Regression of Coronary Atheromatosis in Rhesus Monkeys

By Mark L. Armstrong, M.D., Emory D. Warner, M.D., and William E. Connor, M.D.

ABSTRACT

Rhesus monkeys subjected to the atherogenic stimulus of a high-fat, high-cholesterol diet showed significant coronary atheromatosis at the end of 17 months. Smaller fibrotic lesions with scant stainable lipid were found in animals that were subsequently fed either of two cholesterol-free diets for 40 months. The average cross-sectional area of the lumen was more than 80% greater in regression animals than in monkeys with baseline atherosclerosis. The data support the hypothesis that uncomplicated coronary atheromas may regress in primates in appropriate dietary settings.

ADDITIONAL KEY WORDS

hypercholesterolemia in primates
regression of atheromas
regression diets in atherosclerosis

Experimental atherosclerosis has been induced to a variable extent in nonhuman primates by numerous investigators. This is commonly accomplished by the use of a dietary intake sufficiently rich in cholesterol and fat to cause hyperlipidemia. Whether withdrawal of the atherogenic stimulus permits atheromatous lesions to regress and the diameter of the lumen to enlarge toward its normal dimensions has not been established in any primate.

Regression studies in animals other than primates have differed in results as to the late histologic fate of diet-induced atheromatous lesions. Perhaps the most complete investigations have been made in the aorta. A point of doubt is whether aortic studies adequately portray the process that occurs in the muscular arteries. However, when arterial ramifications such as the coronary arteries have been directly studied under the conditions of induced atherosclerosis and the possibility of subsequent regression has been examined, the results have been conflicting. Regression of coronary lesions has been reported in fowl and dogs and lack of regression found in the rabbit, illustrating a perplexing discrepancy in response to regression regimens among biologic orders remote from man and other primates.

The purpose of this study was to characterize the fate of induced coronary atheromatous lesions in a nonhuman primate fed low-fat and linoleate-enriched regression diets. From a functional standpoint, that of coronary flow, three alternative outcomes of the regression trials were foreseen. Regardless of evolving histologic changes in the vessel wall, the cross-sectional area of the lumen might (a) remain the same, or (b) even decrease, or, on the other hand, (c) become larger because of either decreased encroachment of the intima into the lumen or dilatation of the artery to increase the lumen in spite of an obdurately encroaching intima, or by a combination of a dilated artery and smaller lesion size. The occurrence of smaller lesions among regression animals would strongly suggest that coronary
TABLE 1

Major Characteristics of Diets

<table>
<thead>
<tr>
<th></th>
<th>Atherogenic</th>
<th>Low fat</th>
<th>Corn oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>16</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>43</td>
<td>77</td>
<td>45</td>
</tr>
<tr>
<td>Fat</td>
<td>41</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1.2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Values for protein, carbohydrate, and fat are percent of total calories; cholesterol values are percent by weight.

Atheromas that have advanced beyond the primitive fatty streak (21) can be partially reversed in the species studied. This finding would also raise the larger question of the potential reversibility of atheromatous lesions among the several genera of the primate order.

We present data that support the concept of reversibility of coronary atheromatosis in the rhesus monkey.

Methods and Materials

Forty adult male rhesus monkeys (Macaca mulatta), weighing 8.6 ± 0.54 kg, were given semisynthetic diets (Table 1). Ten of these monkeys (controls) were fed a low-fat, cholesterol-free diet throughout the study. The other 30 monkeys were fed the same cholesterol-free diet for a control period of 6 weeks before being given an atherogenic diet for 17 months. These 30 monkeys were then divided into three groups matched for hyperlipidemia, cutaneous xanthomatosis, and body weight (Table 2). Group 1 was examined at autopsy for baseline atherosclerosis. The other two groups were fed cholesterol-free regression diets that were either low in fat (group 2) or rich in linoleate (group 3) for 40 months.

Only the major characteristics of the diets are shown (Table 1); their detailed composition including the supplements used to make them complete in all essential nutrients have been described in detail previously (22). Linoleate-rich diet was a modification of the low-fat diet by enrichment with corn oil to 40% of total calories.

At autopsy the animals were anesthetized with phencyclidine, 0.5 mg/kg, and exsanguinated from the abdominal aorta exposed by laparotomy. The heart was removed and fixed in formalin before the extramural coronary arteries were dissected free for study. Specimens were taken at five predetermined sites for uniformity of evaluation: the proximal main coronary arteries at 0.5 cm from their ostia, the left anterior descending branch 0.5 cm downstream, and both the left circumflex and right coronary arteries at one-third of the distance between their origins and the posterior interventricular groove. Coded transverse sections were used for quantitative estimation of the cross-sectional area of the lumen and intima. Sections stained with hematoxylin and eosin were projected from a microscope on nonabsorbent paper at a magnification of 325 times; the lumen and the intimal and medial tunics of the vessel wall were then traced, cut, and weighed. The error of reproducibility of measurements by this method was less than 1%. In atherosclerotic arteries the internal elastic membrane was used as the landmark for the original position of the intima in estimating the decrease in luminal area. We used the first section projected from each evaluation site except when technically inadequate preparations required the use of a contiguous section.

TABLE 2

Regimen Change after 17 Months of Atherogenic Diet

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum lipids (mg/100 ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>701 ± 47</td>
<td>712 ± 68</td>
<td>703 ± 37</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>401 ± 32</td>
<td>322 ± 30</td>
<td>379 ± 15</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>71 ± 5</td>
<td>74 ± 8</td>
<td>70 ± 4</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>9.8 ± 1.8</td>
<td>9.7 ± 1.0</td>
<td>9.0 ± 1.0</td>
</tr>
<tr>
<td>Xanthoma grade*</td>
<td>2.4 ± 0.5</td>
<td>2.0 ± 0.4</td>
<td>2.4 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE.

*Cutaneous xanthomatosis was graded 0 to 4 as follows: 0.5, a few xanthomatous nodules; 1, small clusters of nodules; 2, multiple nodules and one or more xanthomatous plaques with estimated total involved area 3 cm²; 3, multiple plaques with total involved area greater than 3 cm²; and 4, widespread plaques and clusters of nodules in numerous anatomic sites. Xanthomas forming in scars were omitted from the grade estimates.
The histochemical characteristics of the arterial lesion were also evaluated by other appropriate stains.

Lipid Analyses.—Blood samples were taken at intervals of 2 weeks to 1 month throughout the study for lipid and lipoprotein analyses. The methods used and the results obtained from blood and tissue during and at the end of induction of atherosclerosis have been described (22, 23).

Other Observations.—Hematocrits (24) and body weights were obtained as indices of general health when blood samples were collected. The animals and their cages were under constant surveillance for evidence of untoward findings. Tuberculin tests, examinations of the urine for glucose, and blood pressure determinations were performed periodically.

Results

MORPHOLOGIC FINDINGS

The average luminal narrowing found at each evaluation site for the three groups of experimental animals is shown in Table 3. The overall narrowing at all sites found in baseline atherosclerosis was 58%. In the regression groups, overall narrowing was 22% in the group fed corn oil and 18% in the group fed a low-fat diet. The differences in luminal narrowing between baseline atherosclerosis and each of the regression groups were significant at the 1% level or less for both the overall values and for the average value found at each of the five sites of evaluation. Although there was a mean difference of approximately 10% in the residual luminal narrowing of the main coronary arteries between the two regression diets, this was nonsignificant for the left coronary artery (P<0.4). For the right main coronary artery the difference was significant at the 1% level; since differences in residual narrowing among the other four sites evaluated were nonsignificant, this isolated suggestion of a difference in result between the regression diets is of necessity considered a random type I effect (25).

Control animals showed much less than 1% average narrowing. Typically a unicellular layer of intima was seen on the luminal side of an intact internal elastic membrane. Only trivial intimal thickening was found in a few sections.

The data in Table 3 have been presented in relative terms to compare the amount of luminal narrowing among arteries of differing size. A critical question is whether the mass of intimal tissue found in regression animals was in fact less, or whether the cross-sectional dimensions of the arteries of animals studied after regression diets were greater than those studied at baseline atherosclerosis, with larger lumens and only an illusion of a decrease in intimal tissue. The data pertaining to this question are shown in Table 4. The absolute mean intimal area was more than three times greater in baseline atherosclerosis than after regression diets. Furthermore, the total lumen-intima-media areas show that dilatation could not be detected by significant differences in

### Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>Luminal Narrowing (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>1</td>
<td>60 ± 8</td>
</tr>
<tr>
<td>2</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>3</td>
<td>23 ± 5</td>
</tr>
</tbody>
</table>

Abbreviations of branch arteries: L.A.D., left anterior descending; L. c'flex, left circumflex; R. dist., distal continuation of right main coronary artery.

Values are means ± SE.

### Table 4

<table>
<thead>
<tr>
<th>Group</th>
<th>Intima (mm³)</th>
<th>Total artery (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.720 ± .102</td>
<td>1.93 ± .350</td>
</tr>
<tr>
<td>2</td>
<td>0.138 ± .026</td>
<td>1.58 ± .379</td>
</tr>
<tr>
<td>3</td>
<td>0.213 ± .029</td>
<td>1.63 ± .155</td>
</tr>
<tr>
<td>Control</td>
<td>&lt;0.010</td>
<td>1.66 ± .272</td>
</tr>
</tbody>
</table>

Values are means ± SE.
FIGURE 1

Top left: Baseline atherosclerosis. Marked luminal occlusion by encroachment of intima showing several of the features found: cellular accumulation including lipid-laden macrophages, fibrous proliferation, and cellular breakdown. Hematoxylin and eosin, × 100. Top right: Baseline atherosclerosis. A downstream section from the artery shown in top left figure after staining for fat. A relatively fat-free collar of fibrous tissue encircles the lumen. Throughout the rest of the intima mixtures of fat-free and fat-bearing cells are found among varying amounts of intimal scarring. Oil-Red-O, × 90. Middle left: Baseline atherosclerosis. Large masses of acellular material are seen at the outer third of the intima. Hematoxylin and eosin, × 70. Middle right: Baseline atherosclerosis. In the larger artery a radial arrangement of the outer fibrous structure of the intima orients a mixed population of cells, the middle portion of the intimal lesion is occupied by a circumferential fibrous collar, and the innermost portion shows cellular hyperplasia. This form of intense arteritis may be seen frequently as a response to the hyperlipidemia. The portion of branch artery shown at the left has a greater acellular portion in the intimal mass. Hematoxylin and eosin, × 110. Bottom left: Regression atherosclerosis. Visible intimal lipid (black) is small within an unusually large residual mass of fibrous

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average total transverse area among the groups. Dilatation or distension cannot be ruled out in baseline atherosclerosis, however, since the average cross-sectional area of the arteries of this group was larger than in the regression animals, although the regression monkeys were more than 3 years older at autopsy.

The decreased narrowing of the lumens of the coronary arteries in animals fed regression regimens was accompanied by several features that would be looked for at the end of a regression study. One was the gross observation of distinctly fewer lesions throughout the accessible arterial tree, including the aorta, in regression animals than were seen in animals with baseline atherosclerosis. This finding will be reported separately. A second observation pertains to the gross appearance of the unopened, intact coronary arteries themselves. Group 1 had beaded, ivory-colored arteries, but the regression groups had smoother, paler arteries suggesting thinner walls. The third, and to us, most notable evidence in favor of

tissue. Sudan IV, × 90. Bottom right: Regression atherosclerosis. The residual intimal lesion is rich in collagen (dark), and collagen scars are seen also in the media. Van Gieson, × 90.

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regression was found in the histologic differences of the coronary sections in the regression groups compared to group 1. Figures 1 and 2 illustrate the nature of this evidence.

**SERUM LIPIDS**

The serum lipid values after 17 months of atherogenic diet are shown in Table 2. Serum cholesterol was the most labile lipid. In Table 5 are shown the cholesterol levels for all groups during the initial period of observation, at the end of the induction of coronary atherosclerosis, and at the end of the regression trials. The average serum cholesterol rose fivefold during the first 5 months of the atherogenic diet and remained at this level for the remainder of the 17-month period. The rise in serum cholesterol was preponderantly due to a diet-induced hyperbetalipoproteinemia; the serum lipid and lipoprotein characteristics have been previously described in detail (2, 4, 22). The cholesterol of control animals remained at the general level of 140 mg/100 ml throughout the study. Within 60 days after the two regression diets were started, the serum cholesterol of the hypercholesterolemic animals had returned to the levels observed at the start of the study with little subsequent change throughout the regression period.

**Discussion**

In the present investigation, monkeys had prominent atheromatous lesions in the coronary arteries after they were given a diet rich in fat and cholesterol, but matched animals fed a sequence of atherogenic and regression diets had smaller fibrotic lesions with scant stainable lipid. The regression diets, cholesterol-free and either low in fat or enriched with corn oil, were approximately equal in effect as judged by the residual intimal narrowing of the lumen. The relatively small numbers of primates assigned to each of the regression diets prevent more than a tentative assessment of their comparative merit. Large sample sizes would be required to discriminate among dietary approaches in the control or reversal of coronary atheromatous lesions in their experimentally induced form.

The concept of reversibility of atheromatous tissue is not new. Years ago Anitschkow described loss of visible lipid from lesions in the rabbit in regression studies (12). He studied aortic lesions, and subsequent experimental data have shown a spectrum of changes in the size of aortic lesions in the rabbit after regression regimens from none (19) to minor shrinkage (13, 14, 26) to increases (27).

Whether atheromatous lesions regress in the coronary arteries may be highly dependent on the species chosen for study. Friedman and Byers concluded that coronary atherosclerosis is irreversible in the rabbit (19). We have presented similar evidence in preliminary form (28). Horlick and Katz described partial reversibility of intimal lesions in the coronary arteries of chickens in a pioneering dietary regression study (15). Peterson and Hirst qualified their regression results in the chicken with the statement that a decrease in coronary atherosclerosis occurred in early lesions but not in older fibrotic ones (16). Bevins et al. found regression of intimal lesions of young dogs at 2 to 4 months after being withdrawn from a hypercholesterolemic regimen of cholesterol and thiouracil (17). Minimal or no coronary lesions were found in their regression animals. Since eight of 17 atherosclerotic animals also had no coronary atherosclerosis, the extent of regression in the coronary arteries is difficult to assess. A notable attempt to cause regression of atherosclerosis in primates was reported by Maruffo and Portman (29). In squirrel monkeys a butter-containing diet initiated coronary atherosclerosis, and a corn oil-containing control diet stopped or partially

### Table 5

<table>
<thead>
<tr>
<th>Group</th>
<th>Start of study</th>
<th>17 months</th>
<th>40 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>138 ± 4</td>
<td>701 ± 47</td>
<td>136 ± 11*</td>
</tr>
<tr>
<td>2</td>
<td>142 ± 9</td>
<td>712 ± 68</td>
<td>136 ± 7*</td>
</tr>
<tr>
<td>3</td>
<td>140 ± 5</td>
<td>703 ± 37</td>
<td>138 ± 7*</td>
</tr>
<tr>
<td>Control</td>
<td>143 ± 7</td>
<td>140 ± 10</td>
<td>135 ± 8</td>
</tr>
</tbody>
</table>

Values are means ± SE.

*At 40 months this group consisted of nine animals. See Discussion.
inhibited the process. Actual decrease of the coronary lesions was not observed during regression periods up to 5 months. Loss of visible intimal lipid occurred, however, and we agree with these investigators that a more protracted trial would have been desirable.

Malnutrition and wasting diseases such as carcinomatosis have been associated with apparent decreases of atherosclerosis in man (30-33). It is reasonable to presume that the same conditions might cause comparable change in atherosclerosis in a nonhuman primate. In the present study stability of body weight, normal hematocrits, absence of diarrhea, and alert spontaneous behavior led to the judgement that the animals were healthy. A single regression animal died after 20 months of the low-fat diet following a short illness found at autopsy to be tuberculosis. The matching animal on the linoleate-rich diet was therefore studied at autopsy to maintain comparability of observation periods between the two diets. The decoded coronary sections showed the monkey with the illness to have had 31% average intimal narrowing of the lumen and the matching animal 20%. The inclusion or exclusion of this pair of animals has no effect on the statistical significance of the regression results. No other extraneous disease was found. The possible relationship between neoplasia and linoleate-enriched diet noted in man by Dayton et al. (34) was not evident in the primate group fed the high-fat regression diet.

Common aggravants of atherosclerosis such as spontaneous diabetes and hypertension are infrequently encountered in the rhesus monkey. Periodic checks for urinary glucose were uniformly negative. The absence of a hypertensive state was demonstrated by both blood pressure and heart weight data. No sustained high blood pressures were obtained by indirect means (35). Consistently normal basal blood pressures were found after recourse to mild sedation with phencyclidine. Sedation was used infrequently, however, because it diminished food consumption for 1 to 5 days, and maintenance of a relatively constant dietary intake was an important feature of the nutritional requirements of the study. Cardiac hypertrophy was not present as a stigma of baseline atherosclerosis; the ratio of heart weight to body weight (g/100 g) in the experimental groups was not significantly different than that observed in controls: group 1, 0.311 ± 0.004; controls, 0.317 ± 0.042; group 2, 0.348 ± 0.044; and group 3, 0.306 ± 0.038.

Uncomplicated atheromas, defined for present purposes as masses of intimal fat covered with a fibrous cap, were the principal lesions under study. The stringent regression diets were designed to test whether excess arterial lipid deposited in atheromatous lesions can be depleted in a primate by dietary means. The possibility that atheromatous connective tissue elements such as collagen might significantly diminish was considered exceedingly remote, and the data of this morphologic study show condensation rather than decrease in the intimal connective tissue after regression.

On the other hand, there is strong evidence that the diets caused a large loss of lipid in the atheromatous tissue under study. How lesions significantly complicated by hemorrhage, ulceration, massive accretion of scleroproteins, necrosis and calcium formation might respond in comparable dietary settings is not known. No ulceration was found in any lesion in this study. Examples of the other complications were scattered among the lesions in baseline atherosclerosis, but were not frequent or prominent enough to permit even a preliminary estimate of their influence on the postulated disappearance of lipid from the arterial wall. Whether intimal lesions that contain large extracellular masses of lipid at their bases can be significantly depleted of this extracellular burden is unknown at present. It has been shown that even severe human atheromas equilibrate with labeled plasma cholesterol (36-38). A crucial question is whether mechanisms exist for an outward flow of sterols and other lipids from the "gruel" in the depths of the classical atheroma. Some cholesterol clefts were noted in baseline atherosclerosis, together with other evidence of extracellular lipid. No such examples were seen in the vessel walls of regression animals, thereby raising
but not answering the question whether the seemingly inert extracellular lipid mass within a plaque may also be mobilized to larger body pools when appropriate conditions are provided.

The mechanisms and most of the local conditions that underlie the potential reversibility of atherosclerosis in any of its stages from the fatty streak to the increasingly advanced plaque are still to be elucidated.

References


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