Aortic Intimal Response to Endothelial Removal in Cebus and Squirrel Monkeys

Gayle A. Hoover and K. C. Hayes

Whereas squirrel monkeys have an inherent susceptibility to atherosclerosis, cebus monkeys are relatively resistant. To assess whether this difference might lie in their response to endothelial injury, the acute morphologic changes in the aortic intima after endothelial removal were examined in the two species. The endothelium of the lower thoracic aorta was removed with an embolectomy catheter, and the intimal response was compared with the uninjured upper thoracic aorta in each monkey. By 21 days after aortic denudation, regrowth of the endothelium (assessed by in vivo Evans' blue dye staining) was significantly greater in squirrel monkeys (90% ± 5% of aortic surface) than in cebus (56% ± 11%). Squirrel monkeys had comparable sudanophilic surface in the nonballooned, control aorta (25% ± 7%) and the ballooned, lower thoracic segment (17% ± 6%). Cebus monkey aortas had no sudanophilia in either segment. The intima/media ratios (IMR) in all regions of the aorta were significantly greater in squirrel monkeys than in cebus, but in both species the IMR of the ventral ballooned segment was two to three times the IMR of the nonballooned control segment. In the dorsal aorta, where endothelial regrowth was more rapid, the IMR was similar to the control aortic segment. By electron microscopy the thickened aortic intima in both species contained a marked increase in modified smooth muscle cells, but lipid accumulation did not result from endothelial removal or regrowth in either species. Thus, although the squirrel monkey aorta had atherosclerotic lesions before endothelial removal, the acute intimal response to endothelial injury was similar in degree and kind in both cebus and squirrel monkeys. This suggests that factors other than those controlling the initial intimal thickening following endothelial injury are responsible for the observed difference in arterial lipid accumulation between cebus and squirrel monkeys. (Arteriosclerosis 4:165-175, March/April 1984)

In studies of nonhuman primate atherogenesis, squirrel monkeys are found to be highly susceptible to both naturally occurring aortic atherosclerosis and to lesions resulting from diet-induced hypercholesterolemia. In contrast, cebus monkeys from the same family (Cebidae) resist the development of atherosclerosis in the wild or in captivity when fed an atherogenic diet. We have recently documented the inherent differences in the aortic wall of these two species at birth and demonstrated differences in susceptibility to naturally occurring atherosclerosis in old age when the cebus monkey aortic intima shows a musculoelastic thickening without lipid accumulation, whereas the squirrel monkey aortic intima is thicker and undergoes changes characteristic of moderately advanced atherosclerosis.

A currently popular theory of atherogenesis proposes that it represents a response of the intima to endothelial damage or dysfunction caused by various injurious stimuli. The hypothesis implies that the difference between squirrel and cebus monkeys in their susceptibility to atherosclerosis stems from differences in the types or amounts of circulating factors inducing endothelial injury, differences in the integrity of the endothelial barrier, or differences in the response of the arterial wall to injury.

Previous studies of diet-induced atherogenesis in these monkeys, cynomolgus monkeys, and patas monkeys have suggested that the circulating lipoproteins are important determinants of the susceptibility or resistance to the disease. This study was designed to examine the possibility that components within the arterial wall or factors controlling them dictate the atherosclerotic response. Accordingly, the endothelium was removed from the lower thoracic aorta in squirrel and cebus monkeys and the intimal response to this injury was compared under conditions of relative normcholesterolemia.
Methods

Monkeys

Eight squirrel and six cebus monkeys, all feral females, were culled from breeding stock eating a commercial diet (Monkey Chow, Ralston Purina Company, St. Louis, Missouri). Their approximate ages, based on dental wear, general body condition, and breeding records, ranged from 10 to 15 years. All were in good health and maintained a constant body weight (the average for squirrel was 620 g, and for cebus, 2060 g). They were caged individually and fed a semipurified diet containing 31% of the calories from corn oil, 19% from protein (lactalbumin), and 50% from carbohydrate without cholesterol for a period of 12 to 14 months before experimentation.

The total circulating cholesterol levels determined in plasma samples 3 weeks before endothelial removal were similar in both species (cebus, 141 ± 25 mg/dl; squirrel, 148 ± 12 mg/dl). The HDL cholesterol levels, assayed in the plasma supernatant after heparin/manganese precipitation of other lipoproteins, was also similar in the two species (cebus, 92 ± 34 mg/dl; squirrel, 78 ± 10 mg/dl). At this time one-half of the monkeys of each species continued to receive the semipurified corn oil diet and one-half were fed a similar diet containing 31% of the calories from coconut oil. Total and HDL plasma cholesterol levels were determined for each monkey at the time of endothelial removal and again at sacrifice. In both cebus and squirrel monkeys, coconut oil feeding induced a moderate increase of the VLDL + LDL cholesterol did not change significantly. Although this increase in plasma cholesterol level was statistically significant (p < 0.05), no significant differences were observed between dietary groups in either species for any of the aortic morphological measures examined. Therefore, in this short-term study, we combined the two dietary groups to compare the arterial response to endothelial removal in the two species independent of diet.

Removal of Aortic Endothelium

To denude the aortic endothelium, the technique of Baumgartner et al., using an arterial embolectomy catheter, was adapted. After an overnight fast, monkeys were prepared for surgery under halothane anesthesia. A thin-walled 4F Fogarty arterial catheter (Edwards Laboratories, Santa Ana, California) for cebus monkeys, or a standard 3F catheter for squirrel monkeys, was inserted via the right femoral artery until the catheter tip was situated in the mid-thoracic aorta. The balloon was inflated with CO₂ to a pressure of 260 mm Hg greater than that required to initiate its distention (approximately 500–600 mm Hg), withdrawn to the bifurcation, deflated, and returned to the mid-thoracic aorta for repetition of the procedure. After the final pass, the femoral artery was ligated and the wound sutured. In preliminary trials with monkeys sacrificed 1 hour after catheter treatment, we determined that five passes in cebus monkeys and eight passes in squirrel monkeys were necessary to ensure complete removal of the endothelium as detected by silver staining of the aorta.

Autopsy Procedure

Based on our preliminary observations of endothelial regeneration in trial monkeys, we chose to evaluate the intimal response 21 days after endothelial removal. This time period allowed for a measurable intimal thickening, yet left a ventral zone of the aorta still uncovered by endothelium to be compared morphologically with the reendothelialized areas.

Each monkey received an intravenous dose of 5 ml/kg of Evan’s blue dye (452 mg/ml in saline, Harvey Laboratories, Incorporated, Philadelphia, Pennsylvania) 40 minutes before sacrifice. Pilot studies with silver nitrate stain indicated that aortic areas excluding Evans’ blue dye (white) were covered with endothelial cells, while areas of dye uptake (blue) lacked an endothelial cover.

A solution of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) was perfused through the left cardiac ventricle for 20 to 30 minutes, followed without interruption by 2% paraformaldehyde and 2.5% glutaraldehyde in the same buffer for 15 to 20 minutes. The aorta was then opened along the ventral surface, dissected from surrounding tissues, and pinned on cardboard. Closeup pictures of each aorta were taken on 35 mm film for color slides (Kodachrome 25, Eastman Kodak Company, Rochester, New York). The pinned aorta was immersed in the second fixative solution overnight at room temperature.

Evans’ Blue Dye Distribution and Endothelial Regrowth

Color slides of the en face aortas in the region between the sixth intercostals and the celiac orifice were projected onto paper at a magnification of × 7 and the outlines of blue areas (denuded) and white areas (nondenuded) were traced, cut, and weighed. The percentage of total surface that was covered with endothelium was thus calculated. The mean values for the two species were compared using Student’s t test after logarithmic transformation of the data.

Microscopic Analysis

Tissue specimens were chosen from the same anatomical sites for each monkey. These tissues included: a full circumference ring from the control, nonballooned aorta just proximal to the orifices of the first intercostal arteries avoiding the ductus arteriosus scar; a full circumference ring at the level of the ninth intercostal arteries (ballooned); and, at the tenth intercostals (ballooned), a longitudinal section through the right orifice that extended half-way up and down to the two adjacent intercostal orifices.
These blocks of aorta were postfixed in 2% (wt/vol) aqueous osmium tetroxide for 90 minutes after several rinses in 0.1 M cacodylate buffer (pH 7.4). They were dehydrated in 2,2-dimethoxypropane and propylene oxide, infiltrated and embedded in Epon 812. Sections 1 μm thick were mounted on glass slides and stained with methylene blue/azure II/basic fuchsin. They were projected by a Zeiss microprojector to a mirror for reflection to the screen of an image analyzer (MOP-3, Zeiss). The outlines of intima and media, separated by the internal elastic lamina (IEL) were traced to obtain the area of the intima relative to the area of the media, or the intima/media ratio (IMR). These data were transformed into logarithms to meet the requirements of normality and linearity for analysis of variance and were tested for statistical significance of the two factors, species and aortic region.

Representative tissue specimens from the nonballooned aorta and from the dorsal and ventral ballooned aorta in each monkey were cut into thin sections and stained with uranyl acetate and lead citrate for study in a Philips EM-300 electron microscope.

**Gross Sudanophilia**

The remainder of each aorta was stained for fat with Sudan IV. The percentage of surface covered by sudanophilic areas in the control, nonballooned region between the level of the first and third intercostals, and in the ballooned region between the level of the sixth intercostal orifices and celiac orifice was determined under a dissecting microscope with the aid of a 10 × eyepiece fitted with a lattice of 121 points. The region between the third and sixth intercostals was not included because the original line of injury in this area could not be detected with certainty after 21 days. The aorta was held between two glass slides and the surface was scanned systematically with the lattice. The number of points hitting a sudanophilic area was divided by the total number of points counted (1500–3000) to obtain the percentage of sudanophilic surface.

**Results**

**Extent of Endothelial Regrowth**

The pattern of aortic endothelial regrowth in both squirrel and cebus monkeys was similar to that described in rats and rabbits. Regenerating endothelial cells spread from uninjured areas in the proximal aorta and dorsally situated intercostal orifices. Regrowth was most rapid in the longitudinal direction parallel to blood flow. In all cebus and one-half the squirrel monkeys, the ventral aortic surface was still uncovered 21 days after balloon embolectomy.

Notably, the regrowth of endothelium in squirrel monkey aortas was significantly faster than in cebus monkeys (Table 1). The thoracic aortas in one-half of the squirrel monkeys were entirely covered by endothelium, while 62% to 90% regrowth occurred in the remainder, with denuded patches observed along the ventral surfaces or in the mid-dorsal regions (Figure 1A). On the other hand, no cebus monkey aorta was fully recovered with endothelium after 21 days.

**Table 1. Reendothelialized Surface and Sudanophilia of Descending Thoracic Aortas 21 Days after Endothelial Removal In Squirrel and Cebus Monkeys**

<table>
<thead>
<tr>
<th>Species</th>
<th>No.</th>
<th>Balloon</th>
<th>Nonballoon</th>
<th>Reendothelialized surface (% thoracic aorta)</th>
<th>Sudanophilic surface (% thoracic aorta)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squirrel</td>
<td>8</td>
<td>90 ±5</td>
<td>17 ±6</td>
<td>25 ±7</td>
<td></td>
</tr>
<tr>
<td>Cebus</td>
<td>6</td>
<td>56 ±11</td>
<td>0 ±0</td>
<td>1 ±1</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE.

*Significant difference between species (p < 0.05).

Figure 1. Photographs (with line drawings placed above photographs for clarity) of thoracic aortic surface depicts distribution and intensity of Evans' blue in squirrel (A) or cebus monkeys (B). Note the uniform regrowth from intercostal arteries typical of cebus at 21 days and the somewhat irregular pattern associated with this particular squirrel monkey. Blood flow in both pictures was from right to left. Bar = 5 mm.

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The extent of endothelial recovery varied widely (20% to 88%) among individual cebus monkeys. A typical cebus aorta is depicted in Figure 1 B.

**Gross Sudanophilia**

Seven of the eight squirrel monkey aortas demonstrated sudanophilia to a variable extent. In individual squirrel monkey aortas the ballooned area had approximately the same or less sudanophilia compared to the nonballooned, proximal thoracic area. However, the extent of sudanophilia was not statistically different between the two areas (Table 1). Furthermore, there was no relationship between still uncovered or reendothelialized intima and sudanophilia in ballooned aortas of either species. In cebus monkeys none of the six aortas was sudanophilic in any region, except for a lightly stained area evident in the vicinity of the first and second intercostal orifices in one monkey.

**Light Microscopic Observations**

**Squirrel: Nonballooned Intima**

The degree of intimal thickening in the control, upper thoracic region of squirrel monkey aortas was greater, but more variable, than in control specimens of cebus monkey aortas. In general, the features of the squirrel monkey nonballooned aortic intima resembled those previously described, consisting of two to four layers of longitudinally oriented smooth muscle cells. These cells were typically surrounded by rings of intensely stained connective tissue. Extracellular lipid vacuoles near the IEL were sometimes evident, and in one aorta numerous cholesterol clefts appeared. Occasionally, lipid vacuoles within smooth muscle cells were also seen. Thus, naturally occurring aortic intimal thickening and lipid accumulation were present to some degree in these squirrel monkeys before endothelial removal.

**Squirrel: Ballooned Intima**

Sections from the balloon-injured thoracic aortas of squirrel monkeys displayed two distinct zones within the intima which was two to three times thicker than the nonballooned control intima (Figure 2, inset). The deeper zone consisted of many tightly packed smooth muscle cells arranged parallel to the vessel axis with densely stained connective tissues between cells. The superficial zone contained a more lightly stained connective tissue matrix in which smooth muscle cells had no particular orientation and their density appeared much less than those in the deeper zone. The relative thickness of the two zones varied around the circumference of the vessel and between sections. In some places, especially the dorsal aortic surfaces that were reendothelialized first, the superficial zone was very thin, whereas in the ventral area it comprised most of the intima. A few smooth muscle cells with lipid vacuoles were noticed in both the superficial and deep zones together with extracellular lipid vacuoles at the IEL, but no relationship between the presence of lipid and any area of aorta could be ascertained.

**Cebus: Nonballooned Intima**

Sections from the nonballooned region of cebus monkey aortas displayed a slight degree of diffuse intimal thickening, consisting predominantly of elastin in thin sheets above the IEL with smooth muscle cells scattered between the elastic layers, as previously described in a similar group of cebus monkeys not subjected to experimental manipulation. In some spots around the circumference, the endothelium lay directly on the thin, but distinct, IEL.

**Cebus: Ballooned Intima**

In contrast to the control area, the balloon-injured region of cebus monkey aortas was characterized by an intimal thickening approximately double that of its nonballooned counterpart (Figure 3, inset). The smooth muscle cells were usually oriented with their long axis parallel to the vessel, but in some places their direction was random. They were surrounded by lightly stained connective tissue. In some sections, layers of thin elastin lay in the deeper portion of the intima above the IEL. The thickness of the intima gradually increased from the dorsal to the ventral area. The dorsal portions, where reendothelialization occurred first, resembled the control area with minimal intimal thickening.

**Intima/Media Ratios**

The visual impression of a thicker intima in the ventral portion of the denuded aortic segment was confirmed by measurements of the IMR. In both species, the mean IMR of the ventral ballooned area was approximately twice that of the dorsal area. The IMRs of the nonballooned control areas (dorsal and ventral) were similar and approximated the thickness of the rapidly covered dorsal ballooned areas (Table 2). In squirrel monkeys aortic sections of all regions, the mean IMR was two to three times greater than in cebus monkeys. However, the increase in the IMR of ballooned ventral areas (i.e., neointima) relative to the original IMR of the nonballooned area was similar in the two species (about 250%), implying that the neointimal thickening following endothelial removal was comparable (Table 2).

**Electron Microscopic Observations**

**Squirrel: Nonballooned Intima**

The numerous intimal smooth muscle cells observed in the nonballooned region of squirrel monkey aortas were rich in myofilaments, sometimes containing lipid droplets, and were surrounded by tightly packed collagen fibers. Electron-dense pleomorphic lipid particles and amorphous lipid droplets were located in the extracellular space near the IEL in many sections.
Figure 2. Squirrel monkey 21 days after endothelial removal. Portion of the intima from the ventral aorta reveals a superficial neointima (top) containing transversely oriented, modified smooth muscle cells (SMC) with many organelles. Basement membrane-like material (BML) in an amorphous ground substance surrounds the cells in this zone. The deeper zone (bottom) contains longitudinally oriented smooth muscle cells with dense myofilaments surrounded by collagen fibers (C) and granular debris (G). Bar = 2 \mu m. Inset. Light micrograph of transverse section illustrates the tightly packed intimal smooth muscle cells in the deeper zone near the internal elastic lamina and the more loosely constructed superficial zone thought to represent neointimal proliferation following endothelial removal. Bar = 10 \mu m.
Figure 3. Cebus monkey 21 days after endothelial removal. Neointima from the ventral aorta shows a marked proliferation of modified smooth muscle cells (SMC) which have many cytoplasmic organelles, especially dilated rough endoplasmic reticulum (RER) and a paucity of myofilaments in the periphery (M). Large amounts of basement membrane-like material (BML) fill the interstitial space. Bar = 2 μm. Inset. Light micrograph depicts the marked intimal thickening. Bar = 10 μm.

Table 2. Intima/Media Ratios (IMR) of Descending Thoracic Aortas 21 Days after Endothelial Removal in Squirrel and Cebus Monkeys

<table>
<thead>
<tr>
<th>Species</th>
<th>No.</th>
<th>Ballooned area (Intima/media ratio)</th>
<th>Nonballooned area (Intima/media ratio)</th>
<th>IMR relative increase (%) (neointima)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squirrel</td>
<td>8</td>
<td>0.26 ± 0.04†</td>
<td>0.15 ± 0.02</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td>Cebus</td>
<td>6</td>
<td>0.09 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.04 ± 0</td>
</tr>
</tbody>
</table>

*Neointima = ballooned ventral IMR/nonballooned control IMR x 100.
†Values represent means ± se. ANOVA indicated that differences between species and areas were significant (p < 0.05). Additional tests for comparisons between means (Student–Neuman–Keuls) indicated that for each species the nonballooned, control IMR was significantly different (p < 0.05) than the Ventral LogIMR, but not the Dorsal LogIMR.
Figure 4. A. Portions of flattened endothelial cells in a white dorsal area of a cebus monkey aorta 21 days after denudation are joined by a junctional complex (arrow). The cytoplasm contains numerous lysosome-like dense bodies (DB) and pinocytotic vesicles (PV). Clusters of microfilaments (stars) are located along the basal margin. The subendothelial space contains abundant amorphous (A) and reticulated (R) basement membrane components. Bar = 0.5 μm. B. Newly formed endothelial cells (ENDO) from the white ventral aorta of a squirrel monkey are rounded and bulge into the lumen. Bar = 1 μm.

Cebus: Nonballooned Intima

In the nonballooned control region of cebus monkey aortas observed by electron microscopy, the intima contained several interrupted layers of electron-lucent elastin between the IEL and the lumen. Occasionally, well-differentiated smooth muscle cells were interspersed between these layers. Collagen fibers filled the rest of the extracellular space. There was no evidence of lipid deposition anywhere.

Squirrel and Cebus: Ballooned Intima

The intimal characteristics of thoracic aortas in both species 21 days after endothelial removal were qualitatively similar by electron microscopy. In white areas the cells lining the lumen had characteristics typical of endothelium (Figure 4 A). They were flat and elongated with interdigitating margins and junctional complexes. The cytoplasm contained fine basal filaments. Electron-dense lysosome-like bodies were also observed. The occasional presence of rod-shaped, tubular (Weibel-Palade) bodies confirmed the identity of these cells as endothelium. In some sections from ventral areas of squirrel monkey aortas, endothelial cells were not flat, but bulged into the lumen (Figure 4 B), a feature characteristic of regenerating endothelial cells.
The intima of ballooned aorta that was still denuded at 21 days had cells lining the lumen resembling smooth muscle cells (Figure 5). These cells were loosely associated with each other, with large gaps between them. They were usually ovoid with the bulk of their nucleus and cytoplasm embedded in the intima (Figure 5 A). Their luminal surface was sometimes smooth, but in other places cytoplasmic processes extended into the lumen. The cells were surrounded by an incomplete basement membrane on all surfaces, including the luminal aspect (although this was not always readily apparent). Single platelets, showing no degranulation and minimal shape changes, loosely adhered to these cells in a few places (Figure 5 B). In squirrel monkey aortas, but not cebus, macrophages were infrequently observed on the surface or in the superficial layers of the intima in denuded areas.

The neointima of cebus monkey aortas and the superficial zone of squirrel monkey aorta consisted of many modified smooth muscle cells containing abundant cytoplasmic organelles, especially rough endoplasmic reticulum, and were surrounded by incomplete basement membranes (Figures 2, 3). Myofilaments were concentrated at the periphery of the cytoplasm. The nucleus often displayed one or more prominent nucleoli. Occasionally, smooth muscle cells were seen in the fenestrae of the IEL. A large amount of fibrillar basement membrane-like material in an amorphous matrix filled the extracellular space (Figures 2, 3). This interstitium presumably contained glycosaminoglycans, as determined pre-
viously by ruthenium red-positive material distributed throughout the connective tissue matrix of aortic intimas in these two species (Westmoreland and Hayes, unpublished). Others27 have demonstrated proteoglycans in the fibriullar connective tissues in proliferative lesions of injured aorta in pigtail monkeys.

In squirrel monkey aortas, deeper intimal zones near the IEL resembled the nonballooned, control intima. Smooth muscle cells showed a predominance of myofilaments throughout the cytoplasm, few organelles, and thick basement membranes (Figure 2). Tightly packed collagen fibers filled the extracellular space in this deeper zone. Lipid vacuoles were sometimes encountered within smooth muscle cells of both the intima and inner media of squirrel monkey aortas, but these were not a regular feature and were not related to denuded or nondenuded areas. Pleomorphic drops of extracellular lipid were often evident in the vicinity of the IEL in squirrel monkey aortas, whereas no lipid was ever detected in sections from cebus monkeys. Because the deep intimal zones of the balloon ed regions in squirrel monkey aortas bore the same morphological features as the nonballooned areas, we assumed these deeper zones represented natural intimal thickening before balloon embolectomy.

Discussion

This study examined the short-term response of the thoracic aortic intima to mechanical endothelial removal in two species of nonhuman primates differing in their inherent susceptibility to atherosclerosis on the premise that disrupting the endothelial barrier might give insights into their differing susceptibility. Three weeks after removal of endothelium in the lower thoracic aorta, the increase in neointima relative to media in both species was approximately two to three times the adjacent, nonballooned control values. As demonstrated in rats26, 28 and rabbits,24 the fibriullar thickening of the intima in both squirrel and cebus monkeys was greater in the ventral regions of the aorta than in the dorsal areas. Since dorsal areas were first to be recovered by regenerating endothelial cells, the prolonged exposure of the ventral intima resulted in greater intimal proliferation.

In these monkeys, intimal thickening was not maximal nor enhanced at the junction between endothelialized and nonendothelialized intima, unlike that reported in the healing rabbit aorta where the thickest, lipid-laden portion of the intima lies under the edge of the regenerating endothelial sheet.29 Qualitatively, the morphological characteristics of the neointima were also similar in cebus and squirrel monkeys and resembled the acute intimal response to mechanical endothelial removal described in other species.24, 26, 28, 30 The characteristics of the nonballooned upper thoracic aortas of these monkeys confirmed our previous observations of naturally occurring atherosclerosis in squirrel monkeys in contrast to the mild, diffuse intimal thickening without lipid deposition in aortas of cebus monkeys.10

Lipid accumulation in the neointima following endothelial removal has not been a usual feature in normocholesterolemic pigs,31 rats,28 rabbits,24 or monkeys,30 although balloon injury in rabbit aortas does enhance cholesterol deposition in reendothelialized intima at the transition zone between blue and white areas under conditions of both normal and elevated serum cholesterol levels.29, 32

In the present study, ballooning and reendothelialization did not induce lipid accumulation in cebus monkey neointima, assessed microscopically or by Sudan staining. Furthermore, similar amounts of lipid observed in ballooned (reendothelialized or denuded) and nonballooned areas of squirrel monkey intima suggest that in this 3-week time interval lipid deposition was not enhanced by endothelial removal-regeneration and may even have been reduced (in the neointima) to the level observed in cebus.

Thus, despite the differences in aortic intimal characteristics before endothelial removal and the dissimilar rates of endothelial regrowth, the intimal response to injury was similar in degree and quality at 21 days in both species under conditions of normocholesterolemia. The implication is that the acute response to injury (at least to the degree imposed by ballooning), as measured by smooth muscle cell proliferation and intimal characterization, did not differ between species and does not appear to explain their differences in atherogenic potential. On the other hand, the comparable nature of their intimal response following endothelial removal suggests that the intact endothelium in these two species may ultimately contribute to their distinct intimal characteristics.

While the aortic intimal response to endothelial removal was similar in the two species, the rate of endothelial regrowth was slower in cebus than in squirrel monkey aortas, where recovery was nearly complete by 3 weeks after removal. This amount of time was shorter than the rate of recovery in the rat, where normal endothelial regrowth is not complete until 6 weeks,26 or in the rabbit, where endothelial regeneration ceases after 2 weeks33 and normal aortic impermeability to Evan's blue dye may not be achieved for as long as 36 weeks after removal of endothelium.24 On the other hand, the rate of endothelial regrowth in the squirrel monkey aorta is comparable to that in pig aortas, which reendothelialize within 28 days.31 Because regenerating endothelial cells may modify the metabolism of the underlying intimal cells, as has been demonstrated in rabbits,24 and lipid and glycosaminoglycans content is greater under newly formed endothelium than in denuded areas,32, 35-37 the implication again is that the endothelial barrier may influence the ultimate, if not the initial, lesion resulting from balloon injury.

The observations in rabbits29, 32 suggest that the process of reendothelialization is associated with in-
creased intimal cholesterol deposition. Thus, one might infer that lipid accumulation in squirrel monkey intima is related to excessive endothelial cell turnover and its observed ability for rapid reendothelialization. However, in this acute response to injury, where squirrel monkey endothelial recovery was more rapid than in cebus and neointimal proliferation was equal to or exceeded that of cebus, no association with visible lipid accumulation was detected. Furthermore, cebus reendothelialize much more rapidly than rabbits, but ordinarily resist experimental attempts to induce intimal lipid accumulation. Accordingly, the endothelial recovery rate is probably not the key determinant of intimal lipid deposition.

By the same token, it has been hypothesized that the extreme susceptibility of the rabbit to atherosclerosis may reflect prolonged intimal exposure due to excessive endothelial cell turnover. However, the opposite would seem true in these primate models where the species experiencing the more rapid reendothelialization, and presumably experiencing a shorter intimal exposure, is documented as being more susceptible to atherosclerosis. As suggested, the key may reside with the "spontaneous" rate of the endothelial cell loss and regeneration and relative limitations in the replicative lifespan of endothelial cells between species. In any event it is likely that a period longer than 3 weeks may be required to elicit species differences among monkeys or that hypercholesterolemia must accompany the injury. It is also possible that mechanisms of atherogenesis in rabbits and primates are not entirely comparable.

Acknowledgments

We are grateful for the helpful discussions and advice of Christopher C. Haudenschild, Marcello Pagano, and Michael B. Stemerman.

References

32. Falcone DJ, Hajar DP, Minnick CR. Enhancement of chole-

Index Terms: endothelium • aortic denudation • atherosclerosis • monkeys

Omission

In the January/February 1984 issue, the abscissa of Figure 4 to the article by Khoo et al. was omitted during the printing process (Khoo JC, Vance JE, Mahoney EM, Jensen D, Wancewicz E, Steinberg D: Neutral Triglyceride Lipase in Macrophages. Arteriosclerosis 1984; 4:34–40). The Editors regret this omission and reprint the corrected figure below.

![Corrected Figure 4](http://atvb.ahajournals.org/)

**Figure 4.** The influence of pH on the activities of triglyceride lipase in homogenates prepared from J774 cells (○), rabbit alveolar macrophages (●), human monocyte-macrophages (▲), and resident mouse peritoneal macrophages (■). The buffer used for the assays was 50 mM Hepes.
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