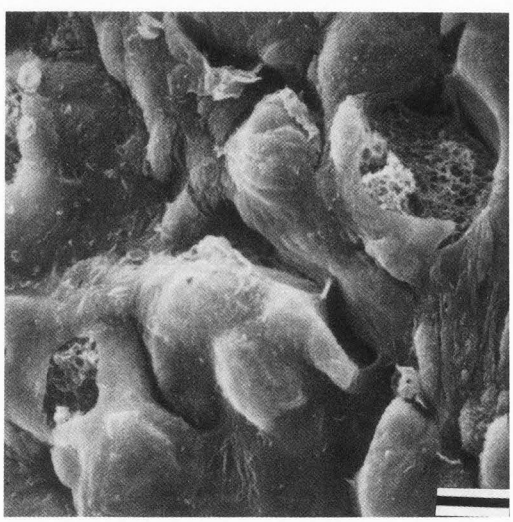

Figure 9: Scanning electron micrograph of the ascending aorta, group III. Areas of the endothelial surface have been lost, revealing a spongelike subendothelium. Bar = 10 μm

appeared that much of the catheter damage involved areas with raised lesions. Thus, it was felt that there was no difference in the amount of atherosclerosis between the group III animals and the group IV animals. As in the group II animals, the areas covered with platelets occasionally had leukocytes associated with them. The atherosclerosis in the ascending aorta, like that in group III, was raised, had a "cobblestone" surface, and had leukocytes associated with it.

The LM coronary artery of the group IV animals had an area of denuded endothelial surface with platelet adhesion involving a mean of 8.6 $\pm$ 9.5% of the surface. Thus, in both groups having coronary angiography there was catheter damage to the endothelial surface of the LM and ascending aorta.

The coronary arteries of the group IV animals, like those of the group III animals, had less atherosclerosis than the ascending aorta. The LM had a mean of 11.7 $\pm$ 10.3% of the surface

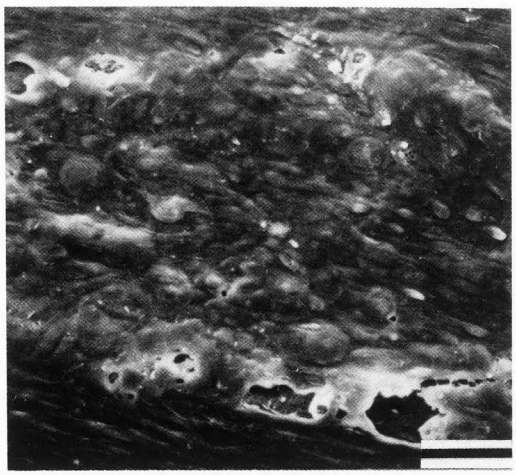

Figure 10: Scanning electron micrograph of the left anterior descending coronary artery, group III. Slightly raised atherosclerotic lesion. The cracking in the lower edge of the plaque may be processing artifact. Bar = 50 μm

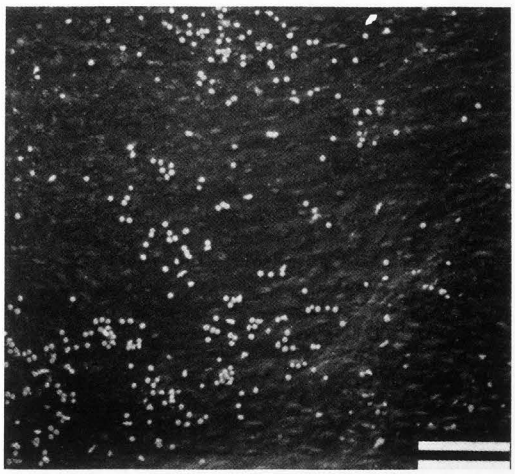

Figure 12: Scanning electron micrograph of the right coronary artery, group III. Scattered cells adherent to the endothelial surface. Bar = 100 μm

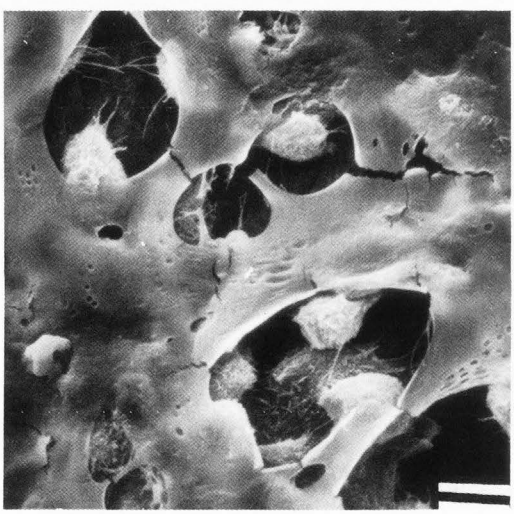

Figure 11: Scanning electron micrograph of the left anterior descending coronary artery, group III. Endothelial surface is torn, revealing cells within the subendothelial space. The cracking also seen in ths figure may be processing artifact. Bar = 10 μm

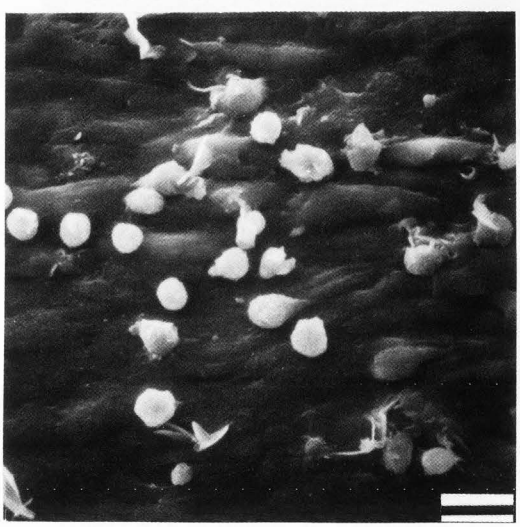

Figure 13: Scanning electron micrograph of the left anterior descending coronary artery, group III. Scattered cells adherent to the endothelial surface. Bar 10 = μm

involved. The RCA had 10.0 ± 17.5%. The LAD had 5.1 ± 5.9%, and the LCX had the least with 2.6 ± 2.4%. The mean surface covered by raised lesions for all 4 coronary arteries was 7.1 ± 9%. As in group III, the atherosclerosis in the coronary arteries was less raised and smoother than that of the ascending aorta. The areas in the coronary arteries where there was no atherosclerosis had an endothelium which was smooth and flat.

Two animals in this group had very little atherosclerosis in the coronary arteries. Unlike the animal in group III, which had no coronary atherosclerotic lesions but adherent leukocytes, neither of these animals had leukocytes adhered to the endothelial surface of the coronary arteries. However, the animal in group IV with the most coronary atherosclerosis had an additional 15% of the surface of the RCA and 30% of the LCX covered with scattered adherent

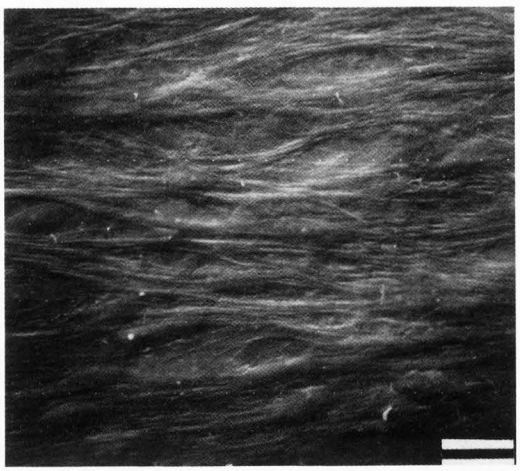

Figure 14: Scanning electron micrograph of the right coronary artery, group III. The endothelial surface is flat with an underlying fibrous appearance. Flow was from left to right. Bar = 20 μm

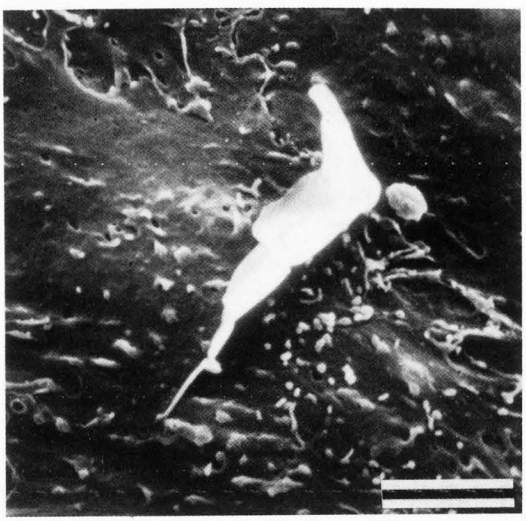

Figure 16: Scanning electron micrograph of an endothelial cell with curled edges, group III. Bar = 5μm

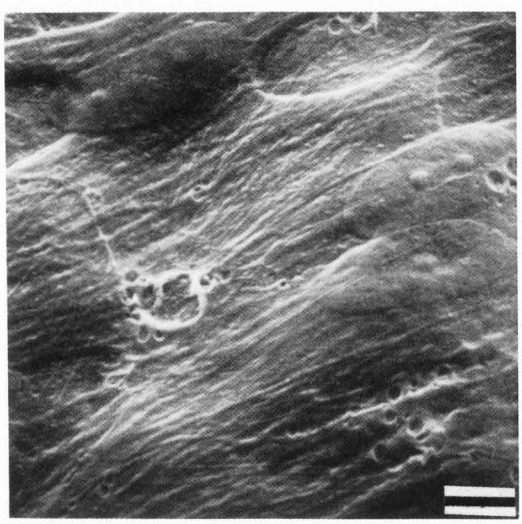

Figure 15: Scanning electron micrograph of the right coronary artery, group III. The endothelial cells are flat with oval nuclei that have one or two prominent smaller elevations. The indentation left of center may be an artifact or represent a hole at an indistinct cell junction. Bar = 5 μm

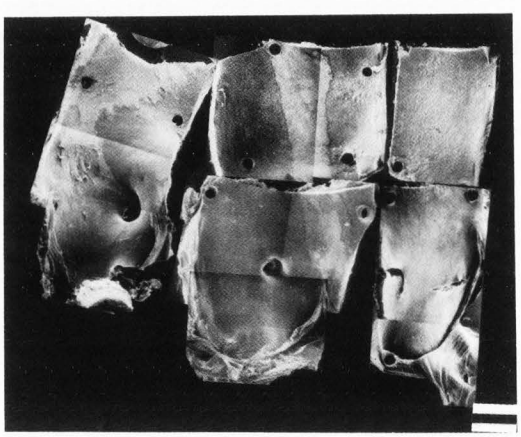

Figure 17: Composite of scanning electron micrographs of the ascending aorta, group IV. The left coronary cusp is located at the left of the photograph, and the right coronary cusp is in the center. There are two streaks of damaged endothelium, one above the left coronary ostium and the other above the right coronary ostium. There is an area of atherosclerosis above the non-coronary cusp. Flow was from bottom to top. Bar = 2 mm

leukocytes. This was a mean of 11% when all 4 coronary arteries were considered.

Except for the ascending aorta and LM, which had damaged endothelium with platelet adhesion, there were no noticeable morphological differences in the endothelium of the group IV animals (atherogenic diet, coronary angiography) from those of group III (atherogenic diet, no angiography). The group IV animals, like the group III animals, had areas with flat endothelium, some areas with curled endothelium and areas of smooth endothelium.

Cell dimensions

The dimensions of the endothelial cells in the ascending aorta are given in Table 3 and those in the coronary arteries are given in Table

4. The areas of the cells in the ascending aorta tend to be slightly larger than those of the coronary arteries (right and left). The axial ratios were considerably different with the cells of the coronary arteries being narrower and more elongate than those of the ascending aorta ($P<0.05$). Within the ascending aorta there were no significant differences in the cell areas or axial ratios between those without angiography (groups I and III) and those with angiography (groups II and IV). There also were no significant differences in these parameters between those fed the control diet (groups I and II) and those fed the atherogenic diet (groups III and IV). Within the left coronary arteries there were no significant differences in the cell areas or axial ratios between those without angiography and those with angiography. There were no significant differences in these parameters between those fed the control diet and those fed the atherogenic diet. Although the cell areas in the right coronary arteries were not different from those of the left coronary arteries, the axial ratios were slightly higher than those of the left.

Discussion

In the present study the normal endothelial surface of the coronary arteries and ascending aorta were described in detail along with the changes that occur from feeding an atherogenic diet and the effects of coronary artery angiography.

The normal surface morphology in the coronary arteries and ascending aorta of M. fascicularis monkeys was very similar to that previously described for other species [5, 6, 15, 30, 31, 36, 47]. The endothelial cells of the coronary arteries were elongate, had few microvilli and the long axis paralleled blood flow. The cells of the ascending aorta were polygonally shaped with numerous microvilli and no distinct orientation of the long axis.

From this study it can be seen that the changes in surface morphology in M. fascicularis monkeys in response to consuming an atherogenic diet are also quite similar to those previously described for other nonhuman primates [11, 12, 30, 47] and other species [9, 19, 43]. The most obvious change was the formation of raised atherosclerotic lesions in the ascending aorta and coronary arteries with adherent leukocytes. This has been commonly reported. A feature of the present study was the disruption of the endothelial surface of raised lesions, exposing a spongy subendothelium. This spongy appearing material is most likely the accumulation of foam cells derived from monocytes as described by Faggiotto et al. [11, 12] in M. nemistrina during the formation of fatty streaks. The denuded surface has been described in several other studies and has been thought to represent degenerative changes or an increased fragility of the endothelial cells in these areas [30, 43, 47]. Recently, areas of endothelial denudation have been described in which platelets were adherent to the exposed foam cells and connective

TABLE 3
CELL DIMENSIONS
ASCENDING AORTA

Group	Number of Cells	Length (μm)	Width (μm)	Area (μm^2)	Axial Ratio
I	274	23.1[a] ±6.1	14.2 ±2.8	261 ±79	1.6 ±0.5
II	39	23.5 ±5.9	15.2 ±3.7	284 ±125	1.52 ±0.3
III	154	24.5 ±5.7	14.5 ±3.1	268 ±71	1.64 ±0.5
IV	52	26.6 ±4.6	15.7 ±3.0	291 ±71	1.7 ±0.4
All groups	519	24.8 ±4.9	14.9 ±3.1	279 ±75	1.63 ±0.4

[a]Mean ± standard deviation

TABLE 4
CELL DIMENSIONS
CORONARY ARTERIES

Group	Number of Cells	Length (μm)	Width (μm)	Area (μm^2)	Axial Ratio
Left coronary arteries					
I	63	39.9[a] ±8.9	9.35 ±2.1	277 ±78	4.9 ±1.5
II	55	37.4 ±7.7	8.59 ±1.7	235 ±54	4.19 ±1.3
III	31	33.5 ±5.3	9.18 ±1.5	235 ±54	3.72 ±1.0
IV	43	39.5 ±7.3	8.11 ±1.8	247 ±78	4.75 ±1.5
All groups	192	38.1 ±7.6	8.83 ±1.8	251 ±67	4.47 ±1.4
Right coronary arteries					
I & II	27	44.4 ±7.1	7.33 ±1.2	250 ±47	5.93 ±1.2
III & IV	48	42.4 ±9.3	9.02 ±1.8	270 ±69	4.72 ±1.3
All groups	75	43.1 ±8.5	8.41 ±1.6	263 ±61	5.16 ±1.3

[a]Mean ± standard deviation

tissue [12]. In the present study these changes were only on raised lesions; and did not have platelets adherent to the surface or conclusive evidence that they occurred in vivo. The argument could be made that they represent artifacts produced during the processing of the tissue caused by the fragility of the endothelium or by underlying lipid.

Although both the aorta and coronary arteries had raised lesions with denuded endothelium, the atherosclerotic lesions in the coronary arteries were not as raised or extensive as those in the ascending aorta. This observation was also made by Jones et al. [30] using rhesus monkeys fed an atherogenic diet for four months. Thus, the progression of atherosclerosis in the coronary arteries of nonhuman primates is apparently delayed from that of the aorta, as is the case in man [10]. This difference in atherosclerosis and the difference in cell shape between the coronary arteries and the aorta, may reflect different intrinsic properties of the cells of these arteries, or they may reflect differing environmental factors such as blood flow or blood pressure. Blood flow in the straight portions of coronary arteries is usually laminar while flow in the ascending aorta may be turbulent or otherwise disorganized. Turbulent flow can create high shear forces and lack of directional flow at the vessel wall [8]. It has been demonstrated that endothelial cell nuclei orient in the direction of flow but are less ordered in the ascending aorta and orifices of major branches [13]. Although studies have been done to examine the relation of cell morphology and atherosclerosis the results have been inconclusive [33].

In addition to the more raised atherosclerotic lesions there were other changes in the endothelium with cholesterol feeding. A large portion of the endothelium of the coronary arteries of the animals fed an atherogenic diet was subjectively smoother and flatter than the endothelium of the control diet-fed animals. Other studies have reported changes in the endothelial cells of animals fed an atherogenic diet occurring prior to actual lesion formation. The endothelial cells have been reported to have an increase in Weibel-Palade bodies [31, 49], an increase in lysosomes and an increase in the number of cytoplasmic filaments [18]. Jones et. al. [30] reported that the endothelial cells became thinner. These alterations in the endothelial cell structure may account for the smooth, very flat surface seen in the present study and may also indicate altered function.

In several animals there were large numbers of leukocytes adherent to otherwise normal appearing endothelium. Several different investigators have identified adherent cells in hyperlipoproteinemic animals as monocytes and postulated that these monocytes migrate through the endothelial surface into the subendothelial space and accumulate lipid [11, 12, 16, 28, 45, 48]. This has led to the suggestion that these monocytes become intimal foam cells and are involved in the atherosclerotic process. In pigeons leukocytes have been associated with the progressive edge of the atherosclerotic lesion. This area is also the area where cell turnover is greatest [27, 29]. A chemo- attractant has been isolated from swine aortic lesions [17]. Whatever the process for attracting leukocytes, it appears their presence represents an altered state of the vessel wall which may predispose it to additional injury.

The main purpose of this study was to evaluate the effect of coronary angiography on surface morphology especially in animals with atherosclerotic vessels and endothelial alterations. An important finding was the denudation of endothelium in the ascending aorta and left main coronary artery caused by passing the catheter to the coronary ostium. The damaged areas in the ascending aorta, the left coronary ostium and the proximal portion of the left main coronary artery correspond to the path of the catheter and therefore represent mechanical injury. In some of these catheter damaged areas leukocytes were present alone with the carpet of platelets. An interaction of platelets and leukocytes in response to catheter injury has been reported in the dog and may be an integral part of the arterial response to injury [42]. The difference in amount of injury between the group II animals and the group IV animals was most likely due to the greater amount of manipulation necessary to position the catheter in these two animals. When all six animals were considered the amount of injury was 25 $\pm$ 5% of the surface of the ascending aorta and 10 $\pm$ 12% of the surface of the LM coronary artery. This damage was considered potentially dangerous because of its location. Although not seen in this study, thrombi forming at the coronary ostium and LM could produce emboli which could lodge in the coronary circulation producing myocardial ischemia. Indeed, the majority of deaths following coronary angiography have occurred in patients with severe left main coronary artery atherosclerosis and have been caused by myocardial infarction [2].

Other than the mechanical damage from the catheter there was no evidence that the clinical angiography procedure produced any injury to vessel surfaces. No injuries were seen in the proximal LAD or LCX indicative of damage from jet forces. There was also no evidence of altered cell morphology in the coronary arteries attributable to angiography even in the setting of hypercholesterolemia. The lack of cell alteration in this clinical setting was most likely due to the short exposure time to contrast media.

There was no clear pattern to indicate that angiography or hyperchloserolemia altered cell sizes or axial ratios. This data must be interpreted cautiously since the number of cells measured from each group was small and the cells measured were not from predetermined locations within each artery.

It is interesting that the response of the atherosclerotic lesions in the ascending aorta to catheter injury was not different from the response of the normal endothelial surface, that is they both responded by forming a platelet

monolayer. It has been reported that injured neointima and the components of the deeper layers of the vessel wall are more thrombogenic than superficially injured normal intima [24, 38]. The early atherosclerotic lesions in this study responded to superficial injury by forming a monolayer of platelets and did not show an increased thrombogenicity. This similarity of response to superficial injury between normal intima and fatty lesions has also been demonstrated in pigs [21]. Once a monolayer of platelets forms following endothelial damage these areas are no longer considered thrombogenic [22, 23]. This implies that at least early fatty streaks may not predispose arteries to thrombosis. Thus the damaged areas produced by the catheter in this study, although potentially dangerous, may heal without serious consequences. However, this study should be interpreted cautiously since patients who usually undergo coronary angiography often have advanced atherosclerosis and may still be at a greater risk of embolization of plaque or thrombosis if deeper components of the vessel wall are exposed.

It should be noted that although there were no detectable changes observed by scanning electron microscopy within the LAD or LCX following selective coronary angiography even in the atherogenic diet group, there still may have been permeability or intracellular changes in the endothelial surface. This study only looked at the surface 24 h after angiography. Damage may have occurred but been mild enough that the endothelium returned to normal by the time of necropsy. This study demonstrates that there are some potentially dangerous areas of endothelial damage from catheter injury following the coronary angiographic procedure. The changes in the endothelial surface which result from consumption of a high cholesterol diet should be considered when undertaking studies involving the endothelium and relating them to the clinical setting.

Acknowledgements

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Discussion with Reviewers

E.A. Spraque: In your introduction you speculate that the rounder, thinner endothelial cells observed over lesions may be more susceptible to damage by catheterization associated with coronary angiography. What physical or biological cell characteristics form the basis of this hypothesis? Would it be more likely that the position of the endothelium on a raised lesion make it a more predictable target for the catheter to encounter?
Authors: We were not only concerned with the areas in the ascending aorta susceptible to catheter damage but also the effects of the contrast media on the endothelium within the coronary arteries. The rounder, thinner endothelium over raised lesions has been shown to be functionally different from normal endothelium in that these areas are more permeable to Evans blue and have a more rapid cell turnover rate. We were speculating that the hypertonic contrast media may have a greater effect on these cells. However, our findings suggest that even these cells are not morphologically altered by the angiographic procedure. Our findings suggest that the endothelium on raised lesions does not make a more predictable target for catheter damage since the group IV animals had less damage in the ascending aorta than the group II animals.

E.A. Spraque: You mention that the "very flat" endothelial morphology observed in coronary arteries may be associated with an increase in lysosomes, Weibel-Palade bodies, or cytoplasmic filaments. It seems also possible that this altered morphology could reflect a change in membrane composition and compliance which would change the response of these cells to hemodynamic wall shear stresses.
Authors: It is likely that the altered morphology could reflect a change in membrane composition due to the hypercholesterolemia. This would be consistent with the increased permeability of endothelial cells following cholesterol feeding.

M. Richardson: Was there more platelet accumulation on the vessels of those animals in which there had been a greater degree of manipulation of the angiography catheter than in those in which manipulation was minimal?
Authors: There was no noticeable difference in the number of platelets on the injured vessel surface of those animals in which there was more catheter manipulation than those in which catheter manipulation was minimal. However, there was a greater percentage of the surface area injured. The animals in group II were the first animals in this study to have coronary angiography and had more catheter manipulation.

M. Richardson: Was the total dose of heparin given during the angiography monitored? Was the effect of this dose of heparin controlled in any experiments on untreated animals?
Authors: Heparin was used to prevent clotting in the angiography catheter and is used in patients during the angiography procedure. We felt its use was appropriate to mimic the clinical setting. The total dose of heparin given was monitored and was about 400 units per animal administered over approximately a half hour period. In a model developed to study coronary artery thrombosis heparin did not alter the response [Foltz et al. Circulation 1976;74:365-370].

A. Faggiotto: Since cholesterol and plasma HDL levels were determined monthly, why did the authors not provide these data? Was there any relationship between the levels and patterns of plasma lipids and the severity of the lesions? One could suspect that the high standard deviations found in all groups could, at least in part, relate to specific variations of response to the two control diets and the atherogenic diet.
Authors: The total and HDL cholesterol levels were determined monthly but this data did not contribute much to the interpretation of the study. The total cholesterol levels rose from an initial level of 123 $\pm$ 34 mg/dl to 505 $\pm$ 241 mg/dl over the first three months and then declined slightly to 478 $\pm$ 198 mg/dl at five months. The HDL cholesterol levels dropped from an initial level of 53 $\pm$ 10 mg/dl to 42 $\pm$ 13 mg/dl at three months and 40 $\pm$ 16 mg/dl at five months. The severity of atherosclerosis appeared to be related to the cholesterol levels but the correlation coefficient was not statistically significant because of the small sample size.

The high standard deviations in cholesterol levels and amount of atherosclerosis reflects the wide variation in the response by individuals fed and atherogenic diet. This variation is well recognized.

The two control diets did not add to the variation. Two animals in group I and both animals in group II were fed a diet modeled on the American Heart Association dietary recommendations. The other two animals in group I were fed a commercial monkey chow for comparison. No differences were seen between those fed the commercial diet and those fed the modeled diet.

A. Faggiotto: Since some monkeys were examined after 6 months, others after 9 months of atherogenic diet, were there morphological or morphometrical differences in animals on the diet for different lengths of time?
Authors: Two of the animals (one in group III and one in group IV) were fed an atherogenic diet for 9 months, the other six were all fed this diet for 6 to 7 months. The two fed for nine months were two animals with low cholesterol levels at the end of the study. It was hoped by feeding the diet for an additional three months these animals would develop more lesions. One of these animals still had the least amount of atherosclerosis.

A. Faggiotto: What kind of saturated fats were used in the atherogenic diet?
Authors: The main sources of lipid in the atherogenic diet were lard (12%), butter (3%), beef tallow (3%) and dried egg yolk (6%).

A. Faggiotto: Since oxidized cholesterol has been proven to be toxic to the arteries of experimental animals by several investigators, were any measures taken to avoid lipid oxidation in the diet?
Authors: After the diets were prepared they were frozen. They were not thawed until just prior to use.

A. Faggiotto: In the six to nine months of atherogenic diet, were any lesions demonstrable in the monkeys that underwent angiography?
Authors: The lesions did not produce enough stenosis to be visible at the time of coronary angiography.

A. Faggiotto: When coronary flow became unintentionally obstructed by the progressing catheter, did the endothelial lesions appear more severe or diffuse by SEM?
Authors: None of the animals which underwent coronary angiography had any noticeable changes in the endothelium including those in which the flow was unintentionally obstructed. The obstruction was for only a couple of seconds and probably not sufficient to produce hypoxic lesions.

A. Faggiotto: In order to provide a clue to distinguish a mechanical from an atherogenic denudation, were the edges of endothelial denudation always characterized by broken cells or were there denuded areas with smooth borders?
Authors: It was not difficult to distinguish areas of mechanical injury. These areas were in pattern across the ascending aorta extending into the coronary ostia. Because the response to the catheter injury was the same for both normal vessel wall and raised lesions, it was not possible to measure with certainty the amount of damaged surface that had been atherosclerotic.

Even in the non-atherogenic diet fed animals the endothelial cells had smooth borders at the edges of the catheter damage. At 24 h post injury broken endothelial cells were not present.

M. Roach and R. Kratky: The micrographs of "fibrous endothelium" are unusual. How often did you see this? Have you observed it under different conditions or in other species?
Authors: The "fibrous endothelium" was only seen in the atherogenic diet fed animals and only where the endothelium had a very smooth appearance. It was present in five of the eight atherogenic diet fed animals and represented less that 20% of the vessel surface area. We have not observed this under different conditions or in different species including pigs and pigeons. Our intention is to reprocess some of these areas for transmission electron microscopy to better characterize these structures. We speculate that these are intimal elastic fibers underlying the endothelium.

M. Roach and R. Kratky: Why did you choose 24 h to assess angiographic damage? Have you, or have others, done studies with catheters alone, with catheter plus saline, with dye alone (e. g. DIVA)? What effect does flow rate have?
Authors: Studies have shown that the changes in endothelium and permeability following exposure to contrast agents are immediate and that the more pronounced changes can still be detected 24 to 48 h later. Part of our goal was to look at the effects on endothelium that may be of significance over a longer period of time. Therefore we selected 24 h to separate those effects that were quickly reversible from those that were more lasting.

We felt a catheter only group would not have added much to this study. The contrast media was injected after the catheter was in position. Therefore damage from the contrast media would have been in the left coronary arteries. The right coronary arteries were not exposed to contrast media and served as an additional control.

If we had seen differences in endothelial morphology in the left coronary arteries following angiography, then a catheter and saline group would have been useful to distinguish changes due to contrast media from changes due to the forces of injection. Since no differences in morphology were seen in the left coronary arteries (other than the catheter damage in the left main) we felt a catheter and saline group would not add much to this study.

Several studies have been done to look at the response of the vessel wall to catheter injury and to contrast agents although not necessarily in the clinical setting of coronary angiography [3, 7, 21, 23, 24, 37, 40, 41, 46, 50] .

Flow rates are an important component in determining the jet forces generated at the catheter tip. Although no injuries could be attributed to the jet forces in this study, flow rates were approximately 5-7 ml/sec. and similar to those reported by Abbott for a hand held syringe capable of producing injury.

M. Roach and R. Kratky: How did you determine the time for pressure fixation? In our experience "fixed" isolated arteries still recoil a bit after 24 h of pressure fixation.
Authors: The time for pressure fixation was determined by past experience with pigeons and other primates. Although we saw some longitudinal relief it was confined to the distal portion of the ascending aorta.

M. Roach and R. Kratky: Have you analyzed lesions at the coronary orifices? In our experience with rabbits this region is very prone to develop lesions.
Authors: The coronary orifices were examined closely since this was an area of potential catheter damage and an area of clinical significance should thrombus formation occur. Raised lesions were frequently seen in this area in the atherogenic diet fed animals.

Reviewer 1: Inconsistent accumulations of leukocytes on the endothelial surface can be brought about by conditions during the procedure

which have nothing to do with injury, for example, anesthesia and hypoxia. Do the authors attribute the accumulations of leukocytes to change in the endothelium associated with any feature of the angiography procedure?
Authors: We do not associate the accumulation of leukocytes on the endothelial surface of the coronary arteries to the angiography procedure. These accumulations were associated with feeding an atherogenic diet and were similar to those seen by others following cholesterol feeding. Leukocytes were also associated with raised lesions as has been frequently reported. The third area in which we saw accumulations of leukocytes was associated with platelet deposition following catheter injury in the ascending aorta. Polymorphic neutrophils have been associated with platelets following denuding injury to the aorta of dogs [42].

E.A. Spraque: Why do the authors predict that catheterization of animals or patients with more severe disease might have more serious effects? Although this sounds logical, it is not supported by the data within this study.
Authors: Although the catheter induced injury in the ascending aorta and left main coronary artery resulted in the formation of a carpet of platelets and not thrombosis in both group II and group IV animals, we do not know if thrombosis or embolization of plaque may occur when more advanced lesions are injured. Thus we wish to caution against extrapolation of these results to more advanced lesions. We speculate that catheter injury to more advanced lesions could expose deeper components of the vessel wall leading to thrombosis or embolization of plaque material.

E.A. Spraque: In describing Figure 15, you describe subendothelial fibrous structures running parallel as well as transverse to the long axis of the coronary arteries from hypercholesterolemic animals. Are these structures unique to hypercholesterolemic animals and do you know that these are subendothelial structures as opposed to stress fibers known to develop within endothelial cells exposed to shear stress?
Authors: The subendothelial fibrous structures described in Figure 15 were seen only in the hypercholesterolemic animals and only in areas with "very flat" endothelium. It is not known with certainty that these are subendothelial structures but they tend to cross cell borders and are consistent with underlying elastic fibers.

M.Richardson: Why were no detectable morphological effects induced by the angiography fluid?
Authors: There are several reasons why the angiographic fluid in this experiment may not have produced detectable morphological effects. Many studies examining the effects of contrast media are designed to compare different products and consequently do not mimic the clinical setting. The procedure used here mimicked the clinical procedure and was not designed to intentionally induce changes. In this setting the contrast agent may be diluted sufficiently to prevent injury or the time of exposure may be so short that damage does not occur. It is also possible that morphological effects occurred at the time of the procedure but had resolved by 24 h.

M. Richardson: Is it possible that the white cells seen in the coronary artery of one treated animal were consequent to angiography?
Authors: We do not believe the white blood cells seen in the coronary arteries of the one treated animal in group IV were a consequence of angiography. This animal had leukocytes in the right coronary artery (which did not have angiography) as well as the left coronary artery. The white cells were most likely a consequence of hypercholesterolemia since they were also present over much of the surface of two animals in group III.