Effect of Dihydrocholesterol and \( \beta \)-Sitosterol on Cholesterol Atherosclerosis in Rabbits

By W. T. Behr, G. D. Baker and W. L. Anthony

While dihydrocholesterol lowered total plasma cholesterol in the rabbit, it did not promote the regression of either the aorta lipid fractions or plaque areas in atherosclerotic animals. A comparative study of the toxicities of \( \beta \)-sitosterol and dihydrocholesterol disclosed that dihydrocholesterol, fed for a 7 month period, resulted in aorta plaque formation and caused the development of liver cirrhosis. On the other hand, \( \beta \)-sitosterol produced no toxic effects.

EXPERIMENTAL evidence has shown that \( \beta \)-sitosterol and dihydrocholesterol are effective in retarding the development of cholesterol atherosclerosis.\(^1\)\(^2\) Further studies have indicated that \( \beta \)-sitosterol is only partially effective in promoting the regression of this condition.\(^3\) Since dihydrocholesterol has been found somewhat more effective in prevention of atherosclerosis, its effect on regression is of interest.

Recent reports have presented evidence that soybean sterols and dihydrocholesterol cause aorta plaque formation,\(^4\) and that dihydrocholesterol causes gall stone formation in rabbits.\(^5\)

This report presents data concerning the effects of dihydrocholesterol on regression of atherosclerosis in the rabbit and the toxic effects of dietary \( \beta \)-sitosterol and dihydrocholesterol.

METHODS

Development. Thirty female albino rabbits, weighing 2000–2500 Gm., were divided into 4 groups;—groups 1 and 2 with 10 rabbits each, and groups 3 and 4 with 5 rabbits each. The normals (group 1) were fed ad lib a diet consisting of pulverized Rockland rabbit ration supplemented with 3 per cent corn oil. For the other 3 groups, 1 per cent cholesterol was added to this basic diet. At the end of 3 months, half of the rabbits in group 1 and all of the rabbits in group 2 were sacrificed by air embolism. The aortic arch, with a segment of thoracic aorta, a liver sample and a blood sample were removed from each sacrificed animal for staining and analysis.

Regression. During the regression study, the remaining group 1 (normal) rabbits and the group 3 (control) rabbits received 97 per cent pulverized Rockland rabbit ration and 3 per cent corn oil. Group 4 (treated) rabbits received 95 per cent rabbit ration supplemented with 3 per cent corn oil and 2 per cent dihydrocholesterol. In order to check the regression of hypercholesterolemia in each rabbit, blood samples were drawn at 1 to 4 week intervals for total cholesterol determination. At the end of 4 months all of the animals were sacrificed as before, and tissue samples stained and analyzed.

Toxicity. Twelve additional normal female albino rabbits were divided into 2 groups of 6 and maintained for a 7 month period on diets composed of 95 per cent Rockland rabbit ration, 3 per cent corn oil, and either 2 per cent dihydrocholesterol (group 5) or 2 per cent \( \beta \)-sitosterol* (group 6). Blood samples were collected periodically for total cholesterol determination. At the end of the experimental period, the rabbits were sacrificed and samples of liver and aorta removed for analysis. Pieces of liver were fixed for histologic examination.

PROCEDURES. The following methods were used:

1. Plasma cholesterol was determined by the Sperry and Webb method.\(^7\)

2. The aortic arches and 5 to 7 cm. of the thoracic aortas were removed, washed in distilled water and fixed overnight in a 9:1 solution of formalin. Subsequently the aortas were stained with Sudan IV and the plaque areas visually estimated.

3. The livers and the stained aortas were dried, dried and weighed. The total lipids were extracted with a 1:1 mixture of methanol and chloroform and determined gravimetrically. Aliquots of the extracts were used for cholesterol determinations by the procedure of Sperry and Webb,\(^7\) and phospholipid analysis by the Fiske and SubbaRow method.\(^8\) The reaction of digitonin and anthrone was used to estimate \( 3\beta \)-hydroxy sterols.

4. For histologic examination, the tissues were fixed in formalin, blocked in paraffin and stained with Harris hematoxylin and eosin.

* The \( \beta \)-sitosterol used in this study was generously donated by Eli Lilly and Company, Indianapolis, Ind.
RESULTS AND DISCUSSION

Regression Studies

Total Plasma and Liver Cholesterol. As shown in figure 1, dihydrocholesterol accelerated the initial rate of total plasma cholesterol regression. However, after 50 days, the regression rates of control and treated animals equalized. By the end of the regression period, the cholesterol values of both these groups were the same, although neither had returned to normal level (table 1). It should be noted that the initial increase in the rate of regression was observed both in this experiment and in our previous experiment on the effects of β-sitosterol.4

Total liver cholesterol, during the development of atherosclerosis, had reached the high values typical of this condition (table 1). At the end of the regression period, the liver cholesterol of the control animals (group 3) decreased to normal (group 1) levels, while that of the dihydrocholesterol treated (group 4) animals remained at elevated levels.

Aorta Lipids. A comparison of the aorta lipids of the normals (group 1) with those of the animals having developed atherosclerosis (group 2) shows a considerable, although non-proportional, increase in the phospholipids, total lipids and total cholesterol. Table 1 also shows that during the 4 month period of the regression study there was actually an increase in the phospholipid and total cholesterol fractions in the control (group 3) as well as in the treated (group 4) rabbits. These increases might be explained by assuming that the excess cholesterol and phospholipid in the blood and other tissues were deposited in the aortas during regression and because of the slow turnover rate in the aortic plaques, accumulated there. On the other hand, total lipid values of the control animals (group 3) remained constant, although the composition of

![Figure 1. Effect of dihydrocholesterol on regression of hypercholesterolemia in rabbits.](image)

**Table 1.—Effect of Dihydrocholesterol on Regression of Plasma, Liver and Aorta Lipid Levels and on Aorta Plaque Area**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. in group</th>
<th>Total plasma cholesterol* (mg. %)</th>
<th>Total liver cholesterol (mg./Gm.)</th>
<th>Aorta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Phospholipid per cent</td>
<td>Total lipid per cent</td>
<td>Total cholesterol per cent</td>
</tr>
<tr>
<td>1. Normals</td>
<td>8</td>
<td>122.7 ± 14.6</td>
<td>8.58 ± 1.67</td>
<td>17.78 ± 4.76</td>
</tr>
<tr>
<td>2. Developed atherosclerosis</td>
<td>9</td>
<td>2709 ± 345</td>
<td>68.3 ± 22.4</td>
<td>3.19 ± 0.925</td>
</tr>
<tr>
<td>3. Regression study, controls</td>
<td>5</td>
<td>230 ± 72</td>
<td>12.05 ± 3.34</td>
<td>6.83 ± 1.09</td>
</tr>
<tr>
<td>4. Regression study, dihydrocholesterol treated</td>
<td>5</td>
<td>262 ± 120</td>
<td>32.6 ± 7.46</td>
<td>7.07 ± 0.41</td>
</tr>
</tbody>
</table>

* Numbers following ± signs are standard deviations.
the lipids must have been altered. The total lipids of the dihydrocholesterol treated rabbits (group 4) increased significantly during this period. These facts would seem to indicate that dihydrocholesterol is unable to prevent the deposition of endogenous cholesterol in the aortas.

Aorta Plaques. The last column in table 1 shows percentages of aorta plaque area. During the regression study period, the plaque areas increased significantly in both groups 3 and 4. This agrees with the increase in the lipid fractions during regression noted in the previous section, but differs from the results obtained in the β-sitosterol regression experiment. Comparison of these two experiments in respect to the values for total plasma and liver cholesterol at the peaks of atherosclerosis (group 2) affords a possible explanation, since these values are much higher in the dihydrocholesterol experiment than in the β-sitosterol study.

Toxicity

Total Plasma Cholesterol. Table 2 shows that both dietary dihydrocholesterol and β-sitosterol significantly reduced plasma cholesterol levels of normal rabbits. This finding correlates with the ability of these substances to reduce the rate of cholesterol absorption.

Liver Lipids. Treatment of normal rabbits with dihydrocholesterol (group 5) resulted in marked changes in the pattern of liver lipid values, such as an elevation in total cholesterol, total β-esters and total lipid, along with a significant decrease in phospholipid. In this group, the excess of β-esters over the total cholesterol is probably due to dihydrocholesterol deposition. The lipid values for the group treated with β-sitosterol (group 6) remained

Table 2.—Effects of Dihydrocholesterol and β-Sitosterol on Plasma, Liver and Aorta Lipid Levels

<table>
<thead>
<tr>
<th>Group</th>
<th>No. in group</th>
<th>Total plasma cholesterol* (mg. %)</th>
<th>Phospholipid</th>
<th>Total lipid</th>
<th>Total cholesterol</th>
<th>Total β-sterols</th>
<th>Aorta plaque area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Liver per cent</td>
<td>Aorta per cent</td>
<td>Liver per cent</td>
<td>Aorta per cent</td>
<td>Liver per cent</td>
<td>Aorta per cent</td>
</tr>
<tr>
<td>1. Normals</td>
<td>8</td>
<td>122.7 ±4.6</td>
<td>7.22 ±1.22</td>
<td>22.0 ±0.17</td>
<td>17.78 ±0.13</td>
<td>0.726 ±0.048</td>
<td>0.202 ±0.048</td>
</tr>
<tr>
<td>5. Dihydrocholesterol</td>
<td>6</td>
<td>53.6 ±4.6</td>
<td>5.47 ±1.22</td>
<td>41.7 ±2.05</td>
<td>27.53 ±0.17</td>
<td>2.05 ±0.048</td>
<td>0.414 ±0.048</td>
</tr>
<tr>
<td>treated</td>
<td></td>
<td>±15.2 ±2.42</td>
<td>±0.794 ±0.17</td>
<td>±0.19 ±0.048</td>
<td>±5.87 ±0.17</td>
<td>±0.351 ±0.048</td>
<td>±0.115 ±0.048</td>
</tr>
<tr>
<td>6. β-sitosterol treated</td>
<td>6</td>
<td>68.7 ±4.6</td>
<td>5.82 ±1.22</td>
<td>24.49 ±0.17</td>
<td>23.28 ±0.17</td>
<td>0.700 ±0.048</td>
<td>0.245 ±0.048</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±5.17 ±2.30</td>
<td>±2.42 ±0.17</td>
<td>±2.52 ±0.17</td>
<td>±5.16 ±0.17</td>
<td>±0.169 ±0.048</td>
<td>±0.142 ±0.048</td>
</tr>
</tbody>
</table>

* Numbers following ± signs are standard deviations.

Fig. 2. Liver sections. A. Dihydrocholesterol treated rabbit. B. Normal. C. β-Sitosterol treated rabbit.
at normal levels, except for a decrease in the phospholipid fraction.

Gross examination of the livers from the β-sitosterol treated rabbits showed no apparent abnormalities. Those from the dihydrocholesterol treated rabbits were fatty, fibrous and contained concretions in the bile ducts. Figure 2 shows representative liver sections from normal, β-sitosterol and dihydrocholesterol treated animals. It is apparent that dihydrocholesterol caused marked cirrhosis, whereas β-sitosterol did not affect liver cells during the seven month period.

Aorta Lipids and Plaque Areas. As shown in table 2, the dihydrocholesterol treated animals exhibited a significant increase in aorta plaque area over the normals, while the β-sitosterol treated rabbits showed no plaques. The increases in the aorta lipid fractions of the dihydrocholesterol treated animals followed, in general, the increase in plaque area. Total lipid, total cholesterol and phospholipid all increased significantly. It is interesting to note that only 50 per cent of the total 3,β-hydroxy sterols can be accounted for as cholesterol. It can be assumed that the balance of the total β-sterols is dihydrocholesterol, but this has not as yet been proven by isolation experiments.

In the case of the β-sitosterol treated animals (group 6), there was no significant increase in any of the lipid fractions with the exception of the phospholipid. The total β-sterol analysis did not differ significantly from the total cholesterol assay.

SUMMARY

After the development of atherosclerosis induced in rabbits by dietary cholesterol, administration of dihydrocholesterol increased the initial rate of plasma cholesterol regression, but hindered the regression of total liver cholesterol. Dihydrocholesterol did not promote the regression of either the aorta lipid fractions or plaque areas.

Dietary dihydrocholesterol, fed to normal rabbits for a 7 month period, resulted in the formation of aorta plaques, and in a general increase in all of the lipid fractions. There is data to suggest that dihydrocholesterol is deposited in the aortas. Extensive liver damage was noted after treatment with dihydrocholesterol. This was characterized by increased total lipid, cholesterol and β-sterols. Histologic examination gave evidence of advanced cirrhosis.

Beta-sitosterol proved to be nontoxic. Its administration did not result in aorta plaque formation or change in liver or aorta lipid fractions. Moreover, it was just as effective as dihydrocholesterol in lowering total plasma cholesterol.

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Dihydrocholesterol and \( \beta \)-Sitosterol


Books received by CIRCULATION RESEARCH are hereby acknowledged. Those of special interest to investigators in basic aspects of the circulation will be reviewed as space permits.


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