Sitosterol Feeding
Chronic Animal and Clinical Toxicology and Tissue Analysis

By R. E. SHIPLEY, M.D., R. R. PFEIFFER, PH.D., M. M. MAR^TT, B.S.
AND R. C. ANDERSON, Sc.D.

Sitosterol has previously been shown to block cholesterol absorption in both animals and
man. Its clinical effectiveness in reducing hypercholesteremia has been repeatedly
demonstrated. For periods exceeding 4 years, large amounts of sitosterol have been
given daily for maintaining reduction of serum cholesterol concentration. The possibility
of adverse side effects resulting from chronic administration has been considered.
Long-term animal and clinical studies indicate that sitosterol has no detectable toxicity
and does not accumulate or deposit in tissues.

In 1951 Peterson and his collaborators1,2 first reported that the addition of soy
sterols to a cholesterol-containing diet fed to chicks largely prevented hypercholesteremia
and cholesterol deposition in the liver and aorta. About the same time Pollak3 reported
that soy sterols prevented hypercholesteremia in cholesterol-fed rabbits. In addition, Pollak
fed crude soy sterols to human beings with elevated serum cholesterol levels and observed
a reduction in hypercholesteremia.4

Since Pollak's initial observations in man there have been many clinical reports confirm-
ing the effectiveness of sitosterol in reducing hypercholesteremia.5-20 The effect
appears to be that of inhibiting absorption of cholesterol from the intestinal tract, thereby
reducing or preventing hypercholesteremia. Proposed mechanisms of action of plant
sterols include (1) formation of a relatively insoluble mixed crystal of sitosterol and
cholesterol,8,21 (2) competition between sitosterol and cholesterol for esterification,22 a
presumed requirement for the absorption of cholesterol, and (3) competition with chole-
sterol for acceptor sites on or within the cell membrane of the gut mucosa.23,24

In contrast to cholesterol, sitosterol is very poorly absorbed from the intestine. In their
classical experiments, Schoenheimer and associates25-28 found that the plant sterols
were not absorbed to a detectable degree, did not accumulate in the animal's tissues, and
that they were, for the most part, excreted unchanged in the feces, as might be expected
of an inert substance.

Using more sensitive radioactive tracer methods, Gould27 has shown that measurable
amounts of sitosterol are absorbed by experimental animals and man. The amounts are
so small as to be indetectable by the most sensitive of Schoenheimer's chemical methods.
Gould also confirmed Schoenheimer's observations that in contrast to cholesterol or dihydro-
cholesterol, sitosterols do not accumulate or deposit in tissues.

More recently, Curran and Costello28 have reported that feeding soy sterols for 4 weeks
led to development of atheromatous plaques in the aorta of each of 4 rabbits. The livers
and aortas of these rabbits contained material that they believed was neither cholesterol nor
saturated sterol and therefore considered to be soy sterols. In a more recent review of
the same experiments Curran29 reaffirms the opinion that soy sterols are "atherogenic" and
that "the use of cholesterol absorption blocking agents such as soy sterols achieves
nothing and is potentially hazardous.''

Because of the widespread and increasing clinical use of sitosterols for reducing hyper-
cholesteremia, the adverse implications drawn in Curran and Costello's reports have
prompted the presentation of extensive evidence which indicates that \( \beta \)-sitosterols
and soy sterols do not accumulate in the tissues as alleged and are not potentially hazardous.

Animal Toxicology Following Prolonged Administration

Before the plant sterols could be considered a safe medication for reducing hypercholesteremia in man, it was necessary to show that they were innocuous when administered to animals over a long period of time. Hence, the following chronic toxicity studies were made.

Methods

Three series of 10 rats each were fed Harlan Rat Chow plus 5 per cent \( \beta \)-sitosterols derived from tall oil for periods of 8, 18 and 22 months respectively and 2 additional groups of 10 rats each were fed a comparable diet containing 5 per cent \( \beta \)-sitosterols derived from cottonseed oil for periods of 18 and 22 months respectively. There was no detectable alteration in growth, blood cell counts, blood urea nitrogen, serum proteins, or in gross or microscopic appearance of any organ or tissue.

Thirteen dogs were fed a basic diet of Friskies (Albers Milling Co.) plus \( \beta \)-sitosterols at doses of 0.5 and 1.0 Gm./Kg. for periods of 8 to 22 months (table 1). At monthly intervals each dog was weighed and hemoglobin, hematocrit, red blood count, white blood count, differential cell count, clotting time, clot retraction time, blood sugar, blood nonprotein nitrogen, urinary albumin and sugar tests were performed. At the time of sacrifice, a complete pathologic study was made, including gross and microscopic examination of heart, aorta, lungs, liver, spleen, kidneys, stomach, intestine, thymus, thyroid, adrenal glands and bone marrow. Blood was examined for serum concentration of total lipid, total sterols, lipid phosphorus, vitamin A level, total protein, albumin, globulin, A:G ratio and ultra-centrifugal lipoprotein pattern.

All dogs maintained weight and had normal values for serum composition and formed blood elements. No gross or microscopic pathologic changes attributable to sitosterol were observed in any dog. There were no gross or microscopic lesions in any of the aortas. Total liver lipids and free and total liver sterol levels determined by the method of Sperry and Webb were not significantly different from those of control dogs.

Six New Zealand white rabbits of both sexes were placed on a diet of Purina Rabbit Chow fortified with 3 per cent cottonseed oil and 4 per cent \( \beta \)-sitosterols derived from cottonseed oil. Each rabbit consumed approximately 100 Gm. of food/day. None of the rabbits showed any gross or microscopic abnormality of the blood vessels or other tissues including heart, thyroid, spleen, liver and intestine after consuming 4 Gm. of sitosterols/day for 348, 488, 593, 665, 842, and 842 days respectively. Total liver and aorta lipid concentration and free and total liver and aorta sterol concentrations were essentially the same as those observed in two control rabbits fed Purina Rabbit Chow only.

Six New Zealand white rabbits of both sexes were placed on a diet of Purina Rabbit Chow fortified with 3 per cent cottonseed oil and 4 per cent soy sterols (Distillation Products, Inc., lot number 11254). Three rabbits were sacrificed after

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**Table 1.—Hepatic Sterol and Total Lipid Concentrations in Dogs Fed \( \beta \)-Sitosterols**

<table>
<thead>
<tr>
<th>Wt. Kg.</th>
<th>( \beta )-sitosterol feeding</th>
<th>Total sitosterol consumed (Kg.)</th>
<th>Liver analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Duration (months)</td>
<td>Daily dose (Gm./Kg.)</td>
<td>Control</td>
</tr>
<tr>
<td>17.8</td>
<td>—</td>
<td>—</td>
<td>Control 7.17</td>
</tr>
<tr>
<td>13.1</td>
<td>—</td>
<td>—</td>
<td>Control 6.79</td>
</tr>
<tr>
<td>12.8</td>
<td>8</td>
<td>1.0</td>
<td>3.1</td>
</tr>
<tr>
<td>10.8</td>
<td>8</td>
<td>1.0</td>
<td>2.6</td>
</tr>
<tr>
<td>9.7</td>
<td>8</td>
<td>1.0</td>
<td>2.3</td>
</tr>
<tr>
<td>10.1</td>
<td>19</td>
<td>0.5</td>
<td>3.0</td>
</tr>
<tr>
<td>8.2</td>
<td>19</td>
<td>0.5</td>
<td>2.3</td>
</tr>
<tr>
<td>9.8</td>
<td>19</td>
<td>0.5</td>
<td>2.8</td>
</tr>
<tr>
<td>8.8</td>
<td>19</td>
<td>1.0</td>
<td>5.3</td>
</tr>
<tr>
<td>7.8</td>
<td>19</td>
<td>1.0</td>
<td>4.4</td>
</tr>
<tr>
<td>10.5</td>
<td>19</td>
<td>1.0</td>
<td>6.0</td>
</tr>
<tr>
<td>7.0</td>
<td>19</td>
<td>1.0</td>
<td>4.2</td>
</tr>
</tbody>
</table>

* Method of Sperry and Webb.
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70 days and the remaining 3 after 212 days. None showed any gross or microscopic abnormality of the blood vessels or other tissues. Values for total liver and aorta lipids and free and total liver and aorta sterol concentrations were not significantly different from values obtained in 2 control rabbits fed Purina Rabbit Chow alone.

Although experiments in these laboratories, which have extended over a period of 4 years, have consistently indicated the safety and effectiveness of sitosterols, unequivocal evidence for the absence of accumulation could not be documented for want of a direct and reliable analytic method for detecting small amounts of sitosterol in the presence of cholesterol. Using a chromatographic method, Swell et al.31 showed the absence of gross sitosterol accumulation in tissues of rats fed soy sterols. Although the validity of the method has been confirmed in these laboratories, its sensitivity appears to be limited to the detection of approximately 5 per cent or more sitosterol in a sterol mixture.

While we were searching for a satisfactory analytic method, the appearance of the paper by Curran and Costello claiming the accumulation of substantial amounts of sitosterol in the liver and aorta of soy sterol fed rabbits prompted a critical examination and appraisal of the indirect method they used.

In the following paragraphs (1) the method employed by Curran and Costello is shown to be inadequate and inapplicable, (2) animal experiments are presented in which the observations reported by Curran and Costello are not confirmed, and (3) a new, direct method of analysis is described. Data obtained by this method indicate that the conclusions of Curran and Costello are erroneous.

**Table 2.—Approximate Solubilities of Sterol Digitonides**

<table>
<thead>
<tr>
<th>Sterol</th>
<th>Amount digitonide (mg)</th>
<th>Solvent</th>
<th>Volume (ml)</th>
<th>Insoluble residue (mg)</th>
<th>Solubility (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>54.5</td>
<td>1:1 Abs. EtOH : Me₂CO</td>
<td>10</td>
<td>38.4</td>
<td>1.6</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>59.7</td>
<td>1:1 Abs. EtOH : Me₂CO</td>
<td>10</td>
<td>44.3</td>
<td>1.5</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>49.8</td>
<td>1:1 95% EtOH : Me₂CO</td>
<td>10</td>
<td>47.5</td>
<td>0.23</td>
</tr>
<tr>
<td>Soy sterols (DPI control 59707)</td>
<td>49.2</td>
<td>1:1 Abs. EtOH : Me₂CO</td>
<td>10</td>
<td>34.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Soy sterols (DPI control 59707)</td>
<td>49.5</td>
<td>1:1 95% EtOH : Me₂CO</td>
<td>10</td>
<td>46.7</td>
<td>0.28</td>
</tr>
<tr>
<td>Soy sterols (DPI control 7349)</td>
<td>47.2</td>
<td>1:1 Abs. EtOH : Me₂CO</td>
<td>10</td>
<td>34.0</td>
<td>1.3</td>
</tr>
<tr>
<td>Soy sterols (DPI control 7350)</td>
<td>50.8</td>
<td>1:1 Abs. EtOH : Me₂CO</td>
<td>10</td>
<td>34.4</td>
<td>1.6</td>
</tr>
<tr>
<td>Soy sterols (DPI control 7350)</td>
<td>42.3</td>
<td>1:1 Abs. EtOH : Me₂CO</td>
<td>10</td>
<td>27.1</td>
<td>1.5</td>
</tr>
</tbody>
</table>

**Examination of the Analytic Method of Curran and Costello for Differentiation of Soy Bean Sterols and Cholesterol**

Using a modification of the method of Pollak and Wadler,32 Curran and Costello analyzed the livers of 4 rabbits fed 9 Gm. of soy bean sterols/week for 4 weeks, and reported28 that soy sterols comprised 44, 36, 32 and 5 per cent of the total liver sterols, respectively, in each of 4 rabbits. Their method was based upon: 1. Their report that the Sperry-Webb procedure30 for cholesterol determination is inadequate for analysis of soybean sterols. The reason for the inadequacy is said to be the solubility of soy bean sterol-digitonin complex in 1:1 ethanolacetone. 2. Their report that, for a more complete precipitation of the soy bean sterols-digonitide, it was necessary to employ a modification of the Pollak-Wadler method, in which methyl alcohol was substituted for ethyl alcohol in the precipitating medium. 3. Their assumption that the value for total digitonin-precipitable sterols (determined by the above modified procedure) minus the value for all Lieberman-Burchard (LB) positive sterols (as determined by the method of Sperry and Webb), and minus the average difference between the two values observed in non-soybean sterol-fed rabbits (assumed to be dihydrocholesterol) was a measure of the amount of soy sterols present.

We have conducted 2 types of experiments to examine the validity of methods and pro-
TABLE 3.—

<table>
<thead>
<tr>
<th>Method</th>
<th>Soy sterols (mg.)</th>
<th>Normal rabbit serum (mg.)</th>
<th>Normal rabbit liver (mg./Gm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperry-Webb</td>
<td>78.5</td>
<td>40.0</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>78.5</td>
<td>22.5</td>
<td>8.5</td>
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<tr>
<td></td>
<td>80.4</td>
<td>33.7</td>
<td>8.3</td>
</tr>
<tr>
<td>Ave. 77.8</td>
<td></td>
<td>Ave. 32.5</td>
<td>7.9</td>
</tr>
<tr>
<td>Turbidimetric</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ethanol-acetone)</td>
<td>77.7</td>
<td>34.6</td>
<td>8.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>33.8</td>
<td>9.2</td>
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<td></td>
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<td>31.4</td>
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<td>Ave. 33.3</td>
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<td>Ave. 8.7</td>
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<tr>
<td>Turbidimetric</td>
<td>78.8</td>
<td>28.0</td>
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<td>27.6</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>29.3</td>
<td>7.7</td>
</tr>
<tr>
<td>Ave. 28.2</td>
<td></td>
<td>Ave. 8.0</td>
<td></td>
</tr>
</tbody>
</table>

* Compared to cholesterol standard and corrected for molecular weight difference.

The next experiment was performed to determine the magnitude of difference among values for sterol content of normal rabbit serum, normal rabbit liver and a sample of soy sterol when extracted and analyzed by the Sperry-Webb method, the Pollak-Wadler procedure with ethanol-acetone and the Pollak-Wadler procedure employing methanol-acetone. The results in table 3 show that there is very little difference among values obtained by the 3 methods, indicating that both the Sperry-Webb and the Pollak-Wadler turbidimetric methods are sensitive and adequate for the determination of soy sterols, and that the modified Pollak-Wadler procedure employing methanol-acetone possesses no discernible advantage.

The reported finding of large amounts of soy sterols in the livers of rabbits fed these substances is based upon a calculation that assumes that all digitonin-precipitable sterols that are not LB positive by the method of Sperry and Webb are soy sterols or dihydrocholesterol. After subtracting the value of 0.5 mg. (for the amount of dihydrocholesterol estimated to be present) per gram of liver, the remainder (5 to 44 per cent) is considered to be a measure of the amount of soy sterols present.

The calculation cannot be accepted as a valid procedure, since soy sterols are LB positive and would be included and measured as LB positive material along with cholesterol by the method of Sperry and Webb.

Although the extraction and recovery were reported to be something less than complete (83 per cent), Curran and Costello showed that the Sperry-Webb procedure did measure soy sterols as LB positive material as shown in their table 1. The data presented in their tables 1 and 3 do not support the interpretation that large amounts of soy sterols were present in the livers of the soy sterol fed rabbits, but on the contrary, they indicate that there was little, if any, present. It is not apparent why Curran and Costello's modification of the Pollak-Wadler method gave appreciably higher values in 3 of the 4 rabbit livers they analyzed.
Animal Feeding Experiments

As stated earlier, none of the rabbits fed soy sterols and β-sitosterols for periods up to 7 months and for over 2 years respectively exhibited any evidence of deposition of these sterols grossly, microscopically, or by chemical analysis of tissues for total sterols by method of Sperry and Webb. Following the report by Curran and Costello that atheromatous deposits were observed in 4 rabbits fed soy sterols for 4 weeks, an experiment was carried out duplicating as closely as possible the conditions of their study. Additional experiments were begun simultaneously in which larger amounts of soy sterols were fed on a continuous schedule for longer periods and to more animals. Terminally, the tissues were examined for pathologic changes and analyzed for sterol concentration.

Methods

New Zealand white rabbits of both sexes were used. Soy sterols (#S-11254) were supplied by Distillation Products Industries who state, "This product was made from similar material as S-9707* and differs primarily in having a few per cent less fatty acid esters." Purina Rabbit Chow was used as the control diet. The soy sterol diets were prepared by dissolving the soy sterols in chloroform and adding the solution slowly to Purina Chow and S-9707* and differs primarily in having a few per cent less fatty acid esters. Purina Rabbit Chow was used as the control diet. The soy sterol diets were prepared by dissolving the soy sterols in chloroform* and adding the solution slowly to Purina Rabbit Chow in a rotary mixer. The food was placed in shallow trays and the chloroform allowed to evaporate for 24 hours at room temperature. One experimental diet was made to contain 3 per cent soy sterols. The same procedure was followed in preparing a second diet to contain 4 per cent soy sterols and 3 per cent cottonseed oil.

Three groups of 6 rabbits each were matched with respect to weight and sex: Group A, 6 rabbits, fed plain Purina Rabbit Chow (controls) Group B, 6 rabbits, fed Purina Rabbit Chow fortified with 3 per cent soy sterols on Monday, Wednesday and Friday, and plain Purina Rabbit Chow on Tuesday, Thursday, Saturday and Sunday (according to the method of Curran and Costello). Group C, 6 rabbits, fed Purina Rabbit

*S-9707 is a Purina Chow product that differs from S-11254 in having a few percent less fatty acid esters. Purina Rabbit Chow was used as the control diet. The soy sterol diets were prepared by dissolving the soy sterols in chloroform* and adding the solution slowly to Purina Rabbit Chow in a rotary mixer. The food was placed in shallow trays and the chloroform allowed to evaporate for 24 hours at room temperature. One experimental diet was made to contain 3 per cent soy sterols. The same procedure was followed in preparing a second diet to contain 4 per cent soy sterols and 3 per cent cottonseed oil.

*One of the 2 lots used by Curran and Costello.

†Chloroform was substituted for ether (used by Curran and Costello).
Chow fortified with 4 per cent soy sterols and 3 per cent cottonseed oil ad lib.
The consumption of food per rabbit per day averaged approximately 100 Gm. for each of the 3 groups.

The results are presented in table 4. The absence of vascular deposits and the absence of accumulation of soy sterols in tissues confirmed the completely negative findings in previous experiments.

Curran and Costello state that all 4 rabbits to which soy sterols were fed for 4 weeks "had atheromatous lesions" of the aorta at autopsy and it was concluded that "soy sterols produced atherosclerosis in these animals." Since the extent of involvement and the nature of the lesions were not described, there is some question whether the aortic lesions resulted from the feeding of soy sterols or were present in the rabbits prior to the beginning of the experiment. The question is prompted by numerous observations that rabbits seldom develop gross atheromatous lesions with cholesterol feeding if the duration of the feeding is 4 weeks or less. This impression received further support from a separate experiment in which no atheromatous lesions were detectable, either grossly or microscopically when (1) cholesterol was fed to 4 rabbits for 4 weeks according to the regimen used by Curran and Costello for soy sterol feeding or (2) cholesterol was administered in capsules to 4 other rabbits in the amount of 3 Gm. 3 times a week for 4 weeks. Since even cholesterol was not manifestly atherogenic within a period of 4 weeks under the specified conditions, it is doubtful that the aortic lesions found by Curran and Costello were atheromatous deposits induced by the feeding of soy sterols for 4 weeks.

Clinical Toxicology
The requirement that every medication for human use be adequately tested for possible harmful effects before it can be made commercially available is particularly appropriate for the sitosterols, which are administered in large amounts for long periods of time. Chronic experiments in which relatively enormous amounts were fed to rats, dogs and rabbits over long periods consistently and repeatedly failed to reveal any alteration in function or composition of any organ or tissue in any animal. With continuous administration of sitosterols to human beings for periods that now exceed 4 years, during which the total amount of sitosterol consumed is, in many instances, greater than one-half the patient's body weight, there has been no evidence of any harmful effect as determined by laboratory tests of kidney and liver function, blood and urine composition, electrocardiogram, and gall bladder visualization. Also, there has not appeared any symptom or sign indicating that the sitosterols were contributing to the formation or progression of vascular lesions.

On the contrary, preliminary reports described objectively measured improvement in vascular status in 2 very small series of patients who received sitosterols continuously over an extended period. Although this improvement may not be attributed solely to the reduction of hypercholesteremia, these observations tend to negate the suggestion that sitosterols are potentially hazardous or harmful. A more direct implication may be drawn from the work of Gould who studied the distribution of tritium-labeled sterol in the tissues of a terminal cancer patient 4 weeks after a single oral administration of tritium-labeled β-sitosterol. In all tissues examined (blood, liver, coronary artery, and aorta) very small amounts of tritium β-sitosterol were found, from which he concluded that "Tritium β-sitosterol can appear in tissue cells but it does not appear to have a predilection for any particular tissue."

Tissue Sterol Analysis: Phase Transition Endpoint Method
While tracer technics are ideally suited for short term experiments and are extremely sensitive for detection of small quantities of sitosterol, they were not feasible for use in

*Reversal of ischemic electrocardiograph pattern induced by measured effort, improved ballistocardiogram, increase in claudication time and increase in skin temperature.
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the present long term studies. Accordingly, an extensive search was aimed toward development of a direct and reliable nontracer analytic procedure for detecting small amounts of sitosterol in the presence of relatively large amounts of cholesterol. After extensive but fruitless exploration of several different approaches, a method was developed that met the desired requirements for selectivity and sensitivity.

In the present study several series of mixtures ranging from pure cholesterol to 50 per cent cholesterol plus 50 per cent sitosterol were prepared dried from methanol solution for examination by x-ray powder diffraction methods. This composition range yields x-ray diffraction patterns of 3 distinct kinds: type A from 0 to 16 per cent sitosterol where the pattern is indistinguishable from that of pure cholesterol, type C a different pattern obtained from compositions close to that of 1:1 mixed crystals, and type B which has the strongest line of type C superimposed on the type A pattern. The change from type A to type B upon increasing the sitosterol content of the samples was noted to take place not only abruptly but also consistently at 16 per cent (+ 1.5 per cent) sitosterol and 84 per cent cholesterol composition under controlled conditions of preparation. This abrupt transition in the nature of the x-ray patterns was regarded as a useful criterion for identifying cholesterol-sitosterol mixtures that had undergone the change in associated composition.

For the present study other series of mixtures were prepared in which sitosterol (derived from cottonseed oil) and cholesterol varied by 2 per cent increments from 0 to 20 per cent sitosterol, to 100 to 80 per cent cholesterol. Other series were made using soy sterols and sitosterols derived from tall oil. Another series was prepared employing sitosterol (derived from tall oil) and cholesterol (nonsaponifiable digitonin precipitable sterol) extracted from normal rabbit livers.

The x-ray diffraction pattern of the soy sterols, and of β-sitosterol derived from cottonseed oil and from tall oil were not detectably different. Also, the pattern for cholesterol that had been repeatedly crystallized was not different from that of cholesterol extracted from normal rabbit livers. In each instance the phase transition occurred at sitosterol concentrations between 15 and 17 per cent.

The following procedure was used to determine the amount of sitosterol, if any, present in a sterol sample. Five milligram samples were dissolved in 10 ml of methanol. Evaporation of the solvent was carried out in an oven at 50 C. under ½ atmosphere pressure to a point of apparent dryness and then for an additional 2 hours at very low pressure. The crystalline residue was lightly ground in an agate mortar and approximately 0.3 mg packed into a cellulose-acetate capillary. The x-ray exposures were made in Norelco powder cameras using chromium K-alpha radiation for 2 hours duration at 35 K. at 10 ma.

If the pattern was an A type, it was evident that something less than 16 per cent sitosterol was present. To the second 5 mg. portion a volume of a methanol solution of sitosterol was added such that the concentration of sitosterol in the sterol mixture was increased by approximately 2 per cent. The entire procedure was repeated and the pattern again examined as many times as necessary to bring the concentration of sitosterol to the point where the type A pattern was replaced by the type B pattern.* Knowing the weight of the original sterol sample and the amount of sitosterol that was added to reach the transition end point, the concentration of sitosterol, if any, in the original sample could be calculated. If the original sample exhibited a pattern other than type A, it was evident that more than 16 per cent sitosterol was present and cholesterol would be added until the B-A transition was observed. The same calculation was made for determining original sitosterol concentration.

To check the validity of the method in practice, a series of cholesterol-sitosterol mixtures containing 0, 3, 7, 12, 15, 17 and 20 per cent sitosterol were prepared, identified only by code number and submitted for analysis by the phase transition end point method, again using approximately 2 per cent increments of added sitosterol (or cholesterol) if the initial pattern so indicated. The concentration of sitosterol was correctly identified in each instance within ± 2 per cent.

Next, sterols extracted and isolated as non-

*It has been assumed for many years that the major sterol component of soy sterols is γ-sitosterol rather than β-sitosterol. As pointed out in Elsevier's Encyclopedia of Organic Chemistry, Series III, Vol. 14 (suppl.) page 18028, this impression appears to have arisen from a misinterpretation of the work of Bonstedt* who isolated a relatively pure γ-sitosterol fraction from soy sterols, the predominant sterol of which is β-sitosterol.

*In cases where the available sample of extracted sterol is insufficient to provide many 5 mg. portions, a portion so examined may be redissolved and sitosterol increments added repeatedly.
saponifiable digitonin precipitable material from the livers of normal rabbits and of rabbits that had been fed either soy sterols or \( \beta \)-sitosterols were examined for the presence of sitosterol by the above method. The sterol samples were from rabbits including one fed soy sterols, 3 Gm./day, 3 times a week for 4 weeks, 2 fed soy sterols 4 Gm. daily for 7 months, and 2 fed \( \beta \)-sitosterol. 4 Gm. daily for 348 and 842 days respectively. In all instances the amount of sitosterol that had to be added to the liver sterols to bring the concentration to the point at which the phase transition occurred was 16 per cent (± 2 per cent) of the total. This demonstrates that the sterols from the livers of both normal rabbits and rabbits fed either soy sterols or \( \beta \)-sitosterol contained less than 2 per cent, if any, sitosterol.

**SUMMARY**

\( \beta \)-sitosterols have been administered to human beings for periods exceeding 4 years without any adverse effects.

No evidence of toxicity was observed in rats, rabbits or dogs given large daily oral dietary supplements of \( \beta \)-sitosterols or soy sterols for periods up to 2 years. No gross or microscopic alterations of any tissue were observed. There was no histologic evidence of deposition of the plant sterols, and chemical analyses of aorta and liver showed no increase in sterol content.

A new analytic procedure using x-ray diffraction technic (phase transition end point method) is described and shown to be adequate and reliable for detecting small amounts of sitosterol in the presence of relatively large amounts of cholesterol. Employing this method, sterols extracted from the livers of rabbits that had received either soy sterols or \( \beta \)-sitosterols for long periods of time were found to contain no detectable plant sterols, within the limits of accuracy of the method (± 1.5 per cent).

A recent report by Curran and Costello that substantial amounts of soy sterols accumulated in the tissues and also produced atherosclerotic lesions in rabbits fed these sterols for short periods of time was not confirmed. Critical examination of the methods employed revealed inadequacies that could explain the apparent finding of accumulation.

**ACKNOWLEDGMENTS**

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**SUMMARIO IN INTERLINGUA**

Sitosteroles \( \beta \) ha essite administrate a humanos durante periodos de plus que 4 annos sin ulle effecto adverse.

Nulle signos de toxicitate esseva observate in ratos, conilos, o canes que recipieva grande diurne supplementos dietari oral de sitosteroles \( \beta \) o de steroles de soja durante periodos de usque a 2 annos. Nulle alterationes macro- o microscopic del histos esseva observate. Esseva trovate nulle indication de depositos de steroles vegetal, e le analyse chimic de aorta e hepate monstrava null augmento del contento sterolic.

Es describite un nove methodo que utilisa un technica a roentgeno-diffraction e que se ha provate adequate e fidel pro le detection de micre quantitates de sitosterol in le presentia de relativemente grande quantitates de cholesterol. Per medio de iste methodo il esseva monstrate que le steroles extrahite ab le hepate de conilos alimentate durante longe periodos de tempore con supplementos de steroles de soja o de sitosteroles \( \beta \) continueva—intra le limites del exactitude del methodo (± 1,5 pro cento)—nulle detegibile amontas de steroles vegetal.

Le recente reporto de Curran e Costello que considerabile quantitates de steroles de soja se accumulava in le histos de conilos e mesmo produciva lesiones atherosclerotic in ille animali post que illos habeva recipite tal steroles durante breve periodos de tempore non esseva confirmate. Un examine critic del methdos empleate per le autores mentionate revelava inadeguates que suffice forsan a explicar le constatation erronee de accumulaciones de steroles vegetal.
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