Regression of Experimental Atherosclerotic Lesions in Rhesus Monkeys Consuming a High Saturated Fat Diet


Atherosclerotic lesions were induced in rhesus monkeys by feeding them a high-fat, high-cholesterol diet for 2 years. Arteries were examined after autopsy of a subgroup of animals (group P) and cholesterol was removed from the diet of the remaining animals. Lesions were examined in other subgroups after 30 weeks (group R1) and after 52 weeks (group R2). A control group (group C) was fed the diet without cholesterol throughout the study. The mean total serum cholesterol concentration before, during, and after lesion induction was 151,390, and 157 mg/dl, respectively. The mean percent of surface area with fatty streak or fibrous plaque and the free and esterified cholesterol content of the artery increased in all six arterial segments examined in group P. The means for percent of surface with fatty streak and for arterial cholesterol content or concentration (but not for extent of surface with fibrous plaque) were consistently less in groups R1 and R2 than in group P, although they remained greater in groups R1 and R2 than in group C. The mean intimal thickness for coronary arteries was 10-fold greater in group P than in group C and 60 percent less in groups R1 and R2 than in group P; there was, however, much variability among animals and these differences among groups were not statistically significant. By using several measures in several arterial systems, we have shown that there was regression of diet-induced atherosclerotic lesions in rhesus monkeys while they were fed a diet high in saturated fat but without cholesterol for 30 or 52 weeks.

(Arteriosclerosis 7:125-134, March/April 1987)

Regression of experimental atherosclerotic lesions was first reported in rabbits by Anitschkow in 1933. Since that time these observations have been extended to many other models of experimental atherosclerosis. A comprehensive review of these studies has recently appeared. These experiments indicate that modification of the experimental conditions can induce reversibility of arterial lesions in all animal models. Although extrapolation of these observations to humans is not totally feasible, they seem to support the idea that controlling some of the risk factors operating in the genesis of coronary heart disease (CHD) will favor regression of atherosclerotic lesions and will be of great value in the prevention of CHD.

In previous studies we have addressed the issue of regression of diet-induced early atherosclerotic lesions in rhesus monkeys after an 8- or 12-week exposure to an atherogenic diet, focusing mainly on the gross extent and qualitative features of aortic atherosclerosis and on changes in the chemical composition of lesions. The present study was undertaken to examine the regression of experimental lesions in several arterial segments when dietary cholesterol is removed after a more prolonged period (104 weeks) of lesion induction with a high cholesterol diet. We also intended to learn more about the association between the response of serum lipids and the evolution and regression of arterial lesions as well as about the changes of intimal cell populations during the progression and regression phases. In this report we will present only findings regarding the extent of lesions determined by gross evaluation and chemical analysis in six arterial segments and morphometrically in coronary arteries. Findings regarding associations between serum lipids and arterial lesions and findings related to changes in intimal cell populations and ultrastructural features will be presented in other reports.

Methods

Animals

A group of 60 young (3 to 6 years of age) male rhesus monkeys (Macaca mulatta) weighing between 4.0 and 8.4 kg were purchased from an animal importer who had conditioned them for a minimum of 60 days after importation. They were initially housed two to a cage at Delta Regional Primate Research Center (DRPRC), Covington, Louisiana, where they underwent the rigorous 90-day quarantine that is routinely given at that institution. Approximately 1 month after this quarantine period, the animals were transferred to individual caging at Louisiana State University Medical Center in New Orleans, where they remained for the duration of the study. All procedures were in accordance with institutional guidelines.

Diet

The animals were fed a commercial monkey food (Purina Monkey Chow-25) given ad lib for the first 2 months of quarantine. They were then fed the basal open formula diet.
shown in Table 1. This diet was formulated to have nutrient composition similar to human diet and provided protein at 15% and fat at 38% of calories with a P/S ratio of 0.35. The cholesterol content of this diet was 0.02 mg/Kcal. This same diet, with cholesterol (USP) and 1.7% dried egg yolk added to provide cholesterol at 0.37 mg/Kcal, was used as the atherogenic diet during the lesion-induction period (see below). During the lesion-regression period, they were again fed the basal diet shown in Table 1. During basal, lesion-induction, and lesion-regression periods, the animals were fed twice a day an amount of food sufficient to maintain normal growth and body weight.

**Plan of Study**

During the quarantine and basal (low cholesterol) diet periods, the tooth eruption patterns were determined and the approximate ages were assessed according to the method of Hurme. The 60 animals were assigned an age rank. Four months after beginning the basal diet (6 months after receipt), one animal was selected from each sextile on age to provide a group of six control animals (group C). These animals remained on the basal diet throughout the study.

The 54 remaining animals were divided into three groups (blocks) by ranking on estimated age and then assigning randomly one animal to each block from each successive group of three animals. Four months after beginning the basal diet, we began feeding the high cholesterol diet to the first of these blocks (block 1). Blocks 2 and 3 began receiving this diet after 6 and 12 weeks, respectively.

At 3- to 6-week intervals throughout the study, the animals were sedated with ketamine (10 mg/kg, intramuscular) for determination of body weight and for blood samples. The total serum cholesterol concentration was determined on all blood samples as described below. One animal died from an undetermined cause about 1 year after beginning the atherogenic diet.

**Terminal Procedure**

After an overnight fast, each animal was sedated with ketamine (10 mg/kg, intramuscular) and a terminal blood sample was drawn. The animal was then killed with an overdose of sodium pentobarbital (65 mg/kg, intravenous) and a complete autopsy was performed.

The heart was removed along with 2 cm of the aortic arch. The aortic arch was cannulated and the heart was perfused with 3% glutaraldehyde at about 100 mm Hg for 30 minutes. The left common and the proximal 10 to 15 mm of the anterior descending and 5 to 10 mm of the circumflex branches of the left coronary artery were taken for electron microscopic evaluation. The heart was fixed by immersion in 3% glutaraldehyde for an additional 90 minutes, then immersed in buffered neutral formalin where it remained until further processing.

The descending aorta was excised and flushed with cold saline. Fat and other adhering tissue were removed from the adventitia. The aorta was opened with a longitudinal incision along the dorsal line and was divided into left and right halves by cutting along the ventral line. Blocks of tissue were taken for electron microscopic and histomorphometric evaluation from three standardized sites (2 thoracic and 1 abdominal) along the dorsal edge of the left lateral half, and the remainder of this half was immersion-fixed in buffered formalin for gross evaluation. The right lateral half of the aorta was divided into descending thoracic and abdominal regions by transection at the first paired intercostal arteries and at the proximal margin of the celiac orifice. After photographs were taken for area determination, these arteries were wrapped carefully in aluminum foil and stored at −30°C prior to chemical analyses.

Other arteries that were excised, opened longitudinally, and fixed in formalin for gross and microscopical evaluation as for the left lateral half of the aorta were: the left brachial, left common iliac and femoral, and left carotid artery to a point just distal to the bifurcation. In addition to the right lateral half of the aorta, the proximal 5 cm of the right brachial, iliac-femoral, common carotid, and bifurcation of carotid arteries were also prepared and frozen for chemical analyses. Samples of other organs and tissues were taken for routine histological examination.

**Measurement of Atherosclerotic Lesions**

After fixation in buffered formalin, the arteries were stained with Sudan IV, sealed into plastic bags, and identi-
ified only with a code number. Study pathologists visually estimated the percent of intimal surface involvement with fatty streaks (FS) and fibrous plaques (FP) for six arterial segments by methods that have been tested extensively\textsuperscript{13,14} for consistency and reproducibility. The six arterial segments evaluated in this fashion were: the left half of the descending thoracic aorta, the left half of the abdominal aorta, the left carotid sinus and internal and external carotid arteries 0.5 cm proximal and distal to the bifurcation, the left common carotid artery and the proximal 5 cm of the left brachial artery and left iliac-femoral artery.

Histologic sections perpendicular to the lumen axis were prepared at 5 mm intervals for the right coronary artery (four sections), and for the remainder of the left anterior descending (five sections) and left circumflex (three sections) coronary arteries after removal of blocks for electron microscopy. These 12 sections, stained with elastica-Van Gieson, were projected onto the surface of a graphic digitizer for morphometry. The following parameters were obtained for each section: the area of the intima, the length (perimeter) of the internal elastic lamina (IEL), and the maximum thickness of the intima. We calculated the percent of lumen area reduction as the percent of area within the IEL (assumed circular) that was measured as intimal area. A measure approximating mean thickness of the intima in each section was calculated as the ratio of area of the intima to the perimeter of the IEL. This measure underestimates the mean intimal thickness especially for thicker intimas. Thus, for a concentric lesion occupying one-third of the original lumen area, this measure is 91% of the true intimal thickness. The maximum lumen area reduction we observed was only 33%; therefore we will refer to this measure as Intimal thickness (IT) in this paper. Finally, the mean of these derived measures and the mean of the maximum intimal thickness over all 12 sections for each animal were calculated and used as measures of coronary atherosclerosis.

### Chemical Analyses

Serum cholesterol concentrations were measured in the Autoanalyzer II (Technicon Instruments Corporation, Tarrytown, New York) using an isopropanol extraction followed by a colorimetric reaction with a modified Liebermann-Burchard reagent.\textsuperscript{15}

The adventitia was carefully stripped from the thoracic and abdominal aortas by blunt dissection under magnification; thus the term intima-media is used to describe the aortic preparations. In the other four arterial segments, the adventitia was carefully stripped of any excess fat or connective tissue and the chemical analyses were performed on the total wall of the artery, including the remaining adventitia.

The arterial preparations were freeze-dried under vacuum and weighed. The dry tissue was minced in an homogenizer with chloroform/methanol (2:1), and the lipid was extracted by the method of Folch et al.\textsuperscript{16} The extracts were evaporated with the use of vacuum. Gas-liquid chromatography of the trimethylsilyl derivatives was used to determine free cholesterol (FC) on one aliquot and, after saponification with a mild base, to determine total cholesterol on another aliquot.\textsuperscript{17} Cholesterol was used as an internal standard.\textsuperscript{18} Esterified cholesterol (EC) was determined by the difference.

### Statistical Analyses

The statistical significance of the differences among groups and among arterial segments in mean values of percent of intimal surface with all lesions and with fatty streak (FS) was obtained by the Bonferroni inequality in an analysis of variance using the ANOVA procedure of the SAS statistical package.\textsuperscript{19} Where necessary, the square root transformation was used to correct for significant deviations from the normal distribution. Because of the extreme deviation from normal distribution, the statistical significance of the differences in FP in the aorta and peripheral arteries and of the measures of intimal thickness and percent reduction in luminal area in coronary arteries was assessed using the nonparametric Wilcoxon rank-sum statistic.\textsuperscript{19} The statistical significance of differences in serum cholesterol concentration between diet periods was determined by a t test of paired observations.

### Results

The mean and range of total serum cholesterol concentration during steady state in each diet period are shown in Table 2. Steady state was attained within 12 to 18 weeks after beginning the high cholesterol diet and again 12 to 18 weeks after return to the basal diet. For every animal in groups P, R1, and R2 there was a statistically significant increase in serum cholesterol concentration between basal and lesion induction periods ($p < 0.001$). There were no statistically significant differences in serum cholesterol concentration among groups P, R1, or R2 during any diet periods.

### Table 2. Mean Serum Total Cholesterol Concentration (mg/dl) in Three Diet Periods by Group

<table>
<thead>
<tr>
<th>Diet period</th>
<th>Group</th>
<th>C (n = 6)</th>
<th>P (n = 27)</th>
<th>R1 (n = 6)</th>
<th>R2 (n = 12)</th>
<th>R (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td></td>
<td>161</td>
<td>154</td>
<td>146</td>
<td>144</td>
<td>145</td>
</tr>
<tr>
<td>Lesion</td>
<td></td>
<td>157</td>
<td>390*</td>
<td>380*</td>
<td>380*</td>
<td>380*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(136-202)</td>
<td>(230-695)</td>
<td>(276-565)</td>
<td>(245-598)</td>
<td>(245-585)</td>
</tr>
<tr>
<td>Induction</td>
<td></td>
<td>152</td>
<td>162</td>
<td>157</td>
<td>159</td>
<td></td>
</tr>
</tbody>
</table>

Groups: C is the control group; P is the progression group; R1 is the 30-week regression group; R2 is the 52-week regression group; R is groups R1 and R2 combined. The numbers in parentheses indicate the range of values.

*Differs significantly from group C ($p < 0.001$).
period. For all animals of groups R1 and R2 the decrease in serum cholesterol concentration after reduction of dietary cholesterol was also statistically significant ($p < 0.001$). In the control group there were no significant changes in serum cholesterol concentration throughout the study.

There were no statistically significant differences between groups R1 and R2 for any measure of lesions ($p > 0.10$); therefore, these have been pooled as group R in Tables 3 through 6. Table 3 shows the mean percent of intimal surface with atherosclerotic lesions in six arterial segments by experimental group and by lesion type. Spontaneous fatty streaks and fibrous plaques were observed in some animals of the control group but, except for fatty streaks in the thoracic aorta, these covered only a small fraction of the surface. The mean for FS was fourfold to 18-fold greater after the 2-year period on high cholesterol diet than in the control group, and these differences were statistically significant ($p < 0.05$) for all arterial segments. The mean for FP was also consistently greater for group P than for group C for all arteries. These differences were statistically significant ($p < 0.05$) for the bifurcation of the carotid and the iliac-femoral arteries and were of borderline significance ($p < 0.10$) for the abdominal aorta and the brachial artery as well. The effect of the low cholesterol diet on FP was not as consistent, with the mean for group R less than that for group P in only four of the six arteries. These differences in fibrous plaque between groups P and R were not statistically significant for any artery ($p > 0.05$).

Examples of the sudanophilic lesions induced in the aortas and carotid arteries are shown in Figures 1 and 2. The arteries selected for groups P, R1, and R2 in these figures are from animals within a narrow range of mean steady state serum cholesterol concentration (375 to 393 mg/dl). These figures illustrate the extent and topographic distribution of the lesions induced and the effect of regression on these lesions. They also show the large variability in the distribution and extent of lesions induced among animals with a similar elevation of serum cholesterol concentration.

The results of the analysis for cholesterol in the arterial wall samples for individual arterial segments by experimental group are shown in Tables 4 and 5. The means are expressed as $\mu g/cm^2$ (content) in Table 4 and as mg/g dry defatted weight (concentration) in Table 5. Both free cholesterol (FC) and esterified cholesterol (EC) content and concentration are consistently greater in group P than in group C for all arterial segments examined ($p < 0.05$), paralleling findings with visually estimated atherosclerosis.

### Table 3. Mean Percent of Arterial Surface with Lesion in Six Arterial Segments

<table>
<thead>
<tr>
<th>Arterial segment</th>
<th>Lesion type</th>
<th>Group</th>
<th>C (n = 6)</th>
<th>P (n = 27)</th>
<th>R1 (n = 6)</th>
<th>R2 (n = 12)</th>
<th>R (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left $\frac{1}{2}$ descending thoracic aorta</td>
<td>Fatty streak</td>
<td>13.1</td>
<td>52.5$^*$</td>
<td>25.3</td>
<td>23.9$^t$</td>
<td>17.9$^t$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fibrous plaque</td>
<td>0.0</td>
<td>2.1</td>
<td>3.0</td>
<td>8.0</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total lesions</td>
<td>13.1</td>
<td>54.6$^*$</td>
<td>28.3</td>
<td>14.9$^t$</td>
<td>19.4$^t$</td>
<td></td>
</tr>
<tr>
<td>Left $\frac{1}{2}$ abdominal aorta</td>
<td>Fatty streak</td>
<td>4.0</td>
<td>45.4$^*$</td>
<td>27.0</td>
<td>22.4$^t$</td>
<td>23.9$^t$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fibrous plaque</td>
<td>0.4</td>
<td>7.5</td>
<td>0.8</td>
<td>4.0</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total lesions</td>
<td>4.4</td>
<td>52.9$^*$</td>
<td>27.8</td>
<td>26.4$^t$</td>
<td>26.8$^t$</td>
<td></td>
</tr>
<tr>
<td>Bifurcation of left carotid artery</td>
<td>Fatty streak</td>
<td>3.0</td>
<td>33.9$^*$</td>
<td>9.5$^t$</td>
<td>5.9$^t$</td>
<td>7.1$^t$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fibrous plaque</td>
<td>2.8</td>
<td>11.9$^*$</td>
<td>2.8</td>
<td>13.6</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total lesions</td>
<td>5.6</td>
<td>45.8$^*$</td>
<td>12.3$^t$</td>
<td>19.5$^t$</td>
<td>17.1$^t$</td>
<td></td>
</tr>
<tr>
<td>Left common carotid artery</td>
<td>Fatty streak</td>
<td>2.2</td>
<td>25.9$^*$</td>
<td>12.6</td>
<td>21.2</td>
<td>18.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fibrous plaque</td>
<td>0.0</td>
<td>0.1</td>
<td>0.6</td>
<td>0.9</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total lesions</td>
<td>2.2</td>
<td>26.0$^*$</td>
<td>13.2</td>
<td>22.1</td>
<td>19.1</td>
<td></td>
</tr>
<tr>
<td>Left brachial artery</td>
<td>Fatty streak</td>
<td>2.2</td>
<td>28.0$^*$</td>
<td>16.8</td>
<td>13.5</td>
<td>14.6$^t$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fibrous plaque</td>
<td>0.3</td>
<td>4.7</td>
<td>6.7</td>
<td>9.0</td>
<td>8.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total lesions</td>
<td>2.5</td>
<td>32.7$^*$</td>
<td>23.5</td>
<td>22.5</td>
<td>22.7$^*$</td>
<td></td>
</tr>
<tr>
<td>Left iliac-femoral artery</td>
<td>Fatty streak</td>
<td>1.0</td>
<td>18.0$^*$</td>
<td>9.3</td>
<td>10.7</td>
<td>10.2$^t$</td>
<td></td>
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<tr>
<td></td>
<td>Fibrous plaque</td>
<td>0.0</td>
<td>9.5$^*$</td>
<td>9.5</td>
<td>5.4</td>
<td>6.8$^t$</td>
<td></td>
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<tr>
<td></td>
<td>Total lesions</td>
<td>1.0</td>
<td>27.5$^*$</td>
<td>18.8</td>
<td>16.1</td>
<td>17.0$^t$</td>
<td></td>
</tr>
</tbody>
</table>

$^*$Differs from group C with $p < 0.05$.
$^t$Differs from group P with $p < 0.05$. 

ARteriosclerosis, Vol. 7, No. 2, March/April 1987
In all arterial segments, the relative increase in EC (averaging 16-fold) exceeded that for FC (averaging three-fold). The mean FC and EC content and concentration were also consistently lower in the group R animals than in the group P animals and this difference was statistically significant ($p < 0.05$) for all except FC in the common carotid and iliac-femoral artery. For all arteries, the mean FC and EC content and concentration were greater in group R than in group C, although this difference was statistically significant only for the EC content or concentration in the abdominal aorta ($p < 0.05$). Again, for all arteries the proportional loss of EC during regression exceeded that of the FC; thus, during the regression period the EC content declined by an average of 74% while FC content declined by an average of only 42%.

The results of the morphometric evaluation of sections of the coronary arteries are given in Table 6. By all three measures (mean intimal thickness, maximum intimal thickness, and percent lumen area reduction) the magnitude of the coronary lesions was greater in group P than in group C with lesions in group R at an intermediate value. There was much variability within all groups so that in spite of the large relative differences among groups C, P, and R, these differences were not statistically significant ($p > 0.10$) when tested with the nonparametric Wilcoxon rank-sum statistic. Examination of the data for individual animals
Figure 2. Sudan-stained carotid arteries from the four treatment groups: C = control group; P = lesion induction or progression group; R1 = 30-week regression group; R2 = 52-week regression group. Arteries in group C were selected to show minimum, median, and maximum lesion extent. Arteries of other groups are from the median in the steady-state serum cholesterol concentration during the lesion induction period. The range of mean serum cholesterol concentration in these latter nine arteries was 375 to 393 mg/dl. Magnification x 0.93.

revealed that a majority of the reduction in mean value resulted from fewer animals with high levels of lesions (see range of values in Table 6); thus it is likely that an appreciable regression occurred for those animals with the most severe lesions.

We examined the potential relationship between the extent of atherosclerosis and artery diameter by calculating the correlation coefficients between the mean intimal area and the diameter of coronary artery within each treatment group and by comparing the mean diameter of arteries in

<table>
<thead>
<tr>
<th>Arterial segment</th>
<th>Cholesterol fraction</th>
<th>Group</th>
<th>C (n = 6)</th>
<th>P (n = 27)</th>
<th>R1 (n = 6)</th>
<th>R2 (n = 12)</th>
<th>R (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left 1/4 descending thoracic aorta</td>
<td>Esterified</td>
<td>25</td>
<td>180*</td>
<td>62†</td>
<td>54†</td>
<td>56†</td>
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<td></td>
<td>Free</td>
<td>75</td>
<td>249*</td>
<td>153</td>
<td>155</td>
<td>155†</td>
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<tr>
<td></td>
<td>Total</td>
<td>100</td>
<td>429*</td>
<td>215</td>
<td>209†</td>
<td>211†</td>
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<tr>
<td>Left 1/4 abdominal aorta</td>
<td>Esterified</td>
<td>16</td>
<td>239*</td>
<td>109†</td>
<td>88†</td>
<td>95†</td>
<td></td>
</tr>
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<td></td>
<td>Free</td>
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<td>250*</td>
<td>135†</td>
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<td>Total</td>
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<td>489*</td>
<td>244</td>
<td>221†</td>
<td>229†</td>
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<tr>
<td>Bifurcation of left carotid artery</td>
<td>Esterified</td>
<td>22</td>
<td>272*</td>
<td>25†</td>
<td>37†</td>
<td>33†</td>
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<td>Free</td>
<td>84</td>
<td>280*</td>
<td>120</td>
<td>116</td>
<td>117†</td>
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<td></td>
<td>Total</td>
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<td>552*</td>
<td>145†</td>
<td>153†</td>
<td>150†</td>
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<tr>
<td>Common carotid artery</td>
<td>Esterified</td>
<td>6</td>
<td>96*</td>
<td>17†</td>
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<td>19†</td>
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<tr>
<td></td>
<td>Free</td>
<td>70</td>
<td>128*</td>
<td>91</td>
<td>89</td>
<td>90</td>
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<tr>
<td></td>
<td>Total</td>
<td>76</td>
<td>224*</td>
<td>108</td>
<td>109†</td>
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<td>Brachial artery</td>
<td>Esterified</td>
<td>5</td>
<td>132*</td>
<td>23</td>
<td>13†</td>
<td>17†</td>
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</tr>
<tr>
<td></td>
<td>Free</td>
<td>59</td>
<td>136*</td>
<td>74</td>
<td>71†</td>
<td>72†</td>
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</tr>
<tr>
<td></td>
<td>Total</td>
<td>64</td>
<td>268*</td>
<td>97</td>
<td>84†</td>
<td>89†</td>
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<tr>
<td>Iliac-femoral artery</td>
<td>Esterified</td>
<td>6</td>
<td>134*</td>
<td>66</td>
<td>46</td>
<td>53†</td>
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</tr>
<tr>
<td></td>
<td>Free</td>
<td>62</td>
<td>162*</td>
<td>109</td>
<td>106</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>68</td>
<td>296*</td>
<td>175</td>
<td>152</td>
<td>160</td>
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</tr>
</tbody>
</table>

*Differs from group C with p < 0.05.
†Differs from group P with p < 0.05.
Table 5. Mean Cholesterol Concentration (mg/g Dry Defatted Weight) In Six Arterial Segments

<table>
<thead>
<tr>
<th>Arterial segment</th>
<th>Group</th>
<th>C (n = 6)</th>
<th>P (n = 27)</th>
<th>R1 (n = 12)</th>
<th>R2 (n = 18)</th>
<th>R (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left 1/4 descending thoracic aorta</td>
<td>Esterified</td>
<td>2.4</td>
<td>16.8*</td>
<td>5.5†</td>
<td>4.8†</td>
<td>5.0†</td>
</tr>
<tr>
<td></td>
<td>Free</td>
<td>7.2</td>
<td>23.4*</td>
<td>13.3</td>
<td>13.8†</td>
<td>13.8†</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>9.6</td>
<td>40.2*</td>
<td>18.8†</td>
<td>18.6†</td>
<td>18.6†</td>
</tr>
<tr>
<td>Left 1/6 abdominal aorta</td>
<td>Esterified</td>
<td>2.3</td>
<td>33.9*</td>
<td>13.2</td>
<td>12.5†</td>
<td>12.8††</td>
</tr>
<tr>
<td></td>
<td>Free</td>
<td>8.9</td>
<td>35.9*</td>
<td>16.5†</td>
<td>18.2†</td>
<td>17.6†</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>11.2</td>
<td>69.8*</td>
<td>29.7†</td>
<td>30.7†</td>
<td>30.4†</td>
</tr>
<tr>
<td>Bifurcation of left carotid artery</td>
<td>Esterified</td>
<td>2.1</td>
<td>21.6*</td>
<td>2.4†</td>
<td>3.6†</td>
<td>3.1†</td>
</tr>
<tr>
<td></td>
<td>Free</td>
<td>8.4</td>
<td>23.0*</td>
<td>11.8</td>
<td>10.4†</td>
<td>10.9†</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>10.5</td>
<td>44.6*</td>
<td>14.2†</td>
<td>14.0†</td>
<td>14.0†</td>
</tr>
<tr>
<td>Left common carotid artery</td>
<td>Esterified</td>
<td>0.5</td>
<td>8.9*</td>
<td>1.8†</td>
<td>1.8†</td>
<td>1.8†</td>
</tr>
<tr>
<td></td>
<td>Free</td>
<td>8.7</td>
<td>12.0</td>
<td>9.5</td>
<td>8.1</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>9.2</td>
<td>20.8*</td>
<td>11.3</td>
<td>9.9†</td>
<td>10.3†</td>
</tr>
<tr>
<td>Left brachial artery</td>
<td>Esterified</td>
<td>0.5</td>
<td>12.9*</td>
<td>2.8</td>
<td>1.4†</td>
<td>1.9†</td>
</tr>
<tr>
<td></td>
<td>Free</td>
<td>8.2</td>
<td>13.4*</td>
<td>8.7</td>
<td>8.2</td>
<td>8.4†</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>8.7</td>
<td>26.3*</td>
<td>11.5</td>
<td>9.6†</td>
<td>10.3†</td>
</tr>
<tr>
<td>Left iliac-femoral artery</td>
<td>Esterified</td>
<td>0.6</td>
<td>11.8*</td>
<td>6.4</td>
<td>4.9</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>Free</td>
<td>5.9</td>
<td>14.4*</td>
<td>11.4</td>
<td>11.3</td>
<td>11.3</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>6.5</td>
<td>26.2*</td>
<td>17.8</td>
<td>16.2</td>
<td>16.7</td>
</tr>
</tbody>
</table>

*Differs from group C with p < 0.05.
†Differs from group P with p < 0.05.

Discussion

In earlier studies, we were able to demonstrate gross, histological, ultrastructural, and chemical evidence of regression of aortic fatty streaks induced during an 8- or 12-week period on an atherogenic diet and followed by a withdrawal of the atherogenic diet for periods up to 64 weeks. We also found that there was a progressive decrease of the EC content in the aortic intima-media with return to near control levels after 64 weeks on the basal diet. These chemical changes in the arterial wall correlated with the histologically and ultrastructurally observed loss of lipid and a shift from the intracellular to extracellular compartments. In contrast to these findings in the aorta, there were only minimal histologically observed intimal lesions induced in the coronary arteries in the progression animals and no evidence of regression of these lesions.

The present study was undertaken to learn more about the regression of lesions induced in the rhesus monkey with a more prolonged period of exposure to atherogenic diet. After 104 weeks on a moderately atherogenic diet, the percent of intimal surface with lesions in the abdominal aorta, brachial, iliac-femoral, and common carotid arteries was greater than that observed following a 12-week period of induction, although there was little difference in the ex-

Table 6. Average Mean and Maximum Coronary Intimal Thickness and Mean Percent Lumen Area Reduction by Atherosclerotic Lesions In All Sections from Each Animal

<table>
<thead>
<tr>
<th>Measure</th>
<th>Group</th>
<th>C (n = 6)</th>
<th>P (n = 27)</th>
<th>R1 (n = 12)</th>
<th>R2 (n = 18)</th>
<th>R (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean intimal thickness (μm)</td>
<td></td>
<td>1.1</td>
<td>10.5</td>
<td>4.1</td>
<td>4.6</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0-4)</td>
<td>(0-73)</td>
<td>(0-10)</td>
<td>(0-16)</td>
<td>(0-16)</td>
</tr>
<tr>
<td>Maximum intimal thickness (μm)</td>
<td></td>
<td>39.8</td>
<td>93.9</td>
<td>79.0</td>
<td>61.1</td>
<td>67.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0-75)</td>
<td>(0-340)</td>
<td>(12-39)</td>
<td>(0-150)</td>
<td>(0-150)</td>
</tr>
<tr>
<td>Lumen area reduction (%)</td>
<td></td>
<td>0.4</td>
<td>4.4</td>
<td>1.5</td>
<td>1.3</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0-1)</td>
<td>(0-33)</td>
<td>(0-3)</td>
<td>(0-7)</td>
<td>(0-7)</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate range of values.
No statistically significant differences (p > 0.05) were observed.
tent of surface with lesions observed for the descending thoracic aorta. The mean for FP in the group P animals of this study was greater in all arterial segments than in animals in the 12-week experiment, suggesting that extent and severity of these more advanced lesions increases with time on the experimental atherogenic diet.

We observed a two- to threefold greater extent of visually detectable lesions and approximately a 75% greater concentration of aortic EC in the control animals of the present study than in the control groups of our earlier study, although in both control groups these measures of lesions were low. One factor that may account for this difference is the difference in diets. In the earlier study, we fed the control groups a commercial monkey food whereas in the present study we fed the high saturated fat diet shown in Table 1. It is possible that the high saturated fat content or other factors such as the source of dietary fiber could have increased the formation of sudanophilic lesions. This possibility is strengthened by the observation that the mean total serum cholesterol in the control group of the present study (150 mg/dl) was somewhat higher than in the control groups of the earlier study (average 130 mg/dl). Other factors such as age or time in captivity might also have affected the extent of the lesions in these control groups.

In our earlier studies we examined the regression of lesions occurring when both the saturated fat and cholesterol content of the atherogenic diet were reduced. In the present study we chose to examine the effects of reducing only the dietary cholesterol concentration. Since pilot studies had shown that in the rhesus monkey a diet high in saturated fat causes little or no elevation of serum cholesterol or lipoproteins and since our earlier studies indicated that the extent of elevation of serum cholesterol was a major factor in determining the extent of lesion formation, we felt that this simpler regimen should also result in an appreciable regression of lesions. The data shows that reduction of dietary cholesterol alone with a return of serum lipids to the baseline values, while still feeding a diet high in saturated fat, will cause a regression of diet-induced atherosclerotic lesions.

After withdrawal of cholesterol from the diet of these rhesus monkeys, there was a decrease in mean for FS in all six arterial segments evaluated grossly and this decrease was statistically significant in all but the common carotid and iliac-femoral arteries. Regression of FS was apparent after a return to the basal diet for 30 weeks and was not appreciably greater after a further 22-week period on the basal diet. This regression was not complete since the mean for FS remained significantly greater in group R than in group C for all six arteries. Thus, it seems that after prolonged induction of atherosclerosis under our experimental conditions, a return to baseline levels of fatty streak was not achieved within 52 weeks by controlling dietary cholesterol alone.

The ratio of FP to percent of surface with all lesions was found by Tracy and Toca to be the best estimate of the rate of conversion of fatty streaks to fibrous plaque in human atherosclerosis. Using this measure, the findings in this study suggest that the rate of conversion of fatty streaks to fibrous plaques varies considerably among arterial segments, being highest for the iliac-femoral artery and near the bifurcation of the carotid arteries.

We found that the mean for FP was consistently greater in the progression group than in the control group, indicating that the high cholesterol diet accelerated the formation of these more advanced lesions. Although we did not show a consistent or statistically significant regression of FP, it is apparent that the accelerated rate of progression did not continue during the 2 years after removal of the dietary cholesterol. With the large variability and the extreme deviation from normal distribution among animals, it is apparent that group sizes larger than 18 to 27 rhesus monkeys or a longer period of regression would be required to show a statistically significant increase or decrease in FP that may occur after removal of dietary cholesterol.

Other studies of regression of atherosclerosis in rhesus monkeys employing this same basic protocol have been reported. Armstrong et al fed a higher level of dietary cholesterol for 17 months to induce a more severe narrowing of coronary artery lumen than we observed in this study. Using measures of cross-sectional intimal area and percent of luminal area reduction obtained from immersion-fixed coronary arteries, they reported a 60% to 80% decrease in lesions after 40 months of feeding a cholesterol-free diet. Vesselinovitch et al and Wissler and Vesselinovitch reported the results of two studies, part of which involved an examination of regression of severe diet-induced aortic and coronary lesions after removal of dietary saturated fat and cholesterol. In the first study, they reported a decrease in surface with lesions from 81% to 31% after return to the basal diet following 18 months of a severely atherogenic diet. They also reported a 70% reduction in extent and a 55% reduction in the severity of microscopically evaluated coronary lesions in these animals. In the second study, they reported a similar reduction in aortic atherosclerosis, but a less significant reduction in coronary atherosclerosis, 12 months after a return to the basal diet following 12 months on a severely atherogenic diet.

The lesions we studied were generally less severe and were induced over a longer time on an appreciably less drastic and somewhat more human-like diet than those employed in these earlier studies, and there were appreciable differences in methods of evaluating lesions; however, where they can be directly compared, the findings of regression reported in this paper are in general agreement with the findings of these earlier studies.

More recently Clarkson et al. and Wagner et al. have published results of a study of regression of lesions induced in 300 rhesus monkeys by feeding a diet high in saturated fat and very high in cholesterol for 19 months followed by alteration in dietary cholesterol content. Their study also differed from ours reported here in a number of ways that make direct comparisons difficult. First, during the regression period, these researchers adjusted dietary cholesterol individually for each animal so as to maintain plasma cholesterol at either 200 mg/dl or 300 mg/dl, whereas we removed dietary cholesterol to allow plasma cholesterol to return to basal levels. As in the earlier studies discussed above, the diet they used caused a
REGRESSION OF EXPERIMENTAL ATHEROSCLEROSIS  Eegen et al.  133

much greater elevation of serum lipids with both mean and maximum plasma cholesterol concentrations almost dou-
ble those attained in our study. Finally, the methods for
evaluating lesions differed appreciably in the two studies.
Although these investigators presented data on gross
evaluation of lesions in a number of arterial systems for the
groups killed after 19 or 38 months lesion induction,26 no
data on these measures were reported for the groups after
regression.21, 22 The data on coronary lesions were pre-
sented as total intimal area in 15 sections and were not
normalized for the length of inner elastic lamina to pro-
vide a measure of mean intimal thickness as we have chosen
to do.

Clarkson et al.20 reported that for the coronary lesions
induced over 19 months there was a regression of intimal
area in the animals with moderate lesions and plasma
cholesterol at 200 mg/dl after 24 months and in animals
with more extensive lesions after 48 months. They ob-
served further progression of lesions after 24 months when
plasma cholesterol levels were 300 mg/dl. They reported21
that coronary lesions induced over 36 months showed a
regression after 48 months but not after 24 months while
the animals were maintained at 200 mg/dl plasma choles-
terol. At 300 mg/dl plasma cholesterol, some animals with
these 38-month lesions showed regression, while others
showed further progression, of coronary lesions. In the
present study, although nonparametric statistical analysis
failed to show a significant regression of coronary lesions
in group R as a whole, examination of the data for individ-
ual animals indicated that there was a regression of the
most severely thickened coronary intimas after 7 or 12
months at basal plasma cholesterol levels.

Clarkson et a1.21 also reported that aortic lesions induced
for 36 months and evaluated by measuring intimal areas
showed little evidence of regression after 24 months either
at 200 or 300 mg/dl plasma cholesterol, while a few ani-
mals showed regression after 48 months at 200 mg/dl.
They also reported that the intimal areas of the carotid
bifurcation or the common carotid arteries showed no evi-
dence of regression in any groups.

Wagner et al. reported on changes in aortic and carotid
and iliac-femoral artery cholesterol after 24 months27 or
after 48 months28 on the two regression regimens follow-
ing 19 months of progression diet. For both periods of regres-
sion, they observed an essentially complete loss of accu-
mulated arterial cholesterol in those animals maintained at
200 mg/dl but only a partial loss in those maintained at 300
mg/dl. Thus in contrast to the morphometric measure-
ments discussed above, this study did show regression of
the arterial cholesterol in the aorta and carotid arteries.
Under conditions of our study, we observed 60% to 88%
reductions of accumulated EC and a somewhat smaller
reduction of FC content in these arteries after 12 months
regression at basal plasma cholesterol levels (about 160
mg/dl). Thus, for this measure of atherosclerosis, our study
agrees with the data reported by Wagner et al.27, 28

In this paper we have documented conclusively that
atherosclerotic lesions induced in rhesus monkey arteries
by feeding dietary cholesterol and saturated fat for 2 years
will regress significantly during 30 or 52 weeks while the
animals are fed the same diet high in saturated fat but
without cholesterol. This regression effect is shown by
gross and chemical analyses in different arterial segments
as well as by morphometric measurements of coronary
artery lesions. Additional analyses of the relationship of
serum lipid and lipoprotein fractions and histomorphomet-
ic and ultrastructural features of aortic lesions will be re-
ported in the future.

Acknowledgments

The authors acknowledge the assistance of Christian R. Abeo
in the acquisition and quarantine of the animals and of Jimmle
Leslie and Bernard Jackson in the care and handling of the
animals.

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Index Terms: cholesterol • dietary cholesterol • arterial cholesterol
Regression of experimental atherosclerotic lesions in rhesus monkeys consuming a high saturated fat diet.

D A Eggen, J P Strong, W P Newman, G T Malcom and C Restrepo

Arterioscler Thromb Vasc Biol. 1987;7:125-134
doi: 10.1161/01.ATV.7.2.125

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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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