Spontaneous and Stress-Induced Myocardial Infarction in Aged Atherosclerotic Dogs

By Harry Sobel, Ph.D., Carl E. Mondon, M.S., and Reuben Straus, M.D.

Atheromatosis may be induced in the dog following daily ingestion of cholesterol and administration of thiouracil1 or I131.2 There appears to be agreement in the following:

Grossly observable lesions occur in blood vessels only when the cholesterol levels exceed 800 mg. per cent for many months. Individual dogs show great variation in the degree of atheromatous change and its distribution, even though comparable serum cholesterol levels are maintained. While fibrotic, proliferative, ulcerative, hemorrhagic, and calcific changes occur, they are relatively mild compared to the massive degree of atheromatosis which may be observed. Although the degree of coronary atheromatosis may be extensive, myocardial infarction is rarely reported. In most experiments, mongrel dogs were used with little attention given to the age of the animal. This seemed unfortunate since aging phenomena could influence the response to this regimen. It was, therefore, decided to investigate this matter.

Lindsay et al.3 reported that spontaneous "arteriosclerosis," characterized by intimal fibrosis and deposition of mucoid ground substance and collagen, occurs in dogs over eight years of age and is "often accompanied by coronary disease with myocardial infarction or fibrosis." Lipids appear to play no role in the genesis of these spontaneous intimal lesions. In order to avoid complications due to the spontaneous lesions, it was accordingly decided to work with dogs which were six to eight years of age.

Methods

Male mongrel dogs, weighing 10 to 18 Kg., were obtained from a Los Angeles City animal shelter. They were housed in a veterinary hospital during the entire experiment. Only those animals whose age was estimated to be six to eight years and who were free of any detectable disease, as judged by a thorough examination administered by two competent veterinarians, were selected for the experiment.

I131 was administered as follows: Dogs were fasted for four days while maintained on distilled water.4 They received 0.5 mc./Kg. of I131 orally followed by three more days of fasting. Three dogs died within two weeks following severe hemorrhagic gastroenteritis probably due to radiation damage.

Friiskies dog meal was mixed with a 20 per cent cholesterol solution in ether, so that for every 100 Gm. of meal, 10 Gm. of cholesterol was present. After uniform mixing, by rotating in a large metal can, the food was spread over a large surface to permit the ether to evaporate. The mixed meal was fed daily to the animals in servings of 7½ Gm. per Kg. of body weight, thus supplying 750 mg. of cholesterol per Kg.

Thiouracil was fed daily at a dose of 50 mg./Kg. mixed with 10 Gm. chopped meat. The remainder of the diet consisted of Friiskies dog meal, with or without 10 per cent lard, fed ad libitum.

Several dogs were treated with DOCA (0.4 mg./Kg. for 10 weeks), injected subcutaneously, and other dogs with ACTH during the experiments as indicated in table 1 and the text.

Two types of provocation were used in attempts to induce myocardial infarction:
1. Injection of 0.3 U. pitressin/Kg. over a 20-second period.
2. Exposure to a stressor.

For the stress experiment, animals were restrained in a modified Pavlov rig as previously described.* This contained a stock to keep the head restrained in a normal position. Loose thongs were applied to the legs and tied to the supports of the rig to restrict movement. Straps were placed around the body of the dog between the fore and hindlimbs to restrict motion further. Both

*Sobel, H., Mondon, C. E., and Harvey H. Unpublished observations.
## TABLE 1

### Fate of Dogs on Atherogenic Regimen

<table>
<thead>
<tr>
<th>Dog</th>
<th>Duration of experiment (weeks)</th>
<th>Treatment</th>
<th>Cholesterol &quot;plateau&quot; level mg. per cent</th>
<th>Degree of coronary atherosclerosis</th>
<th>Provocation</th>
<th>Cause of death</th>
</tr>
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<tbody>
<tr>
<td>G</td>
<td>57</td>
<td>1\textsuperscript{st}, cholesterol &amp; lard for 7 weeks; then thiourea added. 4 doses ACTH</td>
<td>700</td>
<td>49</td>
<td>1</td>
<td>Pitressin</td>
</tr>
<tr>
<td>XB</td>
<td>31</td>
<td>Thiouracil &amp; cholesterol. DOCA 0.4 mg./Kg. for 10 weeks</td>
<td>400</td>
<td>27</td>
<td>0</td>
<td>Pitressin</td>
</tr>
<tr>
<td>XP</td>
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<td>Thiouracil &amp; cholesterol. DOCA 0.4 mg./Kg. for 13 weeks</td>
<td>700</td>
<td>28</td>
<td>0</td>
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</tr>
<tr>
<td>T</td>
<td>34</td>
<td>Thiouracil &amp; cholesterol. DOCA 0.4 mg./Kg. for 10 weeks</td>
<td>800</td>
<td>19</td>
<td>0</td>
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<tr>
<td>TC</td>
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<td>400</td>
<td>31</td>
<td>0</td>
<td>Pitressin</td>
</tr>
<tr>
<td>EC</td>
<td>32</td>
<td>Thiouracil &amp; cholesterol</td>
<td>700</td>
<td>23</td>
<td>0</td>
<td>Pitressin</td>
</tr>
<tr>
<td>NS</td>
<td>30</td>
<td>Thiouracil &amp; cholesterol</td>
<td>400</td>
<td>29</td>
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<td>Pitressin</td>
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<tr>
<td>FT</td>
<td>33</td>
<td>Thiouracil &amp; cholesterol</td>
<td>400</td>
<td>31</td>
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<td>Pitressin</td>
</tr>
<tr>
<td>B</td>
<td>90</td>
<td>1\textsuperscript{st}, cholesterol &amp; lard for 7 weeks; thiourea added; 4 doses ACTH</td>
<td>1,000</td>
<td>77</td>
<td>3</td>
<td>2 stress series; 2 max. stress series</td>
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<tr>
<td>I</td>
<td>53</td>
<td>Thiouracil, lard &amp; cholesterol; 1 dose ACTH</td>
<td>1,200</td>
<td>30</td>
<td>3</td>
<td>2 stress series; 2 max. stress series</td>
</tr>
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<td>L</td>
<td>48</td>
<td>Thiouracil, lard &amp; cholesterol; 1 dose ACTH</td>
<td>1,750</td>
<td>42</td>
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<td>2 stress series; 2 max. stress series</td>
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<tr>
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<td>50</td>
<td>Thiouracil, lard &amp; cholesterol; 1 dose ACTH</td>
<td>1,100</td>
<td>43</td>
<td>1</td>
<td>2 stress series; 2 max. stress series</td>
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<tr>
<td>Z</td>
<td>37</td>
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<td>1,800</td>
<td>23</td>
<td>2</td>
<td>1 stress restraint</td>
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<tr>
<td>PL</td>
<td>47</td>
<td>2 1\textsuperscript{st} (6 weeks apart). Cholesterol for 10 weeks, then thiourea added</td>
<td>1,000</td>
<td>36</td>
<td>1</td>
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<td>4</td>
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<td>2,200</td>
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<td>4</td>
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<td>E</td>
<td>51</td>
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<td>2,500</td>
<td>37</td>
<td>5</td>
<td>2 stress series; 2 max. stress series; pitressin</td>
</tr>
<tr>
<td>K</td>
<td>40</td>
<td>Thiouracil, lard &amp; cholesterol</td>
<td>2,500</td>
<td>25</td>
<td>5</td>
<td>1 stress series; 1 max. stress series</td>
</tr>
<tr>
<td>EP</td>
<td>29</td>
<td>1\textsuperscript{st}, cholesterol &amp; lard for 7 weeks; thiourea added, 1 dose ACTH</td>
<td>2,300</td>
<td>18</td>
<td>5</td>
<td>D&lt;sub&gt;a&lt;/sub&gt;</td>
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<tr>
<td>C</td>
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<td>2,700</td>
<td>26</td>
<td>5</td>
<td>D&lt;sub&gt;a&lt;/sub&gt;</td>
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<tr>
<td>CH</td>
<td>23</td>
<td>Thiouracil &amp; cholesterol</td>
<td>2,200</td>
<td>16</td>
<td>5</td>
<td>D&lt;sub&gt;a&lt;/sub&gt;</td>
</tr>
<tr>
<td>CK</td>
<td>48</td>
<td>2 1\textsuperscript{st} (6 weeks apart). cholesterol for 10 weeks; thiourea added</td>
<td>1,300</td>
<td>17</td>
<td>5</td>
<td>Pitressin</td>
</tr>
<tr>
<td>PS</td>
<td>47</td>
<td>1\textsuperscript{st}, cholesterol for 10 weeks; thiourea added</td>
<td>2,100</td>
<td>17</td>
<td></td>
<td>Pitressin</td>
</tr>
</tbody>
</table>

*Based on average trend when highest values were reached.

†Based on arbitrary scale determined at autopsy. O = no atherosclerosis visible grossly . . . 5 = extreme degree of coronary atherosclerosis.

D<sub>s</sub> — Cause of death unknown.

Du — Cause of death due to stress.

T — Terminated.

D<sub>a</sub> — Died due to asphyxia.

D<sub>b</sub> — Died following administration of pitressin.

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hindlegs were attached to a string which led to a counter so that a record was kept of the number of times either leg was raised. Heart rate was determined with a Stillborn electrocardiographic machine, using encephalographic pins for the electrodes. The animal was maintained in the rig for nine hours (9 A.M. to 6 P.M.). Water was offered frequently ad libitum. There was occasional struggling in attempts to gain release, but in general, this restraint does not elicit violent response. After two days, the test was repeated with the exception that during the hours of 3 to 5, 60 10-second direct current (60 to 110 volts) shocks were applied to a forepaw according to a randomized program. The electrodes were 10-cent pieces, approximately 1/4 inch apart. These shocks were accompanied by individual behavioral patterns which ranged from mild acceptance (accompanied only by lifting of the paw) to vigorous activity and vocalization during the shocks.

Both stressers (i.e., restraint and restraint with the shocks) induced large increases in urinary corticoid excretion. A modification of the latter stresser, consisting of 90 30-second (110 volts) shocks applied to a forepaw according to a randomized program. The electrodes were 10-cent pieces, approximately 1/4 inch apart. These shocks were accompanied by individual behavioral patterns which ranged from mild acceptance (accompanied only by lifting of the paw) to vigorous activity and vocalization during the shocks.

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**Results**

The findings are shown in table 1. They are arranged in groups as follows: group A, serum cholesterol level 400 to 1,000 mg. per cent for 19 to 49 weeks; group B, serum cholesterol level 1,000 to 1,800 mg. per cent for 23 to 77 weeks; group C, serum cholesterol levels exceeding 1,800 mg. per cent for at least 16 weeks. Degree of atherosclerosis was determined after autopsy following various manipulations described below.

**GROUP A**

This group consisted of eight animals. The cholesteral-thiouriacil regimen alone produced relatively little elevation of serum cholesterol and virtually no atheromatosis in seven dogs, even when it was supplemented with desoxycorticosterone injection. Dog G, which had received $^{131}$I thiouriacil, cholesterol, and a diet containing 10 per cent lard, fared no better, however.

**GROUP B**

The six animals in group B exhibited a modest degree of atheromatosis. Dog PL had received two courses of $^{131}$I; cholesterol and, eventually, thiouriacil were added to the diet. Dogs I, L, and M received the thiouracil-cholesterol regimen and 10 per cent lard diet. Dogs Z and B received $^{131}$I in addition.

**GROUP C**

All nine animals exhibited extensive atheromatosis. Dog CH received thiouracil and cholesterol alone; dogs E and K, this regimen with 10 per cent lard in the basal diet; dogs PS, CW, and CK, $^{131}$I, thiouracil, and cholesterol; dogs A, D, and EP, $^{131}$I, thiouracil, cholesterol, and 10 per cent lard diet.

**INFLUENCE OF ACTH AND PITRESSIN**

Rosenfeld et al. reported that several injections of ACTH gel resulted in permanent increase in cholesterolemia in beagles maintained on an atherogenic regimen. In order to study this matter further, intramuscular injections of ACTH gel, at doses of 40 U. daily, were administered to eight dogs on the regimen, over periods of one to four days, as indicated in table 1. Increases in serum cholesterol, ranging from 200 to 2,500 mg. per cent, were observed after 2 to 7 weeks following at least two injections. However, return to preinjection level was observed in all cases after several additional weeks. Our findings definitely establish the hypercholesterolemic effect of ACTH to be transitory. Because of the short duration of these effects, we do not believe that ACTH influenced the outcome of this investigation in those dogs to which it was administered.

In view of the profound effect of relatively small doses of pitressin on coronary blood flow in dogs and the association of the resulting sequelae with those produced by anoxic coronary insufficiency, the effect of vasopressin in atherosclerotic dogs was investigated. The dose selected, 0.3 U. pitressin/Kg., which approximates that utilized by Raskin in clinical application of pitressin in testing for coronary insufficiency, was injected intravenously over a period of 20 seconds.

Two deaths were noted among the 12 dogs on the atherogenic regimen who were injected with pitressin. This followed ataxia, and collapse occurred within two minutes after injec-
tion. The electrocardiogram revealed in 
sequence complete heart block, A-V nodal 
rhythm, ventricular extrasystoles, and eventu-
tal termination in ventricular fibrillation.
Histologically, the myocardium of both dogs 
(belonging to group C) exhibited old infarcts 
and myocardial fibrosis.† This was not the 
 ease in any of the other dogs on the athero-
genic diet or in normal dogs which received 
pitressin.

SPONTANEOUS MYOCARDIAL INFARCTION

During the course of this investigation, 
three dogs died spontaneously from myocar-
dial infarction. They were all in group C. 
The diagnosis of myocardial infarction was 
made by clinical impression and finally by 
autopsy.

The dog C was the first to succumb. This 
animal, following 131 treatment and choles-
terol feeding, developed rapid rise in blood 
cholesterol to a 15-fold increase above the con-
trol level in two and one-half months. Addi-
tion of thiouracil to the diet caused further 
increase to 2,700 mg. per cent. Electrocardio-
graphic recordings taken seven months after 
initiation of treatment demonstrated occa-
sional extrasystoles. Three weeks later, a peak 
cholesterol value of 3,085 mg. per cent was 
observed. Two weeks later, this animal was 
found dead in his run. Autopsy revealed 
extensive atherosclerotic involvement of the 
arterial tree including the coronary arteries.
Microscopically, there was extensive involve-
ment of both the intima and media by lipid-
laden cells. The myocardium revealed areas 
of fibrosis suggestive of old areas of infarc-
tion.

Dog CH exhibited very rapid development 
of hypercholesterolemia on the cholesterol and 
thiouracil supplement. Within three months, 
this animal appeared lethargic. Two and one-
half months later, he died in the run. Autopsy 
revealed acute pulmonary edema.

†Histological studies on the pitressin-treated dogs were carried out approximately four years ago. Due to unavoidable circumstances, these specimens are no longer available, and greater detail cannot be given of the histopathology.

Coronary and aortic atherosclerosis was exten-sive; the abdominal aorta at the bifurca-
tion into the common iliac arteries was 
 extremely narrowed. Extensive old myocar-
dial infarction was present.

In the third animal, dog EP, blood choles-
terol increased very little following 131 
treatment and institution of the cholesterol-
lard regimen. Addition of thiouracil to the 
diet caused a rapid rise from values of 300 
mg. per cent to over 2,000 mg. per cent within 
a month. Approximately four months later, 
this animal appeared ill, refused food, and 
was listless. Electrocardiographic determina-
tion revealed the presence of ventricular 
 ectopic extrasystoles. Four days after the 
appearance of the aberrant electrocardio-
gram the animal was infused with 250 cc. 
of Aminosol since he had refused food.

Subsequently, he became free of extrasystoles. 
Seven days later, ventricular tachycardia re-
turned. He was found dead on the second 
 morning. Autopsy revealed considerable ath-
 eromatous involvement of the coronary arter-
es and aorta, including both the intima and 
media. There was a large area of fibrosis in 
the myocardium suggestive of an old infarct 
and a more recent infarction characterized by 
necrosis of muscle with regional infiltration 
by polymorphonuclear neutrophils and leuko-
cytes.

MYOCARDIAL INFARCTION INDUCED 
BY STRESS

Eight dogs were exposed to nine hours of 
 restraint in the modified Pavlov rig. Although 
 large increases in urinary corticoid excretion 
occur under these circumstances,¹ no patho-
logical effects were observed. They were then 
exposed, on several occasions, to 60 10-second 
shocks applied to a forepaw over a two-hour 
period (table 1). Except for dog K, no ob-
vious changes in heart status were observed. 
Later, 90 30-second shocks were applied over 
the same period: "the maximum stresser." ² 
Two dogs, A and K, died following this treat-
ment; they were in group C. Those that sur-
vived this treatment were groups A and B.

Dog K had developed a rapid rise in cho-
 lesterol, from a basal value of 165 mg. per
MYOCARDIAL INFARCTION IN AGED DOGS

cent to 1,890 mg. per cent within five weeks. Five and one-half months after initiation of the regimen, cholesterol values hovered in the vicinity of 2,500 mg. per cent. The animal was exposed to a series of 60 10-second shock periods. It was observed that following the third exposure, he exhibited occasional ectopic extrasystoles which continued through the next day. This was the only dog in which this occurred under these circumstances. In light of this development, it was decided to continue the 60 10-second electroshock tests to determine whether more profound changes might be elicited. Within one-half hour after conclusion of the next series of shocks, extrasystoles were again noted. These became more pronounced on the following day. Another series of shocks was administered in three days. The electrocardiograms taken two and four hours after the shocks revealed extrasystoles again. Two days later, this aberration was still present. It was also noted, following this test, that the pupil in the left eye was extremely dilated. This suggested a possible cerebral accident. No response to light and darkness was elicited. Hyperemia was noted in the eye grounds, but no retinal aneurysms or gross pathology were observed.

The sixth electroshock test was given eight days following the last. Extrasystoles were no longer present before this test or one day following.

In view of the fact that there was no further progression of electrocardiographic abnormalities, the "maximum stresser" was instituted. This again consisted of 90 intermittent shocks applied for 30 seconds every minute. Several electrocardiographic readings were taken after every 30 shocks. One hour and 15 minutes following the last shock, the animal expired.

Postmortem examination of the body revealed extensive atheromatosis involving the aorta and coronary blood vessels. The lumina of the major aortic branches were almost occluded with the lipid deposits, including their intercostal and lumbar branches. Histological examination of the coronary arteries revealed large atheromatous lesions involving the entirety of the wall of the artery. In places, only a small portion of the outer media was preserved. Portions of the intima were thickened by fibroblastic proliferation and slight atheromatous change. In a few foci, there was evidence of old and recent hemorrhage, cystic degeneration, and slight fibrosis as well as cholesterol crystal deposits. In one section, there was a blood clot without peripheral organization which was compatible with an early thrombus. Smaller arteries in the myocardium showed severe lipoidal lesions involving the intima and media more extensively. The regional myocardium revealed an occasional small focus of fibrosis which suggested old small infarcts (fig. 1). Other areas revealed focal early infarction characterized by loss of cellular detail (fig. 2).
Dog A initially revealed a slow upward trend in cholesterol values following $^{131}$I treatment and institution of cholesterol and 10 per cent lard supplements to the diet. One and one-half months later, thiouracil was added, and cholesterol levels rose to values over 1,000 mg. per cent within two weeks. This soon assumed a plateau at approximately 1,400 mg. per cent where it remained for about 13 months, after which it rose to over 2,000 mg. per cent during the last four and one-half months of life. This dog was exposed to the 60 10-second electroshock stresser on two separate occasions. No gross electrocardiographic changes were noted. It was next exposed to 90 consecutive 30-second intermittent shocks. Electrocardiographic patterns again revealed no pertinent abnormalities. This test was repeated seven weeks later. Following the first 40 shocks, the animal succumbed. The circumstances preceding his death were as follows: After he had received 30 consecutive shocks, an electrocardiographic record was obtained. No apparent pathology was observed. The shocks were continued. The animal soon appeared to be in temporary respiratory distress, presumably as a consequence of active struggling against the stock. Electrocardiographic records at this time revealed cessation of normal sinus rhythm with ectopic ventricular beats in conjunction with respiratory standstill. Institution of artificial respiration resulted in temporary restoration of cardiac rhythm which shortly progressed to increasing intervals of cardiac standstill with isolated ectopic ventricular beats followed by ventricular fibrillation and death. Following extensive examination, rare small areas of focal acute myocardial infarction were found (fig. 3) along with an area of ischemic myocardial atrophy (fig. 4).

Dog B exhibited a progressive rise in serum
cholesterol to 2,500 mg. per cent over a period of 14 weeks. He remained approximately at this level for 37 weeks. One week following the second series of 60 10-second shocks, evidence of stroke appeared with staggered gait, rotation of the head to the right, and ocular manifestations. He recovered and survived two maximum stressers and an injection of pitressin and was eventually terminated (table 1). Postmortem examination of this animal revealed severe atheromatous and atherosclerotic changes in the major coronary arteries and their branches. The lumina were stenosed by intimal fibrous proliferation as well as lipid accumulation. No recent thrombus was identified in these sections. The myocardium, however, did reveal a large, old, healed infarct with replacement fibrosis and fat infiltration. Grossly, a large aneurysm of the left ventricle developed at the site of this old infarct.

Dog B survived two series of maximum stressers without any apparent ill effects. Following the second, he was terminated. Postmortem examination of the animal revealed severe atherosclerotic changes in the coronary blood vessels similar to those found in the previously described animals. The involvement was primarily in the media, but the intima was somewhat thickened by fibrous proliferation and showed much less atheroma development. The lumina of the blood vessels were stenosed, and there was an occasional fresh blood clot which showed no evidence of organization but was compatible with a very recent thrombus. The myocardium showed slight fat infiltration, and there were several small foci of myocardial necrosis suggestive of recent myocardial infarction.

Dog Z died accidentally from strangulation while under restraint in the modified Pavlov rig. Postmortem examination of the animal
FIGURE 6
Dog Z: Portion of aneurysm of the wall with large zone of fibrosis and fat infiltration representing the wall of the aneurysm. The intrinsic branches of the coronary artery here also show atheromatous change.

revealed some of the coronary blood vessels to be essentially uninvolved by the atherosclerotic process, while other areas showed moderate-sized arteries with focal atheromatous change. In some areas, the lumina were stenosed and almost obliterated. In one area there was a moderate-sized scar suggestive of an old infarction (fig. 5). Regional to this area there was a severely involved intramural coronary artery with myocardial aneurysm (fig. 6).

GROUP C DOG WITHOUT PATHOLOGY

Dog D was classified in group C. He had received 120 15-second shocks and later an injection of 0.3 U. pitressin/Kg., but he did not exhibit any pathological sequelae. At autopsy, advanced atherosclerosis was present in the coronary blood vessels, but evidence of myocardial infarction was not apparent.

Thus, eight dogs out of nine in group C developed myocardial infarction: spontaneously (four), after pitressin (two, both exhibiting old infarcts), or following a stresser (two). Myocardial infarction was observed in two (B and Z) of six dogs of group B and none in group A.

ADDITIONAL OBSERVATIONS

A number of additional observations were made of the animals during atherogenesis and while they were in the stresser rig. The findings were incidental to this study, but three items are important enough to mention here:

1. The personality type of the dogs as evaluated by procedures described elsewhere* (See page 971) decreased by approximately ½ unit (i.e., slight reduction in activity, aggressiveness, and anxiety) as compared with the starting state, probably due to the ensuing hypothyroidism.

2. Lee-White clotting times were determined in dogs B, I, L, M, E, A, and K. In all cases, this was reduced to 4 to 8 minutes (average for normal dogs is 10 to 12 minutes). They were not further depressed by the maximum stresser.

3. Catecholamine secretion averaged 7.8 μg over the six-hour period following the first shock and the "maximum stresser," as compared to 3.3 μg for the corresponding control period.

Discussion

Although an extreme degree of canine atherosclerosis may be produced by the experimental diet, myocardial infarction occurs rarely. Jordan et al.10 observed extensive coronary atheromatosis in dogs treated with 131I and cholesterol, but myocardial infarction was not produced.

In a series of more than 200 dogs, maintained on a thioacil-cholesterol regimen, most of whom had extensive coronary atherosclerosis, Kendall observed only one dog with any evidence of myocardial infarction. This group included six dogs, aged seven to ten years, which were maintained for six months.
on the diet and developed severe coronary lesions.

It is our impression that dogs which were employed in our experiment did not differ in degree of cholesterolemia or atheromatosis from those which we have observed previously or which others have reported. The occurrence of five (possibly seven, since the dogs which died following pitressin administration had evidence of old infarcts) instances of spontaneous myocardial infarction appears to be unique. It cannot be accounted for by any particular procedure used in the induction of atherosclerosis. The addition of 10 per cent lard to the diet of some of our dogs represents a departure from that which other investigators used. However, some dogs receiving this supplement did not develop severe atherosclerosis. We must, therefore, point to the age of these dogs and observe that infarction, whether spontaneous or induced, occurred with high frequency in those with cholesterol values exceeding 1,800 mg. per cent for at least 16 weeks.

Age-associated changes occur in the vessel wall as well as in the myocardium. They include increased deposition of fibrocollagenous tissue.

These changes are consistent with a theory of aging under investigation in this laboratory. It is believed that the fibrillar density of connective tissue increases with age and that, as a consequence, the rate of delivery of oxygen and nutrients to the cells gradually decreases so that eventually the supply becomes inadequate to support them. Thus, any change which further interferes with metabolic exchange might provoke pathological sequelae in older animals, whereas the same event would produce little change in the young. Consistent with this theory is the observation that year-old rabbits develop atherosclerosis on a cholesterol regimen. Although an equal degree of hypercholesterolemia develops, this does not happen in young rabbits. Selye observed that the myocardial necrotizing action of a variety of agents administered along with 2a-methyl-9a-chlorocortisol and Na2HPO4 were much more pronounced in old rats than in the young. There is a greater susceptibility to the development of ischemic myocardial lesions in older animals following acetylcholine, pitressin, and isoproterenol.

Age-associated changes in the vasa vasorum may play an important role in influencing the outcome with animals on an atherogenic regimen. Gonzalez et al. reported almost invariable involvement of the vasa vasorum of the aorta with changes similar to those in the parent artery. Often, atheromatous changes were seen in the absence of any intimal alteration of the parent artery. Following withdrawal of the cholesterol-thiouracil regimen, Bevans et al. observed that lipid was rapidly removed from the intima and media but that aggregations of lipid tended to accumulate about the vasa vasorum. Dixon suggested that in aging, narrowing of the vasa vasorum would tend to increase anoxia of the vessels.

The role of stress in inducing acute myocardial death is being given considerable attention currently. There is insufficient information to determine whether the death of the two dogs which were stressed was due to increased cardiac work load, neurogenic stimulation, or release of humoral agents. It is possible that a stresser might induce myocardial infarction by causing the liberation of a vasoactive agent which, in the presence of pre-existing coronary atherosclerosis, would reduce coronary flow still further. Hirsch reported that myocardial infarction could be induced in normal rats by exposure to electric shock. This is probably due to arteriolar constriction. It is well known that pitressin (vasopressin) is released following exposure to a stresser. Furthermore, pitressin has been reported to cause myocardial infarction in patients under a number of circumstances. Quantities as large as 259 mU. have been detected in the urine of smokers. Pitressin caused death within six minutes in two of our dogs with severe atherosclerosis but no detectable permanent change in normal dogs or in dogs with less advanced atherosclerosis.
However, Dr. Pedro Forresti-Domingo, working in this laboratory, assayed blood drawn from the internal jugular vein of anesthetized dogs which were severely shocked for several minutes and from week-old pups pretreated with Aneolysen. Increase in blood pressure was slight. It was calculated that the quantity of vasopressin released under these circumstances was considerably less than the quantity which was used to produce myocardial infarction in the atherosclerotic dogs.

Cross and Oblath24 have studied five of our atherosclerotic dogs which had been exposed to the shocks but did not succumb. They observed that, as compared to normal dogs, coronary flow in the atherosclerotic dogs per unit net propulsive force was unvaried or increased. The ability of the atheromatous coronary tree to increase or decrease its vasmotor tone was markedly curtailed. Pitressin, at a dose of 5 U./L. blood volume, reduced coronary flow so that for each mm. Hg mean coronary driving pressure, 0.2 to 0.4 cc. of blood issued from the coronary veins in normal dogs. In atherosclerotic animals, 0.5 to 1.0 cc. flowed per minute. These data seem to suggest that the pitressin causes myocardial infarction by a mechanism other than that due to vasospasm of the artery or arteriole.

The cause of myocardial infarction in dogs with advanced atherosclerosis following the stresser is thus not clear. Vasopressin release would not account for it. Urine volume was not affected during or following the stresser. We have infused much larger quantities of epinephrine and norepinephrine into atherosclerotic dogs as compared to those which were excreted during the maximum stresser without inducing any apparent permanent effects. The release of these agents, therefore, does not appear to be the cause of the infarctions. A continued search for endocrinological and neurogenic factors seems to be indicated.

The stresser could have induced myocardial infarction by greatly increasing cardiac work due to anxiety. Consistent with this explanation is the fact that heart rate in some dogs increased as much as 250 per cent during the shocks.

Acceleration of Lee-White clotting time in the experimental dogs has not been reported previously. The relationship to the myocardial lesion is not clear, particularly since stress did not further decrease clotting time.

It is well known that the degree of hypercholesterolemia, which is finally achieved in dogs maintained on an atherogenic regimen, ranges widely, as it has here. This difference in response is not understood, although it is usually thought that differences in breed may be important. Because of our interest in the role of personality traits in dogs* (See page 971), an attempt was made (in retrospect) to determine whether there was any correlation between the dog's personality rating with the degree of hypercholesterolemia. It is of interest, incidentally, that the induction of hypothyroidism did not seem to produce marked change in personality rating. It is a definite impression that dogs rated low in aggressiveness, anxiety, and activity exhibited the greatest hypercholesterolemia. This is confirmed by a preliminary experiment with normal dogs fed a diet containing 10 per cent lard. The rate of increase in serum cholesterol levels appeared to be greatest in dogs which were rated low in these traits.‡ Furthermore, dogs under restriction stress who reacted mildly appeared to exhibit a less rapid clearing of lipemia induced by feeding of a fat load.§ All in all, this suggests that the degree of hypercholesterolemia of dogs maintained on an atherogenic regimen may be related to the rate of energy expenditure—the lower this is, the greater will be the cholesterol levels.

Summary

Male, mongrel dogs, six to eight years of age, were placed on an atherogenic regimen which included 131 administration and/or thiouracil with cholesterol feeding and, in some cases, the addition of 10 per cent lard to the diet. As is usually observed in such

‡Sobel, H., and Thomas, H. Unpublished observations.
§Sobel, H., and Thomas, H. Unpublished observations.

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experiments, the degree of hypercholesterolemia and atheromatosis varied greatly. However, in nine out of ten dogs with blood cholesterol levels of 1,800 mg. per cent or above for over 16 weeks, myocardial infarction appeared (a) spontaneously, (b) after pitressin administration, and (c) after exposure to a stresser. Myocardial infarction has been noticed only rarely previously. Reasons are given for the belief that the advanced age of the animals which were used here played a role in the genesis of infarction.

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The experimental animals were maintained in the facilities of Dr. N. A. Rotheuberg, who gave generous help and advice throughout this experiment.

References

Spontaneous and Stress-Induced Myocardial Infarction in Aged Atherosclerotic Dogs
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