The Effect of Soybean Sterols on the Absorption of Cholesterol by the Rat

By RAY H. ROSENMAN, M.D., SANFORD O. BYERS, PH.D., AND MEYER FRIEDMAN, M.D.

Mixed soybean sterols (50 per cent sitosterols) were fed to rats simultaneously with cholesterol in acute and chronic experiments. In acute experiments, concurrent feeding of a test dose of soybean sterols and cholesterol did not diminish the usual fraction of cholesterol absorption into the thoracic duct lymph. In chronic experiments the addition of 2 or 10 per cent soybean sterols to a diet which induces chronic hypercholesteremia in rats failed to inhibit the development or magnitude of chronic hypercholesteremia.

The increasing emphasis placed upon dietary cholesterol in atherogenesis suggests the possibility that reduction of the amount of cholesterol that is absorbed in the intestine may provide a practical prophylaxis against atherosclerosis. In this regard Siperstein and associates recently found that dihydrocholesterol, when added to a high-cholesterol, atherogenic diet, prevented the usual hypercholesteremia and atherosclerosis in birds. We have extended this observation to a mammalian species, the rat, and have shown that the mechanism of this effect of dihydrocholesterol is due to a reduction in the amount of cholesterol absorbed from the intestine.

In similar fashion, Peterson and associates reported that the addition of soybean sterols to the ration prevented the usual hypercholesteremia and atherosclerosis occurring in cholesterol-fed chickens. Hernandez and coworkers found a pronounced decrease in the amount of labeled cholesterol recovered from thoracic lymph of rats fed cholesterol together with soybean sterols, compared with rats fed only cholesterol. Pollak confirmed this observation in the rabbit, although he found that an excess of sitosterols was required to prevent hypercholesteremia and atherosclerosis in the cholesterol-fed rabbit. Pollak also reported that ingestion of substantial amounts of sitosterols led to reduction of blood cholesterol in human subjects. On the other hand, Dreisbach and associates could not confirm this since they found that chronic feeding of soybean sterols failed to alter the hypercholesteremia and atherosclerosis induced in rabbits by high cholesterol diets. Moreover, Wilkinson and associates recently reported that no significant change in blood cholesterol occurred in human subjects fed sitosterols under carefully controlled circumstances.

The present study extends these observations of the effect of soybean sterols upon blood cholesterol to another mammalian species, the rat. The results indicate that, at the concentration fed, soybean sterols do not inhibit the absorption of dietary cholesterol nor reduce the hypercholesteremia affected by chronic feeding of cholesterol and cholic acid in rats.

Acute Experiments

Methods. Adult male (Long-Evans) rats were used. Cholesterol was assayed as Lieberman-Burchard positive material according to our previously described methods.

As described previously, functioning thoracic duct fistulas were established in 14 rats averaging 225 Gm. in weight, and were divided into three groups. Prior to operation all rats ingested stock ration. Forty-eight and 24 hours preoperatively the rats of groups II and III received by stomach tube 100 mg. of soybean sterols dissolved in 3 cc. of olive oil. At the same times, the rats of group I received only 3 cc. of olive oil. This was done to ensure the presence of adequate soy sterols in the intestine in the experimental rats. Group I consisted of six rats that received postoperatively by stomach tube a dose of...
TABLE 1.—Cholesterol Content of Thoracic Lymph

<table>
<thead>
<tr>
<th>No. of Rats</th>
<th>Av. Wt. (Gms.)</th>
<th>0 to 24 Hour Lymph Collection</th>
<th>24-48 Hour Lymph Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cholesterol</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vol. cc.</td>
<td>Cholesterol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Concentration (mg./100 cc.)</td>
<td>24 hr. Content (mg.)</td>
</tr>
<tr>
<td>Group I: Rats Receiving Cholesterol Only</td>
<td></td>
<td>6</td>
<td>293</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28-48</td>
<td>60-86</td>
</tr>
<tr>
<td>Group II: Rats Receiving Cholesterol and 100 Mg. Soy Sterols</td>
<td></td>
<td>5</td>
<td>276</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25-88</td>
<td>27-93</td>
</tr>
<tr>
<td>Group III: Rats Receiving Cholesterol and 500 Mg. Soy Sterols</td>
<td></td>
<td>3</td>
<td>241</td>
</tr>
</tbody>
</table>

100 mg. of cholesterol dissolved in 3 cc. of olive oil. Group II consisted of five rats that received postoperatively 100 mg. of cholesterol plus 100 mg. of mixed soybean sterols* in 3 cc. of olive oil. Group III consisted of three rats that postoperatively received 100 mg. of cholesterol plus 500 mg. of soybean sterols in 3.5 cc. of olive oil. Thoracic duct lymph was collected for two consecutive 24-hour periods in groups I and II and for 24 hours in group III and analyzed for cholesterol. During these intervals the rats ingested only 0.5 per cent NaCl drinking fluid. All animals were autopsied at the end of the collection period and the lymph ducts examined. The 14 rats from which the data was obtained represent those whose lymph ducts were unilat., showing normal unobstructed flow of lymph.

Results. The cholesterol contents of the thoracic lymph collections are presented in table 1. The average volumes of collected lymph were somewhat greater in the rats receiving both soybean sterols and cholesterol. On the other hand, the total cholesterol content of the 24-hour lymph collections was essentially the same in the control, cholesterol fed rats (group I) as in the rats simultaneously fed both cholesterol and soybean sterols (groups II and III).

CHRONIC EXPERIMENTS

Methods. Twenty-five rats were divided into three groups, each of which was fed ad libitum a stock ration containing 2 per cent cholesterol and 1 per cent cholic acid. This supplement has been found capable of inducing chronic hypercholesteremia in rats.15, 16 Seven control rats of group I ingested this diet while eight rats of group II ingested the same diet with addition of 2 per cent mixed soybean sterols (75 to 80 per cent sitosterols). Ten rats comprised group III which was fed the same ration supplemented with 10 per cent mixed soybean sterols (75 to 80 per cent sitosterols). Blood samples were obtained three and nine weeks later and analyzed for total cholesterol concentrations.

Results. The data obtained from these rats are presented in figure 1. The results indicate

* We wish to thank the Distillation Products Industries for the mixed soybean sterols used in these studies, reported to contain 95 per cent sterols, 10 to 15 per cent of which were stigmasterol and 75 to 80 per cent sitosterols.
that the rise of plasma cholesterol induced by supplementing the diet with cholesterol and cholic acid was not inhibited by the addition of soybean sterols to the ration either in a concentration of 2 or 10 per cent. The rats ingesting ration containing 10 per cent soy sterols (group III) had an initial average weight of 188 Gm. and a final weight of 243 Gm. Those ingesting 2 per cent soy sterols (group II) rose from 226 Gm. to 260 Gm. during the experimental interval. The average weight of the control rats (group I) rose from 175 Gm. to 250 Gm. during this period.

**DISCUSSION**

The quantitative effect of a given substance upon the intestinal absorption of cholesterol can be determined by analysis of thoracic duct lymph cholesterol, since previous studies from this laboratory have shown that all of the cholesterol absorbed from the intestinal lumen is transported via the thoracic lymph.\(^{10}\) The present results indicate that the simultaneous administration of soybean sterols (sitosterols) fails to interfere significantly with the entrance of fed cholesterol from the intestinal lumen into the thoracic lymph of the rat. This lack of inhibitory effect was observed even with large dosage of soybean sterols, and may be contrasted with the positive inhibitory effect of dihydrocholesterol upon the absorption of dietary cholesterol under similar circumstances.\(^{3}\) At first glance our results might seem at variance with the recent study of Hernandez and co-workers\(^{4}\) in which they reported a pronounced decrease in the amount of labeled cholesterol recovered from thoracic duct lymph of rats simultaneously fed the same type of preparation of soybean sterols and \(^{14}\)C-labeled cholesterol, when compared with rats fed only cholesterol. In their study a test dose of 4 mg. of cholesterol was employed. On the other hand, ordinary stock laboratory ration (Purina) provides an average daily intake of dietary cholesterol of about 17 mg. For this reason a larger amount of cholesterol (100 mg.) was used as the test dose in our studies. Thus it seems probable that the apparent divergence of our results from those of Hernandez and co-workers may be due to the very small amount of cholesterol (4 mg.) administered by them as their test dose.

We have also studied the effect of feeding both small and large amounts of soybean sterols upon the blood cholesterol of rats ingesting a high cholesterol diet. Previously it was found that the increased cholesterol absorption effect by feeding of cholic acid\(^{13}\) induces chronic hypercholesteremia in rats when cholesterol and cholic acid are simultaneously fed over prolonged intervals.\(^{11, 12}\) As also noted in birds,\(^{1}\) the concomitant feeding of dihydrocholesterol significantly retards the rate of development and the intensity of the hypercholesteremia induced by feeding cholesterol plus cholic acid supplements to rats.\(^{2}\) In contrast, the present data indicate that concomitant feeding of even large amounts of soybean sterols failed to inhibit the rise of plasma cholesterol induced by chronic feeding of cholesterol and cholic acid to a mammalian species, the rat.

**SUMMARY**

The effect of ingested soybean sterols (sitosterols) upon the absorption of dietary cholesterol was studied in the rat. The simultaneous feeding of soybean sterols with cholesterol did not reduce the amount of cholesterol absorbed into the thoracic duct lymph. Chronic hypercholesteremia was induced in rats by feeding a diet containing 2 per cent cholesterol and 1 per cent cholic acid. The addition of either 2 per cent or of 10 per cent soybean sterols to this diet failed to inhibit the rate of development or the magnitude of the induced hypercholesteremia.

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