For 26 months, we fed 60 baboons a high saturated fat, high cholesterol diet that contained very low concentrations of four common pesticides (chlordane, parathion, diazinon, and carbofuran). We detected no effect of pesticides on body weight, serum lipid, or lipoprotein cholesterol concentrations, or experimental atherosclerosis. We then examined the associations of serum lipid and lipoprotein cholesterol concentrations (predictor variables) with arterial lesions (response variables). Among predictor variables, very low density lipoprotein plus low density lipoprotein cholesterol concentration showed a positive association with fatty streaks in the aorta and its major branches, including the coronary arteries, while high density lipoprotein cholesterol concentration showed a consistently negative association. The very low density lipoprotein plus low density lipoprotein/high density lipoprotein cholesterol ratio was more highly associated with lesions than was either value alone. These results are consistent with epidemiologic evidence suggesting that high density lipoprotein cholesterol concentration is inversely related to probability of developing clinically manifest atherosclerotic disease. (Arteriosclerosis 1:3–12, January/February 1981)

Epidemiologic evidence suggests that human atherosclerotic disease is positively associated with low density lipoprotein (LDL) cholesterol concentration, and negatively associated with high density lipoprotein (HDL) cholesterol concentration. Evidence from animal models also indicates an inverse association of experimental atherosclerosis with LDL cholesterol, but there is limited evidence for a negative association with HDL cholesterol.1

We have performed an experiment with baboons to test the effects of four common pesticides on serum lipoproteins and on experimental atherosclerosis. The experiment was stimulated by the observation that workers exposed to chlorinated hydrocarbon pesticides had elevated HDL cholesterol concentrations.2 Feeding a diet enriched in cholesterol and saturated fat to the baboons elevated total serum and lipoprotein cholesterol concentrations to levels similar to those of humans. We detected no effect of pesticides on either serum lipoproteins or experimental atherosclerosis. However, the associations between lipoprotein cholesterol concentrations and the atherosclerosis induced were similar to epidemiologic observations on LDL and HDL cholesterol concentrations and coronary heart disease in humans.

**Methods**

**Subjects and Treatment**

Sixty adult olive baboons (Papio sp.) (30 male, 30 female) were received from Hazelton Prime Laboratories, Farmingdale, New Jersey, in three groups of 20 animals. During the first 3 months of the experiment, the baboons were kept in outdoor cages by sex in groups of 10. During the following 24 months, they were kept in indoor-outdoor cages in groups of three of one sex. Three animals died of causes not related to the diet or pesticides administered.
During a 1-month baseline period, all animals received stock baboon chow containing 5.8% calories from fat. Following this period, they received the high cholesterol, saturated fat (atherogenic) diet (table 1) for 2 months, and thereafter received the same diet with added pesticide (table 2) for 24 months.

The diet contained 1.7 mg/kcal of cholesterol and provided 21% of calories as protein, 41% as fat (mostly lard), and 38% as carbohydrate. Saturated fatty acids provided 17.2%, and polyunsaturated fatty acids 6.0%, of the calories. The estimated daily intake of each pesticide per kilogram of body weight is shown in table 2. The low dose of pesticide was calculated to provide a daily intake per kilogram of body weight several times the recommended allowable daily intake for humans, and the high dose was calculated to provide just under the amount required to cause acute toxicity in baboons as estimated in a preliminary range finding study.

Blood was collected twice during the baseline period and every 2 months while the animals were on the atherogenic diet. After an overnight fast, the blood was drawn under ketamine (Vetalar, Parke, Davis and Company, Detroit, Michigan) anesthesia with no anticoagulant and centrifuged promptly to separate serum. The animals were weighed each time blood was drawn.

**Laboratory Methods**

**Cholesterol and Triglyceride Analysis**

Serum and lipoprotein fractions were analyzed for cholesterol by an enzymatic method on the ABA 100 Bichromatic Analyzer (Abbott Laboratories, South Pasadena, California). Triglycerides were determined by an enzymatic method on the same instrument.

Two serum cholesterol and triglyceride analyses were performed at a 1-month interval while the animals were on the chow diet, and 12 were performed at 2-month intervals while they were on the atherogenic diet.

**Lipoprotein Analyses**

Serum lipoproteins were separated by a modified single-spin, isopycnic, density-gradient ultracentrifugal method. Four ml of serum, brought to a density (d) of 1.21 g/ml by adding NaCl-NaBr crystals, were overlaid with three NaCl solutions (3.8 ml, d = 1.063; 3.3 ml, d = 1.019; and 1.8 ml, d = 1.006) in a 13.2 ml tube and centrifuged for 24 hours at 38,000 rpm and 10°C in a Beckman SW-40 rotor. The isolated lipoproteins (VLDL, LDL, HDL) were analyzed for cholesterol at 2, 6, 10, and 26 months after beginning the atherogenic diet. Since the overall average very low density lipoprotein (VLDL) cholesterol concentration was only 8.5% of the average combined VLDL + LDL cholesterol concentration, we used the combined VLDL + LDL cholesterol values for the statistical analyses.

Determinations of lipoprotein cholesterol by a dextran sulfate-CaCl<sub>2</sub> precipitation method also were performed at 4, 18, 22, and 26 months after beginning the atherogenic diet.

**Removal and Preparation of Arteries**

Complete autopsies were performed on the 57 animals that survived the 27 months of the experiment. We bisected the aorta longitudinally and stored the left half at —20°C for lipid analysis. The right half of the aorta and the longitudinally opened coronary, carotid, innominate, brachial, and iliac-femoral arteries were fixed, stained with Sudan IV, and stored in plastic bags.

Table 1. Ingredients of the Atherogenic Diet

<table>
<thead>
<tr>
<th>Item</th>
<th>Units per kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ralston Purina Special Monkey Chow 25-5045-6</td>
<td>706.00 g</td>
</tr>
<tr>
<td>Lard (with or without premixed pesticide)</td>
<td>128.00 g</td>
</tr>
<tr>
<td>Dried egg yolks</td>
<td>43.00 g</td>
</tr>
<tr>
<td>Cholesterol, USP</td>
<td>5.30 g</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>9.26 mg</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>0.75 mg</td>
</tr>
<tr>
<td>Salt (NaCl)</td>
<td>0.97 g</td>
</tr>
<tr>
<td>Water</td>
<td>117.00 ml</td>
</tr>
</tbody>
</table>

Table 2. Dose of Pesticide by Treatment Groups

<table>
<thead>
<tr>
<th>Groups*</th>
<th>Dose of pesticide</th>
<th>Concentration (μg/g feed)</th>
<th>Estimated daily intake (mg/kg body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorodane</td>
<td>Low</td>
<td>5.0</td>
<td>0.10</td>
</tr>
<tr>
<td>Low</td>
<td>50.0</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>25.0</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>Diazinon</td>
<td>Low</td>
<td>5.1</td>
<td>0.10</td>
</tr>
<tr>
<td>High</td>
<td>20.3</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>Parathion</td>
<td>Low†</td>
<td>7.5</td>
<td>0.15</td>
</tr>
<tr>
<td>High</td>
<td>25.0</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>Carbofuran</td>
<td>Low†</td>
<td>62.3</td>
<td>1.25</td>
</tr>
<tr>
<td>High</td>
<td>250.0</td>
<td>5.00</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.0</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>

*There were three males and three females in each treatment group. Six males and six females comprised the control group. There were a total of 30 males and 30 females in the study.

†One male animal died in each of these groups. Observations on them are excluded from all analyses.
Grading Atherosclerotic Lesions

Two observers (one coauthor, HCM; and a consulting pathologist, J. P. Strong, New Orleans), independently and blinded to sex and treatment, estimated the percent of intimal surface of each artery involved with all types of atherosclerotic lesions, and then estimated the proportion of the involved surface made up of fatty streaks and of fibrous plaques. The graders used definitions identical to those used previously.6,9 Intraclass correlation coefficients between the two sets of grades for fatty streaks ranged from a low of 0.643 in the left brachial artery to a high of 0.939 in the right iliac-femoral artery. The mean of the two gradings was used for statistical analyses.

Analyses of Aortic Intimal Lipids

We dissected the intima from the left half of each aorta on ice and stored it at −20°C. Lipids were extracted from the intima with chloroform-methanol (2:1) and separated from water-soluble components by the procedure of Folch.10 We measured plasmalogens by quantitative gas-liquid chromatography of the dimethyl acetals (DMAs), which were formed by the method of Farquhar11 and separated from fatty acid methyl esters by thin layer chromatography.12 The DMAs were measured with n-octadecane as an internal standard on a 1 meter glass column packed with 3% OV-17 on Gas Chrom Q (Applied Science Laboratories, State College, Pennsylvania) at 160°C.

We measured free and total cholesterol enzymatically by modifying an enzymatic method3 on the ABA-100 Bichromatic Analyzer. After the chloroform-methanol extract was dried and resuspended in redistilled isopropanol, we measured free cholesterol by addition of Auto Flow Cholesterol Reagent (BMC/Boehringer Mannheim, Indianapolis, Indiana) without cholesterol esterase. Total cholesterol was then measured by adding cholesterol esterase to the same reaction mixture. Cholesterol esters were calculated as the difference between the total and free cholesterol concentrations. Triglycerides were measured enzymatically in isopropanol on the ABA 100 Bichromatic Analyzer.4 Phospholipids were measured by the method of Bartlett.13 We computed the amounts of each lipid both as the weight of lipid per unit wet weight of intima and as total amount of each lipid in each intima.

Statistical Methods

Using analysis of variance,14 we tested the effects of the four pesticides and sex on body weight, serum lipid and lipoprotein cholesterol concentrations, arterial lesions, and the amounts and concentrations of lipids in the aortic intima. We divided the observations on the animals into predictor variables and response variables for subsequent analyses. The predictor variables were obtained by averaging all the measurements made on each animal during the basal period while it was on a chow diet, and during the challenge period while it was on the atherogenic diet. As predictor variables, we used body weight, serum triglyceride concentration, and total serum cholesterol concentration during the basal and challenge periods and, in addition, VLDL + LDL cholesterol concentration and HDL cholesterol concentration during the challenge period. We calculated the increment in total serum cholesterol as the difference between challenge and basal total serum cholesterol concentrations; and the ratio of VLDL + LDL cholesterol to HDL cholesterol [(VLDL + LDL)/HDL]. We carried out the statistical analyses with lipoprotein cholesterol values from both ultracentrifugal and precipitation techniques combined, and repeated them using the ultracentrifugal values alone. The results presented in subsequent tables are based on the ultracentrifugal values alone, and these were similar to those based on both precipitation and ultracentrifugal values.

The response variables were the gross evaluations of lesions and chemical analyses of the left half of the aorta. Gross evaluations included the percent surface area involved with fatty streaks in the aortic arch, the right halves of the thoracic aorta and the abdominal aorta, the innominate artery, and averages of the pairs of left and right carotid arteries, brachial arteries, and iliac-femoral arteries. Involvement of any artery with fibrous plaques and involvement of the coronary arteries with fatty streaks were considered as present or absent for statistical analyses. In the carotid, brachial, and iliac-femoral arteries, a grade of “present” was scored if fibrous plaques were found in either the right or left branches. Because of the binary nature of these grades, we considered their relationship with lipids separately.

To compare the results of chemical analyses of aortic intima with surface involvement of the aorta, we computed a single gross grade as the weighted average of intimal surface involvement of the thoracic and abdominal segments of each aorta.

To provide a descriptive summary, we computed partial correlation coefficients (adjusted for linear effects of pesticide and sex)14 among selected predictor and response variables.

To develop prediction equations, we used stepwise (forward selection) multiple regression15 for each response variable separately and examined the order of entry of each predictor variable into the regression equation. From the order of entry of each predictor variable into the equation, we selected the overall best three predictor vari-
ables. With these, we then computed multiple regression coefficients that best predicted each response variable.

In considering the relationships between predictor variables and "present" or "absent" scores of lesions, we classified the animals as either less than or equal to the median, or greater than the median, with respect to VLDL + LDL, HDL, and (VLDL + LDL)/HDL cholesterol concentrations. No adjustments were made for pesticide or sex. The degree and direction of the associations in these 2 × 2 contingency tables were measured by phi coefficients, which are interpreted like correlation coefficients. To develop a prediction equation for the probability of occurrence of a lesion, we used sex, VLDL + LDL cholesterol, and HDL cholesterol in the multiple logistic model of Walker and Duncan.

Results

General Findings
Characteristics of Lesions

The intimal lesions were similar to those described previously in the baboon fed an atherogenic diet for 2 years. Sudanophilic fatty streaks showed intimal thickening with intracellular lipid and connective tissue. Lesions grossly classified as fibrous plaques were made up primarily of smooth muscle cells and connective tissue, accompanied by varying amounts of lipid.

Effects of Pesticides

By analysis of variance, there were no statistically significant differences among the pesticide treatment groups in body weight, serum lipid or lipoprotein cholesterol concentrations, gross extent of lesions in any artery, or amount or concentration of any lipid in the aortic intima. We concluded, therefore, that none of the four pesticides influenced these variables.

Effects of Sex

The overall mean VLDL + LDL cholesterol concentration for males was 91.3 mg/dl, and for females, 89.9 mg/dl (ns). The mean HDL cholesterol concentration for males was 77.5 mg/dl; for females, 72.8 mg/dl (ns). The only significant difference between the sexes in atherosclerosis was in extent of involvement with fatty streaks in the thoracic aorta. Males had 24.0% surface involvement, and females, 41.6% (p < 0.05).

Predictor and Response Variables

A summary of predictor and response variables is given in tables 3 and 4. The partial corre-

Table 3. Serum Lipid and Lipoprotein Cholesterol Concentrations (Predictor Variables) and Extent of Arterial Fatty Streaks (Response Variables)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predictor (mg/dl):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>57.4</td>
<td>28.2</td>
<td>11.5</td>
</tr>
<tr>
<td>Challenge</td>
<td>49.5</td>
<td>22.5</td>
<td>20.1</td>
</tr>
<tr>
<td>Total serum cholesterol</td>
<td>112.8</td>
<td>20.6</td>
<td>74.5</td>
</tr>
<tr>
<td>Basal</td>
<td>204.2</td>
<td>51.1</td>
<td>114.5</td>
</tr>
<tr>
<td>Challenge</td>
<td>91.5</td>
<td>46.5</td>
<td>4.5</td>
</tr>
<tr>
<td>Increment in total serum cholesterol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Challenge</td>
<td>90.5</td>
<td>35.6</td>
<td>32.7</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>75.0</td>
<td>15.1</td>
<td>46.0</td>
</tr>
<tr>
<td>(VLDL + LDL)/HDL cholesterol ratio</td>
<td>1.2</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Response (% surface):</td>
<td>7.0</td>
<td>7.4</td>
<td>0.0</td>
</tr>
<tr>
<td>Aorta, arch</td>
<td>33.3</td>
<td>24.7</td>
<td>0.0</td>
</tr>
<tr>
<td>Aorta, thoracic</td>
<td>9.8</td>
<td>11.4</td>
<td>0.0</td>
</tr>
<tr>
<td>Aorta, abdominal</td>
<td>10.0</td>
<td>9.9</td>
<td>0.0</td>
</tr>
<tr>
<td>Innominate</td>
<td>10.9</td>
<td>11.4</td>
<td>0.0</td>
</tr>
<tr>
<td>Carotid</td>
<td>5.7</td>
<td>4.4</td>
<td>0.0</td>
</tr>
<tr>
<td>Brachial</td>
<td>2.6</td>
<td>3.2</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Abbreviations: VLDL = very low density lipoprotein; LDL = low density lipoprotein; HDL = high density lipoprotein.
Table 4. Gross Extent of Fatty Streaks, Wet Weight, and Lipid Content of Aortic Intima (55 Baboons)

<table>
<thead>
<tr>
<th>Response variable, aorta</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross extent of fatty streaks (% surface)</td>
<td>25.60</td>
<td>18.60</td>
<td>0.00</td>
<td>65.70</td>
</tr>
<tr>
<td>Intima, wet weight (mg)</td>
<td>172.90</td>
<td>65.90</td>
<td>69.10</td>
<td>434.30</td>
</tr>
<tr>
<td>Total cholesterol (mg)</td>
<td>0.96</td>
<td>0.60</td>
<td>0.14</td>
<td>2.62</td>
</tr>
<tr>
<td>Free cholesterol (mg)</td>
<td>0.76</td>
<td>0.44</td>
<td>0.13</td>
<td>2.15</td>
</tr>
<tr>
<td>Cholesterol esters (mg)</td>
<td>0.20</td>
<td>0.18</td>
<td>0.00</td>
<td>0.79</td>
</tr>
<tr>
<td>Triglycerides (mg)</td>
<td>1.43</td>
<td>1.79</td>
<td>0.02</td>
<td>7.48</td>
</tr>
<tr>
<td>Phospholipids (mg)</td>
<td>1.12</td>
<td>0.34</td>
<td>0.37</td>
<td>1.69</td>
</tr>
<tr>
<td>Plasmalogen (mg)</td>
<td>0.24</td>
<td>0.11</td>
<td>0.03</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Partial Correlation Coefficients

Partial correlation coefficients for predictor variables and surface involvement with fatty streaks are shown in table 5. All coefficients for VLDL + LDL cholesterol are positive and are slightly higher than those for challenge serum cholesterol. All coefficients for HDL cholesterol are negative. The coefficients for the (VLDL + LDL)/HDL cholesterol ratio are higher than those for VLDL + LDL cholesterol or for HDL cholesterol alone.

Partial correlation coefficients among predictor variables, gross extent of aortic fatty streaks, and the amount of each lipid in the aortic intima are shown in table 6. The gross extent of aortic fatty streaks is highly correlated with amounts of total, free, and esterified cholesterol in the intima, and weakly correlated with amounts of triglyceride, phospholipid, and plasmalogen. Coefficients for challenge VLDL + LDL cholesterol are positive for total, free, and esterified cholesterol in the aortic intima, and those for challenge HDL cholesterol are negative or close to zero.

We also examined partial correlation coefficients for the predictor variables and concentrations (mass/wet weight) of cholesterol and cholesterol esters in the aortic intima (results not shown). The correlation coefficients of VLDL + LDL cholesterol and (VLDL + LDL)/HDL ratio with concentrations were positive but smaller in magnitude than those of the lipoprotein fractions with amounts. The correlation coefficients of HDL cholesterol with aortic intimal cholesterol concentrations were positive and larger in magnitude than those of HDL cholesterol with amounts.

Table 5. Partial Correlation Coefficients among Serum Lipid and Lipoprotein Cholesterol Concentrations and Arterial Fatty Streaks

<table>
<thead>
<tr>
<th>Artery</th>
<th>Triglycerides</th>
<th>Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Challenge</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aorta, arch</td>
<td>0.37</td>
<td>0.19</td>
</tr>
<tr>
<td>Aorta, thoracic</td>
<td>-0.05</td>
<td>-0.08</td>
</tr>
<tr>
<td>Aorta, abdominal</td>
<td>0.16</td>
<td>-0.15</td>
</tr>
<tr>
<td>Innominate</td>
<td>0.12</td>
<td>-0.05</td>
</tr>
<tr>
<td>Carotid</td>
<td>0.10</td>
<td>-0.09</td>
</tr>
<tr>
<td>Brachial</td>
<td>0.14</td>
<td>-0.07</td>
</tr>
<tr>
<td>Iliac-femoral</td>
<td>0.11</td>
<td>-0.11</td>
</tr>
</tbody>
</table>

Abbreviations: VLDL = very low density lipoprotein; LDL = low density lipoprotein; HDL = high density lipoprotein.
Table 6. Partial Correlation Coefficients among Serum Lipid and Lipoprotein Cholesterol Concentrations and Aortic Intimal Lipid Content (55 Baboons)

<table>
<thead>
<tr>
<th>Response variable, aorta</th>
<th>Triglycerides</th>
<th>Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gross extent of fatty streaks</td>
<td>Basal</td>
</tr>
<tr>
<td>Gross extent of fatty streaks</td>
<td>1.00</td>
<td>0.02</td>
</tr>
<tr>
<td>Intima, wet weight</td>
<td>0.06</td>
<td>0.14</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.72</td>
<td>0.20</td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>0.69</td>
<td>0.22</td>
</tr>
<tr>
<td>Cholesterol ester</td>
<td>0.69</td>
<td>0.13</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0.07</td>
<td>0.21</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>0.37</td>
<td>0.25</td>
</tr>
<tr>
<td>Plasmalogen</td>
<td>0.15</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Abbreviations: VLDL = very low density lipoprotein; LDL = low density lipoprotein; HDL = high density lipoprotein.

Prediction Equations

Upon comparing the order of entry into the regression equation, we selected the VLDL + LDL and HDL cholesterol concentrations as the two best overall predictors. The best single predictor would have been the (VLDL + LDL)/HDL ratio, but we wished to determine the signs of the coefficients for the two lipoprotein fractions separately. Sex was the third predictor selected.

Multiple regression analysis, using these three variables as predictors for extent of fatty streaks in each artery, yielded the results shown in table 7. Sex has an appreciable effect only in the thoracic aorta. All coefficients for VLDL + LDL cholesterol have a positive sign, and those for HDL cholesterol have a negative sign.

Column $R^2$ in table 7 shows that the three selected predictor variables account for 28% to 54% of the variation in lesions, depending on which artery is considered. The column $R^2$Step shows similar values for three predictor variables selected by stepwise regression for each artery. Since $R^2$ and $R^2$Step are nearly identical, the three predictor variables selected on an overall basis explain essentially the same amount of variation as the first three variables selected by the stepwise procedure for each artery separately.

Table 7. Multiple Regression Coefficients for Predictor Variables for Arterial Fatty Streaks

<table>
<thead>
<tr>
<th>Artery</th>
<th>Coefficients</th>
<th>Cholesterol</th>
<th>Residual variance†</th>
<th>$R^2$</th>
<th>$R^2$ Step</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intercept</td>
<td>Sex*</td>
<td>VLDL + LDL</td>
<td>HDL</td>
<td></td>
</tr>
<tr>
<td>Aorta, arch</td>
<td>9.88</td>
<td>2.02</td>
<td>0.10‡</td>
<td>-0.19‡</td>
<td>37.30</td>
</tr>
<tr>
<td>Aorta, thoracic</td>
<td>11.29</td>
<td>16.31‡</td>
<td>0.27‡</td>
<td>-0.36</td>
<td>450.77</td>
</tr>
<tr>
<td>Aorta, abdominal</td>
<td>7.44</td>
<td>-1.70</td>
<td>0.19‡</td>
<td>-0.16</td>
<td>67.46</td>
</tr>
<tr>
<td>Innominate</td>
<td>13.01</td>
<td>1.72</td>
<td>0.12‡</td>
<td>-0.22‡</td>
<td>74.64</td>
</tr>
<tr>
<td>Carotid</td>
<td>10.53</td>
<td>-2.55</td>
<td>0.22‡</td>
<td>-0.21‡</td>
<td>68.08</td>
</tr>
<tr>
<td>Brachial</td>
<td>4.11</td>
<td>0.42</td>
<td>0.08‡</td>
<td>-0.09‡</td>
<td>10.49</td>
</tr>
<tr>
<td>Iliac-femoral</td>
<td>3.99</td>
<td>-1.54‡</td>
<td>0.06‡</td>
<td>-0.06‡</td>
<td>5.14</td>
</tr>
</tbody>
</table>

*Sex has value 1 for males, 2 for females.
†Standard errors for the coefficients may be obtained by multiplying the square root of the residual variance by 0.269 for sex, 0.0037 for VLDL + LDL cholesterol concentration, and 0.0090 for HDL cholesterol concentration.
‡Absolute value of coefficient divided by its standard error equal to or greater than 2.
Abbreviations: VLDL = very low density lipoprotein; LDL = low density lipoprotein; HDL = high density lipoprotein.
Table 8. Multiple Regression Coefficients for Predictor Variables for Aortic Intimal Lipid Content

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>Cholesterol</th>
<th>Residual variance</th>
<th>R²</th>
<th>R² Step</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response variable, aorta</td>
<td>Intercept</td>
<td>Sex*</td>
<td>VLDL + LDL</td>
<td>HDL</td>
</tr>
<tr>
<td>Gross extent of fatty streaks</td>
<td>10.666</td>
<td>10.085‡</td>
<td>0.252‡</td>
<td>-0.308‡</td>
</tr>
<tr>
<td>Intima, wet weight</td>
<td>306.070</td>
<td>-25.909</td>
<td>0.231</td>
<td>-1.524‡</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.529</td>
<td>-0.017</td>
<td>0.010</td>
<td>-0.007</td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>0.557</td>
<td>0.021</td>
<td>0.007</td>
<td>-0.006</td>
</tr>
<tr>
<td>Cholesterol ester</td>
<td>-0.035</td>
<td>-0.001</td>
<td>0.003</td>
<td>-0.001</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0.928</td>
<td>-0.108</td>
<td>0.009</td>
<td>-0.001</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>0.847</td>
<td>0.054</td>
<td>0.004</td>
<td>-0.002</td>
</tr>
<tr>
<td>Plasmalogen</td>
<td>0.169</td>
<td>-0.007</td>
<td>0.001</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*Sex has value 1 for males, 2 for females.
†Standard errors for the coefficients may be obtained by multiplying the square root of the residual variance by 0.272 for sex, 0.0038 for VLDL + LDL cholesterol concentration, and 0.0094 for HDL cholesterol concentration.
‡Absolute value of coefficient divided by its standard error equal to or greater than 2.

Abbreviations: VLDL = very low density lipoprotein; LDL = low density lipoprotein; HDL = high density lipoprotein.

Multiple regression analysis, using the same three variables as predictors for overall gross extent, wet weight, and weights of lipids of the aortic intima, yielded results shown in Table 8. As in Table 7, all of the coefficients for VLDL + LDL cholesterol are positive in sign, and those for HDL cholesterol are negative. The predictor variables account for a much larger proportion of variance in cholesterol and cholesterol esters than in triglyceride phospholipid, or plasmalogen. Similar to the results in Table 7, \( R^2 \) and \( R^2 \text{ Step} \) are nearly identical. All coefficients for VLDL + LDL and HDL cholesterol and concentrations of aortic intimal lipids were positive (results not shown).

Table 9. Correlation Coefficients and Multiple Logistic Function Coefficients for Predictor Variables and Coronary Artery Fatty Streaks

<table>
<thead>
<tr>
<th>Artery</th>
<th>Proportion positive</th>
<th>VLDL + LDL</th>
<th>(VLDL + LDL) HDL</th>
<th>Intercept</th>
<th>Sex</th>
<th>VLDL + LDL</th>
<th>HDL</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right coronary</td>
<td>9/57</td>
<td>-0.04</td>
<td>-0.33</td>
<td>0.15</td>
<td>1.23</td>
<td>-0.7 (0.62)</td>
<td>0.02 (0.01)</td>
<td>-0.06 (0.03)</td>
</tr>
<tr>
<td>Left coronary</td>
<td>18/57</td>
<td>0.39</td>
<td>-0.14</td>
<td>0.39</td>
<td>-2.53</td>
<td>0.20 (0.69)</td>
<td>0.04‡ (0.01)</td>
<td>-0.03 (0.02)</td>
</tr>
<tr>
<td>Anterior descending</td>
<td>16/57</td>
<td>0.25</td>
<td>-0.07</td>
<td>0.32</td>
<td>-0.34</td>
<td>-0.61 (0.66)</td>
<td>0.02‡ (0.01)</td>
<td>-0.03 (0.02)</td>
</tr>
</tbody>
</table>

*Phi coefficients (Conover, 1971).
‡Standard errors in parentheses.
‡Absolute value of coefficient divided by its standard error equal to or greater than 2.
Abbreviations: VLDL = very low density lipoprotein; LDL = low density lipoprotein; HDL = high density lipoprotein.
Table 10. Correlation Coefficients Among Predictor Variables and Fibrous Plaques in Three Arteries*

<table>
<thead>
<tr>
<th>Artery</th>
<th>Proportion positive†</th>
<th>VLDL + LDL</th>
<th>HDL</th>
<th>(VLDL + LDL)/HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta, abdominal</td>
<td>6/57</td>
<td>0.23</td>
<td>-0.11</td>
<td>0.35</td>
</tr>
<tr>
<td>Carotid</td>
<td>6/57</td>
<td>0.23</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Iliac-femoral</td>
<td>18/57</td>
<td>0.09</td>
<td>0.09</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*Selected because 10% or more were positive for fibrous plaques.
†Phi coefficients (Conover, 1971).
Abbreviations: VLDL = very low density lipoprotein; LDL = low density lipoprotein; HDL = high density lipoprotein.

Fibrous Plaques

Only three arteries contained fibrous plaques in 10% or more of the animals. Table 10 shows the phi coefficients for association with lipoprotein fractions in these three arteries. The association is positive with VLDL + LDL cholesterol, (VLDL + LDL)/HDL, and, in two of the three arteries, with HDL cholesterol.

The multiple logistic function coefficients (results not shown) for the three predictor variables for each artery indicated relationships similar to the relationships indicated by the phi coefficients given in table 10. Fibrous plaques in the abdominal aorta had a positive coefficient for VLDL + LDL cholesterol (0.03, SE = 0.01) and a negative coefficient for HDL cholesterol (—0.04, SE = 0.04), and $R^2$ was 0.15. Coefficients for fibrous plaques in the carotid and iliac-femoral arteries were small and positive for both lipoprotein cholesterol fractions, and the two $R^2$ values were smaller.

Discussion

Lack of Effect of Pesticides

The results suggest that ingestion of several times the maximum allowable daily intake of chlordane or the other three pesticides does not significantly alter serum lipoprotein levels or the rate of atherogenesis. The doses of chlordane were considerably less than the doses of other chlorinated hydrocarbon pesticides administered to rats by Ishikawa et al.,19 who found that Arochlor 1254 elevated HDL cholesterol. The intakes of lindane and DDT by the men first observed to have elevated HDL cholesterol were not known.

Relationship of Lipoprotein Cholesterol to Fatty Streaks

The correlation and multiple regression analyses support the idea that VLDL + LDL cholesterol concentration is positively associated with experimental atherosclerosis in all the major arterial branches, and that HDL cholesterol concentration is negatively associated with atherosclerosis. The results also indicate that the ratio of the two lipoprotein cholesterol fractions is more predictive than the absolute levels of either one. The three variables (sex, VLDL + LDL cholesterol, and HDL cholesterol) account for slightly less than one-third to slightly more than one-half of the variation in fatty streaks of the major muscular and elastic arteries. The predictive value is less for lesions in the coronary arteries.

If there were small, undetected effects of the pesticides on either lipoprotein cholesterol concentrations or experimental atherosclerosis, these effects might have confounded the relationships between lipoprotein cholesterol and atherosclerosis. The partial correlation coefficients, however, were adjusted for effects of pesticide, and regression coefficient values seen in the multiple regression analyses, where pesticide effects were ignored, showed the same pattern of relationships.

Relationship of Lipoprotein Cholesterol to Fibrous Plaques

Results with fibrous plaques were less consistent than those with fatty streaks, since only abdominal aortic fibrous plaques showed the positive association with VLDL + LDL cholesterol and the negative association with HDL cholesterol (table 10). Evaluation of fibrous plaques in the carotid arteries is complicated by intimal thickening in the carotid body region. Fibromuscular plaques frequently occur in the iliac-femoral arteries of baboons that are not hyperlipidemic.20 These results indicate that the fibrous plaques of the abdominal aorta are associated with VLDL + LDL and HDL cholesterol as are the fatty streaks, and therefore may be due to progression of fatty streaks under the influence of the altered lipoprotein levels. On the other hand, the fibrous plaques of the carotid and iliac-femoral arteries may be due to a different stimulus.
**Relationship of Triglyceride Concentrations to Atherogenesis**

Average serum triglyceride concentrations were low compared to those of humans, but variability was high (table 3). However, both correlation coefficients and stepwise multiple regression computations indicate that, as with most other animal models, serum triglyceride concentrations are not strongly associated with atherosclerosis.

**Effect of Sex**

In multiple regression analyses, sex emerged as a significant predictor of lesions in the thoracic aorta and iliac-femoral arteries in addition to lipoprotein cholesterol concentrations (table 7). Therefore, it appears that sex affects atherogenesis in some arteries of the baboon, but the effect is not explained by differences in lipoprotein cholesterol.

**Relationship of Lipoprotein Cholesterol to Aortic Intimal Lipid**

The correlation coefficients and predictive capability of serum lipoprotein cholesterol concentrations were higher for amount of total, free, and esterified cholesterol in aortic intima than for concentration of the same lipids in aortic intima. This finding is consistent with the histologic characteristics of aortic fatty streaks produced by moderate hyperlipidemia. These streaks contain smooth muscle and connective tissue as well as intra- and extracellular lipid, and have fewer lipid-filled foam cells than lesions in animals with extremely high serum cholesterol levels.

Cholesterol content of the aortic intima is more closely associated with morphologic lesions than is triglyceride, phospholipid, or phosphatidylcholine (a class of phospholipid reported to be increased in human atherosclerotic lesions). Furthermore, intimal triglyceride and phospholipid are less strongly associated with serum lipoprotein cholesterol concentrations than are cholesterol and its ester.

**Comparison with Findings of Others**

Only a few experiments with nonhuman primates have involved total serum or lipoprotein cholesterol levels in the range encountered in most humans. In 37 male baboons fed for 2 years diets varying in cholesterol and saturation of fat, terminal serum cholesterol levels ranged from 72 to 262 mg/dl. The partial correlation coefficient (computed from the published data and adjusted for linear effects of treatment) for total serum cholesterol concentration and extent of aortic lesions was 0.51, similar to the value of 0.44 (table 6) obtained in the present experiment.

Kritchevsky et al. produced mild hypercholesterolemia (93–208 mg/dl) in 24 baboons by feeding cholesterol-free semisynthetic diets containing various pure carbohydrates. The partial correlation coefficient (computed from data provided by the authors and adjusted for linear effects of treatment and sex) for total serum cholesterol concentration and extent of sudanophilic aortic lesions was 0.36, nearly identical to that found in the present experiment.

Armstrong et al. fed rhesus monkeys small supplements of dietary cholesterol, which elevated the LDL/HDL cholesterol ratio but maintained the total plasma cholesterol concentrations near to those of control monkeys fed chow diets. The monkeys fed cholesterol had greater intimal thickening, more aortic sudanophilia, and higher aortic cholesterol concentrations than monkeys not fed cholesterol. The authors suggested that the concomitant change in the LDL/HDL cholesterol ratio might be a "useful index of the atherogenic potential of plasma," but did not present more detailed analyses of the relationship.

**Correlation of Serum Lipids with Atherosclerosis in Humans**

There have been few attempts to relate serum cholesterol concentrations to atherosclerotic lesions in humans on an individual basis. Several older studies found no association between the two. A few older studies and several more recent ones found positive correlation coefficients between plasma cholesterol concentrations and aortic and coronary artery lesions ranging up to 0.36.

Only one study examined the relationship of HDL cholesterol to lesions. Solberg et al. found a partial correlation coefficient for total serum cholesterol and raised lesions in the coronary arteries of 0.30 (p < 0.01); in the cerebral arteries, 0.31 (p < 0.01). The partial correlation coefficients for HDL cholesterol and raised lesions in the coronary arteries was −0.22 (p < 0.01); in the cerebral arteries, −0.11 (ns). These correlation coefficients are similar in magnitude and direction to those found in the present study.

**Increasing the Predictive Capacity of Lipoprotein Concentrations**

Although up to one-half of the variation can be accounted for by sex, VLDL + LDL cholesterol, and HDL cholesterol in multiple regression equations, there remains considerable residual variation. Differences in LDL size may also be important determinants of atherogenicity. More precise characterization of lipoproteins may lead to even greater predictive power for atherosclerotic lesions in both experimental animals and humans.
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References


Index Terms: baboon (Papio sp.) · experimental atherosclerosis · pesticide · lipoprotein
Relationship of lipoprotein cholesterol concentrations to experimental atherosclerosis in baboons.

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