The Effect of Dihydrocholesterol on the Absorption of Cholesterol by the Rat

By Ray H. Rosenman, M.D., Sanford O. Byers, Ph.D., and Meyer Friedman, M.D.

Simultaneous feeding of dihydrocholesterol and cholesterol to rats significantly reduced the amount of cholesterol absorbed into the thoracic lymph. Hypercholesteremia and hyperphospholipemia developed in rats simultaneously fed cholesterol and cholic acid for 8 weeks. The addition of dihydrocholesterol to this diet also decreased the rate of development and magnitude of the hypercholesteremia, but had no apparent effect upon the rise of plasma phospholipids.

Recent concepts suggest that dietary cholesterol contributes more effectively to atherogenesis than does endogenous cholesterol. This probability has not led to a practical prophylaxis against arteriosclerosis, because the only method known to be effective in reducing absorption of cholesterol has been a diet very low in animal fat.

However, Peterson has shown that simultaneous addition of soybean sterols and cholesterol to the diet of chicks does not produce the rise in plasma cholesterol which is obtained when the diet is supplemented only with cholesterol. In line with this finding Siperstein, Nichols and Chaikoff added dihydrocholesterol to an otherwise atherogenic poultry diet and found that this sterol also will prevent the usual elevation of plasma cholesterol in chickens.

The present paper extends these observations to a mammalian species, the rat, and also, for the first time, presents direct evidence that the mechanism of this action is an inhibition of the absorption of dietary cholesterol. Obtaining such evidence was made possible by the fact that all the cholesterol absorbed from the lumen of the intestine is transported by way of the thoracic lymph. Analysis of the daily lymph collection, therefore, provides quantitative information on the total amount of cholesterol absorbed during the period of the collection.

**Methods**

**Acute Experiments.** Functioning thoracic duct fistulae were established by a previously described technique in Long-Evans strain male rats averaging about 280 Gm. Control rats received by stomach tube a dose of 100 mg. of cholesterol dissolved in 3 cc. of olive oil. Experimental animals received 100 mg. of cholesterol plus 100 mg. of dihydrocholesterol in 3 cc. of olive oil. Lymph was collected for 24 hours and analyzed for cholesterol as described previously for blood. All animals were autopsied at the end of the collection period and the lymph ducts examined. Data is included from only those rats whose lymph ducts were undilated at autopsy, showing normal unobstructed flow of lymph.

**Chronic Experiments.** Control rats were fed ad lib. the stock laboratory diet, supplemented with 2 per cent cholesterol and 1 per cent cholic acid. We have found this supplemented diet capable of inducing hypercholesteremia in rats. Experimental rats consumed the same diet with the addition of 2 per cent dihydrocholesterol. Blood samples were drawn from the tail at the start of the experiment, and every two weeks thereafter for eight weeks. The plasmas were analyzed for total and free cholesterol, by previously described methods.

*We are grateful to the Schering Corporation, Bloomfield, New Jersey, for a generous supply of dihydrocholesterol.
TABLE 1.—Cholesterol Content of Thoracic Lymph

<table>
<thead>
<tr>
<th>No. of Rats</th>
<th>Weight (g.)</th>
<th>Vol. of 24-Hr. Lymph Collection (cc.)</th>
<th>Cholesterol Concentration (Mg./100 ml.)</th>
<th>Total Cholesterol Content (Mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Rats Receiving Cholesterol Only</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average 11</td>
<td>289</td>
<td>50</td>
<td>0.70</td>
<td>33</td>
</tr>
<tr>
<td>S.E. Mean...</td>
<td>±4.8</td>
<td>±2.8</td>
<td>±2.1</td>
<td></td>
</tr>
<tr>
<td>B. Rats Receiving Both Cholesterol and Dihydrocholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average 20</td>
<td>287</td>
<td>54</td>
<td>0.44</td>
<td>23</td>
</tr>
<tr>
<td>S.E. Mean...</td>
<td>±3.5</td>
<td>±2.3</td>
<td>±1.2</td>
<td></td>
</tr>
</tbody>
</table>

and for lipid phosphorus by the method of Stewart and Hendry. Lipid phosphorus was calculated to phospholipid by multiplying by 25.

RESULTS

The cholesterol content of 24-hour collections of thoracic lymph from rats receiving oral cholesterol is presented in Table 1 and is contrasted with that from rats receiving both cholesterol and dihydrocholesterol. The average volumes of lymph collected from each group are almost the same, but the lymph cholesterol concentration in the group receiving dihydrocholesterol is only approximately 0.3 per cent as great as the value for the control group. The total cholesterol content of the 24-hour lymph collection is, of course, correspondingly reduced in the group receiving both cholesterol and dihydrocholesterol.

The data obtained from chronic experiments are presented in Figure 1. In the control group the plasma cholesterol values increased from a predicted average of 47 mg. per cent to a value of 196 mg. per cent at the end of the second week. This value was maintained throughout the experimental period except for an unexplained remission to 150 mg. per cent at the sixth week. In this group receiving dihydrocholesterol a rise in the plasma cholesterol level was not evident until the fourth week, and then increased from a predicted value of 48 mg. per cent only to 79 mg. per cent. The final plasma cholesterol concentration at the end of the eighth week was 196 mg. per cent in the control group and only 99 mg. per cent in the experimental group. No obvious effect is apparent upon the corresponding phospholipid values.

In animals receiving dihydrocholesterol the plasma free cholesterol concentration averaged 14 mg. per cent before the experiment and 18 mg. per cent after eight weeks on the diet. The corresponding figures for the control rats were 13 mg. per cent and 30 mg. per cent. It is therefore apparent that the rise in plasma total cholesterol taking place during the course of the experiment was very largely a rise in esterified cholesterol.

DISCUSSION

Administration of dihydrocholesterol interferes with the entrance of cholesterol into the thoracic lymph of the rat. It seems reasonable to suppose that this interference, demonstrated in the acute experiments, accounts for the effectiveness of dihydrocholesterol in inhibiting the hypercholesteremia in the chronic experiments.

Our findings are thus consistent with those of Peterson and co-workers and of Siperstein, and co-workers, referred to earlier.

SUMMARY

1. Simultaneous feeding of dihydrocholesterol with an equal quantity of cholesterol to rats reduced the amount of cholesterol absorbed into the thoracic lymph during the following 24 hours to 63 per cent of control values.
2. Rats fed a diet containing 2 per cent cholesterol and 1 per cent cholic acid developed hypercholesteremia. The addition of 2 per cent dihydrocholesterol to this diet slowed the rate of development of hypercholesteremia and diminished its intensity.

REFERENCES


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