Protein-Lipid Relationships in Experimental Canine Atherosclerosis

By David P. Barr, M.D., Ella M. Russ, B.S. and Howard A. Eder, M.D., Forrest E. Kendall, Ph.D. and Liese L. Abell, Ph.D.

The applicability of Cohn's method number 10 to the study of dog plasma has made possible this investigation of experimental canine atherosclerosis. Administration of thiouracil which causes hypercholesterolemia but no atherosclerosis in dogs produces no considerable alteration in the distribution of plasma lipoproteins. High cholesterol feeding and administration of thiouracil causes both atherosclerosis and significant alterations in the distribution of lipoproteins.

In the preceding article it was shown that Cohn's microfractionation method accomplishes effective separation of lipoproteins in the plasma of normal dogs. The method has therefore been applied to a preliminary study of protein-lipid relationships before and during the production of experimental canine atherosclerosis.

Subjects

Three male and three female dogs aged 5 to 6 months and weighing between 7 and 14 Kg. were selected from dogs born at the colony maintained for the study of experimental atherosclerosis. After preliminary study, atherosclerosis was induced by the method devised by Bevans, Davidson and Abell with the one modification that after 50 days of thiouracil administration, Estinyl in dosage of 1.0 mg. was added to the diet of each dog. The study included one control period and four experimental periods as follows:

Control Period. Blood was drawn after the dogs had been under supervision on a diet of a commercial dog food moistened with milk.

Experimental Period 1. Blood was drawn again after 50 days during which diet and thiouracil were continued as before, but an oral dose of 1.0 mg. of Estinyl per day was added.

Experimental Period 2. Blood was drawn after an additional 21 days in which diet and thiouracil were continued as before, but an oral dose of 1.0 mg. of Estinyl per day was added.

Experimental Period 3. Blood was drawn at the end of 34 days during which thiouracil and estrogen were continued as before, but with addition of 5 per cent cholesterol to the daily ration which was fed without restriction.

Experimental Period 4. Blood was drawn again at the end of 3 weeks more of the regimen as outlined in Period 3. By this time the dogs had been on the high cholesterol diet for a total of 55 days.

Methods

Blood was drawn from the jugular vein into ACD solution. At the protein laboratory at The New York Hospital-Cornell University Medical Center the plasma was fractionated by a technic identical to that described in the previous paper. Three fractions were obtained under the precise conditions necessary for the isolation in human plasma of Cohn's Fractions IV + V + VI, II, and I + III. Since, however, dog plasma is known from electrophoretic studies to contain some protein components never found in human plasma, the Cohn nomenclature was avoided and the designations A, B, and C + D were substituted respectively for Cohn fractions IV + V + VI, II, and I + III. Protein analyses were made by the standard Kjeldahl and biuret procedures. Cholesterol and phospholipid determinations were performed as described elsewhere. (See Ref. 3, previous paper.) At the same intervals during the experimental period, serum cholesterol determinations were made at Goldwater Memorial Hospital by the method of Schoenheimer and Sperry, and those of serum phospholipids by a method differing little from that employed by the protein laboratory at the New York Hospital. The results of analyses in the two laboratories were in essential agreement throughout. For the sake of brevity in presentation, only those of the New York Hospital laboratory are included in this report.

From the Department of Medicine, The New York Hospital-Cornell Medical Center, New York and Columbia University Research Service, Goldwater Memorial Hospital, Welfare Island, New York.

These studies have been aided by grants from the U. S. Public Health Service, by support from the Public Health Research Institute of the City of New York, Inc., the Commonwealth Fund, and the Albert and Mary Lasker Foundation, and by gifts from Mrs. Katherine Lilly Connolly.

Received for publication, December 2, 1954.

* Estinyl is the trade name for 17-ethinyl estradiol of the Schering Corporation, Bloomfield, N. J.
**Table 1.—Cholesterol, Phospholipids, and Protein in Plasma and Its Fractions Before and During Production of Experimental Canine Atherosclerosis**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Control (av. mg. %)</th>
<th>Period 1 (av. mg. %)</th>
<th>Period 2 (av. mg. %)</th>
<th>Period 3 (av. mg. %)</th>
<th>Period 4 (av. mg. %)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cholesterol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfractionated plasma</td>
<td>203</td>
<td>431</td>
<td>613</td>
<td>2046</td>
<td>1641</td>
</tr>
<tr>
<td>Fraction A</td>
<td>171</td>
<td>350</td>
<td>420</td>
<td>104</td>
<td>245</td>
</tr>
<tr>
<td>Fraction C + D</td>
<td>25.9</td>
<td>85.9</td>
<td>73.7</td>
<td>5.3</td>
<td>17.5</td>
</tr>
<tr>
<td><strong>Phospholipids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfractionated plasma*</td>
<td>442</td>
<td>677</td>
<td>624</td>
<td>969</td>
<td>1065</td>
</tr>
<tr>
<td>Fraction A*</td>
<td>340</td>
<td>465</td>
<td>504</td>
<td>140</td>
<td>426</td>
</tr>
<tr>
<td>Fraction D*</td>
<td>45</td>
<td>166</td>
<td>149</td>
<td>308</td>
<td>518</td>
</tr>
<tr>
<td><strong>Protein</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfractionated plasma (Kjeldahl)</td>
<td>8.71</td>
<td>8.88</td>
<td>8.83</td>
<td>6.48</td>
<td>6.64</td>
</tr>
<tr>
<td>Fraction A (Biuret)</td>
<td>4.04</td>
<td>3.71</td>
<td>3.31</td>
<td>2.00</td>
<td>2.05</td>
</tr>
<tr>
<td>Fraction B (Biuret)</td>
<td>3.60-4.42</td>
<td>3.81-4.38</td>
<td>3.39-4.51</td>
<td>2.17-2.27</td>
<td></td>
</tr>
<tr>
<td>Fraction C + D (Biuret)</td>
<td>1.01</td>
<td>1.45</td>
<td>2.77</td>
<td>2.27</td>
<td></td>
</tr>
</tbody>
</table>

* Values for one male and one female dog only.

**RESULTS**

In Table 1, data from the analyses of cholesterol, phospholipids, and proteins of the unfractionated plasma and the fractions have been assembled. Derived cholesterol–phospholipid ratios have been included. Mean values and ranges are presented separately for male and female dogs. It will be noted not only in the untreated dogs but also in all stages in the production of atherosclerosis that more than 95 per cent of the cholesterol found in the unfractionated plasma is recovered in Fractions A + C + D. Even in the extreme circumstances of the high cholesterol diet, Fraction B is almost free of lipids.

In the untreated animals the characteristics are quite similar to those reported in a previous study.1 The cholesterol and phospholipids are chiefly concentrated in Fraction A. The cholesterol–phospholipid ratios in Fraction A are quite like those found in Fraction IV + V + VI of human plasma, while those of the unfractionated plasma and Fraction C + D are much lower than in Cohn's Fraction I + III in the plasma of humans. Concentration of proteins in Fraction A are lower and in Fraction C + D relatively and absolutely higher than in the corresponding fractions in human plasma.

Following thiouracil administration the cholesterol and phospholipid concentration of the unfractionated plasma is increased although the increment of the phospholipids is less than that of cholesterol. The cholesterol–phospholipid ratios of plasma are greater and the ratios for Fraction A and for Fraction C + D are higher than normal. There is no significant change in the distribution of cholesterol between the fractions.

With thiouracil and estrogen there are further changes in the same direction, but it is impossible to tell whether this is merely a continuation of the thiouracil action or whether estrogen contributes to its development. The results offer no indication of a reversal of thiouracil effect by the action of estrogen.
When the high cholesterol diet is substituted for the normal diet there is, as would be expected, an enormous increase in the concentration of cholesterol. At the same time there is a more moderate increase in the concentration of phospholipid. There is marked elevation of the cholesterol-phospholipid ratios in the plasma and its fractions and also a change in distribution. The amount of cholesterol in Fraction A (alpha lipoproteins) is both relatively and absolutely decreased. In one of the dogs whose normal value was 124 mg. per 100 ml. or 81.4 per cent of the total, the value of 32 mg. per 100 ml. or 1.3 per cent of the total was attained only thirty-four days after the high intake of cholesterol was commenced.

Especially noteworthy are the differences in the chemical accompaniments of developing atherosclerosis in male and female dogs. Preceding the initiation of treatment, cholesterol and phospholipid concentrations are approximately equal in the sexes. Later observations show that at every stage in the production of atherosclerosis, the male animals develop higher concentrations of cholesterol and phospholipids and also higher cholesterol-phospholipid ratios.

Considerable changes in the concentration and distribution of protein occur during the production of the experimental atherosclerosis. The concentration of protein in unfractionated plasma and in Fraction B is within normal limits but the concentration in Fraction A is diminished while that of Fraction C + D is notably increased.

**Discussion**

During the production of canine atherosclerosis there are gradual transformations of protein-lipid relationships. With increasing concentrations of cholesterol and phospholipids in the unfractionated plasma, there is a relatively and finally an absolute reduction of these lipids in Fraction A and a corresponding increase in Fraction C + D. The cholesterol-phospholipid ratios in the plasma are elevated. These changes are similar to those found in the plasma of human atherosclerosis as exemplified by survivors of myocardial infarction and are finally exaggerated to a degree simulating the changes found in cases of hypercholesterolemic xanthomatosis.

The correlation between the development of atherosclerosis and the distribution of cholesterol may be particularly significant. With thiouiracil alone, atherosclerosis does not develop in dogs, and the distribution of cholesterol between the fractions is essentially unchanged. When, however, the feeding of large amounts of cholesterol is added to the thiouiracil dosage atherosclerosis develops rapidly, and it is at this stage that the distribution of cholesterol is radically changed with actual diminution in Fraction A and the greatest accumulation in Fraction C + D.

The cholesterol-phospholipid ratios of both fractions and consequently of the plasma are increased with the administration of thiouiracil and are enormously augmented during the use of the high cholesterol diet. The changes observed in Fraction C + D and in the unfractionated plasma are exaggerated replicas of those observed in human primary hypercholesterolemic xanthomatosis. The elevation of cholesterol-phospholipid ratios in Fraction A which is apparent even during the administration of thiouiracil is unique. Previous experience has shown that even in the most extreme cases of human hypercholesterolemia the cholesterol-phospholipid ratios of Fraction IV + V + VI remain within the normal limits, averaging 0.50. The changes in cholesterol-phospholipid ratios during experimental atherosclerosis indicate the formation of lipoproteins or combinations of proteins and lipids that are grossly deviant from those found in normal canine and human plasma.

The difference in the concentration and distribution of lipoproteins during the production of experimental atherosclerosis in male and female dogs is suggestive of a possible hormonal influence. It is of interest in relation to the observation of Katz, Pick, and Stampler that the cholesterol-induced coronary atherosclerosis which is prominent in cholesterol fed roosters does not appear in the egg producing hen.
LIPOPROTEIN IN CANINE ATHEROSCLEROSIS

SUMMARY

Cohn's microfractionation method number 10 has been used in the study of experimental canine atherosclerosis. It has been shown that the atherogenesis is accompanied by changes in protein-lipid relationships of dog plasma which finally simulate closely those observed in human atherosclerosis. The cholesterol-phospholipid ratios of lipid-bearing fractions suggest the formation of lipoproteins differing in composition from those found in normal human or normal canine plasma.

REFERENCES


Anoxia and Coronary Flow

While it has been demonstrated repeatedly that anoxia increases coronary blood flow—during systole as well as diastole—several questions remain unanswered: How large a change in arterial O₂ saturation is required? Does a slight reduction in arterial O₂ saturation within physiologic ranges act as such a stimulus, or is drastic anoxia required? Does anoxia operate through nervous mechanisms as well as directly on the intact vessels?

Answers to these questions were sought by Bad Nauheim investigators who shunted coronary sinus flow to a brachial vein and continuously recorded aortic pressure, mean coronary sinus flow and pressure, as well as O₂ saturations in the coronary vein, right ventricle and aorta. In control tests, oxygen was used for artificial respiration, thus assuring ca 95% arterial O₂ saturation and 3 to 3.5 percent CO₂ in expired air.

Experiments on 28 dogs under morphine-pernocton anesthesia showed that the least reduction of arterial O₂ saturation effective in increasing coronary flow was 4.17 ± 1.46 percent when saturation levels of arterial blood ranged anywhere between 100 to 47% O₂. As to mechanisms, the conclusion was reached that arterial oxygen saturation and its cardiac utilization coefficient are of greater importance in determining coronary flow than either arterial oxygen capacity or mean arterial pressure, or a combination of the two.

Since increase in aortic pressure incident to hypoxia only partly explained the augmentation of coronary flow, coronary vasodilation was predicated, as is commonly done. All that should have been claimed is that coronary resistance diminished; the possibility that extravascular changes might have been operative needs to be considered. The observation that the effects appeared independent of heart rate changes and persist after denervation of the heart are interesting. The latter indicates that stimulation of chemoreceptors and extra cardiac reflexes were not concerned in their anoxia experiments. However, stimulation of accelerator nerves under normal conditions increased coronary flow and when hypoxia was induced during continued stimulation it caused a further increase, showing that separate superimposed effects can exist.

For details consult two papers by A. Alella, Arch. f. d. gesamten Physiol. 259, 422 and 436, 1954.