Hyperlipemia and Atheromatosis in a Hibernator, Citellus columbianus

By Joseph H. Bragdon, M.D.

Ground squirrels occasionally show extreme hyperlipemia. Triglycerides predominate, but high levels of cholesterol and phospholipids also occur. Evidence is presented indicating that the hyperlipemia results from periods of dietary caloric excess. During hibernation the triglyceride levels are low. Atheromata were found in the aorta and endocardium in those animals with the highest cholesterol levels.

Spontaneous atherosclerosis is extremely rare among mammalian species other than man. In recent years evidence has accumulated that the atherosclerotic process is in some way related to the blood lipids, especially to cholesterol. The observation has occasionally been made that the concentration of plasma cholesterol is higher in man than in any other mammalian species and this has been offered as additional evidence for the etiologic role of the blood lipids in the pathogenesis of atherosclerosis. Because of the wide interest in these relationships, the following observations on the ground squirrel, Citellus columbianus, are reported.

The only previous report found on blood lipid concentrations in a hibernating species is that of Wilber and Musacchia. They analyzed the blood of 10 Alaskan ground squirrels (Citellus barrovicensis) immediately after trapping. The mean values for whole blood were: total cholesterol 224 mg. per 100 cc., phospholipid 615 mg. per 100 cc., and total fatty acids 833 mg. per 100 cc. They found no significant seasonal differences.

Materials and Methods

Observations were made on 23 animals, about equally divided by sex. They had been trapped in Montana in the spring, several months before arrival in this laboratory. In the interval they had been on a vegetable diet low in fat. The animals were maintained in this laboratory from 3 to 15 months before they were sacrificed. They were kept in individual cages, and food and water were offered ad libitum. Several different diets were utilized: (1) a low-fat vegetable diet consisting essentially of sweet potato and kale, but in which cabbage or apple was occasionally substituted; (2) a high-fat vegetable diet, in which raw peanuts were offered in addition to the above foods; (3) commercial rabbit pellets; (4) rabbit pellets with 1 per cent cholesterol.*

Blood samples were obtained either by cardiac puncture or from the aorta. The animals were not fasting, except as noted. The serum was extracted in ethanol-acetone (1:1), and aliquots of the extract were used for the following determinations: total and free cholesterol; lipid phosphorus* (lipid phosphorus multiplied by 25 equals phospholipid); and total fat by a colorimetric method following dichromate oxidation. Triglyceride values were obtained by difference. It is realized that this single extraction carries through certain nonlipid reducing substances, but the amount is relatively small. Lipoprotein concentrations were determined in the analytic ultracentrifuge at a saline density of 1.21.† Flotation rates, however, have been converted to the more familiar terminology of Lindgren, Elliott, and Gofman.

At postmortem examination the heart and thoracic aorta were irrigated with isotonic saline solution, fixed in formalin, embedded in gelatin, sectioned with the freezing microtome, and stained with oil red 0 and hematoxylin. Routine examination included 10 sections through the arch of the aorta and 10 sections through the heart at the levels of the aortic and mitral valves.

Observations

Serum Lipids. Eleven animals were bled after being on the low-fat vegetable diet for periods of one to three months. All but two

* The cholesterol was dissolved in ether and the solution sprayed over the pellets.
† I am indebted to Dr. Edwin Boyle for these determinations.
Table 1.—Serum Lipid and Lipoprotein Levels in Squirrels on Different Diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>No. of animals</th>
<th>Avg. wt. gain or loss (Gm.)</th>
<th>Lipids in mg. per cent</th>
<th>Lipoproteins in mg. per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total Cholesterol</td>
<td>Free Cholesterol</td>
<td>Phospholipids</td>
</tr>
<tr>
<td>Low fat</td>
<td>11</td>
<td>+51</td>
<td>215</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(462-291)</td>
<td>(61-100)</td>
<td>(423-750)</td>
</tr>
<tr>
<td>High fat</td>
<td>10</td>
<td>+236</td>
<td>324</td>
<td>169</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(142-641)</td>
<td>(50-377)</td>
<td>(309-1150)</td>
</tr>
<tr>
<td>Pellet</td>
<td>4*</td>
<td>-102</td>
<td>159</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(138-185)</td>
<td>(41-55)</td>
<td>(257-463)</td>
</tr>
<tr>
<td>1% cholesterol</td>
<td>4</td>
<td>-10</td>
<td>277</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(202-361)</td>
<td>(57-113)</td>
<td>(391-670)</td>
</tr>
<tr>
<td>Hibernating</td>
<td>5</td>
<td>-101</td>
<td>273</td>
<td>72</td>
</tr>
</tbody>
</table>

* One hyperlipemic animal omitted.

had gained weight. In most cases the serum was turbid, that is, lipemic. The triglyceride content of the serum averaged 740 mg. per 100 cc. and the total cholesterol averaged 215 mg. per 100 cc.

Ten animals were bled after being on the high-fat vegetable diet for periods of two to eight months. All gained weight; some became obese. All serums were turbid. The triglyceride levels averaged 3,890 mg. per 100 cc. The mean total cholesterol was 324 mg. per 100 cc. The data for other determinations are presented in table 1.

The effect of fasting on the visible lipemia of the serum was determined. Four animals on the high-fat diet were bled without anesthesia from a leg vein. All serums were turbid. Food was removed from the cages and 24 hours later they were again bled. In two cases some slight turbidity remained. At 48 hours the serums of three were clear; no blood was obtained from the fourth animal because it had gone into hibernation. Food was then presented, and 24 hours later the serums of the three active animals were again turbid. At each bleeding less than 1 ml. of blood was removed.

Five animals were placed in a cold room at 5 C. and food was withheld. Hibernation was successfully induced in all. Ten weeks later, when the body weights had dropped approximately 22 per cent, they were bled. The serum triglyceride levels were very low, averaging 137 mg. per cent. The mean cholesterol level was, however, higher than that found in animals on the low fat diet.

Vegetable diets are free of cholesterol. As some species respond with hypercholesterolemia when cholesterol is added to the diet, the effects of such treatment were studied in this species. In order to accustom them to the change in diet, five animals were first offered rabbit pellets exclusively. After four weeks, during which time all lost weight, four of the animals had relatively low lipid levels, but one revealed the highest values encountered in this study. Serum triglycerides were 9,480 mg. per 100 cc. and total cholesterol was 1205 mg. per 100 cc. The animal was killed at this time. Data on this animal are omitted from table 1.

The remaining four animals were then offered pellets containing 1 per cent cholesterol and were bled four weeks thereafter. Their weights were relatively constant during this period. The serum cholesterol averaged 277 mg. per 100 cc.

Anatomic Observations. No animal died spontaneously. Twenty-one of the 23 animals were examined post mortem. The only gross abnormality encountered was fatty infiltration of the liver, the severity of which correlated roughly with the serum triglyceride concentration. In some cases the liver weight was as much as 6 per cent of the body weight; in one animal triglycerides comprised 44 per cent of the wet weight of the liver.

Microscopic examination of the aorta showed the normal intima to be extremely thin, compa-
rable to that of the rat, rather than that of the rabbit or man. Focal deposits of sudanophilic material were seen in the intima in eight of the animals. They occurred only in animals on the high fat diet and in the one animal with severe hyperlipemia on the pellet diet. The fat existed in small intracellular droplets and extracellularly, in more finely divided form, along the inner surface of the internal elastic lamella. In most cases there was no tissue reaction. In two cases, however, there were focal accumulations of foam cells beneath the endothelium. These masses protruded into the lumen of the vessel. No anisotropism, necrosis, or fibrosis could be demonstrated in these lesions. Of seven animals sacrificed while on the low fat diet, none showed sudanophilic material in the aortic intima.

In the heart, fatty deposits were frequently seen in the commissures between the aortic cusps, occasionally in the mitral leaflets, and rarely in the walls of coronary arteries. These were extracellular and elicited no tissue reaction. Their presence bore no relation either to diet or to terminal serum lipid concentrations. The heaviest such deposit was in fact found in an animal after 10 weeks of hibernation. In one heart, from an animal on the high fat diet, an atheromaleike lesion, similar to those described in the aorta, was present on the endocardium of the left ventricle.

Lipoproteins. The serums of 14 animals were analyzed for lipoproteins in the ultracentrifuge. Most of these serums scattered light intensely, indicating the presence of large lipoproteins of very low density. These are the classes which, in man, have been shown to be rich in triglycerides.

It should be appreciated that lipoprotein quantitation is less reproducible than is the chemical quantitation of serum lipids. Many methodologic difficulties are encountered, particularly with extremely lipemic serum. The most characteristic finding was the presence of large amounts of alpha lipoproteins. All animals also showed measurable amounts of S\textsubscript{f} 2-8, and these were significantly higher in the animals on the high fat diet. Lipoproteins of S\textsubscript{f} 10-20 were either absent or present in relatively low concentrations. S\textsubscript{f} classes 20-80 varied from 0 to 210 mg. per 100 cc.

**DISCUSSION**

The most unusual feature of these observations is the extraordinary amount of fats which these animals at times carry in their blood. Triglycerides comprise the major portion of the lipids and are also the most labile. The turbidity of the serum was found to be proportional to the triglyceride concentration, thus confirming Lever's observation in human serum. Some authors have inferred that the turbidity of serum depends on the phospholipid/cholesterol ratio. The ratio of phospholipid to cholesterol in these animals was high, averaging 2.8 (1.7 to 3.8) on a weight basis.

Figure 1 shows the ratio of free to total cholesterol plotted against the triglyceride content. The values for all 49 bleedings are included. The scattergram shows that this species resembles man in that the lipoprotein classes with a high content of triglyceride contain relatively more of their cholesterol in the free state than do the more "normal," high density lipoproteins.

These animals also show relatively high cholesterol levels. On the low fat diet values corresponded both in mean and in range with

![Fig. 1. Scattergram of all bleedings, showing a positive correlation between the ratio of free to total cholesterol and the triglyceride content of the serum.](http://circres.ahajournals.org/)

FIG. 1. Scattergram of all bleedings, showing a positive correlation between the ratio of free to total cholesterol and the triglyceride content of the serum.
so-called normal human values. On the high fat diet many animals showed concentrations that would be hypercholesterolemic in man. It should be noted that the hibernating animals, presumably burning mostly fat, had cholesterol levels between those observed in the low fat and high-fat dietary groups.

What produces these high lipid levels, at times exceeding 10 gm. per 100 ml. of serum? No correlations with sex could be established. The animals on the high fat diet revealed higher serum lipids than those on the low fat diet, but it must be recalled that the highest concentrations encountered were in an animal that had been fed rabbit pellets alone for the preceding month. These contain relatively few calories in the form of fat. It is unfortunate that data are not available for food consumption and for short-term weight changes immediately preceding bleedings. An attempt was made to correlate serum triglyceride concentrations with body weight for each diet. In the case of three animals on the high fat diet, two or more determinations were available. Each animal showed rising levels with increasing weight, and the curves were quite parallel, but the total data were inadequate to be significant. It seems probable that these animals convert their food to fat at a rate which often exceeds the rate at which the deposits can store the fat. The fatty livers observed during hyperlipemia tend to confirm this interpretation. The hyperlipemia might thus result from periods of dietary caloric excess. Other hibernating species might manifest the same metabolic phenomenon.

It is hazardous to compare anatomic lesions which have presumably taken weeks to develop with a single determination of serum lipids or lipoproteins, which may be subject to rather rapid fluctuation. It should be noted, however, that the three animals which showed atheromatous lesions had at death the three highest cholesterol concentrations encountered. It appears significant that none of the animals on the low fat diet showed any sudanophilic material in the aortic intima, whereas such deposits occurred in 7 of the 10 animals on the high fat diet. It appears that the fat deposits seen in the endocardium are less readily reversible. They were found in 14 of the animals and bore no relation to either diet or to serum lipids. The mere presence of sudanophilic material without tissue reaction cannot be taken as evidence of the atherosclerotic process under these circumstances.

The concentrations of alpha lipoprotein are among the highest reported for any species. The Sr 2-8 class of beta lipoproteins was significantly elevated in the group on the high fat diet compared with the group on the low fat diet. Differences in the other measurable beta lipoprotein classes were not significant. All these beta lipoproteins have been suspected of playing a role in the pathogenesis of human atherosclerosis.

Summary

The ground squirrel, Citellus columbianus, when maintained in the laboratory, frequently exhibited extremely high serum lipid levels. Triglycerides predominated and were the most labile. Values up to 10 gm. per 100 cc. were encountered. Cholesterol and phospholipid levels were also among the highest recorded for any mammalian species. Although a high-fat vegetable diet produced higher serum lipids than did a low fat diet, this may have resulted from the higher caloric intake rather than from the nature of the food. Ultracentrifugal analyses of the serum showed that alpha lipoproteins and very low density beta lipoproteins predominated. The Sr 2-8 class was significantly higher in the group on the high fat diet. Atheromatous lesions were found in the aorta or heart of the three animals with the highest serum cholesterol concentrations.

Hibernation resulted in very low triglyceride concentrations. The feeding of cholesterol did not produce hypercholesterolemia.

References

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*Circ Res.* 1954;2:520-524
doi: 10.1161/01.RES.2.6.520

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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