Reversibility of Atherosclerosis in Cholesterol-Fed Rabbits

By Walter M. Bortz, M.D.

ABSTRACT

Previous studies have attempted to demonstrate the reversibility of rabbit atherosclerosis after discontinuance of cholesterol feeding or by other measures. These attempts have been largely unsuccessful. In all the studies, atherosclerosis was induced by cholesterol feeding for several months. At the end of these prolonged periods, the animals had advanced aortic lesions. The present study sought to determine whether a shorter exposure to an elevated blood cholesterol might produce lesions that were reversible.

Rabbits were fed cholesterol until their serum levels reached at least 1000 mg/100 ml; cholesterol feeding was then discontinued. Serum cholesterol values rapidly returned to normal levels. Some were killed at the time of discontinuance of the cholesterol diet and the others at periods up to 300 days thereafter. The aortas were removed and analyzed chemically for cholesterol content. The results demonstrate that cholesterol feeding of short duration produces a significant lesion whose cholesterol content may decrease markedly some weeks after the serum cholesterol has returned to normal levels.

ADDITIONAL KEY WORDS cholesterol feeding aortic cholesterol

Studies have indicated measures which, with reasonable certainty, wholly or partially prevent the development of experimental atherosclerosis in animals (1-3). There is less certain evidence, however, regarding reversibility of atheromata once they have been established experimentally. Demonstration of isotopic equilibrium between cholesterol in the serum and in the vessel wall (4, 5) indicates that an elevation in serum cholesterol will be followed eventually by an increased amount of cholesterol in the vessel wall. Conversely, reduction in serum cholesterol should be followed by a reduction in cholesterol in the vessel wall if the concept of a totally miscible pool is valid. Attempts to verify this hypothesis were generally unsuccessful.

To explore further the possibility of reversing atherosclerosis in cholesterol-fed rabbits, the following simple experimental protocol was devised.

Methods

The rabbits used were females of the New Zealand white strain; they weighed approximately 1.5 kg at the beginning of the study. The control diet was Purina rabbit chow. Cholesterol was fed by coating the chow pellets with cholesterol, 1% by weight. Blood was obtained by cardiac puncture during and after the cholesterol diet to determine the level of serum cholesterol. The cholesterol was determined by the method of Abell et al. (6).

In the first study 16 rabbits were fed the cholesterol diet for 28 days and then returned to normal chow diet; two or three of these rabbits were killed at intervals thereafter up to 182 days. Three other rabbits were fed the high cholesterol diet for 14 days and then killed at intervals up to 70 days. Three control animals were maintained on normal chow diet and killed after 100 days. Seven others were fed the high cholesterol diet continuously and killed at intervals up to 382 days. It was concluded by gross visual grading, possibly erroneously, that 28 days represented too lengthy a feeding period for the observance of reversibility. Consequently a second study was designed.

In this second study eight rabbits were fed the cholesterol diet for 14 days, and six others were fed the diet for 21 days. These animals were then killed at intervals up to 56 days. Only those animals whose serum cholesterol reached 1000 mg/100 ml or more regularly developed atherosclero-
BORTZ

Days of diet before sacrifice

FIGURE 1
Cholesterol content of aorta of rabbits that were fed cholesterol. The mean value of aortas of three rabbits fed a control diet was 0.006 mg cholesterol/mg protein.

The mean plasma cholesterol was 2157 ± 108.6 mg/100 ml in control rabbits, 721 ± 75.7 mg/100 ml in animals that were fed a 1% cholesterol diet and had serum cholesterol levels of 1000 mg/100 ml or more before discontinuance of the diet, and 172 ± 30.8 mg/100 ml in animals that were fed a normal diet until their plasma cholesterol levels reached 1000 mg/100 ml or higher. We conclude that the cholesterol diet was effective in producing hypercholesteremia and was reversible in most animals in whom it was applied.

Results and Discussion

Table 1 represents a composite response of the serum cholesterol to the different dietary changes. The 12 control rabbits (three in study 1 and nine in study 3) never developed any evidence of hypercholesterolemia. The eight rabbits with continued cholesterol feeding (seven in study 1 and one in study 3) had sustained hypercholesterolemia. Two of these animals were followed for longer than 300 days, and their serum cholesterol was consistently over 1500 mg/100 ml. In contrast, in the 54 other animals (19 in study 1, 14 in study 2, and 21 in study 3), upon discontinuance of the cholesterol diet, the plasma cholesterol fell quickly and by 6 weeks had reached the original control values. This observation is dissimilar to that of Friedman and Byers who found that serum cholesterol did not fall to basal levels until 5 months after stopping the cholesterol diet (7). They attributed the persistent elevation to mobilization of excess cholesterol from the tissues into the blood (8). However, they were fed a diet containing 2% cholesterol for 3 months.

In our first two studies, the cholesterol diet was fed for arbitrarily chosen periods before discontinuance. As a result, the blood cholesterol values in some animals did not reach 1000 mg/100 ml before the diet was discontinued; atherosclerotic lesions were not present in cell aortas, possibly a result of these various cholesterol values. But in the third study in which the cholesterol diet was continued until the blood cholesterol reached a level in excess of 1000 mg/100 ml, atherosclerosis occurred in all animals.

Earlier experience indicated that there is close agreement between the degree of atherosclerosis as judged by gross visual grading and by quantitative chemical analysis (9). However, for our third study we measured the cholesterol content of the aorta and related it to nitrogen content.

The cholesterol content of the aorta at the end of the cholesterol dietary period averaged 0.058 ± 0.008 (SE) mg cholesterol per mg protein (Fig. 1); in aortas of seven animals on a control diet it was 0.006 ± 0.0015 mg cholest-

<table>
<thead>
<tr>
<th>Number of weeks</th>
<th>Serum cholesterol (mg/100 ml)</th>
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<tbody>
<tr>
<td></td>
<td>54 rabbits whose diet was discontinued</td>
</tr>
<tr>
<td>0</td>
<td>1757 ± 108.6 (36)</td>
</tr>
<tr>
<td>1</td>
<td>721 ± 75.7 (11)</td>
</tr>
<tr>
<td>2</td>
<td>373.8 ± 63.4 (11)</td>
</tr>
<tr>
<td>3</td>
<td>172 ± 30.8 (8)</td>
</tr>
<tr>
<td>4</td>
<td>106 ± 17.5 (9)</td>
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<tr>
<td>5</td>
<td>98.5 ± 12.6 (10)</td>
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<tr>
<td>6</td>
<td>87.6 ± 33.5 (8)</td>
</tr>
<tr>
<td>9</td>
<td>2190 (1)</td>
</tr>
</tbody>
</table>

Values are the means and their standard errors. Numbers in parentheses are the number of samples represented by each value.
terol per mg protein. The aortas of rabbits killed 14 to 42 days after discontinuance of the diet had a higher cholesterol concentration than those killed immediately after the diet was stopped, despite the rather prompt decline of the serum cholesterol toward normal values (Table 1). At later time intervals, however, the aortic cholesterol content fell until at the longest time interval studied, 300 days, the aortic cholesterol content was 0.020 ± .003 (SE) mg cholesterol per mg protein (approximately one-fifth the maximal observed values). This contrasted with aortic cholesterol content of 0.091 mg/mg of protein at 300 days in the animal in which cholesterol feeding was continued.

Histologic examination of the aortas of two experimental rabbits (killed 200 to 300 days after return to normal diet) showed sudanophilic material still widely scattered throughout the intimal, subintimal, and medial layers of the artery in both particulate and conglomerate patterns (Fig. 2). There were some areas of destruction of normal architecture with fibrous tissue proliferation without sudanophilic material. These presumably represented areas of previous cholesterol deposition. Small foci of calcification were rarely seen.

Several decades ago, Anitschkow claimed that arterial lesions of rabbits showed regression when the serum cholesterol returned to normal, but that this process took an extremely long time (10). More recently, Rodbard, Pick, and Katz showed that thyroid treatment, but not starvation or estrogen administration, induced regression of aortic lesions in the chick (11). Buchwald found that...
creation of an ileal bypass shunt resulted in a 30% regression of lipid deposit in rabbits still maintained on a high cholesterol feeding program (12). Friedman and Byers recently reported the lowering of cholesterol content of the thrombo-atherosclerotic plaque in rabbits with an implanted aortic coil by discontinuance of cholesterol feeding (13). However, other investigators were unable to confirm regression of aortic atherosclerosis in the rabbit. In fact, lesions seemed generally to be worse at the time of final observation than at the time of discontinuance of cholesterol feeding (7, 14). Kritchevsky tried, without success, to add various fats and thyroid compounds in an attempt to demonstrate that vascular lesions regress (3, 15).

Wilens reported the reversibility of traumatically induced cutaneous xanthomata in the rabbit upon discontinuance of cholesterol feeding (16). Friedman and Byers demonstrated in the rabbit the reversibility of atherosclerosis of an aortic segment surgically transplanted into the anterior chamber of the eye when cholesterol diet was withdrawn (17). Both of these reports indicated, however, that in situ aortic atherosclerosis was unaffected by such treatment.

In the foregoing experiments cholesterol was fed over periods of several months. The present experiment utilized a much shorter feeding period, but moderate to advanced atherosclerosis did result, consisting of more than foam cell or fatty streak lesions. These positive results, when contrasted with the previous negative ones, lead to the conclusion that there is an early reversible phase before fibrous tissue reaction is so severe as to prevent resolution of the fatty deposit.

Reversibility of human atherosclerosis has not been proved. Isotope experiments have demonstrated that there is an exchange of arterial and blood cholesterol, indicating potentiality of regression of the lesions, but evidence on this point has been either anecdotal or indirect.

Summary

This study demonstrates the reversibility of aortic atherosclerosis in the cholesterol-fed rabbit. The experimental design was such that the exposure of the vessel wall to extreme hypercholesteremia was short. Reversibility under these conditions suggests that the early lesion, even of rather marked degree, is still susceptible to reversal. Failure of others to demonstrate this was probably due to the extended period of cholesterol diet used.

Acknowledgments

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References

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WALTER M. BORTZ

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