Atherosclerosis of Cynomolgus Monkeys Hyper- and Hyporesponsive to Dietary Cholesterol
Lack of Effect of Vasectomy

Thomas B. Clarkson, Nancy J. Alexander, and Timothy M. Morgan

A moderately atherogenic diet was fed to young adult cynomolgus macaque males that were observed to be either hypo- or hyperresponsive to dietary cholesterol and who were randomized into groups to be either vasectomized or sham-vasectomized. The extent of atherosclerosis was found to be considerably greater at all arterial sites studied for the monkeys that were hyperresponsive to dietary cholesterol. The differences in atherosclerosis development among the hyperresponder monkeys occurred primarily in the proximal portions of the coronary arteries, the proximal and distal portions of the common carotid arteries, and only in the most proximal portions of the femoral arteries. There were no significant effects of vasectomy or sham vasectomy on atherosclerosis extent in either the hyper- or the hyporesponding groups, although there was a suggestion of somewhat larger lesions in the left circumflex coronary artery of hyperresponder monkeys that were vasectomized and somewhat smaller atherosclerotic lesions in the left common carotid arteries of vasectomized monkeys. The data presented here do not support our first report of worsened atherosclerosis among cynomolgus monkeys fed diets high in cholesterol. The findings of the current study are consistent with recent epidemiological studies of vasectomized and nonvasectomized human males.

(Arteriosclerosis 8:488–498, September/October 1988)

The metabolic aspects of hyper- and hyporesponsiveness to dietary cholesterol have been studied extensively in squirrel monkeys (Saimiri sciureus),1-3 rhesus monkeys (Macaca mulatta),4-7 and baboons (papio species).8,9 The pathologic consequences when hyper- and hyporesponding monkeys continue to consume cholesterol-containing diets for long periods have been studied only in squirrel monkeys. Hyporesponding squirrel monkeys fed cholesterol-containing diets for long periods had minimal atherosclerotic lesions, while hyperresponding squirrel monkeys fed the same diets had markedly exacerbated atherosclerosis.10,11 While we have reported on the occurrence of hyper- and hyporesponsiveness to dietary cholesterol among cynomolgus macaques and its strong parental influence, the significance for atherogenesis of vasectomy had not previously been examined.12

The repeated injection of animals with foreign antigens exacerbates atherosclerosis.13 There are similarities between the repeated injections of a foreign antigen and the body's reaction to sperm after vasectomy. Sperm continue to be produced at prevasectomy rates, soluble antigens from sperm enter the circulation, the sperm are seen as foreign proteins by the body, and antisperm antibodies develop.14,15,16 Because of the immune response to vasectomy, there has been concern over the past several years about possible long-term health effects of the procedure. Some of the concern arose from our report nearly a decade ago that vasectomized cynomolgus macaques fed diets high in cholesterol developed more extensive atherosclerosis than did sham-vasectomized monkeys fed the same diet.15 After that report, several case-control epidemiological studies were undertaken in an attempt to determine whether there was any evidence that coronary heart disease increased among vasectomized men17-22; there was no evidence that vasectomy increased the incidence of coronary heart disease in the general population. The apparent difference in vasectomy effects on arteries between humans and monkeys has been unclear.

We and others have considered the possibility that our first observation of vasectomized animals was based on too small a sample and that an exacerbating effect of vasectomy might not be seen if much larger groups of monkeys were studied. Additionally, we were concerned with the extreme atherogeneity of the diet that was used (inducing total plasma cholesterol concentrations of 600 to 800 mg/dl).

More recently, we reported on the results of a study designed to examine the effect of a moderately athero-
in the response to vasectomy when plasma cholesterol concentrations were either slightly elevated (hyporesponders) or considerably elevated (hyperresponders). Consistent with our studies of squirrel monkeys, we found atherosclerosis to be more extensive among hyper- as compared to hyporesponder cynomolgus monkeys. Additionally, there were suggestions that vasectomy may have resulted in slightly reduced amounts of atherosclerosis at some arterial sites; however, we could not determine whether such small differences were due to somewhat lower total plasma cholesterol concentrations and low density lipoprotein cholesterol concentrations. In general, it is our conclusion that vasectomy had no significant effects on the extent of atherosclerosis among either hyper- or hyporesponding cynomolgus monkeys.

Methods

Animals

The animals used were 66 male cynomolgus monkeys imported as adults from Malaysia (average age 7.5 years, estimated by dentition). They were selected from a population of about 300 adult male Macaca fascicularis that had been fed a diet containing approximately 0.34 mg/ kcal of cholesterol for 2 months. Blood samples for determination of total plasma cholesterol and high density lipoprotein (HDL) cholesterol concentrations were taken from all animals at weeks 6 and 8 during this pretest period. The 34 animals selected as being hyporesponsive to dietary cholesterol had an average plasma cholesterol concentration of 222 mg/dl. In contrast, the 32 animals selected as being hyperresponsive to dietary cholesterol had an average plasma cholesterol concentration of 422 mg/dl. After selection, the animals were brought to the Bowman Gray School of Medicine, the diet was continued, and plasma cholesterol concentration tests were repeated to confirm their hypo- and hyperresponsiveness.

Design and Allocation of Monkeys to Groups

The design of the experiment was a stratified randomized trial in which animals either hypo- or hyperresponsive to dietary cholesterol were randomized to be

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of monkeys</th>
<th>TPC</th>
<th>HDLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperresponders</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vx (Group 1)</td>
<td>17</td>
<td>221 ± 11.6</td>
<td>55 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>(116 to 287)</td>
<td>(39 to 78)</td>
<td></td>
</tr>
<tr>
<td>Sham Vx (Group 2)</td>
<td>17</td>
<td>222 ± 11.4</td>
<td>55 ± 4.1</td>
</tr>
<tr>
<td></td>
<td>(123 to 293)</td>
<td>(40 to 79)</td>
<td></td>
</tr>
<tr>
<td>Hyporesponders</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vx (Group 3)</td>
<td>16</td>
<td>423 ± 18.7</td>
<td>45 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>(313 to 615)</td>
<td>(24 to 72)</td>
<td></td>
</tr>
<tr>
<td>Sham Vx (Group 4)</td>
<td>16</td>
<td>426 ± 19.2</td>
<td>47 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>(308 to 597)</td>
<td>(23 to 72)</td>
<td></td>
</tr>
</tbody>
</table>

Data are the means (mg/dl) for the groups ± SE of the mean; the range of values is in parentheses.

Vx = vasectomy, Sham Vx = sham vasectomy, TPC = total plasma cholesterol concentration, HDLC = high density lipoprotein cholesterol concentration.

The design of the experiment was a stratified randomized trial in which animals either hypo- or hyperresponsive to dietary cholesterol were randomized to be

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>gm/100 gm</th>
<th>Protein (gm)</th>
<th>Fat (gm)</th>
<th>Carbo (gm)</th>
<th>Calories/100 gm of diet</th>
<th>Chol (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein USP</td>
<td>9.0</td>
<td>9.0</td>
<td>—</td>
<td>—</td>
<td>36.0</td>
<td>—</td>
</tr>
<tr>
<td>Lactalbumin</td>
<td>8.0</td>
<td>6.0</td>
<td>—</td>
<td>—</td>
<td>32.0</td>
<td>—</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>33.5</td>
<td>3.5</td>
<td>0.33</td>
<td>28.5</td>
<td>131.0</td>
<td>—</td>
</tr>
<tr>
<td>Dextrin</td>
<td>10.0</td>
<td>—</td>
<td>—</td>
<td>10.0</td>
<td>40.0</td>
<td>—</td>
</tr>
<tr>
<td>Sucrose</td>
<td>3.6</td>
<td>—</td>
<td>—</td>
<td>3.6</td>
<td>14.4</td>
<td>—</td>
</tr>
<tr>
<td>Applesauce</td>
<td>0.7</td>
<td>0.003</td>
<td>trace</td>
<td>0.168</td>
<td>0.68</td>
<td>—</td>
</tr>
<tr>
<td>Butter</td>
<td>23.50</td>
<td>0.10</td>
<td>19.00</td>
<td>0.10</td>
<td>171.90</td>
<td>70.5</td>
</tr>
<tr>
<td>Hegsted salt mix</td>
<td>3.80</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Complete vitamin mix</td>
<td>2.60</td>
<td>—</td>
<td>—</td>
<td>2.6</td>
<td>10.4</td>
<td>—</td>
</tr>
<tr>
<td>Alphacel</td>
<td>5.22</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.08</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>80.0</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>20.60</td>
<td>19.33</td>
<td>44.97</td>
<td>436.38</td>
<td>150.5</td>
</tr>
</tbody>
</table>

Equivalent to 0.35 mg cholesterol/kcal of diet.

Protein = 19% of calories, fat = 40% of calories, and carbohydrate = 41% of calories.

Table 1. Allocation of Monkeys to Groups and Pre-experimental Plasma Lipid Concentrations

Table 2. Composition of Moderately Atherogenic Diet
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The double-ligation technique, and the other half were sham-vasectomized by subjecting them to the same surgical procedure but without ligation or resection of the vas deferens.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Baseline</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>HO-VX</td>
<td>5.17 ± 0.22</td>
<td>5.37 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>HO-SHAM</td>
<td>4.97 ± 0.13</td>
<td>5.30 ± 0.18†</td>
</tr>
<tr>
<td></td>
<td>HP-VX</td>
<td>4.83 ± 0.12</td>
<td>5.22 ± 0.12†</td>
</tr>
<tr>
<td></td>
<td>HP-SHAM</td>
<td>4.81 ± 0.19</td>
<td>5.19 ± 0.19†</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dl)</td>
<td>HO-VX</td>
<td>15.53 ± 0.71</td>
<td>12.65 ± 0.73†</td>
</tr>
<tr>
<td></td>
<td>HO-SHAM</td>
<td>14.82 ± 0.87</td>
<td>12.58 ± 0.51†</td>
</tr>
<tr>
<td></td>
<td>HP-VX</td>
<td>15.25 ± 0.81</td>
<td>12.53 ± 0.87†</td>
</tr>
<tr>
<td></td>
<td>HP-SHAM</td>
<td>16.50 ± 0.96</td>
<td>13.40 ± 1.09†</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>HO-VX</td>
<td>0.96 ± 0.03</td>
<td>1.04 ± 0.04‡</td>
</tr>
<tr>
<td></td>
<td>HO-SHAM</td>
<td>0.98 ± 0.04</td>
<td>1.06 ± 0.03*</td>
</tr>
<tr>
<td></td>
<td>HP-VX</td>
<td>1.00 ± 0.04</td>
<td>1.13 ± 0.04*</td>
</tr>
<tr>
<td></td>
<td>HP-SHAM</td>
<td>1.04 ± 0.05</td>
<td>1.15 ± 0.04</td>
</tr>
</tbody>
</table>

*Mean during experimental period significantly (p<0.05) different from the baseline mean value. †Mean during experimental period significantly (p<0.001) different from the baseline mean value. ‡Hyperresponders significantly (p<0.05) different from the hyporesponders.


Table 3. Body Weight and Clinical Pathologic Observations

Health Profile Observations

Antisperm Antibody Measurements

Both sperm agglutination and sperm immobilization antibody assays were used to assess the immune response to vasectomy. Results from sperm agglutination antibody (SpAgg) assays are reported in integers representing the lowest titer of serum from a test animal that would agglutinate a given volume of normal sperm. Values ranged from 0 to 8 and represent serum dilutions of 1:10, 20, 40, 80, 160, 320, 640, or 1280 with 0 being <1:10. Animals with high SpAgg concentrations would

Surgical Procedures

After 6 months of consumption of the cholesterol-containing diet, half of the animals in each dietary cholesterol response group were vasectomized by the double-ligation technique, and the other half were sham-vasectomized by subjecting them to the same surgical procedure but without ligation or resection of the vas deferens.

Diet

A moderately atherogenic diet designed to mimic the composition of diets often consumed by North American humans who are at high risk for coronary heart disease was used in this study (Table 2). The diet contained 40% of calories from fat and 0.35 mg cholesterol per calorie. The diet was mixed with 24 ml of H2O per 100 grams dry weight constituents, and 100 grams was fed to each animal twice a day. Both hypo- and hyperresponsive animals consumed all of the diet. Body weight gains were recorded on monkeys sedated with ketamine hydrochloride by using a Doppler ultrasound apparatus (Arteriosonde 1010, Roche, Cranberry, NJ). Blood pressures were determined at 10 different intervals during the course of the study.

Blood Pressure

Systolic and diastolic blood pressure measures were recorded on monkeys sedated with ketamine hydrochloride by using a Doppler ultrasound apparatus (Arteriosonde 1010, Roche, Cranberry, NJ). Blood pressures were determined at 10 different intervals during the course of the study.

Plasma Lipid Concentrations

Blood samples for determination of total plasma cholesterol and HDL cholesterol concentrations were taken at 2-month intervals during the course of the study. Total plasma cholesterol determinations (in mg/dl) were done by using the AutoAnalyzer II procedure. HDL cholesterol concentrations (in mg/dl) were assessed by the heparin manganese precipitation procedure, as described in the Lipid Research Clinics manual. All plasma lipid evaluations were evaluated in our Lipid Analytic Laboratory, which is in complete compliance with the Cooperative Lipid Standardization Program of the U.S. Department of Health and Human Services. Animals were fasted 24 hours before sampling, and during the sampling procedures they were restrained with ketamine hydrochloride (15 mg/kg).

Health Profile Observations

During the course of the pre-experimental and experimental periods, body weights were determined at 2-month intervals. At 6-month intervals, total serum protein concentrations, blood urea nitrogen concentrations, serum creatinine concentrations, urinary protein concentrations, hematoctits, hemoglobin concentrations, and enumeration of erythrocytes/leukocytes were measured in the Clinical Pathology Laboratory of the Department of Comparative Medicine using standard quality-controlled procedures.

either vasectomized or sham-vasectomized. The animals were allocated to the two groups so they would be as equal as possible within each stratum in terms of total plasma cholesterol concentrations, HDL cholesterol concentrations, and age. The design of the experiment and the plasma lipid concentrations of the four groups of animals are shown in Table 1.

Figure 1. Schematic illustration of sources of coronary artery blocks from morphometric evaluations.

Diet

A moderately atherogenic diet designed to mimic the composition of diets often consumed by North American humans who are at high risk for coronary heart disease was used in this study (Table 2). The diet contained 40% of calories from fat and 0.35 mg cholesterol per calorie. The diet was mixed with 24 ml of H2O per 100 grams dry weight constituents, and 100 grams was fed to each animal twice a day. Both hypo- and hyperresponsive animals consumed all of the diet. Body weight gains were the same (see Table 3).

To ensure that plasma cholesterol concentrations remained within desired ranges, it was necessary to make two reductions in the amount of dietary cholesterol. At month 4 of the 18-month study, the amount of dietary cholesterol was reduced to 0.25 mg/kcal. At month 11, a further reduction was made to 0.18 mg/kcal, and this amount was fed for the remainder of the experiment.

Surgical Procedures

After 6 months of consumption of the cholesterol-containing diet, half of the animals in each dietary cholesterol response group were vasectomized by the double-ligation technique, and the other half were sham-vasectomized by subjecting them to the same surgical procedure but without ligation or resection of the vas deferens.

Table 3. Body Weight and Clinical Pathologic Observations

RCA

Aorta

LCX

LAD

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have a high dilution factor and thus a high integer value. Numbers for the complement dependent sperm immobilization (Splmm) antibody assays are the result of the following equation:

\[
\text{Splmm} = 1 - \frac{\% \text{ motile sperm in test serum}}{\% \text{ motile sperm in control serum}}
\]

The range of possible values is 0 to 1, with 0 representing low antibody concentration in the experimental serum and 1 representing high antibody titers in test animals.

**Measurements of Atherosclerosis**

**Thoracic and Abdominal Aorta**

The thoracic and abdominal aorta were dissected from each animal. The arteries were cleaned of excess adventitia, were opened longitudinally, were mounted flat on cardboard, and were immersion-fixed in 10% neutral buffered formalin for 1 week. After fixation, the arteries were stained with Sudan IV in isopropyl alcohol. To evaluate the percent intimal involvement, the arteries were photographed and the photographs were then digitized to calculate the percentage of total surface area affected with plaque and/or fatty streaks.

To evaluate lesion extent, five standard blocks (whole width of aorta) were cut from each artery by using a celluloid template for block selection. The blocks were 3 mm long and were cut perpendicular to the long axis of the artery. After embedding in paraffin, a 5 μm section was cut from each block and was stained with Verhoeff van Gieson stain.

The stained artery cross-sections were then projected with a projection microscope onto a digitizer tablet. Using a hand-held stylus and a computer-assisted digitizer, we measured the cross-sectional intimal area of each artery section.

**Coronary Arteries**

At necropsy, the heart was removed and the coronary arteries were perfused with 10% neutral buffered formalin under a pressure of 100 mm Hg. After pressure fixation, 17 tissue blocks (each 3 mm in length) were cut perpendicularly to the long axis of the coronary arteries. Six of these were serial blocks from the left circumflex, six were from the left anterior descending artery, and five were from the right coronary artery (Figure 1). Two sections were cut from each block and were stained with either hematoxylin and eosin or Verhoeff van Gieson stains.

Each of the cross-sections stained with Verhoeff van Gieson were projected, and the area occupied by intima and/or intimal lesion was measured. The extent of coronary artery atherosclerosis for each animal was expressed as the cross-sectional intimal area (in mm²) of each of the sections of each of the coronary arteries. Coronary artery sections were also projected and digitized by a second investigator; a comparison of cross-sectional intimal areas based on the two sets of measurements yielded a reliability coefficient of 0.96.

### Table 4. Hematologic Observations

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Baseline</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte counts</td>
<td>HO-VX</td>
<td>14.13 ± 1.53†</td>
<td>13.05 ± 0.76‡</td>
</tr>
<tr>
<td>(x10⁹/mm³)</td>
<td>HO-SHAM</td>
<td>12.53 ± 1.05</td>
<td>10.85 ± 0.69</td>
</tr>
<tr>
<td>HP-VX</td>
<td>14.57 ± 1.12‡</td>
<td>13.99 ± 0.83§</td>
<td></td>
</tr>
<tr>
<td>HP-SHAM</td>
<td>11.08 ± 0.84</td>
<td>10.00 ± 0.34</td>
<td></td>
</tr>
<tr>
<td>Total monocyte count</td>
<td>HO-VX</td>
<td>4.45 ± 0.59</td>
<td>3.04 ± 0.40‡</td>
</tr>
<tr>
<td>(x10³/mm³)</td>
<td>HP-VX</td>
<td>4.68 ± 0.70</td>
<td>2.57 ± 0.34‡</td>
</tr>
<tr>
<td>HP-SHAM</td>
<td>5.68 ± 1.17</td>
<td>2.92 ± 0.44§</td>
<td></td>
</tr>
</tbody>
</table>

*Mean during experimental period significantly (p<0.05) different from the baseline mean value. †Vasectomized animals significantly (p<0.05) different from the sham-vasectomized animals. §Vasectomized animals significantly (p<0.001) different from the sham-vasectomized animals.

**Iliacofemoral and Carotid Arteries**

The iliacofemoral and common carotid arteries, including the carotid bifurcations, were removed at necropsy, were opened longitudinally on the anterior surface, and were placed in 10% neutral buffered formalin for immersion fixation. For both the iliacofemoral and common carotid arteries, five standardized blocks were taken, and the prepared sections were stained with hematoxylin and eosin and with Verhoeff van Gieson stains. Cross-sectional intimal areas were determined for each of the arteries as described for coronary arteries. The size of the carotid bifurcation prevented taking more than a single standardized block. The carotid bifurcation was processed as described for the common carotid arteries, and the extent of atherosclerosis was determined as the cross-sectional intimal area.

### Table 5. Blood Pressure Observations

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Baseline</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure</td>
<td>HO-VX</td>
<td>96.47 ± 3.21</td>
<td>95.33 ± 3.27</td>
</tr>
<tr>
<td>(mm Hg)</td>
<td>HO-SHAM</td>
<td>96.47 ± 3.33</td>
<td>98.38 ± 4.12†</td>
</tr>
<tr>
<td>HP-VX</td>
<td>93.56 ± 3.22</td>
<td>92.11 ± 3.36</td>
<td></td>
</tr>
<tr>
<td>HP-SHAM</td>
<td>95.12 ± 1.73</td>
<td>93.76 ± 2.31</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>HO-VX</td>
<td>51.65 ± 1.92</td>
<td>52.20 ± 1.82</td>
</tr>
<tr>
<td>(mm Hg)</td>
<td>HO-SHAM</td>
<td>52.18 ± 1.88</td>
<td>51.04 ± 1.97</td>
</tr>
<tr>
<td>HP-VX</td>
<td>50.00 ± 1.98</td>
<td>48.98 ± 2.18</td>
<td></td>
</tr>
<tr>
<td>HP-SHAM</td>
<td>50.96 ± 2.38</td>
<td>50.92 ± 2.35</td>
<td></td>
</tr>
</tbody>
</table>

*Mean during experimental period significantly (p<0.05) different from the baseline mean value.

**Statistical Analyses**

Comparisons of variables with multiple measurements for each monkey were performed by univariate repeated measures analysis of variance (ANOVA) using BMDP3V software which does not exclude cases with incomplete repeated measures. Repeated measures of blood pressure were measured across time, and repeated measures of intimal areas were done on multiple sections. There were no missing repeated observations: 2% in body weight,
Results

Health Profile Observations

In Table 3 we have summarized the results of the most important clinicopathologic observations taken both before the initiation of the experiment (baseline) and during the course of the experiment (experimental). Generally, the animals remained in good health, and there were no major differences in the health profile data between hypo- and hyperresponder animals. There was a modest gain in body weight during the course of the study, an average of half a kilogram.

Because we recognized that there was some possibility that the vasectomized animals might develop glomerulonephritis as a result of their circulating immune complexes (sperm antigen/antibody), multiple measurements were made of the blood urea nitrogen and plasma creatinine concentrations. The blood urea nitrogen concentrations of all animals in all four groups decreased during the course of the experiment. The decreases were clinically unimportant, statistically significant, and have often been noted in the improved health of feral animals. There were small increases in plasma creatinine concentrations during the experimental period in all groups. Although some of the increases were statistically significant, the magnitude of the increase was of no clinical significance, and there was no indication of a relation between hyper- or hypo-responsiveness to dietary cholesterol or vasectomy.

There were no effects of hyper- or hypo-responsiveness to dietary cholesterol on leukocyte counts. We have no explanation for the small differences among the groups during the baseline period. During the experimental period, the vasectomized animals had higher total leukocyte counts than did the sham-vasectomized animals, and...
there was no difference in the extent of this elevation between the hyper- and hyporesponding vasectomized animals (Table 4). Small sperm granulomas developed in the majority of the vasectomized animals, and it was our conclusion that the elevated leukocyte counts related to local low-grade infections associated with fistulous tracts that developed from the sperm granulomas.

Monocyte adherence to endothelium is an important part of atherogenesis. We have reported that there are decreased numbers of monocytes adhering to the endothelial surfaces of the major arteries of vasectomized cynomolgus monkeys fed a moderately atherogenic diet. For that reason it was of interest to us to determine whether there were differences between vasectomized and sham-vasectomized hyper- and hyporesponder animals in the extent of total monocyte count decrease. As noted in Table 4, there were large decreases in total monocyte counts during the experimental period. There was also a significant effect of vasectomy on the magnitude of the decrease in monocyte counts. In keeping with our earlier observation that vasectomy was associated with reduced numbers of monocytes adhering to arterial surfaces, it was of interest to note that the vasectomized animals had significantly higher total monocyte counts. Whether the lower total monocyte counts of the sham-vasectomized animals and the higher counts of the vasectomized animals related to differences in arterial wall monocyte adherence is uncertain.

During the course of this experiment, we also measured total serum protein concentrations, urinary protein concentrations, hematocrits, hemoglobin concentrations, and enumerated erythrocytes. There were no differences that related to hyper- or hyporesponsiveness or to vasectomy, and we did not present those data in Table 4.

In Table 5 we have summarized our observations on blood pressure. There were no effects of hyper- or hyporesponsiveness to dietary cholesterol on either systolic or diastolic blood pressure. Similarly, there were no effects of vasectomy on blood pressure, although the hyporesponder sham-vasectomized animals had statistically significantly lower systolic blood pressures; the magnitude of that change is of no clinical significance.

Antisperm Antibodies

Both sperm agglutinating and sperm immobilizing antibody levels were measured in this study. The results indicate that there was a pronounced immune response to vasectomy. The degree to which hyper- and hyporesponding monkeys developed antisperm antibodies after vasectomy was...
The extent of coronary artery atherosclerosis observed in the left anterior descending (LAD), left circumflex (LCX), and right coronary (RCA) arteries. The data are the average intimal areas (IA) in mm² for each of the blocks studied. Hyporesponder vasectomized group (○), hyporesponder sham-vasectomized group (●), hyperresponder vasectomized group (▲), and hyperresponder sham-vasectomized group (△).

Figure 6. The extent of coronary artery atherosclerosis observed in the left anterior descending (LAD), left circumflex (LCX), and right coronary (RCA) arteries. The data are the average intimal areas (IA) in mm² for each of the blocks studied. Hyporesponder vasectomized group (○), hyporesponder sham-vasectomized group (●), hyperresponder vasectomized group (▲), and hyperresponder sham-vasectomized group (△).
Figure 7. Examples of the coronary artery atherosclerosis seen in the proximal portions of the left circumflex coronary arteries. A. From a hyporesponsive vasectomized monkey. B. From a hyporesponsive sham-vasectomized monkey. C. From a hyperresponsive vasectomized monkey. D. From a hyperresponsive sham-vasectomized monkey. The examples shown are neither the least nor the most affected but were chosen because they were near the group median. All sections were stained with Verhoeff van Gieson and were photographed at x 40.

The effect of hyper- and hyporesponsiveness to dietary cholesterol on the ratio of total plasma cholesterol to HDL cholesterol concentrations are presented in Figure 5. As expected, there were major differences between hyper- and hyporesponse animals in the ratio of total plasma cholesterol to HDL cholesterol concentrations. The ratio seen among the hyporesponse animals was generally about 4 and did not change markedly with reductions in dietary cholesterol. In contrast, in the hyperresponse animals, the ratio increased to about 12 and decreased with each of the decreased dietary cholesterol regimens, reaching a final ratio of about 7. There were no significant effects of vasectomy among either the hyper- or hyporesponse animals.

Atherosclerosis Development

Thoracic and Abdominal Aorta

The evaluation of thoracic and abdominal aorta atherosclerosis was done in two ways. First, we estimated the percent of the intimal surface affected with lesions, and then we determined the lesion extent expressed as average intimal area of those lesions (Table 6) In both the thoracic and abdominal aorta there were significant differences between the hyper- and hyporesponse animals in both percent involvement and lesion extent. There were no effects of vasectomy. In the thoracic aorta, the hyperresponse animals developed markedly more atherosclerosis than did their hyporesponse counterparts. Consistent with our recent earlier study, we found smaller lesions among the vasectomized animals of both the hyper- and hyporesponse groups. The thoracic aortic differences did not reach statistical significance (p = 0.34); however, the differences in the abdominal aorta were significant (p = 0.005).

Coronary Arteries

For the three main coronary arteries, the descriptor used for the amount of atherosclerosis was the mean cross-sectional intimal area recorded for each of the blocks as illustrated in Figure 1. In Figure 6 we have summarized all of those observations for the left anterior descending, left circumflex, and right coronary arteries. In all three of the coronary arteries there was a significant effect of hyperresponsiveness on the development of coronary artery atherosclerosis (left anterior descending, p = 0.019; left circumflex coronary artery, p < 0.001; and right coronary artery, p = 0.006). There were no significant effects of vasectomy or sham vasectomy among the hyper- and hyporesponse groups, although there was a
suggestion of larger (~0.1 mm²) lesions in the left circumflex coronary arteries of hyperresponder monkeys that were vasectomized. The differences noted in the coronary arteries were quantitative, not qualitative (Figure 7).

As expected, the extensiveness of atherosclerosis decreased from the proximal toward the more distal portions of each of the coronary arteries (left anterior descending, p<0.001; left circumflex, p<0.001; and right coronary artery, p<0.001).

It was of particular interest to us to note that the more extensive coronary artery atherosclerosis seen among hyperresponsive animals occurred primarily in the most proximal one or two blocks. With the exception of the left circumflex coronary artery, the difference in the extent of atherosclerosis between hyper- and hyporesponding animals was minimal in the more distal blocks.

**Left and Right Common Carotid Arteries**

Diet-induced common carotid artery atherosclerosis in the cynomolgus monkey tends to occur primarily in the most proximal portions and in the more distal portions just before the carotid bifurcation. In Figure 8 we have illustrated our observations on the extent of atherosclerosis in the left and right common carotid arteries. In both the left and right common carotid arteries there was a highly significant effect of hyperresponsiveness for both arteries (p<0.001). The worsened atherosclerosis seen among the hyperresponder animals occurred primarily in the proximal and distal portions, with only minimal differences in the extent of atherosclerosis in the mid-portion of the common carotid arteries.

In the left common carotid artery, the vasectomized animals had significantly less atherosclerosis (p=0.037). The effect was not seen in the right common carotid artery (p=0.209). The finding of small atherosclerotic lesions in...
the left common carotid artery is consistent with our recent observations of vasectomized cynomolgus monkeys fed moderately atherogenic diets. The differences are relatively small, are of no clinical significance, and may have occurred by chance, although there is evidence for diminished atherosclerosis in the more distal blocks of the right common carotid artery. Certainly there is no evidence for worsened atherosclerosis due to vasectomy among either the hyper- or hyporesponding animals.

**Carotid Bifurcation**

In Table 7 we have summarized our observations on carotid bifurcation atherosclerosis among the hyper- and hyporesponding vasectomized and sham-vasectomized monkeys. The carotid bifurcation of cynomolgus monkeys is an unusual arterial site in that considerable atherosclerosis develops among feral animals consuming natural diets. Further, the association between plaque progression and the plasma lipid concentrations of cynomolgus monkeys is inconsistent. In this experiment there was a highly significant effect of hyperresponsiveness in the right carotid bifurcation (p<0.001), but there was no such effect in the left carotid bifurcation. The lack of an effect of hyperresponsiveness at the left carotid bifurcation probably relates to the rather large lesions found among sham-vasectomized hyporesponding animals. At the right carotid bifurcation, the lesions seen among the vasectomized animals tended to be smaller, but the effect was not statistically significant (p=0.090). Among the hyporesponding animals, the findings of smaller carotid bifurcation plaques is again consistent with our recent report.

**Left and Right Femoral Arteries**

Our observations on the extent of atherosclerosis in the left and right femoral arteries are summarized in Figure 9. There was a significant effect of hyperresponsiveness at both of these arterial sites (left femoral artery, p=0.015; right femoral artery, p=0.013). Such significant effects of hyper- and hyporesponsiveness relate almost entirely to the worsened atherosclerosis found in the most proximal block of those arteries. In subsequent blocks, the effect of hyperresponsiveness was minimal. There was no effect of vasectomy (left femoral artery, p=0.36; right femoral artery, p=0.14). There were large differences in susceptibility to diet-induced atherosclerosis between the proximal and distal portions of the femoral artery, and this finding suggests that these arterial sites may be useful for research on regional differences.

**Discussion**

The extent of atherosclerosis was found to be considerably greater at all arterial sites studied for the monkeys that were hyperresponsive to dietary cholesterol. The differences in atherosclerosis development among the hyperresponder monkeys occurred primarily in the proximal portions of the coronary arteries, the proximal and distal portions of the common carotid artery, and only in the most proximal portion of the femoral arteries. Among the hyperresponder animals, there was no evidence that vasectomy increased atherosclerosis extent, although there was a suggestion of larger lesions in the left circumflex coronary artery but not in the left anterior descending and right coronary arteries. There was no effect of vasectomy on coronary artery atherosclerosis of the hyporesponder animals.

The data presented here do not support our first report of worsened atherosclerosis among vasectomized cynomolgus monkeys fed diets high in cholesterol. This study differs from the first reported study in several ways. First, much larger groups of animals were used. Second, the atherogenic diet fed to the animals was more typical of that consumed by North American human beings in fat and cholesterol content. Third, current morphometric methods for assessing lesion extent permit the collection of more extensive and precise observations of the arteries being studied. Finally, the large morphometric data bases lend themselves to more sophisticated statistical analyses. The finding in the current study that vasectomy has no effect on atherosclerosis in either hyper- or hyporesponding monkeys is consistent with recent epidemiological studies of human beings.

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**References**


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Atherosclerosis of cynomolgus monkeys hyper- and hyporesponsive to dietary cholesterol.
Lack of effect of vasectomy.
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