Lowering of Serum Cholesterol by the Administration of a Plant Sterol

By Maurice M. Best, M.D., Charles H. Duncan, M.D., Edward J. Van Loon, Ph.D., and Joan D. Wathen, B.S.

The plant sterol, beta-sitosterol, has been administered to nine subjects on unrestricted diet. A sustained reduction of serum total cholesterol and a lowering of the ratio of cholesterol to lipid phosphorus occurred. Interference by sitosterol with the absorption of cholesterol, both dietary and that excreted into the gastrointestinal tract, is presumably responsible for this hypcholesterolemic effect. No toxic or undesirable side effects were observed. A means of studying the effects of a sustained lowering of serum cholesterol on atherosclerotic states would now seem to be available.

The sitosterols are the most widely distributed of the plant sterols, or phytosterols. This sterol fraction is now known to contain at least five components, alpha₁, alpha₂, alpha₃, beta, and gamma sitosterols. Beta and gamma sitosterols, the most extensively studied members of the group, are stereoisomers and differ only in the spatial configuration of the C-17 side chain. Beta-sitosterol is the main sterol of cottonseed oil, tall oil, and wheat-germ oil; gamma-sitosterol is the principal sterol of soybean oil.

Beta and gamma sitosterols are closely related to cholesterol in chemical structure (fig. 1), but differ in that they are not appreciably absorbed from the digestive tract. Peterson and co-workers have studied the effects of the administration of sitosterol to chicks maintained on high fat and high cholesterol diet. They noted a markedly lower plasma and liver level of cholesterol and a highly significant decrease in the incidence of atherosclerosis. This was interpreted to indicate that sitosterol interferes with the absorption of cholesterol. A lowering of serum cholesterol in human subjects fed a usual diet plus crude soy sterol, presumably largely gamma sitosterol, has been reported.

Methods

Nine subjects were selected for treatment. Two (subjects H-1 and H-2) had serum cholesterol levels within the normal range and were without evidence of cardiovascular or renal disease. Seven patients were hypercholesterolemic. Included were three with previous myocardial infarction (patients H-3, H-6, H-7), two with chronic renal disease (patients H-8, H-9), one with peripheral vascular disease (patient H-4), and one with a labile hypertension (patient H-5). A major factor in the selection was the willingness of the subjects to cooperate and adhere to the schedule of treatment.

Beta-sitosterol was administered orally in dosage of 5 to 6 Gm. immediately before the ingestion of food. There was no restriction as to type or amount of food. The preparation was in liquid form, 30 ml containing 3 Gm. of 98 per cent pure beta sitosterol. A placebo preparation, similar in appearance, taste, and consistency, was also employed in the study. At no time did the patient know whether he was taking sitosterol or placebo, and the majority remained unaware that a placebo was ever used. This precaution was taken to avoid possible influence of such knowledge on the patients' selection of diet, particularly with regard to fat and cholesterol content. To minimize the chance of other possible bias the initial eight weeks of the study were conducted by the double-blind method, in which neither subject nor doctor knew which preparation was sitosterol and which was placebo.

The period of study ranged from 13 to 29 weeks, with an average of 22 weeks. Included were two periods of placebo administration in all patients, and one or more periods of sitosterol administration. All determinations of serum cholesterol are included in the data presented except those during the two-week period immediately after discontinuation of
**RESULTS**

Table 1 summarizes the effects of beta-sitosterol administration on serum total cholesterol (Abell) in the nine subjects. In all there was a reduction in serum total cholesterol, the mean fall ranging from 13 to 224 mg. per 100 ml. In terms of per cent of control level, the fall ranged from 6.7 per cent to 20.0 per cent. Eight of the nine patients showed a reduction in cholesterol significant at the 5 per cent point value or below, as determined by the t test.

Changes in serum total cholesterol during alternate periods of placebo and sitosterol administration in patient H-4 are shown in figure 2. Apparently spontaneous fluctuations in the serum cholesterol during the initial placebo and sitosterol periods emphasize the necessity for repeated determinations in studies of this type.

A consistent difference was noted in absolute values of serum total cholesterol by the three methods employed. Figure 3, showing the hypcholesterolemic effect of sitosterol administered to patient H-6, illustrates this difference. The parallelism demonstrated by this patient was noted in all subjects.

The hypocholesterolemic effect of sitosterol is manifest at the end of one week, but there is further decline during the succeeding four weeks of administration (fig. 4). No tendency toward "escape" has been noted, and at the end of up to 18 weeks of continuous administration the cholesterol level has remained below control levels.

Significance of the mean difference of paired

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**Table 1.—Effect of Beta-Sitosterol on Serum Total Cholesterol (Abell)**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Mean Serum Total Cholesterol (mg./100 ml.)</th>
<th>% Fall</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treatment</td>
<td></td>
</tr>
<tr>
<td>H-1</td>
<td>196 ± 11</td>
<td>183 ± 10</td>
<td>6.7</td>
</tr>
<tr>
<td>H-2</td>
<td>215 ± 10</td>
<td>199 ± 16</td>
<td>7.9</td>
</tr>
<tr>
<td>H-3</td>
<td>260 ± 11</td>
<td>230 ± 14</td>
<td>11.6</td>
</tr>
<tr>
<td>H-4</td>
<td>261 ± 17</td>
<td>223 ± 16</td>
<td>14.7</td>
</tr>
<tr>
<td>H-5</td>
<td>270 ± 10</td>
<td>241 ± 13</td>
<td>10.7</td>
</tr>
<tr>
<td>H-6</td>
<td>283 ± 6</td>
<td>248 ± 12</td>
<td>12.1</td>
</tr>
<tr>
<td>H-7</td>
<td>305 ± 3</td>
<td>249 ± 23</td>
<td>18.1</td>
</tr>
<tr>
<td>H-8</td>
<td>276 ± 4</td>
<td>240 ± 13</td>
<td>13.1</td>
</tr>
<tr>
<td>H-9</td>
<td>1122 ± 35</td>
<td>898 ± 200</td>
<td>20.0</td>
</tr>
</tbody>
</table>

* Significant at 5 per cent point value for n = 1 degrees of freedom.

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**FIG. 1.** The structural formula of beta and gamma sitosterols differs from that of cholesterol only in the presence of an ethyl group on carbon-24.

**FIG. 2.** A fall in serum total cholesterol is noted during beta sitosterol administration, with return to the initial range when a placebo was substituted. The concentrations of S1 10-30 and 30-100 classes of lipoproteins at the end of 7 and 12 weeks of sitosterol administration were approximately one-half those during the placebo period. This subject has continued to take sitosterol, and at 31 weeks his serum total cholesterol is 212 mg. per 100 ml.
values (mean control and mean sitosterol for each patient) is shown in table 2 for each cholesterol method. The per cent reduction will be seen to be quite similar by all three, although the absolute reduction is somewhat greater as measured by the method of Zlatkis. In our laboratory the method of Abell gives results in good agreement with Schoenheimer-Sperry; the modified methods give higher values, that of Pearson about 30 mg. per 100 ml., and that of Zlatkis about 63 mg. per 100 ml. The reduction of serum total cholesterol of 12.8 per cent is based on all determinations made during sitosterol administration. As can be seen from figure 4, the reduction would be appreciably greater if determinations during

Table 2.—Mean Reduction Serum Total Cholesterol

<table>
<thead>
<tr>
<th>Method</th>
<th>mg./100 ml.</th>
<th>%</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abell</td>
<td>53.0</td>
<td>12.8</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Pearson(mod.*)</td>
<td>56.5</td>
<td>12.0</td>
<td>.001</td>
</tr>
<tr>
<td>Zlatkis(mod.*)</td>
<td>63.3</td>
<td>11.9</td>
<td>.001</td>
</tr>
</tbody>
</table>

* To be published.

the first two weeks of sitosterol administration were eliminated. A less significant reduction ($p < .02$) in the ratio of serum total cholesterol to lipid phosphorus was observed in the nine patients. In three patients (H-2, H-4, and H-7) the fall in the ratio of 20.4, 25.6, and 16.2 per cent, respectively, was statistically significant. The reduction in the ratio of serum total cholesterol to lipid phosphorus was due largely to the fall in serum total cholesterol, lipid phosphorus showing no consistent change.

Lipoprotein concentrations were determined by means of the ultracentrifuge at the completion of placebo and sitosterol treatment periods in patients H-4, H-6, and H-7. In these patients the concentrations of Sr 10–30 and 30–100 classes of lipoproteins at the end of the period of sitosterol administration were approximately one-half that of the placebo period.

**DISCUSSION**

The level of plasma cholesterol would seem to be regulated by a rather efficient homeostatic mechanism, ordinarily varying only within narrow limits. This relative constancy is the result of a dynamic equilibrium rather than the existence of plasma cholesterol as a stagnant pool. The average life of a molecule of plasma cholesterol (regeneration time) is approximately 12 days. Thus cholesterol is continuously being excreted or metabolized, and replaced by either synthesis or absorption. Although most tissues have been shown to be capable of synthesizing cholesterol from acetate, the liver appears to be the only appreciable source of endogenous plasma cholesterol, at least in the dog. It has been estimated that the hepatic synthesis of cholesterol in man is about 1.2 Gm. per day.

The cholesterol content of the average Amer-
ican diet varies from a low value of 0.2 to 0.3 Gm. per day to a high of 0.7 to 0.8 Gm., depending upon the foods ingested.\textsuperscript{14} Dietary cholesterol, being derived from cellular material, is largely in the free (non-esterified) state. The mechanism of cholesterol absorption is incompletely understood, but there is some evidence that esterification by pancreatic esterase is involved.

Cholesterol presented to the gut for absorption includes not only that in the diet, but also the estimated 0.5 to 1.5 Gm. excreted daily by the liver. The cholesterol in the bile is in the same free state as that in the diet,\textsuperscript{19} and there is no reason to suspect that the gut differentiates between cholesterol molecules from these two sources.

From these considerations it seems probable that a substance which interferes with the absorption of cholesterol would act upon both dietary cholesterol and that present in the bile. The effects of sitosterol administration would thus not necessarily be the same as those of restriction of dietary cholesterol.

The mechanism by which sitosterol interferes with the absorption of cholesterol has not been established. Pollack has proposed that the admixture of sitosterol and cholesterol results in the formation of nonresorbable crystals.\textsuperscript{16} Hernandez and associates have reported that in rats sitosterol interferes with the esterification of cholesterol, and suggest that this effect may be responsible for the interference with absorption.\textsuperscript{17} The time relationship of the administration of sitosterol to ingestion of food is important; administration immediately before meals facilitates admixture of the plant sterol with the cholesterol in the food and that contained in the bile.

Schoenheimer has presented direct evidence that the plant sterols are not absorbed in experimental animals,\textsuperscript{18} but the evidence that beta sitosterol is not absorbed in man is as yet indirect. The cholesterol methods used all give color development with beta-sitosterol. As compared with the optical density given by an equal amount of pure cholesterol, that of beta sitosterol has been determined to be 60.5 per cent by the method of Abell, 71.5 per cent by that of Pearson, and 79 per cent by that of Zlatkis. Furthermore, in vitro addition of betasitosterol to serum reveals no interference with color development, the optical density being the exact sum of those obtained when the cholesterol in the serum and the added beta-sitosterol are determined separately. Any appreciable absorption of beta-sitosterol thus seems unlikely, in view of the observed fall in serum cholesterol, as determined by these methods.

The sustained lowering of serum cholesterol by dietary means alone is difficult. Keys has shown that the fall in serum cholesterol is dependent upon the absence of both cholesterol and neutral fat from the diet.\textsuperscript{19} Such a rigid dietary regimen is unacceptable to patients for any prolonged period. Failure to achieve a sustained lowering of serum cholesterol by dietary means may also be due to an increase in endogenous cholesterol production by the liver or intestinal mucosa. It has been shown by Gould and Taylor that the level of exogenous cholesterol ingestion inversely influences the rate of endogenous synthesis from acetate in the dog and rabbit.\textsuperscript{20}

Preliminary results of studies now in progress indicate that the administration of beta-sitosterol is accompanied not only by a lowering of serum total cholesterol, but also by changes in other serum lipids. A decrease in the level of the "atherogenic" Sf 10-100 classes of lipoproteins and a fall in serum neutral fat\textsuperscript{21} have been noted in the subjects so studied. Gofman has observed a reduction in Sf 12-100 classes of lipoproteins in some subjects following limitation of caloric intake or of fat in the diet.\textsuperscript{22} The changes incident to sitosterol administration cannot be attributed to negative caloric balance, since weight loss did not occur. Whether or not there is any interference with fat absorption remains to be determined.

The administration of beta-sitosterol has not been accompanied by toxic reactions or undesirable side effects. The preparation is not unpleasant and is suitable for long-term administration. While the evaluation of any measure directed toward atherosclerosis is a difficult problem because of the lack of criteria
for determining the extent or rate of progression of the disease, beta-sitosterol would seem to offer a means of testing the value of a sustained reduction in serum cholesterol.

Conclusions

1. The administration of beta-sitosterol to human subjects on an unrestricted diet is accompanied by a significant reduction in serum total cholesterol.

2. The ratio of serum total cholesterol to lipid phosphorus is reduced following the administration of beta-sitosterol. The reduction is due largely to the change in cholesterol, since lipid phosphorus is not consistently altered.

3. A reduction in the "atherogenic" S₇, 10–100 classes of lipoproteins has been observed in the three subjects whose serum have been studied by the analytic ultracentrifuge.

4. Toxic or unpleasant side effects have not been observed in subjects receiving sitosterol.

5. Beta-sitosterol, by interfering with the absorption of dietary cholesterol and with the reabsorption of that excreted into the gastrointestinal tract, offers a means of studying the effects of a sustained lowering of serum cholesterol in atherosclerotic states.

SUMARIO ESPAÑOL

1. La administración de beta-sitosterol a sujetos humanos en una dieta no restringida fue acompañada por una significativa reducción en el colesterol total del suero.

2. La proporción del colesterol total del suero al fósforo lipídico se reduce luego de la administración del beta-sitosterol. La reducción se debe grandemente al cambio en colesterol ya que el fósforo lipídico no se altera consistentemente.

3. Una desviación de la clases S₇, 10–100 "atherogénica" de lipoproteínas fue observado en los tres sujetos cuyos sueros fueron estudiados por la ultracentrifuga analítica.

4. Efectos tóxicos o indeseables no han sido observados en los sujetos durante la administración del sitosterol.

5. El beta sitosterol ofrece un medio de estudio para los efectos de una reducción sostenida del colesterol del suero en sujetos ateroescleróticos, mediante la interferencia a la absorción del colesterol dietético y en la reabsorción de aquel eliminado en el tubo digestivo.

Acknowledgment

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References


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