Effect of Experimental Renal Hypertension on Experimental Thiouracil-Cholesterol Atherosclerosis in Dogs

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With the collaboration of Mrs. Gloria Fein and Mr. Frank Kojikawa

Experimental renal hypertension accelerated the onset and accentuated the severity of thiouracil-cholesterol atherosclerosis in dogs. Increased blood pressure and other hemodynamic changes appear to be responsible for these effects. Hypertension and sex were without significant effect on serum cholesterol, cholesterol: phospholipid ratio and low density lipoproteins. Sex had no significant effect on the incidence or severity of this experimental atherosclerosis, including coronary involvement.

There is clinical evidence indicating that hypertension accelerates the onset and accentuates the progress of atherosclerosis. Thus the incidence of atherosclerosis is higher in human hypertensives than in normotensives and there is other circumstantial evidence relating the arterial pressure level to the development of atheromatous plaques in man. Since final proof is lacking, however, controlled studies of the effect of hypertension on atherosclerosis in experimental animals should yield information of value. Kendall and associates showed that experimental cholesterol atherosclerosis, similar in anatomic distribution and other characteristics to human atherosclerosis, can be produced in the dog by thiouracil-cholesterol feeding. Accordingly we decided to combine this form of experimental atherosclerosis with experimental hypertension produced by the classic method of Goldblatt. When we began work in 1948 there was only one report of a similar study in animals, that of Dill and Isenhour who found that experimental renal hypertension increases the incidence and severity of spontaneous atherosclerosis in rabbits. During the course of our work, Triantafilo, Lira, and Mardones reported that rabbits with experimental renal hypertension developed more severe and extensive lesions of experimental cholesterol atherosclerosis than did normotensive controls. On the other hand, Stamler and Katz found that salt hypertension did not significantly augment the lesions of spontaneous atherosclerosis in chickens, although DCA significantly increased the atherosclerotic lesions of cholesterolized, salt-hypertensive chickens.

A preliminary report of our research offered evidence for an accelerating and accentuating effect of experimental renal hypertension on experimental thiouracil-cholesterol atherosclerosis in dogs. Since then, Moses reported a similar finding from a comparable study which had the advantage of using paired litter mate dogs for the experimental and control groups. Unfortunately, his work was prematurely terminated by an epidemic of fatal distemper-hepatitis.

This final report of our research confirms our preliminary conclusion and to our knowledge describes the most severe lesions of experimental cholesterol atherosclerosis thus far produced in animals.

Methods

After a control period of 2 to 3 months, hypertension was produced in 22 dogs by bilateral renal artery constriction with a 3 weeks interval between constrictions. The technic used has invariably produced hypertension in more than 500 dogs. One to 2 months after the second renal artery constriction, 17 of the 22 hypertensive dogs were given 0.1 Gm./Kg. of thiouracil and 1.0 Gm./Kg. of cholesterol daily and orally. The cholesterol was mixed with prepared dog food (Pard) and milk. The dogs were continued on thiouracil and cholesterol until sacrificed. Of the 5 remaining hypertensive dogs,
after at least 5 months of hypertension, 1 was placed on thiouracil-cholesterol for 5 months and then taken off 5 months prior to sacrifice, 2 were given thiouracil without cholesterol, and 2 cholesterol without thiouracil. Seventeen normotensive control dogs were subjected to sham renal operations and treated with thiouracil and cholesterol until sacrificed. The 17 renal artery constricted and 17 normotensive control dogs varying in age from 1-4 years and in weight from 7-16 Kg, were selected and matched in pairs for maximum similarity as to age, sex, weight, size, body fat, and breed mixture.

Direct mean femoral artery pressures were obtained semiweekly; serum cholesterol and lipid phosphorus were determined biweekly; clinical examinations and weighings were made monthly or oftener when indicated; urinyses were carried out bimonthly or more often; blood urea nitrogen determinations were made when indicated; and electrocardiograms were recorded at the beginning of the experiment and shortly before sacrifice.

At intervals of approximately 1 month, 1 or more pairs of matched renal artery constricted and normotensive control dogs were sacrificed after 2 to 7 months on thiouracil-cholesterol. One pair each was sacrificed after 9 and 13 months on thiouracil-cholesterol. Sacrifice was by pentobarbital anesthesia and exsanguination. Gross atherosclerotic lesions were graded from 0.5 (small, scattered, occasional plaques in the arterial tree) to 5 (numerous, large, coalescing, ulcerative and/or calcified lesions widely distributed through the arterial tree). Grading was done independently by two observers without knowledge of the history of the animals examined. Dissection of the arterial tree extended to the smallest arteries possible. A thorough necropsy was performed in relation to other structures and organs. Tissue sections were obtained from various parts of the arterial tree and from the heart, brain, kidney, pancreas, liver, thyroid, stomach, intestine, spleen, and adrenal. Sections were stained with hematoxylin and eosin and with Sudan IV.

RESULTS

Atherosclerotic (and other) Lesions. Table 1 summarizes results with the 17 pairs of renal artery constricted and normotensive control dogs after 2 to 13 months on thiouracil-cholesterol. The renal artery-constricted animals are listed primarily in order of increasing severity

<p>| Table 1.—Experimental Thiouracil-Cholesterol Atherosclerosis in Chronic Renal Hypertensive Dogs (nos. 18-34) and Normotensive Dogs (nos. 1-17) |
|---|---|---|---|---|---|---|---|</p>
<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Sex</th>
<th>Serum cholesterol (mg. %)</th>
<th>Serum lipid F (mg. %)</th>
<th>Cholesterol: phospholipid ratio</th>
<th>Normotension (mm. Hg)</th>
<th>Hypertension (mm. Hg)</th>
<th>Ath. Ind.</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>M</td>
<td>215 ± 15</td>
<td>900 ± 184</td>
<td>12 ± 1.0</td>
<td>24 ± 1.7</td>
<td>122 ± 2.0</td>
<td>133 ± 3.0</td>
</tr>
<tr>
<td>19</td>
<td>F</td>
<td>164 ± 17</td>
<td>578 ± 113</td>
<td>14 ± 1.7</td>
<td>18 ± 1.6</td>
<td>133 ± 2.1</td>
<td>130 ± 2.0</td>
</tr>
<tr>
<td>20</td>
<td>M</td>
<td>171 ± 8</td>
<td>620 ± 63</td>
<td>12 ± 1.5</td>
<td>19 ± 1.7</td>
<td>135 ± 2.2</td>
<td>132 ± 2.0</td>
</tr>
<tr>
<td>21</td>
<td>M</td>
<td>215 ± 95</td>
<td>1018 ± 256</td>
<td>10 ± 3.6</td>
<td>32 ± 3.6</td>
<td>140 ± 5.1</td>
<td>140 ± 5.1</td>
</tr>
<tr>
<td>22</td>
<td>F</td>
<td>284 ± 27</td>
<td>970 ± 219</td>
<td>16 ± 2.4</td>
<td>24 ± 2.7</td>
<td>138 ± 4.5</td>
<td>137 ± 5.5</td>
</tr>
<tr>
<td>23</td>
<td>F</td>
<td>326 ± 62</td>
<td>1386 ± 1292</td>
<td>20 ± 4.3</td>
<td>30 ± 7.5</td>
<td>141 ± 6.5</td>
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</tr>
<tr>
<td>24</td>
<td>M</td>
<td>320 ± 47</td>
<td>1132 ± 376</td>
<td>19 ± 2.8</td>
<td>40 ± 4.0</td>
<td>141 ± 7.4</td>
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<tr>
<td>25</td>
<td>F</td>
<td>246 ± 19</td>
<td>948 ± 352</td>
<td>15 ± 5.2</td>
<td>32 ± 2.1</td>
<td>142 ± 7.2</td>
<td>142 ± 7.2</td>
</tr>
<tr>
<td>26</td>
<td>F</td>
<td>210 ± 23</td>
<td>1308 ± 114</td>
<td>12 ± 1.0</td>
<td>32 ± 2.8</td>
<td>144 ± 7.0</td>
<td>144 ± 7.0</td>
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<tr>
<td>27</td>
<td>M</td>
<td>187 ± 15</td>
<td>1165 ± 165</td>
<td>15 ± 3.3</td>
<td>27 ± 5.2</td>
<td>145 ± 8.0</td>
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<tr>
<td>28</td>
<td>M</td>
<td>201 ± 27</td>
<td>927 ± 263</td>
<td>13 ± 2.2</td>
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<tr>
<td>29</td>
<td>F</td>
<td>241 ± 65</td>
<td>1485 ± 151</td>
<td>15 ± 6.3</td>
<td>32 ± 3.3</td>
<td>149 ± 7.1</td>
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<tr>
<td>30</td>
<td>M</td>
<td>107 ± 6</td>
<td>2075 ± 693</td>
<td>9 ± 3.8</td>
<td>35 ± 9.5</td>
<td>148 ± 7.1</td>
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<tr>
<td>31</td>
<td>M</td>
<td>130 ± 10</td>
<td>1062 ± 238</td>
<td>13 ± 5.6</td>
<td>27 ± 4.1</td>
<td>152 ± 7.1</td>
<td>152 ± 7.1</td>
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<tr>
<td>32</td>
<td>M</td>
<td>207 ± 41</td>
<td>1782 ± 304</td>
<td>14 ± 1.5</td>
<td>34 ± 5.7</td>
<td>157 ± 7.1</td>
<td>157 ± 7.1</td>
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<tr>
<td>33</td>
<td>F</td>
<td>260 ± 53</td>
<td>1256 ± 194</td>
<td>17 ± 4.3</td>
<td>29 ± 2.8</td>
<td>161 ± 7.1</td>
<td>161 ± 7.1</td>
</tr>
<tr>
<td>34*</td>
<td>M</td>
<td>150 ± 6</td>
<td>1257 ± 389</td>
<td>16 ± 1.6</td>
<td>33 ± 6.7</td>
<td>165 ± 7.1</td>
<td>165 ± 7.1</td>
</tr>
<tr>
<td>Av.</td>
<td>219</td>
<td>1165</td>
<td>14</td>
<td>27</td>
<td>118</td>
<td>172</td>
<td>156</td>
</tr>
<tr>
<td>1-172</td>
<td>Av.</td>
<td>225</td>
<td>1185</td>
<td>14</td>
<td>27</td>
<td>118</td>
<td>124</td>
</tr>
</tbody>
</table>

* Combination renal hypertensive and buffer nerve hypertensive dog.
† Ath. Ind. = Atherosclerosis Index (graded 0-5).
‡ Detailed tabulation of pertinent data may be obtained from authors on request.
of atherosclerosis and secondarily in order of increasing period on thiouracil-cholesterol. Serum cholesterol and lipid phosphorus averages before thiouracil-cholesterol are each based on 4 to 6 determinations and during thiouracil-cholesterol on 4 to 10 determinations, usually after a hypercholesterolemic plateau was reached at the end of the second month of thiouracil-cholesterol. Blood pressure averages are based on a minimum of 8 readings when the time interval was as short as 1 month and at least 12 to 18 readings when the interval was longer.

The atherosclerotic lesions averaged 2.1 in the 17 renal artery constricted dogs and 0.5 in the 17 normotensive controls. The difference between means as tested by the Wilcoxon rank test is statistically significant at the 1 per cent level. Two normotensive control dogs had gross lesions graded 2 and 4, whereas 9 renal artery constricted dogs were graded 2 to 5. Conversely, 11 normotensive control dogs were graded 0, whereas this was true for only 3 renal artery constricted dogs. Gross lesions did not appear until after 4 months on thiouracil-cholesterol in the normotensive control dogs whereas gross lesions appeared in hypertensive dogs after 2 and 3 months.

A normotensive control dog typical for grade 1 (no. 15) showed a few atherosclerotic plaques in the abdominal aorta and in the external and internal iliac arteries. This dog also showed scattered 1-2 mm. plaques in the superior thyroid, adrenal, and coronary arteries and scattered 3-4 mm. plaques in the renal and middle sacral arteries. Fig. 1 shows atherosclerotic lesions in the aorta and its branches of the only normotensive control dog graded 4 (no. 17). Extensive ulcerative plaques can be seen in the abdominal aorta extending into the external and internal iliac arteries. There are numerous lesions in the coeliac, superior mesenteric, renal and ureteric arteries. The left anterior cerebellar artery showed a small (1 mm.) plaque and the intercostal, diaphragmatic, coronary, splenic, hepatic, cholecystic, inferior mesenteric, cystic, and middle sacral arteries and their branches showed numerous plaques.

A hypertensive dog typical for grade 2 (no. 26) showed involvement of the abdominal aorta and coeliac, superior mesenteric, ureteric, iliac, and thyroid arteries. Hypertensive no. 32 typical for grade 5, showed heavy plaque formation and ulceration in the abdominal aorta, extending into the external and internal iliac arteries and into the coeliac, superior mesenteric, renal and inferior mesenteric arteries. The coronaries and the circle of Willis and their branches showed extensive involvement. Practically every artery of the systemic circulation showed atheromatous plaques.
unusual locations as the mammary arteries and anterior chest wall muscle arteries. All 4 heart valves, especially the mitral, showed plaque formation. Even the pulmonary artery and its main branches showed several small (1–2 mm.) plaques. Figure 2 shows a portion of the left ventricle of this animal with extensive coronary atheromatosis. Hypertensive dogs 33 and 34 also graded 5 showed similar lesions with the addition of a thrombus in the left external iliac artery of each animal.

Scattered plaques occurred in the cerebral circulation of all dogs graded 4 and 5 and in approximately half of the dogs graded 1 to 3. All of the dogs graded 3, 4, and 5 showed atheromatosa of the coronary arterial system but electrocardiograms on these animals were normal. All 11 dogs graded 2 or more showed atheromatosa of the renal artery which were equally extensive proximal and distal to the renal clamp in the 9 renal artery-constricted dogs. Four of these 9 dogs showed a significant increase in their hypertensions during the last month or 2 before sacrifice (fig. 3), 2 showed a questionable increase, and 3 showed no change in hypertensive level. Urinalyses and blood urea nitrogen values remained normal in these and the other renal artery constricted and normotensive control dogs. Small, scattered atheromatous plaques were found in the pulmonary artery and its main branches only of the 3 dogs graded 5.

Hypertrophy and hyperplasia of the thyroid and lipidosis of the liver, spleen, and kidney were similar in the normotensive control and renal artery constricted dogs and have already been adequately described for normotensive dogs on thioctic acid-cholesterol.2 Microscopic examination of the brain, heart, liver, thyroid, spleen, stomach, bowel, pancreas, kidney, and adrenal glands showed no areas of fibrosis or tissue atrophy, even in the dogs with grade 5
HYPERTENSION AND THIOURACIL-CHOLESTEROL ATHEROSCLEROSIS

atherosclerosis. The microscopic characteristics of the atherosclerotic lesions were similar to those already described by Kendall's group.2, 13, 14

Relation of Blood Pressure and Hypertension to Atherosclerosis. There was a slight but not significant correlation between atherosclerosis in the normotensive control dogs and their blood pressure levels ($r_s = 0.34^*; p > .05$) but there is a significant correlation between the normotensive levels of the control dogs times months on thiouracil-cholesterol and the atheromatous lesions ($r_s = 0.63; p < .01$). The only normotensive control dog graded 4 (no. 17) had an average blood pressure of 143 mm. Hg which is just below the minimum for spontaneous hypertension in the dog.16 Table 1 shows a modest correlation between the blood pressure levels of the renal artery constricted dogs during thiouracil-cholesterol and the lesions ($r_s = 0.49; p < .05$), and a similar correlation between the blood pressure levels of these dogs times months of thiouracil-cholesterol feeding and experimental atherosclerosis ($r_s = 0.52; p < .05$). Table 1 also shows a correlation between the increases in blood pressure over normotension (net hypertensions) during thiouracil-cholesterol in the renal artery constricted dogs and the lesions ($r_s = 0.51; p < .05$) which is improved by taking the months on thiouracil-cholesterol into account ($r_s = 0.64; p < .01$).

Table 1 further shows that all 17 dogs developed significant hypertensions following renal artery constriction and prior to thiouracil-cholesterol. However, 4 of the dogs showed sufficient reductions in their hypertensions during thiouracil-cholesterol to result in non-significant average blood pressure increases of 1, 8, 13, and 14 mm. Hg over normotension (nos. 19, 20, 25, and 27). Four other dogs showed low hypertensions of 25–28 mm. Hg while on thiouracil-cholesterol feeding (nos. 18, 22, 28, 29). The remaining 9 dogs showed hypertensions varying from 31 to 135 mm. Hg. When our research group used the renal artery constricting technic on more than 500 dogs not fed thiouracil-cholesterol, approximately 40 per cent showed a decrease of 20 to 40 mm. Hg in their hypertensions during the third to the fifth months of hypertension, presumably due to the development of collateral renal circulation. Of these, one-half or 20 per cent were left with low hypertensions of 25–29 mm. Hg and none with insignificant blood pressure increases. The obvious possibility that thiouracil-cholesterol feeding had an antihypertensive effect in 4 of the 17 hypertensive dogs is supported by the blood pressure findings on renal hypertensive dog 35 which was given thiouracil-cholesterol for 5 months and then taken off thiouracil-cholesterol for 5 months before sacrifice (fig. 4). This dog showed a decrease to normotension during thiouracil-cholesterol and a return toward the original hypertensive level during the months following thiouracil-cholesterol. Thiouracil-cholesterol feeding had no significant effect on the blood pressure of the normotensive, sham-operated control dogs.

Table 1 also shows that 6 of the 17 renal artery constricted dogs had normotensive blood pressure levels of 115 to 143 mm. Hg during thiouracil-cholesterol and were graded 0, 0, 0.5, 1, 2, and 3. Thirteen of the 17 normotensive control dogs had average blood pressure levels of the same range during thiouracil-cholesterol and were graded 0 (9 animals), 0.5, 1, 2, and 4. The difference between these two groups is not statistically significant ($p = 0.171$; Fisher's exact one-tail test for probability). On the other hand, the 11 renal artery constricted dogs with hypertensions averaging 148 to 260 mm. Hg during thiouracil-cholesterol, with one exception (dog 18 graded 0), were graded 1 to 5.

Relation of Hypercholesterolemia, Cholesterol: Phospholipid Ratio, and Serum Lipoproteins to Atherosclerosis. Table 1 shows a significant relationship between the hypercholesterolemia
and the severity of the lesions of the renal artery constricted dogs (r = 0.74; p < .01) which is decreased if the months of thiouracil-cholesterol feeding are considered (r = 0.61; p < .01). The average serum cholesterol was 900 mg. per cent or less in the 3 renal artery constricted dogs which showed no gross lesions and more than 900 in the remaining 14 dogs which showed gross lesions. The correlation coefficient for the hypercholesterolemia and the lesions of the normotensive control dogs was not significant (r = 0.31; p > .05) but suggestive of a relationship.

The relation between the incidence and severity of the gross lesions and the cholesterol:phospholipid ratio of the renal artery constricted dogs during thiouracil-cholesterol is less than for the hypercholesterolemia (r = 0.54; p < .05). For the normotensive control dogs the relation between the lesions and the ratio is better than that between the lesions and the hypercholesterolemia (r = 0.46; p < .05). It is noteworthy that with one exception (dog 21), none of the 14 renal artery constricted dogs and none of the 6 normotensive control dogs which showed atherosclerosis had ratios of less than 1.52 on thiouracil-cholesterol, whereas the 3 renal artery constricted and 6 of the 11 normotensive control dogs which showed no gross lesions had ratios of 1.23 to 1.49. The remaining 5 normotensive control dogs had ratios of 1.67 to 2.70 but were on thiouracil-cholesterol for only 2 to 4 months.

Simultaneous serum lipoproteins by the Gofman technique and serum cholesterols were determined on 2 renal artery constricted dogs and 2 normotensive control dogs once before and once during thiouracil-cholesterol and on 3 renal artery constricted and 2 normotensive control dogs twice at an interval of 3 months during thiouracil-cholesterol. These fragmentary results suggest that thiouracil-cholesterol feeding increases the Sf 10-20 and 20-30 lipoproteins similarly in the renal artery constricted and normotensive control dogs and that the increases in Sf 10-20 and 20-30 lipoproteins roughly parallel increases in serum cholesterol.

There were no significant differences between the levels of serum cholesterol, lipid phosphorus, cholesterol:phospholipid ratio, and serum lipoproteins of the renal artery constricted and the normotensive control dogs.

Relation of Sex to Atherosclerosis, Hypercholesterolemia, and Cholesterol-Phospholipid Ratio. Sex had no effect on the incidence of atherosclerosis in the 17 normotensive and 17 renal artery constricted dogs since 3 of the 8 normotensive females and 3 of the 9 normotensive males, and 6 of the 7 renal artery constricted females and 8 of the 10 renal artery constricted males developed atherosclerosis. The lesions averaged 0.38 in severity in the normotensive females and 0.61 in the normotensive males, and 1.9 in the renal artery constricted females and 2.4 in the renal artery constricted males. These differences are not significant (p > .6 and > .6). Coronary atherosclerosis was found in 0 of the 8 normotensive females and 0.61 in the normotensive males, and 1.9 in the renal artery constricted females and 2.4 in the renal artery constricted males. There is likewise no evidence of any difference in the two sexes in relation to coronary atherosclerosis.

The average hypercholesterolemia was 1166 mg. per cent for the normotensive females, 1202 for the normotensive males, 1133 for the renal artery constricted females and 1188 for the renal artery constricted males. The cholesterol:

*While our primary intent was the study of the effect of experimental renal hypertension on thiouracil-cholesterol atherosclerosis, we also were interested in relations between the hypercholesterolemia and the cholesterol:phospholipid ratio on the one hand, and the incidence and severity of the lesions on the other. Such correlations are not statistically independent of the relation of primary interest. The probability levels of such non-independent correlation coefficients are not known and any implications must be accepted with caution. Rank correlation coefficients were calculated for these secondary associations to facilitate study and comprehension of possible interdependency of factors regardless of true statistical significance or non-significance in the conventional sense.

† Serum lipoproteins were determined through the courtesy of Dr. John F. Gofman of the Division of Medical Physics of the University of California.
phospholipid ratios during thiouracil-cholesterol feeding for the corresponding groups of dogs were 1.67, 1.69, 1.64, and 1.64 respectively. These differences are not significant.

Miscellaneous. The two renal hypertensive dogs fed thiouracil but not cholesterol for 7 months showed no gross lesions. Their serum cholesterols averaged 338 and 393 mg. per cent and their cholesterol:phospholipid ratios .71 and .83 during thiouracil feeding. The two renal hypertensive dogs fed cholesterol but not thiouracil for 7 months were graded 0.5 and 1. Their serum cholesterols averaged 310 and 403 mg. per cent and their cholesterol:phospholipid ratios .89 and .89 during cholesterol feeding.

Discussion
The results confirm our preliminary conclusion that experimental renal hypertension accelerates the onset and accentuates the progress of experimental thiouracil-cholesterol atherosclerosis in dogs. In contrast to the 17 renal artery constricted dogs with average atherosclerotic lesions graded 2.1 and the 17 normotensive controls graded 0.5, 500 renal hypertensive dogs which have been necropsied by our group during the past 20 years after 2 to 84 months of renal hypertension without thiouracil-cholesterol, were graded 0. We and others have observed no gross atherosclerotic lesions at necropsy in many normotensive dogs of various ages, although some workers have observed minimal lesions in old dogs which would be graded less than 0.5 on our scale.

That blood pressure is important in the atherosclerotic potentiating effect of experimental renal hypertension, is suggested by the correlations between the blood pressure levels and net hypertensions of the renal artery constricted dogs and the incidence and severity of their lesions. This is also suggested by the lack of significant difference between the incidence and severity of lesions in the normotensive control dogs and the normotensive renal artery constricted dogs on thiouracil-cholesterol. Moreover, only the 3 dogs graded 5 showed scattered pulmonary artery plaques, and while no pulmonary artery pressures were obtained, conceivably these dogs might have had some degree of left heart failure with increased pulmonary pressures.

The lesions in our dogs, particularly those graded 4 and 5, were similar in their distribution and gross and microscopic appearance to severe human atherosclerosis. The ulcerative lesions of the grade 5 dogs were more severe than any previously seen in experimental cholesterol atherosclerosis; and to our knowledge, thrombosis of a major atherosclerotic artery has not been described in the dog. In spite of involvement of the coronary arteries and their branches in all dogs graded 3, 4, and 5, the electrocardiograms were normal. No areas of fibrosis were found in the myocardium which is contrary to the observation of Steiner and Kendall, and may be due to their dogs being on thiouracil-cholesterol longer than ours.

The significant increase in hypertension 1 to 2 months before sacrifice, in 4 of the 9 renal artery constricted dogs showing renal artery plaques may have been due to further alteration in renal hemodynamics produced by plaque narrowing, with resulting increase in renal pressor mechanisms. If this occurred, it was not associated with any change in urinalysis, blood urea nitrogen, or microscopic appearance of the kidney of these 4 dogs. The fact that the plaques were equally numerous and severe proximal and distal to the clamp suggests a hemodynamic factor other than blood pressure as also operative in the production of the lesions, since our research group found several years ago that similar constriction of the renal artery produced a decrease of 20-70 mm. Hg beyond the clamp.

The mechanism of the apparent antihypertensive effect produced by thiouracil-cholesterol in 4 of the 17 renal hypertensive dogs of table 1 and in dog 35, is unknown. No antihypertensive effect was observed in the 2 renal hypertensive dogs on cholesterol alone or in the 2 on thiouracil alone. Actually cholesterol has been reported to have a hypertensive effect but this has been denied by most investigators. Thyroidectomy has been reported to be without significant effect on experimental renal hypertension in dogs.

This study confirms previous reports that there is a relation between the degree and duration of the hypercholesterolemia, the
concentration of low density serum lipoproteins, and the cholesterol:phospholipid ratio on the one hand and the incidence and severity of the lesions of experimental cholesterol atherosclerosis on the other. Compared with normotension, experimental renal hypertension did not significantly change the serum cholesterol, cholesterol:phospholipid ratio, or low density serum lipoproteins before or during thiouracil-cholesterol feeding, even in the dogs which showed increased hypertension shortly before sacrifice. Our findings confirm Baker and co-workers\textsuperscript{20} who previously reported increased concentrations of low density serum lipoproteins in dogs on thiouracil-cholesterol.

Sex had no significant effect on the incidence or severity of the lesions in the normotensive or renal artery constricted dogs. This was likewise true for coronary atherosclerosis. Thus thiouracil-cholesterol atherosclerosis in the dog appears to differ from cholesterol atherosclerosis in the chicken where the greater coronary involvement of the male is well-known.\textsuperscript{21} Sex likewise had no significant effect on the hypercholesterolemia or the cholesterol:phospholipid ratios. This finding is at variance with that of Barr and co-workers\textsuperscript{22} who found higher concentrations of serum cholesterol and phospholipids and greater cholesterol:phospholipid ratios in normotensive male dogs as compared with female dogs on thiouracil-cholesterol.

The 2 renal hypertensive dogs which developed grade 0.5 and 1.0 atherosclerotic lesions on cholesterol alone despite modest serum cholesterol levels (310 and 403 mg. per cent) and low cholesterol-phospholipid ratios (.89) are the first dogs reported to develop significant atherosclerotic lesions with cholesterol levels under 450 mg. per cent. These results further attest the potentiating effect of hypertension in experimental thiouracil-cholesterol atherosclerosis.

The incidence and severity of the lesions in these renal hypertensive dogs should encourage further studies of the combination of experimental renal hypertension (or other experimental hypertension) and experimental thiouracil-cholesterol atherosclerosis in dogs. Even though our limited studies of the heart and kidney showed no significant functional changes and no areas of fibrosis were noted in the myocardium or other organs, observations over a longer period may demonstrate important functional and structural changes in the heart, kidney, brain, and other organs of severely atherosclerotic hypertensive dogs similar to those of human atherosclerosis.

**Summary**

Experimental renal hypertension accelerates the onset and accentuates the progress of experimental thiouracil-cholesterol atherosclerosis in dogs. The gross lesions are similar to those of human atherosclerosis in distribution and appearance, including ulceration, calcification, and thrombus formation with the severest lesions.

The potentiating effect of experimental renal hypertension on thiouracil-cholesterol atherosclerosis in the dog appears to be due to increased blood pressure and possibly other hemodynamic factors. The hypertension was without effect on the serum cholesterol, cholesterol-phospholipid ratio, or low density lipoproteins.

There was no evidence of significant difference in the incidence and severity of coronary atherosclerosis between male and female dogs. Sex likewise had no significant effect on the hypercholesterolemia or cholesterol:phospholipid ratio during thiouracil-cholesterol feeding.

Cholesterol alone for 7 months produced atherosclerosis in 2 renal hypertensive dogs with plasma cholesterols below 450 mg. per cent.

The possibility of producing experimental chronic myocardial insufficiency in dogs on an atherosclerotic, hypertensive basis, as well as functional deficiencies of other organs, is suggested.

**Acknowledgment**

We are grateful to Dr. H. C. Batson, Professor of Biostatistics, University of Illinois College of Medicine, for advice in relation to certain statistical analyses of this report.

**Summario in Interlingua**

Experimental hypertension renal accelerat la declaration e accentuare le progresso de experimental atherosclerosis a thiouracil-cholesterol in canes. Le examine grosse, le lesiones se monstra simile al lesiones in atherosclerosis in


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