The Effect of Beta-Sitosterol Upon Intestinal Absorption of Cholesterol in the Rat

By MEYER FRIEDMAN, M.D., RAY H. ROSENMAN, M.D. AND SANFORD 0. BYERS, PH.D.

Thoracic duct lymph was collected from groups of rats fed varying doses of β-sitosterol concomitantly with cholesterol. The results indicated that β-sitosterol exerts only a slightly inhibiting effect upon intestinal absorption of cholesterol in the rat.

In an earlier study, the administration of mixed soybean sitosterols was found to be ineffective in retarding both the intestinal absorption of dietary cholesterol and the occurrence of hypercholesteremia in rats under the experimental conditions employed. Hernandez and associates, employing both chemical and tracer techniques also have studied this same problem but their results have been contrary to ours. Hernandez and Chaikoff believed that the explanation for the seeming variance might be in our employment of the Liebermann-Burchard (L-B) reaction in the chemical analysis of lymph cholesterol.

When a pure sitosterol (β-sitosterol)* became available to us, it was decided to restudy the entire problem with particular attention devoted to both the dosages of cholesterol employed and the possible influence of both sitosterol and olive oil upon the cholesterol content of intestinal lymph as determined by the L-B reaction. The results of this second study do not suggest that β-sitosterol is much more effective than mixed sitosterols in impeding the intestinal absorption of cholesterol in the rat.

METHODS

1. Determination of Possible Effect of Olive Oil and β-Sitosterol on Cholesterol Content of Lymph (As Determined by the Liebermann-Burchard Reaction)

A. Olive Oil. In order to determine whether the possible absorption of olive oil itself into intestinal lymph gives a significant Liebermann-Burchard reaction, hence an incorrectly high cholesterol determination in any experiment in which it was employed as a vehicle, a series of nine rats was given 0.5 ml. of olive oil plus 2.5 ml. of HzO by stomach tube and a second series of 9 rats was given 3.0 ml. of olive oil. A third control series of 6 rats was given 3.0 ml. of HzO. Intestinal lymph was collected for 24 hours and analyzed for total cholesterol according to the Saifer-Kainmer method which utilizes the Liebermann-Burchard reaction. The rats were starved for 10 hours before and 24 hours after the experiment.

The collection of intestinal lymph of the rat is an erratic and difficult procedure as usually performed because of the frequent occurrence of fibrin clots in the collecting cannula or in the lymph already collected in the receiving flask. In order to overcome this difficulty, we have inserted a small plastic catheter (OD = 0.024 inches) within the lumen of the lymph collecting cannula (ID = 0.045 inch). The small catheter constantly delivers 0.3 ml. of Na citrate solution (1.5 per cent) per hour by means of a specially designed injection apparatus. This citrate solution mixes with the lymph just as it enters the larger catheter and the lymph-citrate solution then flows by gravity in the space between the two catheters down into the collecting flask. This particular improvement adopted several years ago completely prevents any clotting and in so doing makes the experiment reliable and consistent. It cannot be stressed too strongly that without this modification, collection and measurement of intestinal lymph is an erratic experimental procedure.

B. Sitosterol. A series of eight rats was given 3.0 ml. of olive oil containing 100 mg. of β-sitosterol and their intestinal lymph was collected for 24 hours and analyzed for total cholesterol as described above.

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2. Determination of Effect of \( \beta \)-Sitosterol on Intestinal Absorption of Cholesterol

Four different series of rats were given different amounts of cholesterol dissolved in olive oil by stomach tube with and without 100 mg. of sitosterol. The first series of 23 rats received 15 mg. of cholesterol dissolved in 0.75 ml. of olive oil. Ten of these rats also received 100 mg. of sitosterol with the cholesterol. The second series of 31 rats received 25 mg. of cholesterol dissolved in 0.75 ml. of olive oil and 15 of these rats also received 100 mg. of sitosterol. The third series of 27 rats received 50 mg. of cholesterol dissolved in 1.5 ml. of olive oil and 13 of these rats also received 100 mg. of sitosterol. The fourth series of 29 rats received 100 mg. of cholesterol in 3.0 ml. of olive oil and 16 of these rats also received 100 mg. of sitosterol. All rats were starved for 15 hours before and 24 hours after the stomach intubation.

The intestinal lymph was collected for 24 hours and analyzed for total cholesterol. The total lipid content of the lymph also was analyzed in the fourth series by the method of Bragdon.

RESULTS

1. Possible Effect of Olive Oil and \( \beta \)-Sitosterol on Lymph Cholesterol as Determined by the Liebermann-Burchard Reaction

The control rats given only three ml. of \( H_2O \), apparently absorbed an average of 10 mg. of cholesterol in the 24 hours collection of their intestinal lymph (table 1). Since no cholesterol was fed to these starved rats, the lymph cholesterol represents, in part, a very fixed increment derived from the blood but mostly that cholesterol which previously was excreted into the lumen of the gut either via the bile or by the intestinal wall itself and subsequently reabsorbed. Some of this intestinal cholesterol, however, still escapes absorption because when excess cholic acid is given, the absorption may be increased to 15.0 mg. per 24 hours.7

The rats given 0.5 ml. of olive oil showed an average of 15 mg. of cholesterol via the intestinal lymph (table 1). This increase of 50 per cent above the control value however could not have been due to a falsely high L-B reaction caused by the lymphatic absorption of olive oil itself because the total volume of olive oil fed (0.5 ml.) contained only 1.4 mg. of cholesterol-like substance (as determined by the Liebermann-Burchard reaction). It is far more probable that the ingestion of olive oil did what fat is known to do, namely, increase the intestinal absorption of cholesterol.8, 9, 10

The failure of ingested olive oil itself to influence the Liebermann-Burchard reaction observed in lymph was clearly shown in the series of rats given 3.0 ml. of olive oil. The average total cholesterol in the 24 hour collection of lymph in these rats was 14.0 mg. (table 1), a value about the same as that found in rats given only 0.5 ml. of olive oil. If how-

<table>
<thead>
<tr>
<th>No. of Rats</th>
<th>Average weight (gm.)</th>
<th>Volume (ml.)</th>
<th>Intestinal Lymph (24 hour collection)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Cholesterol</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>mg./100 ml.</td>
</tr>
<tr>
<td>Rats given 3.0 ml. of H(_2)O</td>
<td>6</td>
<td>265</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Range:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S.E. Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rats given 0.5 ml. of olive oil</td>
<td>9</td>
<td>320</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Range:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S.E. Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rats given 3.0 ml. of olive oil</td>
<td>9</td>
<td>304</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Range:</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>S.E. Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rats given 3.0 ml. olive oil + 100 mg. of sitosterol</td>
<td>8</td>
<td>343</td>
<td>39</td>
</tr>
</tbody>
</table>
ever, possible lymphatic absorption of olive oil itself gave a falsely high cholesterol value to the lymph (as determined by the L-B reaction), those rats given 3.0 ml. of olive oil or six times as much as the previous series, should exhibit a greater cholesterol content in their lymph. These results therefore indicate that the use of olive oil as a vehicle does not lead a falsely high Liebermann-Burchard value because of its own absorption.

Similarly, the series of rats given 100 mg. of β-sitosterol in addition to 3.0 ml. of olive oil, exhibited no greater apparent cholesterol content in their 24 hour lymph collection than either series of rats fed cholesterol alone (table 1). This result indicates that the mere ingestion of sitosterol does not lead to a falsely high lymph cholesterol as determined by the Liebermann-Burchard reaction. The result also suggests that sitosterol administration did not appear to impede the usual amount of cholesterol entering the lymph of the starved animal.

### Table 2.—The Effect of Administration of Sitosterol on the Absorption of Dietary Derived Cholesterol

<table>
<thead>
<tr>
<th>Amount of Sitosterol Fed (mg.)</th>
<th>No of Rats</th>
<th>Average weight Gm.</th>
<th>Av. vol. (ml.)</th>
<th>Total cholesterol mg/100 ml.</th>
<th>Total cholesterol mg/24 hrs.</th>
<th>Intestinal Lymph (24 hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Rats given 5 mg. of cholesterol 100</td>
<td>10</td>
<td>Range: 286 (242-320)</td>
<td>31 (22-49)</td>
<td>54 (40-92)</td>
<td>16 (12.0-23.0)</td>
<td>±0.78</td>
</tr>
<tr>
<td>Rats—Control 0</td>
<td>13</td>
<td>S.E. Mean 285 (240-315)</td>
<td>35 (17-62)</td>
<td>56 (36-86)</td>
<td>18.0 (13.0-24.0)</td>
<td>±0.98</td>
</tr>
<tr>
<td>B. Rats given 25 mg. of cholesterol 100</td>
<td>15</td>
<td>Range: 316 (280-370)</td>
<td>40 (28-59)</td>
<td>45 (27-66)</td>
<td>17.0 (12.0-24.0)</td>
<td>±0.78</td>
</tr>
<tr>
<td>Rats—Control 0</td>
<td>16</td>
<td>S.E. Mean 293 (242-350)</td>
<td>40 (22-71)</td>
<td>59 (27-95)</td>
<td>22.5 (18.0-32.5)</td>
<td>±1.57</td>
</tr>
<tr>
<td>C. Rats given 50 mg. of cholesterol 100</td>
<td>13</td>
<td>Range: 269 (230-290)</td>
<td>42.0 (25-77)</td>
<td>62 (26-92)</td>
<td>24 (15-30)</td>
<td>±1.36</td>
</tr>
<tr>
<td>Rats—Control 0</td>
<td>14</td>
<td>S.E. Mean 276 (252-300)</td>
<td>37 (16-55)</td>
<td>85 (44-170)</td>
<td>27 (19-35)</td>
<td>±1.58</td>
</tr>
<tr>
<td>D. Rats given 100 mg. of cholesterol 100</td>
<td>16</td>
<td>Range: 317 (290-348)</td>
<td>30 (25-57)</td>
<td>79 (42-109)</td>
<td>30 (16-47)</td>
<td>±2.5</td>
</tr>
<tr>
<td>Rats—Control 0</td>
<td>13</td>
<td>S.E. Mean 314 (285-342)</td>
<td>34 (19-54)</td>
<td>108 (55-202)</td>
<td>35 (20-51)</td>
<td>±2.8</td>
</tr>
</tbody>
</table>

2. The Effect of Sitosterol on the Intestinal Absorption of Cholesterol

As table 2 indicates, the administration of 100 mg. of β-sitosterol was of little or no value in retarding the intestinal absorption of cholesterol except possibly in those rats fed a dose of 25 mg. of cholesterol.

In the experiment, in which rats were fed 15 mg. of cholesterol, the concomitant administration of 100 mg. of sitosterol reduced the average 24 hour lymph cholesterol from 18 to 16 mg., an apparent reduction of 11 per cent. However, calculation of the standard error of the difference of means indicates that it is only 1.45 times the actual difference of means. This, of course, suggests that actually there is good agreement in the results of both series.

Rats given 25 mg. of cholesterol plus 100 mg. of sitosterol had an average of 17.0 mg. of cholesterol in their 24 hour lymph—a reduction of 23 per cent as compared to the controls. Calculation and application of the S.E. of the
difference of the means to these results suggests that a significant difference exists.

Rats given 50 mg of cholesterol plus 100 mg of sitosterol showed an average of 24 mg of cholesterol in their lymph, a possible reduction of 11 per cent as compared to the controls. Statistical evaluation of these results, however, indicates that again good agreement rather than a difference exists.

Similarly, in the series of rats given 100 mg of cholesterol plus 100 mg of sitosterol, although their average cholesterol content of lymph was 30 mg, as compared to 35 mg found in the controls (a reduction of 14 per cent), the S.E., of the Difference of Means was only 1.3 times the actual Difference of Means, indicating no essential difference between the results of the two series.

**DISCUSSION**

In a previous study from this laboratory the administration of 100 mg of a mixture of sitosterols (soybean sterols) was found to be ineffective in impeding the absorption of an equivalent amount of cholesterol. Furthermore, the addition of a sufficient amount of this sitosterol preparation to make it 10 per cent of a diet, also containing 2 per cent cholesterol and 1 per cent cholate, was found to be completely ineffective in preventing the occurrence of hypercholesterolemia usually observed in animals given this type of high cholesterol high-cholate diet. Although this diet was monstrously high in cholesterol and cholate, it was similarly so in regard to sitosterol. Certainly the results of both types of experiments made us believe that sitosterol was a relatively ineffective agent in the prevention of cholesterol absorption in the rat.

Recently Hernandez and Chaikoff have suggested that failure to observe a significant retardation in cholesterol absorption following the administration of the above type of sitosterols might have been due to a falsely high Liebermann-Burchard color reaction obtained on lymph, possibly due to the absorption of the Liebermann-Burchard color producing substances in olive oil or sitosterols. However, in their own experiments, administration of sitosterols to animals also fed olive oil, gave less apparent cholesterol (as determined by the Liebermann-Burchard reaction) in the 24 hour lymph than in their control animals fed olive oil alone. This of course suggests that no falsely high cholesterol values could have been caused by the possible absorption of sitosterol. These authors did find that olive oil administration increased the apparent cholesterol content of the 24 hour lymph, but as the presently reported experiments indicate, and as is well known, olive oil facilitates the absorption of cholesterol. It is of interest, too, that when these authors compared the cholesterol absorption with and without sitosterol administration, they found that sitosterol depressed the cholesterol absorption 32 per cent as determined by analysis of cholesterol by the Liebermann-Burchard reaction and 39 per cent as determined by their tracer method. In other words, the failure of the Liebermann-Burchard reaction to be influenced by possibly absorbed sitosterol and olive oil contaminants has been verified by their tracer experiments.

Our above results following the administration of a purified preparation of β-sitosterol indicate that, although a difference in cholesterol absorption could be found consistently in series of rats given different amounts of cholesterol, if sitosterol were added, this difference did not appear to be significant (except in the series given 25 mg of cholesterol) when submitted to statistical analysis. The conclusion therefore seems inescapable that in the rat, under the conditions of the present experiment, sitosterol cannot be considered as a truly effective agent in the impedance of absorption of either dietary-derived or intestinally-excreted cholesterol.

The presently reported failure to demonstrate a marked or perhaps significant effect of β-sitosterol upon intestinal absorption of cholesterol in the rat does not necessarily indicate that this substance may not act in a different fashion, to prevent or reduce human hypercholesteremic states. The ingestion of large amounts of sitosterol has been reported to induce a lowering of the serum cholesterol of human subjects. This has not been confirmed by Wilkinson and associates.
Moreover, it is important to point out that the administration of β-sitosterol over prolonged periods of time has not been found to reduce the average serum cholesterol of hypercholesteremic patients more than approximately 16 per cent,13,14 and about five per cent in the normocholesteremic subject.13,15 Whether this degree of reduction in serum cholesterol in the human subject is of practical significance is, of course, open to considerable question. It is of interest that although the feeding of sitosterols with cholesterol to rabbits resulted in a lesser rise of serum cholesterol of approximately eight per cent than occurred in control animals fed only cholesterol, the authors13 did not consider this significant and the extent of aortic atherosclerosis in the rabbits receiving sitosterols and cholesterol was identical with that observed in the control rabbits fed only cholesterol.

**Summary**

The possible effect of administration of β-sitosterol upon the cholesterol absorption of the rat given various amounts of dietary cholesterol was studied. The results obtained suggest that although β-sitosterol possibly exerts a slight retarding effect upon cholesterol absorption at all dosages of cholesterol studied, it did not appear to be a very effective agent for this purpose in the rat.

**Summary in Interlingua**

Esseva investigate le possibile effecto de β-sitosterol super le absorption de cholesterol in ratto che habeva recipite varie quantitates de cholesterol dietari. Le resultatos obtenite pare indicar que in ratto β-sitosterol—ben que illo exerce un leve effecto retardante super le absorption de cholesterol in omne doses studiate—non es un multe efficace agente pro ille objectivo.

**References**

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