Development of Atherosclerosis in Dogs with Hypercholesterolemia and Chronic Hypertension

By Campbell Moses, M.D.

Hypercholesterolemia was induced in pure-bred dogs by cholesterol feeding and propylthiouracil or radioiodine. Hypertension induced by the injection of silica into the renal artery increased the severity of aortic atherosclerosis. Marked hypercholesterolemia increased the severity of aortic atherosclerosis even in normotensive dogs.

While it is a well-founded clinical observation that the presence of sustained arterial hypertension is attended by an increase in the rate of development of arteriosclerosis, at the time these experiments were initiated, there was little experimental evidence available on this relationship. Recently, Wakerlin and associates have demonstrated in dogs the acceleration of arteriosclerosis in the presence of hypertension.

The experiments reported here were designed to determine the role, if any, of chronic hypertension on the rate of development of atherosclerosis in dogs with hypercholesterolemia and to relate such role to variations in the cholesterol partition, cholesterol lipid-phosphorus ratio and the lipoprotein pattern of the serum.

METHODS

The dogs used in this study were pure-bred Basenjis from stock obtained from the Jackson Memorial Laboratory at Bar Harbor, Maine. To avoid genetic variations, no mongrel dogs were included. Prior to beginning the experiment, all dogs were prepared with an exteriorized carotid artery to facilitate the determination of systolic blood pressure without anesthesia by palpation of the artery distal to an occluding pneumatic cuff. All animals had systolic blood pressure determinations at weekly or biweekly intervals throughout the study. Groups I and III were made up of dogs approximately one year of age at the start of the experiment. Group II animals were six months old at the outset.

After a three-month control period, hypercholesterolemia was produced by feeding 5 Gm. of cholesterol mixed with horse meat daily. In addition, all animals received approximately one-half pound of beef suet and one-half pound of cooked pork liver twice each week. Friskies mash in ad lib quantities provided the basic diet. Group I animals also received 0.4 Gm. propylthiouracil daily throughout the experiment. Group II animals received, in addition to 5 Gm. of cholesterol daily, a single dose of 1 milliunit of radioiodine ($^{131}$I) per pound of body weight at the beginning of cholesterol feeding. Group III animals were not given any antithyroid preparation but were kept on 5 Gm. of cholesterol daily throughout the experiment.

Hypertension was produced in three animals from each group six months after the onset of cholesterol feeding by the injection of finely-divided silica (0.02 to 0.05 micra) into both renal arteries at laparotomy. A preliminary study of this method of producing arterial hypertension$^2$ has now been extended to 34 dogs. Littermates in each group were paired as hypertensive and non-hypertensive animals. The nonhypertensive littermates were sham-operated and the renal arteries were exposed but no silica was injected.
At monthly intervals for three months prior to cholesterol feeding (while the exteriorized carotids were healing) and at biweekly intervals thereafter, free and total serum cholesterol and lipid phosphorus determinations were made. Just prior to the onset of cholesterol feeding and at irregular intervals thereafter, the ultracentrifuge lipoprotein pattern, using the technic of the National Heart Institute Cooperative Study, was determined through the cooperation of Dr. Martin Hanig of the University of Pittsburgh, Department of Biophysics. The nonprotein nitrogen, serum albumin and globulin, and Bromsulphalein retention were also determined at intervals during both the control and the experimental period.

Termination of this experiment was planned by sacrifice of the hypertensive and nonhypertensive littermates in each group at intervals varying from 6 to 18 months after the onset of hypertension. Depending upon the preliminary data available from the animals included in this report, additional littermate pairs of Basenjis were to be added to the program until data on at least ten pairs in each group were available.

However, approximately five months after the injection of silica, a nonimmunized dog from the colony of another laboratory was improperly transferred to the animal quarters housing the Basenjis, and a violent epidemic of distemper-hepatitis flared. Attempts to control and to prevent the spread of the disease failed; and in a period of six weeks all the Basenjis, including both the control and the hypertensive groups, and dogs being prepared for the study, either died or were sacrificed in

<table>
<thead>
<tr>
<th>Table 1.—Group I (Cholesterol Plus Propylthiouracil). Monthly Mean Values for Cholesterol, Lipid Phosphorus, Lipoprotein and Systolic Blood Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mo.</strong></td>
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<tr>
<td><strong>Control period—6 paired littermates</strong></td>
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<tr>
<td>1</td>
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<tr>
<td>2</td>
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<td>3</td>
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<tr>
<td><strong>Cholesterol 5 Gm. plus propylthiouracil 0.4 Gm. daily</strong></td>
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<td>4</td>
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<tr>
<td>5</td>
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<td>8</td>
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<tr>
<td>9</td>
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<tr>
<td><strong>Bilateral renal artery silica injection or sham operation</strong></td>
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<tr>
<td><strong>Silica</strong></td>
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<td>10</td>
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<tr>
<td>11</td>
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<td>12</td>
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<td>13</td>
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<tr>
<td>14</td>
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<tr>
<td><strong>Aortic atherosclerosis</strong></td>
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<tr>
<td><strong>Silica</strong></td>
</tr>
<tr>
<td>3+(A)</td>
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<tr>
<td>4+(B)</td>
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<td>3+(C)</td>
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</table>
Table 2.—Group II (Cholesterol Plus $^{131}$I). Monthly Mean Values for Cholesterol, Lipid Phosphorus, Lipoprotein and Systolic Blood Pressure

<table>
<thead>
<tr>
<th>Mo.</th>
<th>Total Cholest. mg.%</th>
<th>Free Cholest. mg.%</th>
<th>Cholest. Esters mg.%</th>
<th>Lipid Phosph. mg.%</th>
<th>Lipoproteins mg.%</th>
<th>Systolic Blood Press. mm. Hg</th>
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<tr>
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<td>Control period—5 paired littermates</td>
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<tr>
<td>1</td>
<td>100</td>
<td>45</td>
<td>55</td>
<td>14.4</td>
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<tr>
<td>2</td>
<td>98</td>
<td>50</td>
<td>48</td>
<td>17.2</td>
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<tr>
<td>3</td>
<td>104</td>
<td>50</td>
<td>59</td>
<td>15.1</td>
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<tr>
<td></td>
<td>$^{131}$I mc. per lb. plus 5 Gm. cholesterol daily</td>
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<tr>
<td>4</td>
<td>124</td>
<td>60</td>
<td>64</td>
<td>24.0</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>180</td>
<td>75</td>
<td>105</td>
<td>18.6</td>
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<tr>
<td>6</td>
<td>220</td>
<td>84</td>
<td>136</td>
<td>22.0</td>
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<tr>
<td>7</td>
<td>245</td>
<td>90</td>
<td>155</td>
<td>24.0</td>
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<tr>
<td>8</td>
<td>250</td>
<td>100</td>
<td>160</td>
<td>26.0</td>
<td>11</td>
<td>45</td>
</tr>
<tr>
<td>9</td>
<td>300</td>
<td>88</td>
<td>212</td>
<td>26.2</td>
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<tr>
<td></td>
<td>Bilateral renal artery silica injection or sham operation</td>
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<tr>
<td></td>
<td>Silica</td>
<td>Sham</td>
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<td>Sham</td>
<td>Silica</td>
<td>Sham</td>
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<tr>
<td>10</td>
<td>270 300</td>
<td>70</td>
<td>90</td>
<td>200</td>
<td>210</td>
<td>24.0</td>
</tr>
<tr>
<td>11</td>
<td>285 324</td>
<td>78</td>
<td>102</td>
<td>207</td>
<td>222</td>
<td>23.0</td>
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<tr>
<td>12</td>
<td>274 330</td>
<td>90</td>
<td>16</td>
<td>184</td>
<td>232</td>
<td>24.6</td>
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<tr>
<td>13</td>
<td>290 346</td>
<td>74</td>
<td>100</td>
<td>190</td>
<td>246</td>
<td>24.0</td>
</tr>
<tr>
<td>14</td>
<td>205 340</td>
<td>90</td>
<td>128</td>
<td>167</td>
<td>212</td>
<td>25.0</td>
</tr>
</tbody>
</table>

|     | Aortic atherosclerosis |                   |                     |                   |                  |                           |
|     | Silica                | Sham              |                     |                   |                  |                           |
| 2+  | (A)                   | 0(A')             |                     |                   |                  |                           |
| 1+  | (B)                   | 1+(B')            |                     |                   |                  |                           |
| 2+  | (C)                   | 1+(C')            |                     |                   |                  |                           |

extremis. Most animals developing this infection died within 48 to 72 hours after the onset of symptoms. A few, however, were lethargic and anorexic for 5 to 10 days before entering an abrupt terminal phase lasting 24 to 48 hours.

Because of the serious illness of the animals in this terminal phase, valid laboratory data on the blood findings prior to sacrifice or death are not available. However, autopsies were performed on these animals, and the extent of the atherosclerotic lesions of the aorta was graded on a 1 to 4 plus basis, depending upon the extent and severity of the aortic lesions. This grading was done first on the fresh aorta and then repeated after several weeks of formalin fixation by observers not having access to the experimental regimen of the animal under study.

Since this experiment terminated unsatisfactorily, a similar experiment using a colony of mongrel dogs was attempted. However, the spontaneous variations in blood pressure and cholesterol partition obtained during the preliminary control period, and the inability to obtain accurate ages and littermate pairs for the experiment, influenced our decision not to continue the program.

Results

Tables 1, 2, and 3 record the monthly mean values for cholesterol, lipid phosphorus, lipoproteins and systolic blood pressure obtained. These data indicate that during the control period the animals in all groups had similar systolic pressures, cholesterol and lipid phosphorus values. After two months of 0.5 Gm. of cholesterol daily into the diet, group I animals (also receiving 0.4 Gm. of propyl-
ATHEROSCLEROSIS IN DOGS

TABLE 3.—Group III (Cholesterol Feeding Only). Monthly Mean Values for Cholesterol, Lipid Phosphorus, Lipoprotein and Systolic Blood Pressure

<table>
<thead>
<tr>
<th>Mo.</th>
<th>Total Cholest. mg.%</th>
<th>Free Cholest. mg.%</th>
<th>Cholest. Esters mg.%</th>
<th>Lipid Phosph. mg.%</th>
<th>Lipoproteins mg.%</th>
<th>Systolic Blood Press. mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td>Sf 12-20</td>
<td>Sf 20-100</td>
</tr>
<tr>
<td>1</td>
<td>120</td>
<td>57</td>
<td>63</td>
<td>15.1</td>
<td>6</td>
<td>7</td>
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<td>2</td>
<td>124</td>
<td>50</td>
<td>74</td>
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<td>3</td>
<td>110</td>
<td>50</td>
<td>60</td>
<td>15.1</td>
<td>12</td>
<td>64</td>
</tr>
</tbody>
</table>

Control period—6 paired littermates

<table>
<thead>
<tr>
<th>Cholesterol feeding 5 Gm. daily</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silica Sham</td>
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<tr>
<td>10</td>
</tr>
<tr>
<td>11</td>
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<td>12</td>
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<td>13</td>
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<td>14</td>
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</table>

Bilateral renal artery silica injection or sham operation

Aortic atherosclerosis

<table>
<thead>
<tr>
<th>Silica Sham</th>
</tr>
</thead>
<tbody>
<tr>
<td>1+(A) 0(A')</td>
</tr>
<tr>
<td>2+(B) 0(B')</td>
</tr>
<tr>
<td>1+(C) 1+(C')</td>
</tr>
</tbody>
</table>

The group I hypertensive dogs demonstrated the most marked atherosclerosis and had mean total cholesterol levels two to four times higher than those in groups II and III. The group I animals also had much higher levels of Sf 12-20 and Sf 20-100 lipoproteins. Conversely, the group III animals with lowest mean total cholesterol levels evidenced the least atherosclerosis at autopsy. The hypertensive animals in groups II and III with moderate and minimal hypercholesterolemia respectively all demonstrated greater atherosclerosis than did their normotensive littermates.

SUMMARY

Paired, littermate, pure-bred Basenjis were utilized to determine the role, if any, of chronic hypertension on the development of aortic...


Table 4.—Summary of Cholesterol, Blood Pressure and Aortic Atherosclerosis Data

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean total cholesterol at 3 months mg.%...</td>
<td>120</td>
<td>104</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>Cholesterol plus Thiouracil</td>
<td>Cholesterol plus I131</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>Mean total cholesterol at 9 months mg.%...</td>
<td>699</td>
<td>300</td>
<td>150</td>
</tr>
<tr>
<td>Mean total cholesterol at 14 months mg.%...</td>
<td>700</td>
<td>522</td>
<td>340</td>
</tr>
<tr>
<td>Mean b.p. mm.Hg</td>
<td>198</td>
<td>134</td>
<td>188</td>
</tr>
<tr>
<td>Mean aortic atherosclerosis. . .</td>
<td>3.3+</td>
<td>1.7+</td>
<td>1.3+</td>
</tr>
</tbody>
</table>

Atherosclerosis in dogs with hypercholesterolemia.

Varying levels of hypercholesterolemia were produced by feeding 5 Gm. of cholesterol in horsemeat daily to dogs (a) receiving 0.4 Gm. of propylthiouracil daily (group I); (b) having received 1 millicurie of I131 per pound of body weight at 6 months of age (group II); and (c) receiving no antithyroid medication (group III).

After a six-month control period of cholesterol feeding, reasonably comparable levels of hypertension were produced in three animals in each group by the injection of a suspension of 5 mg. of white silica into both renal arteries. In each group, sham-operated littermates of the hypertensive dogs were subject to the same feeding regimen.

Five months after the induction of hypertension, all animals in this study died or were sacrificed in extremis as a result of uncontrollable distemper-hepatitis epidemic. At autopsy the degree of atherosclerosis was graded from 1 to 4 plus, depending upon the extent and severity of the lesions.

As summarized in table 4, animals with hypertension demonstrated significantly more atherosclerosis than did their normotensive littermates. Marked hypercholesterolemia was associated with more severe aortic atherosclerosis in both hypertensive and normotensive dogs than in those with mild or moderate elevations of cholesterol.

Conclusions

Atherosclerosis in dogs is enhanced by hypertension induced by the injection of white silica into the renal arteries.

Atherosclerosis is furthered by marked hypercholesterolemia induced by propylthiouracil and cholesterol administration in both normotensive and hypertensive dogs.

Acknowledgments

The author wishes to express his gratitude to Dr. R. S. George, Dr. F. R. Franke, Mrs. G. L. Rhodes and Mrs. R. Aldisert for their assistance in this study.

References

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CAMPBELL MOSES

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