Characteristics of the Aortic Intima in Young and Old Cebus and Squirrel Monkeys


To document naturally occurring aortic intimal changes with age in squirrel and Cebus monkeys, the aortic lipid class composition, histology, and fine structure were quantitatively compared in the two species at birth and in old age. The aortic intima plus inner media in the young squirrel monkey contained more lipid, particularly in the phospholipid and cholesterol ester fractions than the young Cebus. The lipid class composition of the old Cebus monkey aorta resembled that of the young Cebus. In the old squirrel monkey aorta, cholesteryl ester, and to a lesser extent, free cholesterol were increased over young levels, while the phospholipid concentration tended to be lower. The aortic cholesteryl ester:phospholipid ratio increased with age in both species, but the old Cebus monkey aorta maintained the ratio below unity at 0.3, whereas the old squirrel monkey aorta ratio was 2.5. The abdominal aorta of the old squirrel monkey tended to have more lipid in each class than the thoracic segment. Morphologically, the old Cebus monkey aortic intima was similar to the young Cebus in terms of the intima:media ratio, intimal cellularity, and the distribution of intimal components determined by points in electron micrographs. In both age groups the Cebus monkey aorta was characterized by diffuse intimal thickening without lipid deposits. In contrast, the old squirrel monkey aorta had a much greater intima:media ratio, especially in the abdominal aorta, and a greater intimal cellularity than the young squirrel monkey. The distribution of intimal components in electron micrographs of the old squirrel monkey aorta shifted to a predominance of extracellular lipid, smooth muscle cells, and collagen. Deposits of small dense granules, presumably the products of cellular breakdown, were observed in aortic intimas and medias of both species in old age. Thus, differences between Cebus and squirrel monkey aortic intimas were evident at birth. By old age, the Cebus monkey aortic intima remodeled without accumulating lipid, whereas the squirrel monkey developed aortic intimal lesions resembling human atherosclerosis. (Arteriosclerosis 2:252–265, May/June 1982)
In contrast, Cebus monkeys, from the same family (Cebidae) as squirrel monkeys, resist developing aortic sudanophilic or raised lesions, in the wild or when fed a basal diet in captivity. The normal aortic intima of the Cebus monkey is characterized by a benign musculoelastoc thickening. Although these intimal characteristics are not readily changed by diets containing saturated fat and cholesterol, atherosclerotic lesions have been induced by feeding large amounts of cholesterol with saturated fat for a prolonged period of time. Yet age also appears to be a factor, since mature but not juvenile Cebus monkeys develop lesions under these dietary conditions. Yet the extent and severity of diet-induced aortic atherosclerosis is much less in Cebus than in squirrel monkeys with comparable degrees of hypercholesterolemia.

To systematically document specific naturally occurring aortic intimal changes with age in Cebus and squirrel monkeys, the present study quantitatively compared the lipid class composition, histology, and fine structure of the aortic intima in infant and old monkeys. The findings indicate that inherent differences existed in the aortas of the two species at birth and that, by old age, the squirrel monkey aorta developed intimal lesions resembling human atherosclerosis, while the aorta of the Cebus monkey retained characteristics of diffuse intimal thickening.

Methods

Monkeys and Diets

Young monkeys were obtained from our primate colony and included 12 Cebus (Cebus albifrons) and 11 squirrel (Saimiri sciureus) monkeys of both sexes, ranging in age from near term fetuses to 5 months postnatal. These monkeys were either still with their nursing dams or were maintained on Similac, Isomil (Ross Laboratories, Columbus, Ohio), or a semipurified liquid formula diet containing corn oil as the fat source for 45% of the calories. The old animals included eight Cebus (Barranquilla) and 11 squirrel (Leticia) monkeys, all females culled from the breeding colony. They had been eating Monkey Chow (Ralston Purina Company, St. Louis, Missouri), which has 12% of its calories in fat, mainly soybean oil, but some had been switched for less than 1 year from Monkey Chow to a semipurified basal diet with 31% of the calories from corn oil. Their ages, based on dental wear, general body condition, and breeding records, ranged from 12 to 20 years.

Aortic Lipid Composition Analysis

Monkeys available for aortic lipid quantitation and free of clinically or grossly detectable kidney or other disease were sacrificed under sodium pentobarbital anesthesia (30 mg/kg). Aortas were dissected and rinsed in physiological saline. With the aid of a dissecting microscope, the adventitia and outer media layers were carefully stripped away. The remaining intima-inner media layers of adult monkey aortas were divided into thoracic and abdominal segments, whereas the small young aortas were left whole. All aortas were stored at -4°C under nitrogen atmosphere until analyzed.

Aortas were homogenized in saline and aliquots taken for lipid extraction by the addition of 10 ml of methanol followed by 20 ml of chloroform to yield a final solvent to sample ratio of 40:1. The mixture was centrifuged at 2000 × g for 15 minutes at 4°C, the supernatant transferred to another tube, and the pellet reextracted four times with chloroform:methanol (1:1). After each centrifugation, the combined supernatants were filtered through Whatman No. 1 filter paper (Fisher Scientific, Medford, Massachusetts), evaporated under nitrogen gas, and resuspended in benzene. To ensure only minimal lipid loss, aqueous rinses of the lipid extract were not included.

Lipid classes were separated on thin-layer chromatographic plates, which were developed in the following solvent systems: 1) hexane to top of plate, 2) benzene to top of plate, and 3) hexane:diethyl ether:acetic acid (70:31:1) to within 8 cm of the top. The third solvent was utilized to ensure complete separation of the cholesteryl ester fraction from hydrocarbons.

After development, the free cholesterol, free fatty acid, triglyceride, and cholesteryl ester spots were visualized by iodine vapor, scraped into test tubes, and quantified by the charring method of Kritchevsky et al. The cholesteryl ester fraction of young aortas, which was too low to be analyzed by the above method, was quantitated by thin-layer chromatography charring densitometry. Phospholipid spots, which remained at the origin, were quantitated as lipid phosphorus by the method of Bartlett using a factor of 25 to convert to phosphatidylcholine.

Morphological Analysis

Epon-embedded aortic tissues were collected from monkeys killed over several years. Aortas were selected with concern for age of monkey, diet fed, and freedom from obvious disease. For some monkeys, records included total cholesterol values determined in plasma samples collected at the time of sacrifice.

The method for preserving tissue was as follows: monkeys were sacrificed under sodium pentobarbital anesthesia by perfusion via the left cardiac ventricle with a solution of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) (quarter-strength Karnovsky’s fixative). Pressure was maintained at 120 mm Hg for 15 minutes followed by a second 10- to 15-minute perfusion of half-strength Karnovsky’s fixative. The aorta was then opened, carefully dissected free, and pinned on...
cardboard for an additional 2-hour fixation in half-strength Karnovsky's fixative. In the case of a few neonatal monkeys that could not be fixed by perfusion, the fresh aorta was quickly opened, removed, pinned to in vivo dimensions on cardboard, and immersed in half-strength Karnovsky's fixative for 3 hours at room temperature.

After fixation, four (for a young monkey) or eight (for an old monkey) 3 x 5 mm blocks of tissue, in both longitudinal and transverse orientations, were taken from the upper thoracic aorta (arch and proximal descending thoracic), and a similar number were taken from the abdominal aorta below the renal arteries. The blocks (encompassing approximately 50% of the areas sampled) were chosen to avoid branch orifices and the ductus arteriosus scar, but otherwise were selected arbitrarily without regard to surface characteristics, which were relatively uniform in all cases. Gross evidence of atherosclerosis, such as fatty streaks or raised lesions, were not detectable in the unstained aortas.

The tissue blocks were washed overnight in 0.1 M cacodylate buffer, postfixed for 90 minutes in 1% (w/v) osmium tetroxide in 0.1 M cacodylate buffer, rinsed in distilled water, dehydrated in graded concentrations of ethanol, equilibrated in propylene oxide, and embedded in Epon 812.

Light Microscopy

Sections of 1μ thickness mounted on glass slides were stained with methylene blue-azure II-basic fuchsin. Photomicrographic transparencies were taken of each section using a 10X objective. These were projected by a standard procedure onto paper to trace outlines of intima and media. The intima was defined as that area of the wall extending from the lumen to the internal elastic lamina (IEL). In the case of sections from the upper thoracic aorta, where the IEL was often indistinct and multiple layers of intimal elastin were observed, the intima was identified by the orientation of the smooth muscle cells in a direction perpendicular to those of the media. The media included the area between the IEL and the adventitia. Tracings of intima and media were cut out and weighed and the ratio of the weights taken as an index of intimal thickness. The area of each intima (measured in grams) was converted to units of (100 μ)² by use of a conversion factor obtained by weighing a paper "cutout" of a known area. Smooth muscle cell nuclei in the intima of each defined area were counted at a magnification of 100X. Intimal cellularity was thus calculated as the total number of smooth muscle cell nuclei per section divided by the intimal area of that section.

To describe the size of the wall, the thickness of the media of each section was measured with a micrometer in the eyepiece of the microscope, and the number of medial lamellar units per section were counted at 2 points and averaged.

Electron Microscopy

Based on the light microscopic findings, we chose one block from the upper thoracic aorta and one block from the lower abdominal aorta of each monkey that had an intima:media ratio and a smooth muscle cell density closest to the mean of all blocks from the respective aortic area. Silver sections were cut from these blocks on a Sorvall Porter-Blum MT-1 ultramicrotome with a diamond knife and double-stained with uranyl acetate and lead citrate for viewing in a Phillips EM-300 electron microscope at an acceleration voltage of 60 kV.

For point counting purposes, the entire length of intima of each section was photographed on four to five plates at a magnification of approximately 3000X. The complete width of the intima was photographed on each plate unless it was too wide to be covered by a single plate, in which case overlapping pictures were taken to cover the full thickness and a composite picture constructed for counting. Plates were enlarged 2.5 times for printing at a final magnification of 7500X. A calibration grid was photographed with each set of plates to ensure a constant magnification. A lattice similar to the multipurpose test system described by Weibel30 consisting of points positioned at half-inch intervals was superimposed over each electron micrograph. Counts were made of points hitting the various components of the intima from the lumen up to and including the internal elastic lamina identified in the 1 μ section by light microscopy. Counts from all micrographs were summed (approximately 1000 points per section) to calculate percentages of each intimal component.

Statistical Analysis

To compare the aortic lipid composition of the two age groups, a weighted average of the thoracic and abdominal aortic concentrations of each lipid class was used as an estimate of the total aortic lipid concentrations for old monkeys. Aortic lipid class data were assessed by analysis of variance31 in a two-factor design comparing species and age group, or species and aortic segment. The data derived from measurements on 1 μ sections and electron micrographs were analyzed by multiple regression. To meet the requirements of normality and linearity for these analysis, all data were first transformed into logarithms. Examination of the residuals indicated that this transformation provided the best fit of the data to the model.

Results

Even though all monkeys were not eating the same basal diet, the diets were sufficiently alike so that differences due to diet could not be detected in any of the measures assessed.
**Plasma Cholesterol**

Within the young monkey group, mean total plasma cholesterol value of four Cebus (153 ± 23 mg/dl) was similar to that for eight squirrel monkeys (190 ± 42 mg/dl). These values were comparable to plasma cholesterol levels from four old Cebus (153 ± 27 mg/dl) and three old squirrel monkeys (160 ± 33 mg/dl).

**Aortic Lipid Composition**

Statistical analysis revealed significant differences between the two species and between the two age groups in lipid concentrations of the total aorta (table 1). Young squirrel monkey aortas contained twice the mean concentration of phospholipid and four times the level of cholesteryl ester of aortas in young Cebus monkeys. In old squirrel monkeys, the mean aortic cholesteryl ester concentration was six times the young squirrel monkey level. In addition, the aortic phospholipid concentrations of old squirrel monkeys was approximately half that of the young level, resulting in a mean cholesteryl ester:phospholipid ratio of 2.46. These findings were in marked contrast to those in old Cebus monkey aortas, in which mean cholesteryl ester concentration was only minimally increased over young values, thus keeping the cholesteryl ester:phospholipid ratio well below unity. Although both species in the old group had higher free cholesterol concentrations than in the young group, the difference was most pronounced in old squirrel monkey aortas where the mean content was more than double the young average. Free fatty acid levels of aortas were reduced in old age in both species, but the triglyceride concentration remained unchanged, squirrel monkey aortas having more triglycerides than Cebus in both age groups.

Comparison of thoracic and abdominal lipids within the old group (table 1) revealed a similar composition in both segments in Cebus monkey aortas. On the other hand, abdominal aortas of squirrel monkeys had higher lipid concentration in all classes than the thoracic, but due to wide individual variation these differences were not statistically significant.

**Light Microscopic Observations**

Examination of 1 μm sections from the upper thoracic and lower abdominal aortas revealed marked differences between Cebus and squirrel monkeys in both young and old groups. Young squirrel monkeys had intimas of endothelial cells resting directly on the IEL or, as encountered in the upper thoracic segment, separated from the IEL by a thin layer of connective tissue containing an occasional smooth muscle cell (figure 1, inset). Intimas in the upper thoracic aortas of young Cebus monkeys were thickened by as many as four layers of elastin and smooth muscle cells (figure 2A). The abdominal aortic intimas of young Cebus monkeys sometimes contained one or two layers of smooth muscle and elastin above a prominent IEL. Although aortic intimas in young Cebus and squirrel monkeys were similar in young and old groups, the difference was most pronounced in old squirrel monkey aortas where the mean content was more than double the young average. Free fatty acid levels of aortas were reduced in old age in both species, but the triglyceride concentration remained unchanged, squirrel monkey aortas having more triglycerides than Cebus in both age groups.

Comparison of thoracic and abdominal lipids within the old group (table 1) revealed a similar composition in both segments in Cebus monkey aortas. On the other hand, abdominal aortas of squirrel monkeys had higher lipid concentration in all classes than the thoracic, but due to wide individual variation these differences were not statistically significant.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol (mg/g wet wt)</th>
<th>Free fatty acid (mg/g wet wt)</th>
<th>Triglyceride (mg/g wet wt)</th>
<th>Cholesteryl ester (mg/g wet wt)</th>
<th>Phospholipid (mg/g wet wt)</th>
<th>Total lipid (mg/g wet wt)</th>
<th>Cholesteryl ester:phospholipid ratio</th>
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</tr>
<tr>
<td>Young Cebus</td>
<td>6</td>
<td>2.07 ± 0.40</td>
<td>2.62 ± 0.80</td>
<td>0.37 ± 0.06</td>
<td>0.28 ± 0.15</td>
<td>2.24 ± 0.30</td>
<td>7.57 ± 1.34</td>
</tr>
<tr>
<td>Squirrel</td>
<td>7</td>
<td>2.30 ± 0.24</td>
<td>2.10 ± 0.56</td>
<td>0.51 ± 0.08</td>
<td>1.17 ± 0.57</td>
<td>5.56 ± 1.25</td>
<td>11.65 ± 2.14</td>
</tr>
<tr>
<td>Old Cebus</td>
<td>4</td>
<td>3.23 ± 0.59</td>
<td>0.45 ± 0.04</td>
<td>0.33 ± 0.10</td>
<td>0.90 ± 0.44</td>
<td>2.32 ± 0.65</td>
<td>7.22 ± 1.57</td>
</tr>
<tr>
<td>Squirrel</td>
<td>7</td>
<td>5.78 ± 1.26</td>
<td>1.24 ± 0.21</td>
<td>0.45 ± 0.04</td>
<td>6.46 ± 2.71</td>
<td>2.64 ± 0.35</td>
<td>16.07 ± 3.41</td>
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<tr>
<td>Significant variables (p &lt; 0.05)</td>
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<td>A</td>
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<td>S</td>
<td>A</td>
<td>S</td>
<td>S</td>
<td>A</td>
</tr>
</tbody>
</table>

Values represent means ± SE. A = age; S = species; AS = aortic segment; NS = no significant difference; × denotes interaction.
Figure 1. Young squirrel monkey thoracic aorta. The internal elastic lamina (IEL) is scalloped on the luminal aspect with bundles of amorphous elastin (AE) scattered among collagen fibers (C). The endothelial cells have numerous organelles. Two to three layers of interrupted endothelial basement membrane (BM) are apparent. Two pleomorphic membranous sacs (arrows) are evident in the otherwise clean, narrow intima. x 16,200.  
Inset. Light micrograph depicts the close association of endothelium with the internal elastic lamina. x 800.

Figure 2. A. Young Cebus thoracic aorta. Longitudinally oriented thick section illustrates the arrangement of the intimal elastic layers parallel to the long axis of the vessel as opposed to the transverse direction of the media lamellae. x 1000.  
B. Old Cebus thoracic aorta. The muscleelastic thickening of the intima, like that in the neonate, is distinguished from the media by the orientation of smooth muscle cells and elastic laminae. x 1000.  
C. Young Cebus thoracic aorta. The intima is thickened by layers of smooth muscle cells (SMC) separated by discontinuous layers of amorphous elastin (AE). The modified smooth muscle cells display abundant amounts of rough endoplasmic reticulum (RER) and other organelles. In the extracellular space are collagen fibers (C) and fibrillar elastin (FE) in an amorphous ground substance. The top of the internal elastic lamina (IEL) is evident in the lower part of the micrograph. x 5800.  
D. Old Cebus thoracic aorta. The intimal thickening bears characteristics similar to that of the young Cebus except that connective tissue components are more compacted, the amorphous elastin (AE) is present as continuous bands and the intimal cells have more typical smooth muscle cell characteristics (SMC). Aggregates of extracellular dense bodies (arrows) are observed along the luminal aspect of amorphous elastic bands. The absence of extra- and intracellular lipid is noteworthy.  
C = collagen; IEL = internal elastic lamina. x 5800.
Figure 3. Old squirrel monkey abdominal aorta. This transverse section reveals a cap of smooth muscle cells (SMC) and connective tissues covering necrotic debris and extracellular lipid, both in pools (L) and pleomorphic electron dense particles, adjacent to the internal elastic lamina (not shown). Minimal amounts of intracellular lipid (IL) are present. A halo of fibrillar elastin and collagen (arrows) surrounds the reactive smooth muscle cells. $\times$ 3600. Inset. The thickness of the intima with its many smooth muscle cells and connective tissue contrasts sharply with the narrow intima of the young squirrel monkey in figure 1. $\times$ 800.
bus monkeys were thicker than in young squirrel monkeys, the size of intima relative to media (intima: media ratio) was small in both species and in both segments of the aorta (table 2). However, the layers of smooth muscle cells in Cebus monkey aortic intimas of the upper thoracic segment resulted in a threefold greater value for mean intimal cellularity than in young squirrel monkeys and a 10-fold greater value in the lower abdominal segment where squirrel monkey aortic intimas contained virtually no smooth muscle cells (table 2).

Intimas of old Cebus monkeys, like those of young Cebus, showed a musclo-elastic thickening in both thoracic and abdominal aortas, and the intima:media ratios in both regions were similar to the infant monkey values (figure 2B). In the upper thoracic segment, the mean intimal cellularity of old Cebus monkey aortas tended to be lower than in young Cebus, but in the abdominal region intimal cellularity was similar in the two age groups (table 2).

In old squirrel monkeys, the mean intima:media ratio and intimal cellularity of upper thoracic aortas were more than twofold greater than young monkey values. An even greater difference between age groups was noted in abdominal segments, where the mean intima:media ratio of old squirrel monkey aortas was 11-fold and the intimal cellularity was 13-fold, greater than values for young squirrel monkey aortas (table 2). The thickened intimas of old squirrel monkey aortas contained abundant connective tissue surrounding smooth muscle cells (figure 3, inset). In most sections extracellular lipid vacuoles were prominent adjacent to the IEL and often encroached upon the media (figure 4, inset). The IEL was frequently fragmented in areas where lipid accumulated. Furthermore, some elastic layers in the media of the abdominal region appeared to have dissociated, which may have accounted for the slight drop in the average number of medial lamellar units as compared with young squirrel monkey aortas (table 2).

As shown by the significant statistical interactions among the variables of age, species, and aortic segment, these observations indicate that the manifestations of intimal aging differed between the two species and that aging affected the thoracic and abdominal segments differently in squirrel monkey, but not in Cebus monkey aortas (table 2).

In both species and for both age groups, the media of the upper thoracic aorta was approximately 1.5 times the width and has 1.5 times the number of lamellae in the lower abdominal aorta area (table 2). The thoracic and abdominal aortic wall grew with age by increasing the width rather than the number of medial lamellae. The values for medial thickness measured in microns may have been slightly overestimated for young monkey aortas that were immersed in fixative and, therefore, more contracted than those perfused under pressure with fixative.

**Electron Microscopic Observations**

The relative distribution of aortic intimal components, as determined by point counting of electron micrographs, is recorded in table 3. Again, there were statistically significant interactions among the three variables of age, species, and aortic segment. The components of the narrow intimas in young squirrel monkeys were endothelial cells and amorphous (electron lucent) elastin. The latter component

### Table 2. Light Microscopic Morphometry of Thoracic and Abdominal Aortas from Young and Old Cebus and Squirrel Monkeys

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Intima: media (ratio)</th>
<th>Intimal cellularity (nuclei/100 μ)²</th>
<th>Medial thickness (μ)</th>
<th>lamellae</th>
</tr>
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<tbody>
<tr>
<td>Young</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Thoracic</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Cebus</td>
<td>6</td>
<td>0.07 ± 0.01</td>
<td>33 ± 2</td>
<td>310 ± 20</td>
<td>30 ± 2</td>
</tr>
<tr>
<td>Squirrel</td>
<td>4</td>
<td>0.05 ± 0.01</td>
<td>11 ± 4</td>
<td>130 ± 15</td>
<td>19 ± 3</td>
</tr>
<tr>
<td>Abdominal</td>
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<td></td>
</tr>
<tr>
<td>Cebus</td>
<td>5</td>
<td>0.05 ± 0.01</td>
<td>23 ± 4</td>
<td>210 ± 15</td>
<td>18 ± 1</td>
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<tr>
<td>Squirrel</td>
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<td>0.04 ± 0.01</td>
<td>2 ± 1</td>
<td>90 ± 20</td>
<td>13 ± 2</td>
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<td>Old</td>
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<td>Cebus</td>
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<td>0.05 ± 0.01</td>
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<td>400 ± 30</td>
<td>31 ± 2</td>
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<tr>
<td>Squirrel</td>
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<td>0.13 ± 0.04</td>
<td>23 ± 6</td>
<td>280 ± 30</td>
<td>20 ± 2</td>
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<tr>
<td>Abdominal</td>
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</tr>
<tr>
<td>Cebus</td>
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<td>0.04 ± 0.01</td>
<td>25 ± 7</td>
<td>260 ± 15</td>
<td>18 ± 1</td>
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<td>Squirrel</td>
<td>5</td>
<td>0.44 ± 0.23</td>
<td>26 ± 5</td>
<td>140 ± 15</td>
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Significant variables

<table>
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Values represent means ± se of means of four to eight tissue blocks per monkey. A = age; S = species; AS = aortic segment; × denotes interaction.
Figure 4. Old squirrel monkey abdominal aorta. Reactive smooth muscle cells near the internal elastic lamina contain lipid inclusions and are partially encapsulated by fibrous connective tissues. Lipid droplets (L), aggregates of pleomorphic, electron-dense lipid, and the empty cleft of a cholesterol crystal (arrow) are major components of the extracellular intimal space. C = collagen. × 7000. Inset. The pocket of debris is located by the arrow. Note the fragmentation of the internal elastic lamina and lipid accumulation in the upper media. × 400.

The distribution of components in the thickened aortic intimas of old squirrel monkeys shifted to a predominance of smooth muscle cells, collagen, and extracellular lipid in the form of homogeneous droplets, electron dense pleomorphic figures, and cho-
Table 3. Relative Distribution of Intimal Components in Electron Micrographs of Thoracic and Abdominal Aortas from Young and Old Cebus and Squirrel Monkeys

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Endothelium</th>
<th>Smooth muscle</th>
<th>Collagen</th>
<th>Elastin (%)</th>
<th>Amorphous ground substance</th>
<th>Lipid</th>
<th>Calcium granules</th>
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<tr>
<td></td>
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<td></td>
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<td>Amorphous</td>
<td>Fibrillar</td>
<td>Intra-cellular</td>
<td>Extra-cellular</td>
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</tr>
<tr>
<td>Cebus</td>
<td>6</td>
<td>10±4</td>
<td>18±3</td>
<td>16±3</td>
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<td>Squirrel</td>
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<td>22±2</td>
<td>2±2</td>
<td>10±5</td>
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<td>15±4</td>
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<tr>
<td>Cebus</td>
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<td>13±2</td>
<td>15±3</td>
<td>4±2</td>
<td>44±5</td>
<td>12±3</td>
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<td>Squirrel</td>
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<tr>
<td>Cebus</td>
<td>4</td>
<td>9±2</td>
<td>9±1</td>
<td>28±6</td>
<td>34±6</td>
<td>13±4</td>
<td>6±2</td>
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<td>5±0</td>
<td>15±5</td>
<td>31±12</td>
<td>7±2</td>
<td>9±4</td>
<td>2±2</td>
<td>1±1</td>
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<tr>
<td>Cebus</td>
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<td>7±2</td>
<td>22±4</td>
<td>2±2</td>
<td>42±4</td>
<td>16±1</td>
<td>10±2</td>
<td>0</td>
</tr>
<tr>
<td>Squirrel</td>
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<td>3±1</td>
<td>25±7</td>
<td>25±7</td>
<td>4±1</td>
<td>11±5</td>
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</tr>
<tr>
<td>Significant variables</td>
<td></td>
<td>A, S, A×S</td>
<td>S</td>
<td>A, S, NS</td>
<td>S</td>
<td></td>
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<tr>
<td>(p &lt; 0.05)</td>
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Values represent means ± se. A = age; S = species; AS = aortic segment; NS = significant difference; × denotes interaction.

Lesterol crystal clefts (table 3, figures 3 and 4). The relative amount of extracellular lipid varied among individual squirrel monkey aortic sections from approximately 12% to over 50%. In sections with extensive lipid debris, the inner layers of the media were usually involved, the IEL fragmented, and collagen fibers scattered extensively in areas of extracellular lipid (figure 4). Smooth muscle cells occasionally contained lipid droplets (figures 3 and 4) and frequently showed prominent rough endoplasmic reticulum (figures 4 and 5 A). Typically, these cells were surrounded by a ring of densely packed colla-

Figure 5. The connective tissue components common to the intimas of all monkeys are identified by high magnification as amorphous elastin (AE), fibrillar elastin (FE), collagen (C), amorphous ground substance (G), and basement membrane (BM). A, A reactive smooth muscle cell in an old squirrel intima contains prominent rough endoplasmic reticulum (RER). × 29,300. B, A portion of an old Cebus intima illustrates the thickened endothelial basement membrane which was characteristic of this group of monkeys. × 48,600.
Figure 6. Medial section from the thoracic aorta of an old squirrel monkey reveals heterogeneous collection of electron dense granules situated on either side of a fenestration in an elastic lamina (EL). × 15,400. Inset. A calcium granule is surrounded by lipid debris, collagen fibers, and fibrillar elastin in the intima of an old squirrel monkey. × 14,400.
gen, fibrillar elastin, and basement membrane-like material. Necrotic cells were also observed. Extracellular space not included in other categories was labelled as amorphous ground substance due to its uniform, electron lucent quality (table 3, figure 5). This component was very low in old squirrel monkey aortic intimas, where connective tissues were tightly packed together.

In striking contrast to old squirrel monkey aortas, aortic intimas of old Cebus monkeys showed no evidence of either intra- or extracellular lipid. The distribution of aortic intimal components in old Cebus monkeys was essentially the same as in young Cebus (table 3). In both cases, layers of amorphous elastin occupied one-third or more of the intima. These layers were often less compact in the young (figure 2 C) than in the old intima (figure 2 D). Although smooth muscle cells were present in similar numbers in both age groups of Cebus monkeys, those in the infant were often modified. The latter cells were surrounded by basement membrane and had numerous pinocytotic vesicles at the plasma membrane, but there was a noticeable lack of myofilaments and an abundance of intracellular organelles, especially dilated rough endoplasmic reticulum (figure 2 C). A noteworthy feature in old Cebus monkey intimas was the greatly thickened endothelial basement membrane in both thoracic and abdominal sections (figure 5 B).

Aging aortas in both species contained numerous electron-dense bodies that appeared to be debris from lysed cells (figure 6). These bodies were clustered in the interstitium of intima and media and were heterogeneous in size and appearance, ranging from granular to amorphous. Some were membrane-bound; others had a crystalline structure characteristic of calcium granules (figure 6). Although these deposits were observed in the intima, they were more abundant in the inner media and infrequent in the outer media. Calcium granules were noted less frequently in Cebus than in squirrel monkey aortic intimas, where they attained large sizes and numbers (table 3, figure 6).

**Discussion**

Comparison of aortas from Cebus and squirrel monkeys demonstrated that intrinsic differences existed between intimas of the two species in infancy and that aging elicited further differences. The musculoelastic thickening of the aortic intima previously described in wild Cebus monkeys was present to some extent in infant Cebus monkeys. The characteristics of the intimal smooth muscle cells in young Cebus monkeys suggests that these cells were metabolically active and rapidly synthesizing connective tissue. By old age, the Cebus monkey aortic intima had grown in proportion to the media. Furthermore, the distribution of intimal components remained similar to that in the young aorta. Even in the human aortic intima grossly free of lesions, lipid accumulates around fibrous components with advancing age. However, in the aged Cebus monkey aortic intima there was no sign of perifibrous lipid nor was the concentration of aortic lipid fractions different from the young Cebus monkey. This lack of either intra- or extracellular lipid accumulation in the aged Cebus monkey supports the argument that diffuse intimal thickening does not necessarily predispose to atherosclerosis. The fact that the deep layers of the intima were sometimes indistinguishable from the inner media, except by their lighter staining intensity and the orientation of the elastic bands and smooth muscle cells, suggests that this intimal thickening represented a remodeling process that eventually created a new IEL several layers above the original, and does not necessarily represent degenerative change.

The aortic intima of the young squirrel monkey consisted of minimal amounts of loose connective tissues between the endothelium and the IEL. By late adult life, the squirrel monkey aortic intima had thickened to a degree disproportionate to the growth of the media and contained elements characteristic of atherosclerosis including an accumulation of smooth muscle cells, connective tissues, extensive intra- and extracellular lipid, necrosis, and calcium granules. Like advanced human atherosclerosis, these naturally occurring lesions in the squirrel monkey tended to be more severe in the abdominal than in the thoracic aorta, as opposed to diet-induced lesions which are expressed more rapidly in the thoracic aorta of the squirrel monkey.

In the present investigation, the ratio of aortic cholesteryl ester:phospholipid in each group of monkeys paralleled the amount of lipid observed morphologically. Young monkeys of both species and old Cebus monkeys had an aortic cholesteryl ester:phospholipid ratio less than 1.0 and showed no evidence of lipid accumulation by electron microscopy, whereas old squirrel monkey aortas had a ratio greater than 2.0, corresponding to the atherosclerosis observed in that group. A reexamination of lipid composition data from human aortas collected by Smith revealed a similar pattern, with the cholesteryl ester:phospholipid ratio increasing with greater severity of the lesions. In lesion-free aortic intima of humans, all lipids increase with age, but cholesteryl ester undergoes the greatest increase. Thus, the cholesteryl ester:phospholipid ratio rises from approximately 0.3 in young human aortic intima to 1.6 in the old, corresponding to the increase in perifibrous lipid observed microscopically. In atherosclerotic lesions, including fatty streaks and fibrous plaques, the cholesteryl ester:phospholipid ratio reaches 3–4. A similar trend can be demonstrated in the data of Pangana et al., where the ratio of cholesteryl ester:phospholipid increases from approximately 1 in normal human intima to 2–4 in fatty streaks and fibrous plaques.
The clusters of dense bodies observed in the intima and media of old monkeys were interpreted as products of cell necrosis and lysis. In spite of this amount of debris, neither macrophages nor monocytes were observed in the intima or media. Deposits with similar characteristics have been described in the aortic media of aging rats and in the coronary arteries of rats. Joris and Majno suggested these abnormal deposits represent cellular debris which accumulates with increasing age due to an inadequately phagocytosis. Some of the dense bodies in our aging monkeys resembled the granulovesicular bodies described in the arterial wall of several species by Trillo and Haust. These authors speculated that the bodies may be formed and secreted by the smooth muscle cells to play a role in remodeling elastic fibers. This hypothesis could explain the close association between some of the bodies and amorphous elastic fibers in the intimas of our aging monkeys, particularly the Cebus, where a remodeling process of the intima appeared to occur. However, because granules were not observed in intimas of young Cebus monkeys, even though these were also thickened with smooth muscle cells and connective tissues and presumably remodeling, our impression is that the dense bodies represent the dispersed components of necrotic cells trapped against the luminal aspect of elastic lamellae.

The interpretation of these data must take into account that they were derived from aged monkeys from culled female breeding stock. Middleton et al. found a greater prevalence and extent of naturally occurring aortic fatty streaks in female than in male squirrel monkeys in the wild. Rats, which are normally very resistant to arterial intimal change, develop severe atypical atherosclerosis characterized by intimal fibrosis with calcification as a result of repeated breeding. In light of these observations, the present group of culled female breeders was selected on the assumption that they would be most likely to show signs of naturally occurring atherosclerosis. Even under these conditions, the Cebus monkey aortic intima was not subject to the degenerative processes observed in the squirrel monkey aortic intima.

A further consideration of the relative susceptibility to atherosclerosis in these two species is the prevalence of chronic nephritis in squirrel monkeys, especially with advancing age. On the other hand, glomerulonephritis in Cebus monkeys has not been reported, nor has it been a problem in our experience with this species. Although kidney lesions occur naturally in squirrel monkeys, they appear to be exacerbated by dietary cholesterol and hypercholesterolemia. Because renal disease is associated with a high incidence of atherosclerosis, caution needs to be exercised while interpreting the present results. However, squirrel monkeys with evidence of renal disease were excluded from this study as were monkeys with idiopathic aortitis, another relatively common problem in squirrel monkeys that exacerbates atherosclerosis. Aortitis has not been observed in Cebus monkeys.

Considerable variability among individual squirrel monkeys was noted in both the aortic lipid composition data and the morphological set of data of the present study. This variation is consistent with results of other investigators that indicate wide individual variation in the hypercholesterolemic response of squirrel monkeys to an atherogenic diet and in the extent of atherosclerosis developing as a consequence. The similarity in "basal" plasma cholesterol levels for all groups of monkeys indicates that this value was not a useful predictor of susceptibility to naturally occurring atherosclerosis in these species. However, more important than total plasma cholesterol in predicting risk for cardiovascular disease in man is the relative amount of cholesterol carried by high density lipoprotein (HDL) and low density lipoprotein. In the present group of monkeys, HDL cholesterol levels were available for only three squirrel monkeys. Two of the three values were normal (i.e., two-thirds of the total plasma cholesterol was carried as HDL) but in the third monkey the HDL cholesterol level was one-third normal and the intima:media ratio was threefold greater than the other two. A prospective study to examine this relationship would be informative. Analysis of plasma from a similar group of culled female breeders suggested that levels of HDL cholesterol were similar in the two species (Hoover and Hayes, unpublished data). Cebus and squirrel monkeys in the wild also have similar levels of cholesterol in HDL. This does not eliminate the possibility that other aspects of lipoprotein character and metabolism contribute to their relative susceptibility or resistance to atherosclerosis. In addition to circulating plasma factors, the nature of the arterial endothelium, smooth muscle cells, or connective tissue components may be important in determining susceptibility to this disease.

In summary, this study documents, both quantitatively and qualitatively, the differences in Cebus and squirrel monkey aortic intimal characteristics at birth and in old age. Whereas the squirrel monkey develops aortic intimal lesions resembling human atherosclerosis, the Cebus monkey aortic intima remodels without accumulating lipid. Studies to determine those factors responsible for this difference will be important for a further understanding and prevention of primate atherogenesis.

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G A Hoover, R J Nicolosi, R R Camp and K C Hayes

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