Estrogens and Experimental Atherosclerosis in the Baboon (Papio cynocephalus)

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SUMMARY One hundred twenty-six adult female baboons (Papio cynocephalus) were hysterectomized and all except 18 were ovariec-
tomized. The animals were fed a moderately atherogenic diet (40% calories from hydrogenated vegetable oil, 1.5 mg cholesterol/kcal) for two years. Ovariectomized-hysterectomized animals received es-
trone sulfate, ethinyl estradiol, or diethylstilbestrol orally in daily doses similar to those given humans. An ovariectomized-hysterect-
tomized group and a hysterectomized group received no drug. The average total serum cholesterol concentration rose from 136 mg/dl to 223 mg/dl and declined to 186 mg/dl. Concentrations of cholesterol,
triglyceride, and phospholipid in whole serum, low density lipopro-
teins and high density lipoproteins showed no consistent statistically
significant differences among the groups. Triglyceride and phospho-
lipid concentrations were higher in the estrogen-treated and intact-
ory groups than in the ovariectomized nonestrogen treated group,
but not all pairwise comparisons were statistically significant. There
were no consistent statistically significant differences in athero-
sclerotic lesions among the groups. Neither ovariectomy nor estrogen
replacement influence diet-induced experimental atherosclerosis in
the baboon within two years.

THERE ARE CONFLICTING OBSERVATIONS on the relationship
tween estrogens and atherogenesis in both humans and experimental animals. Premenopausal white
women consistently have a lower incidence and prevalence of
myocardial infarction and a lessened extent of advanced
atherosclerotic lesions in the coronary arteries than
do white men of the same age. However, some studies
show that women ovariectomized before the natural meno-
pause lose their relative immunity to coronary heart dis-

ease, while others find no effect of ovariectomy. One
study finds atherosclerosis more severe in ovariectomized
women, but another finds no difference. According to
observations from The Framingham Study, postmeno-
pausal women have a higher incidence of coronary heart dis-

ease than age-matched premenopausal women. Despite a
prevailing impression that the menopause increases risk of

coronary heart disease, age and sex specific mortality rates
do not increase after the menopause more than would be
expected with age. Attempts to reduce the incidence of

coronary heart disease in postmenopausal women by est-

rogen replacement therapy also have yielded conflicting
results. The results of animal experiments on the relationship
between estrogens and atherogenesis are as inconsistent as
those from human studies. Estrogens induced hyperlipi-
demia in chickens but inhibited coronary artery lipido-
sis in cockerels; reduced the size of plaques in White
Carneau pigeons in high doses; produced a variety of

changes in arteries of rats; and, augmented, inhibited, and
failed to affect diet-induced hypercholesterolemia and
atherosclerosis in rabbits.

In the hope of resolving some of these conflicting observa-
tions, this experiment was planned to examine the effects of
ovariectomy and estrogen replacement therapy on serum
lipids and atherogenesis in the baboon, whose reproductive
physiology, estrogen and cholesterol metabolism, greater
prevalence of aortic fatty streaks in the feral female com-
pared to the feral male, and response to an atherogenic diet are
more human-like than are those of chickens, rats, and
rabbits. In order to induce moderate hyperlipidemia and
experimental atherosclerosis, the animals were fed a mildly
atherogenic diet for two years. The estrogens tested were es-
trone sulfate, a biologically active conjugate of estrone and a
major circulating estrogen in humans; 17α-ethynyl estradiol,
a highly potent, orally effective synthetic estrogen struc-

turally similar to estradiol; and diethylstilbestrol, a non-
steroidal compound differing in chemical structure from
the natural estrogens but having estrogenic activity.

Methods and Materials

Subjects

The subjects were 126 adult female baboons (Papio cyno-
cephalus) ranging in estimated age from 5 to 15 years. The
animals were of feral origin, but many had been in the
Southwest Foundation for Research and Education (SFRE)
breeding colony for several years. During this experiment,
they were individually caged. All animals were hysterec-
tomized to eliminate the uterine bleeding that occurs with
continuous estrogen therapy. All but 18 also were ovari-
tomized at the time of hysterectomy. Eight animals died
during the experiment from causes unrelated to the experi-
mental procedure. On the average, these did not differ in
age, serum lipids, or weight from the remaining 118.

Experimental Design

Although the original principal investigator (LRA) did not use a strict randomization procedure for the assignment of
treatments to animals, we analyzed the results as a com-

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Department of Pathology, The University of Texas Health Science Center at
San Antonio, San Antonio, Texas.

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Lung and Blood Institute.

Dr. Leonard R. Axelrod, who designed and initiated this experiment, left
the Southwest Foundation for Research and Education on May 1, 1973, to
become Director of the Criteria and Evaluation Division and Chief Scientist
of the Office of Pesticide Programs, Environmental Protection Agency,
Washington, D.C. The experiment was completed in his absence. Dr. Ax-

elrod died on July 31, 1975. The analyses and interpretation of data contained
in this report were completed after Dr. Axelrod’s death.

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Texas 78284.

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Table 1. Selected Characteristics of Experimental Animals, Ovarian Status, and Estrogen Treatment by Experimental Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of baboons</th>
<th>Start</th>
<th>End</th>
<th>Estimated age, years</th>
<th>Weight, kg</th>
<th>Serum cholesterol</th>
<th>Days after operation</th>
<th>Ovarian status</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Duration, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>16</td>
<td>17</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>17</td>
<td>17</td>
<td>11.6 ± 2.3</td>
<td>14.3 ± 1.9</td>
<td>1.9</td>
<td>131.3 ± 19.9</td>
<td>126.8 ± 9.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>11.1 ± 2.2</td>
<td>14.3 ± 1.6</td>
<td>1.6</td>
<td>138.1 ± 26.4</td>
<td>157.5 ± 8.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>17</td>
<td>17</td>
<td>11.5 ± 2.0</td>
<td>14.0 ± 2.3</td>
<td>2.3</td>
<td>139.2 ± 29.1</td>
<td>146.1 ± 7.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>18</td>
<td>15</td>
<td>15</td>
<td>11.8 ± 4.1</td>
<td>14.9 ± 2.1</td>
<td>2.1</td>
<td>130.6 ± 34.9</td>
<td>155.4 ± 9.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>18</td>
<td>16</td>
<td>16</td>
<td>11.3 ± 4.7</td>
<td>14.0 ± 1.7</td>
<td>1.7</td>
<td>129.6 ± 21.8</td>
<td>104.0 ± 6.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>11.2 ± 1.1</td>
<td>14.7 ± 1.4</td>
<td>1.4</td>
<td>136.9 ± 19.0</td>
<td>61.7 ± 13.9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ES = estrone sulfate; EE = 17α-estradiol; DES = diethylstilbestrol.

Estrogen Dosage and Administration

Estrogen doses approximated, on a weight basis, the usual human doses for replacement therapy. Drugs were administered by embedding tablets in fig bars. The rate of failure to take the drug ranged from 0.1% of treatment days in group 2 to 1.5% in group 3. The overall omission rate for all groups was 0.64%, and only one animal had an omission rate greater than 5%.

Baboons in groups 1 and 2 received 0.625 mg/day, or about 45 μg/kg/day, of Ogen (Abbott Laboratories) of pure crystalline estrone sulfate (ES) stabilized with piperazine. This dose in baboons may equal a higher dose in humans since plasma concentrations of estrogenic steroids are lower in baboons than in humans.34

Baboons in groups 3 and 4 received 17 μg/day, or about 1.2 μg/kg/day, of Estinyl (Schering Corporation) as the source of 17α-estradiol (EE).

Baboons in group 5 received 62.5 μg/day, or about 5 μg/kg/day, of diethylstilbestrol, USP (DES) (Eli Lilly and Co.).

Diet

The baboons had been fed SFRE baboon chow35 (Ralston Purina Co.) from the time of their arrival at SFRE until beginning the atherogenic diet for this experiment. This diet (table 2) was prepared by mixing ground baboon chow, hydrogenated vegetable oil (Crisco, Procter and Gamble Co.), frozen egg yolk, cholesterol, and 6 ml water per 100 g.

Its caloric value was about 3.8 kcal/g wet weight. Each baboon received 360 g per day, about 90 kcal/kg/day, for the first 487 days of the experiment. Because many animals gained excess weight, the amount offered each day thereafter was reduced by 20%. The final diet mixture contained 5.62 mg/g (wet weight) cholesterol, 0.12 mg/g campesterol, and 0.40 mg/g β-sitosterol by gas-liquid chromatographic analysis. Thus, the diet contained about 1.5 mg cholesterol per kcal.

Blood Collection and Serum Lipid Analyses

Thirty ml of blood were collected at 4 month intervals in Vacutainers without anticoagulant (Becton Dickinson Co.), with the animal under Sernylan anesthesia, about 0.8 mg/kg (phencyclidine hydrochloride) (Bio-Ceutic Laboratories, Inc.)

Four lipoprotein classes, separated from 5 ml serum samples by the preparative ultracentrifugal method of Lindgren and associates,37 were analyzed for cholesterol, triglyceride, and phospholipid.

The Bioregional Reference Laboratory, Inc. analyzed whole serum and lipoprotein fractions for cholesterol, triglyceride, and phospholipid. Cholesterol determinations were performed early in the study on a SMA 12/60 Autoanalyzer (Technicon Instruments Corp.) with a Liebermann-Burchard reagent, and later in the study on an ABA-100 (Abbott Laboratories Diagnostic Division) by an enzymatic method. Triglycerides were analyzed by extraction, silicic acid chromatography, and the colorimetric method of Foster and Dunn.38 Quality control was maintained by repeated analyses of commercial pooled serum samples. The coefficient of variation for the cholesterol analyses was 2.4% and for triglycerides, 8.7%. Both methods met the criteria established for clinical laboratories and received a satisfactory score of 3 from the Center for Disease Control. Phospholipids were determined by the method of Zilversmit and Davis,39 with a coefficient of variation of 4% for repeated analyses.

Table 2. Composition and Nutrient Value of the Atherogenic Diet per 100 g Dry Weight

<table>
<thead>
<tr>
<th>Component</th>
<th>Total g</th>
<th>Protein</th>
<th>Fat</th>
<th>Carbohydrate</th>
<th>Fiber</th>
<th>Ash</th>
<th>Cholesterol mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baboon chow</td>
<td>67.1</td>
<td>14.1</td>
<td>3.7</td>
<td>32.9</td>
<td>2.8</td>
<td>5.2</td>
<td>4</td>
</tr>
<tr>
<td>Crisco</td>
<td>11.7</td>
<td>0</td>
<td>11.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Egg yolk, frozen</td>
<td>20.8</td>
<td>3.1</td>
<td>5.7</td>
<td>2.1</td>
<td>0</td>
<td>0</td>
<td>239</td>
</tr>
<tr>
<td>Cholesterol, USP</td>
<td>0.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>366</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>17.2</td>
<td>21.1</td>
<td>35.0</td>
<td>2.8</td>
<td>5.2</td>
<td>609</td>
</tr>
</tbody>
</table>
Autopsy Procedures

We sedated the animals with an intramuscular injection of Sernylan and performed complete autopsies, including gross and histologic examination of all major viscera. The iliac, femoral, innominate, brachial, carotid, and coronary arteries were opened longitudinally and prepared by methods used in the International Atherosclerosis Project. The aorta from the ligamentum arteriosus to its bifurcation was divided along anterior and posterior midlines. The right half of the aorta was frozen for chemical analysis, and the left half, after being sampled for electron microscopy, was prepared for grading like the other arteries.

Grading of Atherosclerotic Lesions

Two experienced pathologists (one of the authors, H.C. McGill; and a consultant, J.P. Strong) independently graded the stained arterial specimens by the method of the International Atherosclerosis Project. Graders were not aware of treatment group or other data, but all the arteries from each animal (except the coronary arteries) were in the same plastic bag. Product moment correlation coefficients between grades of different arterial segments did not exceed 0.34, a value which indicated that the grades of some segments did not unduly influence the grades of other segments.

The intraclass correlation coefficient between estimates by the two pathologists of percent intimal surface area involved with fatty streaks for the aorta ranged from 0.77 to 0.84, values indicating acceptable interobserver variability of the grading. Independent regrading of 20 sets of specimens at a later time indicated that variability within pathologist was similar to variability between pathologist.

We compared aortic fatty streaks among treatment groups on the basis of extent of intimal surface involved. Because grades of fibrous plaques in all arteries and of fatty streaks in some arteries frequently were small or zero, we compared these lesions among treatment groups on the basis of prevalence rather than extent. Specimens were scored as positive only if both pathologists independently classified the specimen as positive.

Aortic Intimal Cholesterol

A technician stripped the intima from the unfixed half of each aorta. Lyophilized and saponified tissue was analyzed by gas-liquid chromatography. A technician measured the total intimal surface area of the unfixed half of the aorta from full size photographs using an electronic digitizer (Wang 662). The coefficient of variation after retracing a randomly selected sample of 20 photographs was 2.0%. From the area and weight of the intima and the concentration of cholesterol, we estimated cholesterol per unit area of intimal surface.

Statistical Methods

Responses were analysed in separate univariate analyses, usually by analysis of variance (ANOVA). For ANOVAs that resulted in a statistically significant overall F test, all pairwise contrasts between treatment group means were computed and evaluated with Tukey's t-test. Analyses by the Kruskal-Wallis ANOVA for ranks confirmed the results of the parametric ANOVAs. In some instances, as noted, analysis of covariance (ANCOVA) was used. For data such as presence of lesions, we tested the null hypothesis of independence between treatment groups and presence of lesions by a Chi-square test.

Results

Evidence of Estrogen Stimulation

All baboons in the group with intact ovaries (7) continued normal menstrual cycles throughout the experiment as indicated by periodic turgescence of the sex skin. The ovariectomized group (7) showed no turgescence of the sex skin. Baboons in groups which received estrogens (1–5) showed moderate sustained turgescence of the sex skin, an observation indicating that they were receiving estrogenic stimulation.

Body and Organ Weights

The group receiving EE from the start of the experiment (3) gained less weight than other groups, and the group receiving EE in the second year (4) also gained less weight after hormone treatment began. The DES treated group (5) lost weight steadily after about day 300 of the experiment; the weight loss began before the daily diet ration was reduced. Mean weights of all groups fluctuated over time in a manner apparently unrelated to treatment. Mean body weights at autopsy of the two EE treated groups (3 and 4) were lower than those of all other groups, and pairwise comparisons by ANOVA with initial body weight as the concomitant variable were statistically significant for these two groups as compared to the group treated with ES for two years. Organ weights at autopsy, analyzed by ANCOVA with total body weight at autopsy as the concomitant variable, showed no real differences.

Serum Lipids and Lipoproteins

The mean total serum cholesterol concentration of all baboons rose in the first three months of the experimental period from 136 mg/dl to 223 mg/dl, declined during the following 18 months to 186 mg/dl, and increased slightly near the end of the experiment. We compared serum lipid and lipoprotein concentrations among groups by ANOVA, using values from two blood samples drawn between 653 and 814 days after the start of the experiment. We reasoned that long term effects of the drugs should be apparent during this period, and that they would not be masked by the large changes associated with beginning the atherogenic diet. Table 3 shows results for cholesterol, triglyceride, and phospholipid in whole serum, LDL, and HDL. Chylomicrons and VLDL (not included in table 3) contained only a minor fraction of total lipids and showed no consistent differences among groups.

Neither cholesterol nor phospholipid concentrations in lipoprotein fractions or in whole serum differed between the ovariectomized group (6) and the group with intact ovaries (7). Total triglyceride, LDL triglyceride, and HDL triglyceride concentrations were higher in all estrogen treated groups (1–5) and in the group with intact ovaries (7) than in the ovariectomized group (6). However, statistically significant pairwise comparisons were limited to groups 1 and 3. Phospholipid concentrations were slightly higher in the
groups treated with ES and EE for 2 years (1 and 3) than in controls or other treatment groups, and several of the differences were statistically significant.

The decrease in caloric intake after day 487 on the atherogenic diet showed no relationship to changes in cholesterol, triglyceride, or phospholipid concentrations in whole serum or in lipoprotein fractions.

Characteristics of Arterial Lesions

Table 4 shows overall measures of fatty streaks and fibrous plaques by artery. Grossly, the lesions resembled those previously described in the baboon. Although some coronary artery lesions were elevated and resembled fibrous plaques, none produced significant stenosis.

Microscopically, all lesions showed intimal thickening with smooth muscle cells. Lesions classified grossly as fibrous plaques were principally smooth muscle cells with varying amounts of lipid in the subintimal layer, and occasionally a core of necrotic debris and extracellular lipid. Electron micrographs from the thoracic and abdominal aorta and coronary arteries of several animals from each experimental group showed that most intimal lipid was within smooth muscle cells, but some was extracellular and some was in macrophages. We detected no qualitative differences among the experimental groups in histologic sections or electron micrographs.

Aortic Fatty Streaks

Table 5 shows treatment group means of percent surface involvement with fatty streaks by aortic segments. The component of variance within animal (due to interobserver difference in grading) was less than the component of variance between animals. Only two pairwise comparisons were statistically significant — in the abdominal aorta, DES treated (5) and ovarietomized untreated (6) groups were more extensively involved by fatty streaks than the untreated group with intact ovaries (7).

In five of the other arteries, the null hypothesis of independence between treatments and presence of fatty streaks was rejected ($P < 0.05$). These five were further examined by partitioning the degrees of freedom.7 The group treated with EE for two years (3) had a significantly lower prevalence of fatty streaks in the right carotid, left carotid, left brachial, and left circumflex coronary arteries. The group treated with ES in the second year (2) and the ovarietomized group (6) had a significantly higher prevalence of fatty streaks in the left coronary circumflex artery than other groups. The group treated with ES from the beginning (1) and the group with intact ovaries (7) had a significantly lower prevalence of fatty streaks in the left anterior descending coronary artery than did all other groups.

Concentration of Cholesterol in Aortic Intima

The overall means of concentrations of cholesterol in the stripped aortic intima were 25.0 mg/g and 111.9 mg/cm².

Table 3. Statistical Comparisons of Serum Lipids and Lipoproteins

<table>
<thead>
<tr>
<th>Fraction of Serum</th>
<th>Treatment group means</th>
<th>Overall mean</th>
<th>Between animal</th>
<th>Within animal</th>
<th>F</th>
<th>Significant pairwise comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL</td>
<td>92.6 77.8 82.1 78.3 91.9 79.3 83.4 83.4 251.3 473.8 1.25</td>
<td>None</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>70.6 62.0 67.4 58.1 59.5 64.0 66.4 64.0 272.3 387.4 0.70</td>
<td>None</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>199.3 165.2 182.5 158.3 180.3 174.1 175.6 176.5 1114.6 379.6 2.25</td>
<td>1/4</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td>34.1 27.9 33.6 31.2 23.8 21.5 27.7 28.7 76.8 78.5 3.11</td>
<td>1-6,3-6</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>29.9 26.9 29.9 24.5 24.4 20.9 26.9 26.3 7.9 74.9 3.63</td>
<td>1-6,3-6</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>94.4 81.6 98.6 82.7 82.2 79.4 79.1 84.4 472.3 172.7 2.77</td>
<td>3-6</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Phospholipid</td>
<td>96.6 76.4 93.3 64.1 74.3 66.9 78.1 78.5 113.6 636.6 5.86</td>
<td>1-4,1-3-4,3-6</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>165.5 132.2 168.0 138.4 161.5 154.3 154.7 153.1 241.0 455.6 6.55</td>
<td>1-2,1-4,2-3,3-4,5-6</td>
<td>1</td>
<td></td>
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<tr>
<td>Total</td>
<td>292.7 259.1 292.6 244.9 266.8 256.8 265.6 268.7 954.0 508.9 4.67</td>
<td>1-3,4-3-6</td>
<td>1</td>
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</tr>
</tbody>
</table>

N = 118 baboons, approximately 2 values/animal between 650 and 820 days.

*Statistically significant at 0.05 level.

**Statistically significant at 0.01 level.

Table 4. Percent of Intimal Surface Involved with Fatty Streaks and Prevalence of Fatty Streaks and Fibrous Plaques by Arterial Segment

<table>
<thead>
<tr>
<th>Arterial segment</th>
<th>Intimal surface involved with fatty streaks, percent</th>
<th>Prevalence, percent positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Fatty streaks</td>
</tr>
<tr>
<td>Aortic arch</td>
<td>11.8 0. 73.1 91.5 3.4</td>
<td></td>
</tr>
<tr>
<td>Thoracic aorta</td>
<td>35.1 1. 86.6 96.6* 5.1</td>
<td></td>
</tr>
<tr>
<td>Abdominal aorta</td>
<td>18.6 0. 81.7 97.5 13.6</td>
<td></td>
</tr>
<tr>
<td>Right carotid</td>
<td>7.1 0. 54.5 90.7 5.9</td>
<td></td>
</tr>
<tr>
<td>Left carotid</td>
<td>6.7 0. 27.0 90.7 9.3</td>
<td></td>
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<tr>
<td>Right brachial</td>
<td>6.1 0. 28.4 84.7 2.5</td>
<td></td>
</tr>
<tr>
<td>Left brachial</td>
<td>11.3 0. 45.5 93.2 9.3</td>
<td></td>
</tr>
<tr>
<td>Right iliac-femoral</td>
<td>3.7 0. 42.1 79.7 15.3</td>
<td></td>
</tr>
<tr>
<td>Left iliac-femoral</td>
<td>3.3 0. 42.1 78.0 16.1</td>
<td></td>
</tr>
<tr>
<td>Right coronary</td>
<td>0.4 0. 17.3 7.6 0.8</td>
<td></td>
</tr>
<tr>
<td>Left coronary, ant. desc.</td>
<td>1.2 0. 17.3 31.4 2.5</td>
<td></td>
</tr>
<tr>
<td>Left coronary, circumflex</td>
<td>0.7 0. 17.9 18.6 0.0</td>
<td></td>
</tr>
</tbody>
</table>

N = 118 baboons.

*Prevalence less than 100% although lowest percent surface involvement is not 0 because range is from the mean of independent estimates and prevalence is based on both observers scoring specimen as positive.

†Mean of independent estimates by 2 observers.
and overall estimated standard deviations were 17.8 and 60.3. No real differences among the treatment groups were apparent by either measure.

Fibrous Plaques

The null hypothesis of independence between treatments and presence of fibrous plaques was rejected (P < 0.05) only for the left iliac-femoral artery. The group treated with ES for the second year (2) and the group treated with DES (5) had a significantly higher prevalence of fibrous plaques as indicated by partitioning the degrees of freedom.

Discussion

Serum Lipids and Lipoproteins

The slight and consistent, but not always statistically significant, elevations in serum triglyceride and phospholipid concentrations in the estrogen treated groups and in the group with intact ovaries compared to ovariecuntomed untreated controls are consistent with the reported effects of estrogenic compounds in humans. The difference we observed, however, is small, and slight elevations of triglyceride and phospholipid concentrations in the absence of increased cholesterol levels would not be expected to augment atherogenesis. Furthermore, no major shifts in distribution of lipids among the lipoproteins appear to result either from ovariecuntomy or estrogen replacement. The failure to find a higher serum cholesterol concentration in ovariecuntomed untreated baboons than in those with intact ovaries or in those receiving estrogens is not consistent with the elevation in serum cholesterol associated with surgical or natural menopause in humans. Detailed comparison of these results with observations on other animal species shows some similarities but many differences.

Atherosclerosis

The comparisons of arterial lesions in this experiment indicate no consistent effect of estrogen deficiency or estrogen replacement on extent or quality of atherosclerotic lesions. The results, therefore, are consistent with reports that ovariecuntomed women have no greater atherosclerosis at autopsy and experience no higher incidence or prevalence of atherosclerotic disease than women with intact ovaries. They are consistent with reports that estrogen replacement therapy does not influence coronary heart disease prevalence in postmenopausal women. They also are consistent with reports that physiological doses of estrogens do not reduce spontaneous atherosclerosis in pigeons or experimental atherosclerosis in rabbits. We have found no reports of the effects of estrogens on diet-induced atherosclerosis in nonhuman primates.

The extent of fatty streaks exceeded that produced in other baboons by similar moderate levels of hyperlipidemia. This difference may be due to age or sex, since most experiments with atherogenic diets in baboons have used young adult males rather than females. The abundant smooth muscle and fibrous tissue in fatty streaks may be typical of the reaction of nonhuman primates to moderate prolonged hyperlipidemia, as observed in rhesus monkeys. These lesions differ from the foam cell lesions typical of experimental animals with higher serum cholesterol levels. Multiple pregnancies also may have caused smooth muscle proliferation, since Wexler has described nonlipid containing intimal thickening in the arteries of breeder female rats. Most of the baboons were multiparous, but because they were feral, accurate assessment of parity was not possible.

Lesions classified grossly as fibrous plaques resembled fibromuscular cushions described in the coronary arteries and aortas of normal rhesus monkeys and feral baboons autopsied immediately after capture. We believe that our baboons probably had developed many of these plaques prior to the experiment. The relatively high frequency of fibrous plaques in the femoral arteries may have been due to trauma to the arteries during venipuncture. Hyperlipidemia may have augmented fibrous plaques, and probably was responsible for lipid deposition within them as reported for hyperlipemic rhesus monkeys. Whatever their origin, they do not appear to have been affected either by ovariecuntomy or estrogen replacement.

Conclusions

In the female baboon fed a moderately atherogenic diet for two years, neither ovariecuntomy nor estrogen replacement after ovariecuntomy produces consistent and statistically significant differences in serum lipid or lipoprotein concentrations or in atherosclerosis. There is a trend (not statistically significant) for baboons with intact ovaries or ovariecuntomed baboons receiving exogenous estrogens to have slightly higher serum triglyceride and phospholipid concentrations than ovariecuntomed baboons. The results are consistent with epidemiologic studies of humans which show no effects of either estrogen deficiency or estrogen replacement therapy on serum lipids or lipoprotein concentrations or on atherosclerosis.

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