Aneurysmal enlargement of the aorta during regression of experimental atherosclerosis

Christopher K. Zarins, MD, Chengpei Xu, MD, and Seymour Glagov, MD
Chicago, Ill.

We explored the relationship between regression of diet-induced atherosclerosis and aneurysmal enlargement of the aorta in cynomolgus monkeys. Atherosclerotic plaques were induced in 17 monkeys by feeding them a diet containing 2% cholesterol and 25% peanut oil for 6 months (group I, n = 6; group III, n = 6) or 12 months (group II, n = 5). Regression was induced in group III by feeding a regression diet consisting of 0.25% cholesterol and 15% corn oil in a standard chow diet, for 6 months after the 6-month induction period. Serum cholesterol was 788 ± 80 mg/dl after 6 months of induction, 508 ± 53 mg/dl after the 12-month induction period, and 198 ± 15 mg/dl in the regression group at 12 months. Aortas were fixed in situ under conditions of controlled pressure perfusion, and transverse sections of the unopened vessels were taken at standard levels in the midthoracic and abdominal aortic segments. The area encompassed by the internal elastic lamina was taken as a measure of artery size. Plaques were abundant in abdominal and thoracic sections after the 6- and 12-month induction periods, and no significant difference was observed in lumen area or artery size between the groups. The ratio of abdominal to thoracic aortic plaque area was markedly reduced in the regression group (0.3 ± 0.2 for regression compared with 0.6 ± 0.3 for 6-month induction and 1.3 ± 0.2 for 12-month induction animals; p < 0.05 for both). A twofold increase was observed in abdominal aortic lumen area in the regression group (10.0 ± 1.5 mm² for regression compared with 5.6 ± 0.7 mm² for the 6-month and 4.2 ± 0.7 mm² for the 12-month induction groups; p < 0.05 for both) as well as a twofold increase in internal elastic lamina area (10.5 ± 1.5 mm² compared with 6.0 ± 0.7 mm² for the 6-month and 5.9 ± 0.8 mm² for the 12-month induction group; p < 0.05 for both). Aortic enlargement in the regression group was accompanied by a reduction in media thickness in the abdominal aorta. No significant vessel enlargement or alteration in media thickness occurred in the thoracic aorta. One of six regression animals (17%) had a threefold enlargement of the abdominal aorta and was thought to have a manifest aneurysm. The evidence of early aneurysmal dilation of the abdominal aorta in monkeys undergoing dietary cholesterol lowering supports the hypothesis that the atherosclerotic process plays a significant role in the pathogenesis of aneurysm formation. Plaque regression and thinning of the aortic wall may initiate aneurysmal enlargement. We conclude that regression of atherosclerosis results in aneurysmal dilation of the abdominal aorta in this experimental model. Localization of this finding to the abdominal aorta is consistent with the known pattern of distribution of aortic aneurysms in man. (J VASC SURG 1992;15:90-101.)

The association between atherosclerosis and abdominal aortic aneurysm formation has long been recognized in humans. However, the mechanisms by which the atherosclerotic process may be associated with aneurysmal dilation are not well defined. A number of other etiologic mechanisms have been proposed, including increased proteolytic enzyme activity, disturbances of trace metal metabolism, and genetic abnormalities leading to deficiencies in connective tissue structure and function. Although some investigators have questioned the role of atherosclerosis in aneurysm formation, evidence for its importance is increasing.
Previous investigations from our laboratory demonstrated aneurysm formation in cynomolgus and rhesus monkeys undergoing atherogenic dietary manipulation for prolonged periods of time. Most of the animals were on regression regimens that resulted in cholesterol lowering with or without drugs. Histologic evidence of thinning of the media, plaque atrophy and loss of normal aortic wall architecture, suggested a relationship between plaque regression and aneurysm formation. To investigate this possibility further, we designed a controlled trial of cholesterol lowering in primates by diet alone to test the hypothesis that regression of atherosclerosis was related to aneurysm formation.

**MATERIAL AND METHODS**

Atherosclerosis was induced in 17 male cynomolgus monkeys (macaca fascicularis) weighing 3 to 5 kg by feeding a diet containing 2% cholesterol and 25% peanut oil in standard monkey chow. This atherogenic diet has been in use in our laboratory for many years. Regression has been successfully achieved in this model by subsequent reversion to a normal or a "prudent" human diet with or without the addition of cholesterol lowering drugs.

For the present study, six animals were fed the atherogenic diet for 6 months (group I), and five were fed the same diet for 12 months (group II). Six animals (group III) were fed the atherogenic diet during a 6-month induction period and were then fed a regression diet consisting of 0.25% cholesterol and 15% corn oil in a standard chow. This diet reflects a prudent human diet. Body weight, serum cholesterol, and lipid levels were determined at monthly intervals. Animal care standards were in compliance with the “Principles of Laboratory Animal Care” (formulated by the National Society for Medical Research) and the “Guide for the Care and Use of Laboratory Animals” (NIH Publication No. 80-23, revised 1985).

At the termination of the experiment, each animal was killed by an overdose of pentobarbital. To permit quantitative assessment of plaque and aortic wall morphology and to assess aortic dimensions, the aorta was pressure-perfusion fixed in situ. Aortic rings were excised at standard locations in the thoracic aorta (midway between the left subclavian artery and the celiac artery) and in the abdominal aorta (midway between the renal arteries and aortic bifurcation). Histologic preparations were stained with hematoxylin and eosin as well as by the Gomori-trichrome-aldehyde fuchsin and Weigert van Gieson stains for connective tissue. Sections were projected onto a digitizing plate and contours of the lumen, internal elastic lamina (IEL) and outer media were traced. Lesion area, lumen area, media thickness and the area encompassed by the IEL (IEL area) were computed. The IEL area was taken as a measure of artery size. Histologic features of lesions, as well as architectural modifications of the media were noted.

Results are expressed as the mean ± SD and the mean ± SEM. Differences between groups were assessed using the Student t test. Differences were considered significant if p values were less than 0.05.

**RESULTS**

**Cholesterol levels**

The initial serum cholesterol level for all animals was 107 ± 5 mg/dl. After initiation of cholesterol feeding, serum cholesterol level rose rapidly in all animals (Fig. 1). After 6 months of the atherogenic diet, the cholesterol level was 788 ± 80 mg/dl in group I animals, 861 ± 118 mg/dl in group II animals, and 810 ± 52 mg/dl in group III animals. A moderate reduction of the initial high peak is normally seen at 4 to 6 months with this dietary regimen. Group II animals continued to have high cholesterol values throughout the dietary period. Animals in group III on the “regression” diet had a marked decline in their serum cholesterol to 198 ± 15 mg/dl at the time they were killed from a level of 810 ± 52 mg/dl at the end of the 6-month induction period. Although markedly reduced, the serum cholesterol level at 12 months in group III was still somewhat higher than control values.

**Atherosclerotic plaque.** The atherogenic diet resulted in plaque formation in both the thoracic and abdominal aorta in all animals. The morphometric data are shown in Tables I, II, and III. Atherosclerotic plaque area in the abdominal aortic sections was 0.4 ± 0.1 mm² after 6 months of the induction diet (group I) and 1.1 ± 0.4 mm² after 12 months on the diet (group II). No difference was observed in thoracic aortic plaque area after 6 or 12 months of induction (Table I). After 6 months of the regression diet (group III), no evidence was seen of plaque area reduction in the thoracic aorta (Table I). In the abdominal aorta, however, plaque area after 6 months of regression (group III) was the same as after 6 months of induction (group I).

In view of the wide individual variance in susceptibility to plaque formation, plaque area in the abdominal aorta (A) was compared to plaque area in the thoracic area (T) as a ratio (A/T). After 12 months of diet induction (group II) A/T was
Fig. 1. Cholesterol levels for the three groups. There is a marked reduction for group III regression animals at 12 months, but not to the baseline level.

Table I. Atherosclerotic plaque

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Thoracic aorta</th>
<th>Abdominal aorta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plaque area (mm²)</td>
<td>Media thickness (mm)</td>
</tr>
<tr>
<td>I 6-mo diet</td>
<td>0.7 ± 0.1</td>
<td>0.27 ± 0.02</td>
</tr>
<tr>
<td>II 12-mo diet</td>
<td>0.8 ± 0.1</td>
<td>0.28 ± 0.04</td>
</tr>
<tr>
<td>III 6-mo diet/6-mo regression</td>
<td>1.5 ± 0.4</td>
<td>0.24 ± 0.02</td>
</tr>
</tbody>
</table>

Mean ± SEM.
*p < 0.05 compared with 6-month diet.
**p < 0.05 compared with 6 month diet and 12 month diet.

Table II. Abdominal aortic dimensions

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Lumen area (mm²)</th>
<th>IEL area (mm²)</th>
<th>Abdominal/thoracic lumen area ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>I 6-mo diet</td>
<td>5.60 ± 1.60</td>
<td>6.03 ± 1.79</td>
<td>0.45 ± 0.13</td>
</tr>
<tr>
<td>II 12-mo diet</td>
<td>4.22 ± 1.61</td>
<td>5.90 ± 1.61</td>
<td>0.42 ± 0.12</td>
</tr>
<tr>
<td>III 6-mo diet/6-mo regression</td>
<td>9.99 ± 3.74*</td>
<td>10.45 ± 3.78*</td>
<td>0.64 ± 0.30</td>
</tr>
</tbody>
</table>

Mean ± SD.
*p < 0.05 compared with 6-month diet and 12-month diet.

increased twofold to 1.3 ± 0.2 compared with 0.6 ± 0.3 for group I (p < 0.05) indicating a relative increase in plaque in the abdominal aorta over the second 6-month diet period (Table I). This is consistent with the known pattern of progressive lesion formation in the abdominal aorta with time in this experimental model. In group III (regression animals), A/T was 0.3 ± 0.2, which was significantly less than A/T for group I or II (p < 0.05 for each) and indicates regression of abdominal aortic plaque.

Media thickness. In the thoracic aorta media thickness was the same in all three experimental groups. In the abdominal aorta, however, media thickness was reduced in group III regression animals
Fig. 2. Abdominal aortic dimensions for each animal. In group III four of the six animals had larger lumen areas and IEL areas than any of the animals in groups I or II. One animal in group III had a threefold increase in lumen and IEL area compared with group I and II animals and an abdominal aorta 1.2 times larger than the thoracic aorta and was considered to have a manifest aneurysm.

Table III. Wall stress

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Thoracic aorta</th>
<th></th>
<th>Abdominal aorta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lumen radius</td>
<td>Wall thickness</td>
<td>Wall stress</td>
</tr>
<tr>
<td></td>
<td>(cm)</td>
<td>(cm)</td>
<td>(dyne/cm² × 10⁴)</td>
</tr>
<tr>
<td>I 6-mo diet</td>
<td>0.20 ± 0.01</td>
<td>0.03 ± 0.001</td>
<td>6.9 ± 1.7</td>
</tr>
<tr>
<td>II 12-mo diet</td>
<td>0.18 ± 0.02</td>
<td>0.04 ± 0.004</td>
<td>5.6 ± 1.8</td>
</tr>
<tr>
<td>III 6-mo diet/6-mo</td>
<td>0.23 ± 0.02</td>
<td>0.03 ± 0.004</td>
<td>8.1 ± 3.9</td>
</tr>
</tbody>
</table>

Mean ± SEM.
*p < 0.05 compared with 6-month diet and 12-month diet.
**p < 0.05 compared with 12-month diet.

compared with the 6-month diet induction animals, p < 0.05 (Table I). Overall wall thickness (0.18 ± 0.03 mm) (plaque thickness plus media thickness) for the abdominal aorta in regression animals was significantly less than in 12-month diet induction animals (0.29 ± 0.06 mm), p < 0.05 (Table III).

Aortic dimensions. Lumen area of the abdominal aorta after 6 months of induction diet (group I) was 5.60 ± 1.60 mm² and the IEL area was 6.03 ± 1.79 mm². Continuation of the diet for 6 additional months (group II) did not result in any change in lumen area or in IEL area (Table II). However, 6 months of cholesterol lowering in group III animals resulted in a twofold increase in both lumen area (9.99 ± 3.74 mm², p < 0.05) and IEL area (10.45 ± 3.78 mm², p < 0.05).

Abdominal aortic dimensions for each animal along with the mean and standard deviation of each group are shown in Fig. 2. Four of the six regression animals in group III had larger lumen and IEL cross-sectional area than any of the nonregression animals in groups I or II. The animal with the largest lumen area and IEL area in group III exceeded the standard deviation and had an IEL area three times larger than the mean cross-sectional area for group I or II. This animal was considered to have a manifest abdominal aortic aneurysm. In this animal the abdominal/thoracic lumen area ratio was 1.2 indicating that the abdominal aorta was 1.2 times larger.
Fig. 3. Sections of abdominal aortas of group I (a), group II (b) and group III (c). There was moderate plaque formation after the 6-month induction diet (a). Plaques were much larger and the media was slightly thinner after 12 months on the induction diet, but the artery size (IEL area) did not change significantly (b). After 6 months of the regression diet (c) plaques were significantly smaller and absent in some regions. The media was thin and artery size (IEL area) was significantly increased. (Weigert van Gieson stain; original magnification ×10.)

Fig. 4. Sections of thoracic aortas of group I (a), II (b) and III (c). There were no significant differences in artery size or media thickness among the groups. (Weigert van Gieson stain; original magnification ×10.)

than the thoracic aorta providing further support for classifying this aorta as truly aneurysmal. The A/T ratio for this animal was well above the standard deviation for group III and considerably larger than the mean and standard deviations of A/T for groups I and II (Table II). It was the only animal with a ratio greater than 1.0. The incidence of manifest aneurysm formation was thus one of six animals in group III (17%).

Wall stress. Tangential wall stress was calculated from the formula $T = Pr/t$, where $T$ is the circumferential (hoop) tension, $P$ is the distending pressure, $r$ is the radius, and $t$ is the wall thickness.\(^{19}\) In group III animals, lumen radius was increased and wall thickness was decreased in the abdominal aorta, resulting in a significant increase in mural tensile stress (Table III).

**Histologic characteristics.** Representative sections of the abdominal aorta in each of the three groups are shown in Fig. 3. There is enlargement of the external diameter and thinning of the media in the regression animal. In the thoracic aorta, no significant differences were observed among the groups in plaque area, lumen area, IEL area, media thickness,
Fig. 5. Histologic changes in abdominal aorta of each group. After 6 months of the induction diet, group I (a), moderate plaque formed with characteristic foamy cell prevalence and little change in the media. In group II (b), after the 12-month induction diet, plaques were complex with formation of fibrous caps and evidence of necrosis and cholesterol accumulation. Media appeared normal with clearly stained elastic lamellae and smooth muscle cells. After 6-month regression diet, group III (c), plaques were much smaller and largely fibrotic. Media was thinned and elastic lamellae were largely inapparent. (Weigert van Gieson stain; original magnification ×75.)

lesion thickness, wall thickness, or wall stress. Representative cross sections of the thoracic aorta for each group are shown in Fig. 4.

After 6 months of the atherogenic diet (group I), the abdominal aorta had well-developed plaques consisting mainly of characteristic foam cells (Fig. 5). After 12 months of the induction diet, the plaques were thicker and more complex with development of a fibrous cap containing collagen and elastin fibers as well as underlying focal zones of necrosis and cholesterol crystal deposition. The media underlying the plaques appeared slightly thinned but there was no overall reduction in media thickness on quantitative morphometry. After 6 months of diet and 6 months of regression, plaque size was similar to that noted after 6 months of the induction diet, but differed markedly in composition. The plaques were devoid of foam cells or necrosis and contained collagen and numerous fusiform cells. Regions of the media were markedly thinned, particularly where plaque was entirely absent suggesting that these may have been regions of plaque regression. Elastin lamellae were absent or greatly reduced in prominence. By contrast, histologic features in the thoracic aorta were remarkably similar in the three groups with little or no striking plaque or medial changes in group III (Fig. 6).

DISCUSSION

Regression of experimental atherosclerosis by dietary lowering of serum cholesterol after a hypercholesterolemic induction period was associated with aneurysmal dilation of the abdominal aorta. This effect did not occur during a corresponding experimental period with continuation of the atherogenic diet. Compared with findings after 6 months of induction, administration of the atherogenic diet for 12 months resulted in increased abdominal aortic plaque formation, whereas discontinuation of the atherogenic diet with marked reduction of serum cholesterol levels resulted in regression of the lesions. This effect was accompanied by a reduction in media thickness, a reduction in overall wall thickness, and enlargement of the aorta. These findings were confined to the abdominal aorta and were not found in the thoracic aorta. Since tangential tensile stress is proportional to radius and inversely related to wall thickness, these changes resulted in an increase in tangential tensile stress and would be expected to contribute to continuation of the enlargement process.

The effect was not uniform for all of the animals. A wide range of susceptibility to atherosclerotic plaque formation, well recognized in diet-induced experimental atherosclerosis in primates, was also
noted in the present study. Genetic differences may account for this variance. Nevertheless, despite a wide variance in plaque formation and artery enlargement during the regression regimen, statistically significant enlargement was demonstrated only in the regression group. One animal had a threefold enlargement of the abdominal aorta during the 6-month regression period. This resulted in an abdominal aorta that was larger than the thoracic aorta and could be considered to be a manifest aneurysm rather than the somewhat lesser but definite dilations noted in the other regression animals.

Review of our experience over the past 15 years with diet-induced experimental atherosclerosis in nonhuman primates had revealed a 13% incidence of grossly evident aneurysms in the cynomolgus monkey and a 1% incidence of aneurysms in the rhesus monkey.\textsuperscript{12} No aneurysms developed in 44 animals fed a standard chow diet, indicating that aneurysms do not occur naturally in this species. No aneurysms were noted among 193 cynomolgus monkeys with 12 months or less exposure to the atherogenic diet. The aneurysms occurred only in a group of 31 animals that had been under study for longer than 16 months, and three of the four animals that developed aneurysms were included in trials of combined diet- and drug-induced regression. Histologic evidence of plaque atrophy and aortic wall thinning in the aneurysms suggested that regression of atherosclerotic plaques associated with aortic wall thinning predisposed to artery dilation and eventual aneurysmal enlargement.

The earlier study was,\textsuperscript{12} however, retrospective and included animals from a number of different dietary and drug regimens as pointed out in a letter discussing our previous report.\textsuperscript{20} To test the hypothesis that cholesterol lowering and regression of atherosclerosis was related to aneurysm formation, the present study was designed to induce plaques by means of a standard atherogenic regimen. Regression was produced by diet alone, thus avoiding possible confounding effects that could be attributable to cholesterol-lowering pharmacologic agents.\textsuperscript{20} To address the question of early aneurysmal enlargement by quantitative morphometric analysis, we paid particular attention to preserving in vivo aortic dimensions. Pressure-perfusion fixation was therefore performed in situ, and the midthoracic and midabdominal aortic segments were sampled at anatomically defined standard levels,\textsuperscript{17} taking care to avoid sections that could include branch points or bifurcations that might result in misinterpretations of vessel dimensions.\textsuperscript{20} The 17% incidence of actual aneurysm formation under these conditions in the present study proved to be consistent with our previous finding of a 13% incidence of grossly visible aneurysm in the long-term studies.

Aneurysm formation in experimental diet-induced atherosclerosis has been reported in several species of monkeys and canines.\textsuperscript{12,21-23} The absence of spontaneous aneurysm formation in these species supports the concept that aneurysm formation may occur as a consequence of atherosclerosis. Furthermore, aneurysms have been observed only after
extended exposure to atherogenic regimens, suggesting that aneurysm formation occurs at a later stage in the atherosclerotic process. This situation is consistent with the observation that patients undergoing operation for abdominal aortic aneurysm are approximately 10 years older than those undergoing operation for aortic occlusive disease. Tilson\textsuperscript{10} and Cohen et al.\textsuperscript{11} have noted a 16% familial incidence of aneurysms in patients and conclude that the tendency to aneurysm formation is probably due to a genetically determined predisposition. On the other hand, 85% of patients with aneurysms have no familial history of the disorder. Since most patients with abdominal aortic aneurysm have atherosclerosis, individual susceptibility to aneurysm formation may reflect an interaction between genetic and environmental atherogenic risk factors. In addition, genetically determined differences in tissue responses and in inflammatory cell reactions\textsuperscript{25} may also explain individual and species differences in the tendency to aneurysm formation.

Histologic observations in the present study as well as those characteristic of human aneurysms tend to support the mechanisms suggested by our previous study.\textsuperscript{12} Since developing atherosclerotic plaques are often associated with atrophy of the underlying media, both in human and in the experimental disease,\textsuperscript{12-15} progressing or stable atherosclerotic plaques overlying atrophic media may be expected to provide structural reinforcement, particularly in association with the fibrogenesis and cell proliferation that characterizes plaque formation. If late in the disease or during regression the plaque is reduced in size and/or altered in composition, tensile support may be insufficient and aneurysm formation may ensue. The increase in radius caused by dilation and the thinning of both plaque and media would tend to increase tensile stress further favoring a transition from dilation to aneurysm. An associated lytic effect on the media during regression possibly exaggerated by increased inflammatory cell activity cannot be excluded, since thinning of the media occurred mainly with regression.

The abdominal aorta is particularly susceptible to plaque formation, plaque involution and aneurysm formation in humans as it was in the present experimental study. Differing susceptibilities of the thoracic and abdominal aorta to atherosclerosis and to aneurysmal dilation may be due to differences in architecture, composition, and nutrition of the artery wall as well as to differences in the distribution of mechanical stresses.\textsuperscript{24} The thoracic aorta is thicker and has a greater number of medial lamellar units than the abdominal aorta, in keeping with its greater diameter and tangential wall tension.\textsuperscript{25} The thoracic aorta contains a greater relative proportion of elastin and a lower proportion of collagen than the abdominal aorta.\textsuperscript{17,25} The increased stiffness of the abdominal aorta is associated with an elevated pulse pressure that could result in altered medial smooth muscle metabolism and increased susceptibility to plaque deposition.\textsuperscript{24,25} In addition, in the human, differences exist with respect to wall nutrition that could result in different propensities to atherogenesis and to different responses of the media to mechanical stress.\textsuperscript{17,25-27}

The outer two-thirds of the thoracic aortic media is supplied by intramural vasa vasorum, whereas the inner 30 medial lamellar units, corresponding to a thickness of 0.5 mm, are presumably nourished by diffusion from the lumen. The human abdominal aorta lacks intramural vasa vasorum and is greater than 0.5 mm in thickness.\textsuperscript{26} The abdominal aorta would therefore appear to be at a relative metabolic disadvantage compared to the thoracic segment. The early formation of intimal plaques in this region would be expected to increase wall thickness, exceeding even further the 0.5 mm usual diffusion distance from the lumen, thereby promoting lipid trapping, further plaque formation, and media atrophy.

These considerations suggest that abdominal aortic aneurysm formation may complicate the atherosclerotic process under special experimental and human clinical conditions. It appears at a relatively late phase of plaque evolution, when plaque regression and media atrophy predominate, rather than at earlier phases when cell proliferation, fibrogenesis, and lipid accumulation characterize plaque progression. Evidences of plaque progression and plaque regression have been observed in different lesions of the same individual during human cholesterol lowering regression trials,\textsuperscript{28} suggesting that local factors as well as general metabolic conditions may be determinants of plaque evolution, vessel enlargement, and aneurysm formation, as suggested by our data and considerations by others.\textsuperscript{26,30}

Our previous study revealed aneurysm formation in cynomolgus monkeys during long-term continued exposure to the atherogenic diet and in relation to regression.\textsuperscript{12} In keeping with those findings and the findings that form the basis of this report we have recently observed abdominal aortic aneurysm formation in several patients treated with cholesterol lowering drugs (personal observation). Thus, individual differences in plaque evolution, reflecting
differences both in rate and duration of plaque formation and plaque regression, and in tissue and cell responses to the atherogenic process are likely to be major determinants of individual susceptibility to aneurysm formation. Microarchitectural differences in artery wall structure as well as local mechanical conditions related to geometry, blood flow, and blood pressure are likely to be major determinants of aneurysm localization. That these factors may be conditioned and/or modulated by genetic predispositions and by local abnormal or injurious hemodynamic and tensile stresses is evident.9,11,20,28,29 Further investigation of long-term plaque evolution, including features of progression and regression and the accompanying individual cellular responses under various mechanical conditions should help to further elucidate the mechanisms underlying the relationship between atherogenesis and aortic aneurysm formation.

REFERENCES

DISCUSSION

Dr. David Tilson (New York, N.Y.). This experiment by Dr. Zarins and colleagues shows that extreme hypercholesterolemia followed by a 6-month period of no dietary cholesterol feeding has produced aortic ectasia and perhaps an early aneurysm in a group of cynomolgus monkeys, keeping in mind that two of the six monkeys in group III had dimensions that were comparable to controls. One question for the authors is whether the no-cholesterol diet is any different than ordinary monkey food. If so, the experiment does not rule out the possibility that the no-cholesterol diet is deficient in some nutrient that is essential for the health of the aortic matrix.

Another question relates to the issue of whether a diet containing 25% peanut oil induces an inflammatory variant that may not be a good analogy to routine atherosclerosis in humans. We and others are beginning to appreciate the potential importance of inflammatory cells in abdominal aortic aneurysm pathogenesis, as they are particularly conspicuous in the adventitia of aneurysms by comparison to aortas with atherosclerotic occlusive disease. Thus, I wonder if this is what one might call a “mixed model” in that whereas it results in myointimal thickening and plaque, at the same time it may also induce features that are typical of aneurysm disease.

At least 15 reports have been published in the last few years suggesting that aneurysm disease has genetic determinants. Many reports document imbalances of matrix metalloproteinases and antiproteases and also differences in the biochemistry of occlusive disease and aneurysm disease. If this is a semantic discussion over whether all arteriopathies have to be considered “atherosclerosis,” I do not object as long as it is recognized that aneurysm disease in humans has different genetics and different biochemistry in many cases.

Dr. Zarins states in his paper that individual susceptibilities are likely to reflect interaction between genetic and environmental influences. I agree with this formulation, and I believe that continuing dialogue and experimentation will place our knowledge of aneurysm disease on a solid basis.

Dr. Christopher Zarins. Thank you very much Dr. Tilson for your comments. Standard monkey chow containing all essential nutrients was fed to all three groups including the low cholesterol regression group. To avoid confounding variables, we did not use cholesterol-lowering drugs to induce regression.

I agree that destruction of the media, including a possible role for inflammatory cells, underlies the pathogenesis of aneurysm formation. There may, however, be species and individual differences in susceptibility to the determining factors. We have found, for example, a striking difference in the incidence of aneurysm formation between rhesus and cynomolgus monkeys subjected to the same cholesterol lowering regimen. Furthermore, under the same dietary conditions individuals within a species may develop very different levels of plasma cholesterol and the degree of plasma cholesterol elevation is not always related to the degree of plaque formation. Thus genetic factors may be important determinants of the natural history of atherosclerosis. Familial hypercholesterolemia is a well-known genetic disorder resulting in early and severe atherosclerosis, but a positive family history is common in patients with coronary heart disease without familial hypercholesterolemia. Clearly, we need to clarify the relationship between specific environmental inciting factors and the genetic determinants of susceptibility. The important point to note, however, is that in this experiment aneurysmal enlargement was induced by exogenous influences, namely cholesterol lowering in a species with no genetic trait for aneurysms. This underscores the importance of atherogenic risk factors in aneurysm formation.

Dr. Ralph DePalma (Washington D.C.). The results of this experiment are important because they promise further progress in the understanding and control of atherosclerosis and aneurysm development.

I have two comments. When we first observed abdominal aortic aneurysms, this was accomplished after a 5-year regression attempt in dogs. We attributed these results to failure of regression because the cholesterol remained elevated at about 235 to 400 because of thyroid ablation in the dog, much as occurs in the cynomolgus monkey. Similar to your experiment, coexisting stenotic lesions were present in the carotids and other places. In a rhesus model, which is different from the one you used, we simply fed sugar and eggs and no tropical oils whatever. We never saw aneurysms nor inflammatory nor necrotic changes, as the Chicago model amply demonstrates. In the cynomolgus monkey, the elevated cholesterol falls very slowly. It takes months and never does get back to baseline, whereas this is not true in the rhesus monkey.

In this experiment, cholesterol remained elevated during regression and never fell to normal because the cynomolgus monkey stores lipid in the liver and spleen and continues to deliver it. Nonetheless, clearly at 12 months the arteries are bigger in the regression group. You have published before that atherosclerosis causes enlargement of arteries, so why would you not interpret this as a failure of lipid reduction or a failure of regression?

My second question is could you discuss, in more detail, the adventitial and medial inflammatory changes that you do see in your model.

Finally, I agree with you that I have seen progression of aneurysmal disease in people taking a low cholesterol diet or losing an enormous amount of weight. What kind of message do you want to deliver to us about dietary advice? Perhaps you can extrapolate from this very interesting experiment.

Dr. Jon Cohen (New Hyde Park, N.Y.). This study has provided us with some very interesting observations to be considered in the evolving yet puzzling story for the
identification of the pathophysiologic event that results in aortic aneurysm formation.

Most interesting, of course, is the observation that elastin in the media was less prominent in the regression animals. These data, along with observations of others, once again suggests that degradation of elastin and not collagen is the critical factor for the development of aortic aneurysms. The question, of course, is what causes an increase in elastin degradation during the regression diet. The authors have suggested that altered medial smooth muscle cell metabolism may be the primary source for the events that occur. We believe that the key to this disease lies within the lack of control of the release of proteases from the aortic smooth muscle cell or the neutrophil.

As Dr. Zarins knows, he and I are in agreement that atherosclerosis is probably the initiating event for the development of aneurysmal disease. However, the aberrant course taken by the patient who develops an aortic aneurysm once they develop atherosclerosis is yet to be defined.

We continue to believe that aneurysmal disease like so many other diseases is the result of a strong interaction between environmental influences and genetic coding. In aneurysmal disease, the environmental factors that favor atherosclerosis such as diet and smoking in the patient with a genetic propensity to develop aneurysm probably result in aneurysmal disease.

My questions are, one, we all know patients with aortic aneurysms are certainly not on any sort of regression diet at the time that they seek treatment. And if anything, I would suppose that 99.9% of them are on high-cholesterol, high-fat diets at the time their aneurysm is discovered. How then would you relate this model to the clinical situation in most cases?

Two, if you believe, like we do, that the medial smooth muscle cell is the major offender in this disease, can you speculate further on what you believe is the underlying physiologic event that differs in the smooth muscle cells between patients with aneurysms and aortic occlusive disease?

**Dr. Phillip Bendick** (Royal Oak, Mich.). Is this truly a regression model? You may have arrested progression of disease after a 6-month diet of cholesterol elevation, but if you look at the two groups that both had a 6-month diet of cholesterol-elevating food, they both develop virtually identical lesion sizes in the abdominal aorta. So is this truly a regression program, or have you simply arrested progression with resumption of a normal diet?

The second question relates to your hypothesis of medial thinning as a response to the support generated by the structure of the plaque itself. Group II, which showed a significant generation of additional plaque over groups I and III, did not show any significant difference in the medial thickness compared to group one; I invite your comments on that aspect of your study. Finally, the medial thinning that you notice in group III corresponds directly to the diameter enlargement. If you simply look at conservation of mass, the medial thinning corresponds exactly to what that increase in diameter would predict; basically you have an increase diameter and medial thinning without any proliferative response. Should we not be looking at the structural composition and the biochemical responses to these rapid large swings in serum cholesterol within the cellular elements of the medial wall itself? It would appear that what happens in the lumen reflects the wall biochemistry and not vice versa.

**Dr. Roger Greenhalgh** (London, England). This is a very worthy topic, one of course which interests many of us greatly. But, I ask myself what these experiments in animals tell us about the formation of aortic aneurysm in the human. A number of requirements are needed to be able to come up with a hypothesis.

First of all, as I agree with many comments made before this discussion, atherosclerosis must be regarded as a prerequisite in the formation of most aortic aneurysms. We know, for example, that in the human 90% of patients with aortic aneurysm smoke, but not all our patients with atherosclerosis develop aortic aneurysms, so we must explain that.

Family history for aneurysm is stronger than for pure atherosclerosis. We also know that there is a difference between stenosing arterial disease and dilating in the sense that for aneurysms there is a greater degree of inflammation in every case. This may be associated with the loss of elastin, which has been demonstrated and documented by our own group and others, and in the increased activity of human aortic elastase in the wall of the artery. Smooth muscle cells are lost, more in the abdominal aorta than the thoracic aorta, and we know that these cells produce the elastin. In other words, when you have lost your elastin, you have lost it permanently.

What I would like to know, from your experimental work, is whether you are suggesting the postulate that for all human aortic aneurysm that atherosclerotic regression is occurring in each case to the extent that we should therefore logically, according to your experiments, feed our patients cholesterol and recommend that they should continue to smoke to make certain that their atherosclerosis does not regress?

**Dr. Zarins.** Drs. DePalma and Bendick asked about the nature of the regression reaction. Clearly there was an increase in lumen cross-sectional area as well as a trend toward reduction in intimal plaque area. This is consistent with arrest of progression rather than regression. However, histologic changes would clearly support the notion that regression was taking place. The abdominal aorta demonstrated definite quantitative plaque regression relative to the thoracic aorta, and the abdominal aorta is where aneurysmal dilation was seen.

In these short-term experiments, cholesterol levels did not return to normal after 6 months due in part to the fact that our regression diet contained 0.25% cholesterol. This diet is designed to mimic the so-called "prudent" American diet. In our previous retrospective study of more prolonged experiments, serum cholesterol was reduced to baseline levels often with drugs, and frank aneurysm formation was
seen after 12 months of regression. In this experiment we wanted to study the influence of cholesterol lowering alone on the early stage in which the initiating dilational changes of aneurysmal enlargement were occurring. We were surprised that one animal, even in this short experimental period developed a frank aneurysm, once again underlining the role of individual differences in response.

Dr. Bendick points out that the thinning of the media in the regression group may have been caused by the aortic enlargement and lack of proliferation to increase media mass. This is certainly possible. However, the combination of increased diameter and reduced wall thickness, caused by both a decrease in media and plaque thickness, resulted in a twofold increase in wall stress in regression animals. This would act to promote progressive aneurysmal enlargement.

The changes underlying the compromise of artery wall integrity are likely to be complex. The effect of plaque accumulation, and the associated inflammatory and cellular metabolic reactions, may alter the function of the smooth muscle cells with a direct effect not only on elastin, but also on collagen content. Enlargement requires changes in both of these structural fibers. Under normal conditions collagen content and orientation are major determinants of mural tensile strength.

Drs. Greenhalgh and DePalma have raised the question of relevance of our findings to the human situation. Our findings strongly suggest that plaque progression and regression in the course of the evolution of atherosclerosis are major determinants of the mechanical integrity of the artery wall. These processes would appear to be occurring at different rates at different times in relation to alterations in risk factors and in relation to location. In our previous experiments we noted that monkeys developed aneurysms both on progression and regression regimens and that the histologic features of plaque and media atrophy were more closely associated with aneurysm formation than the serum cholesterol level. In patients undergoing cholesterol lowering therapy, plaque regression and progression may be occurring simultaneously at different sites and at different rates. Since these changes take time and are probably associated with changes in life style and exposure to risk factors, as well as to genetic differences, it is not surprising that aneurysm patients are 10 years older than those with occlusive disease and that not all patients with atherosclerosis develop aneurysms.

With regard to the role of diet, I believe that the importance of cholesterol intake is probably exaggerated. There is little evidence that progression and regression are directly determined by cholesterol level particularly beyond age 60 years when, according to the Framingham data, there is an inverse correlation between cholesterol level and longevity. Dr. Cohen states that all patients with aneurysms are hypercholesterolemic. Actually, almost all of our own patients with aneurysms have normal or near normal serum cholesterol levels at the time we operate on them. It is more likely that individual determinants of plaque evolution may be more important than cholesterol levels as such.

The advisability of cholesterol lowering, particularly for the elderly, is unclear, and more information is needed on the effects of such intervention on the plaque and artery wall.