The Hypocholesterolizing Effect of Gallogen and its Potentiation by Soybean Sterols

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Gallogen (the diethanolamine salt of the mono-(+)-camphoric acid ester of α,4-dimethylbenzyl alcohol) and soybean sterols have been tested for their effect, singly and in combination, on hypercholesterolemia and aortic cholesterol in cholesterol-fed cockerels. Gallogen alone was found to impede the rise of serum and aortic cholesterol, and also to potentiate these effects of soybean sterols.

The purpose of this communication is to report on the value of Gallogen* alone and in combination with soybean sterol in the treatment of experimentally induced atherosclerosis. Peterson and co-workers observed that the addition of soybean sterols to the diet of chicks, on a high fat-high cholesterol diet, lowers cholesterol levels in blood and liver more than when the soybean sterols were omitted. This observation has been elaborated upon and confirmed by these same workers. Hernandez and co-workers working with rats fed C14-labeled cholesterol, reported that either soybean sterols or β-sitosterol reduce the recovery of radioactive cholesterol and esterified cholesterol from the lymphatic duct. Alfin-Slater and co-workers found that plant sterols have no effect on the plasma cholesterol levels in cholesterol-fed rats, but reduce the total lipids and total cholesterol of the liver. Poljak has reported successful prevention of induced hypercholesterolemia and atherosclerosis in the rabbit and significant reductions of blood cholesterol levels in man by the use of soybean sterols. Other workers have confirmed and prolonged the lowering of human blood cholesterol levels by sufficient soybean sterols or β-sitosterol.

The results of sitosterol administration are much more significant than those of dietary restriction of cholesterol intake. It has been suggested that sitosterol renders cholesterol unavailable for absorption or reabsorption. Consequently, Gallogen was combined with soybean sterol to determine if an increase in the excretion of biliary cholesterol could potentiate the hypocholesterolizing effect of soybean sterols by making more endogenous cholesterol available to this blocking action.

Gallogen has been shown to be a "true" choleretic in that it stimulates an increased excretion of biliary cholesterol with an unchanged specific gravity and bilirubin concentration, as opposed to the hydrocholeretic effect of dehydrocholic acid, for example.

METHOD

Day-old single-comb White Leghorn cockerels were fed Purina Growing Mash from the time of hatching until they were four weeks old. They were then divided into three groups of 10 birds each and one group of 7 birds and placed on the experimental diets (table 1), freshly prepared each week. The chickens were allowed to consume the experimental ration ad libitum for 10 weeks. Weekly 1 ml. blood samples were obtained from each bird and the serum cholesterol levels determined. The blood samples were drawn by cardiac puncture for the first three weeks, and from the alar vein throughout the remainder of the experiment. All serum cholesterol determinations were done by the method of Zlatkis and co-workers.

At autopsy, a 2.5 to 3.0 cm. portion of the thoracic aorta was removed from the heart posteriorly to the vicinity of the diaphragm. Adherent connective tissue was dissected away, and the aortas dried at 105 C. for three hours. The dried tissue was ground in a mortar with 2 ml. glacial acetic acid and a little sand. The mixture was transferred to a centrifuge tube and the mortar rinsed with 1 ml. acetic acid. After centrifuging, the clear supernatant was made

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* Trade name for the diethanolamine salt of the mono-(+)-camphoric acid ester of α,4-dimethylbenzyl alcohol.
TABLE 1.—Type of Experimental Ration and Number of Birds in Each Group

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
<th>No. of Birds</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-1</td>
<td>Purina Mash+ 4% Cottonseed Oil 1% Cholesterol</td>
<td>7</td>
</tr>
<tr>
<td>G-2</td>
<td>Purina Mash+ 4% Cottonseed Oil 1% Cholesterol 1% Soybean Sterol*</td>
<td>10</td>
</tr>
<tr>
<td>G-3</td>
<td>Purina Mash+ 4% Cottonseed Oil 1% Cholesterol 0.05% Gallogen</td>
<td>10</td>
</tr>
<tr>
<td>G-4</td>
<td>Purina Mash+ 4% Cottonseed Oil 1% Cholesterol 1% Soybean Sterol* 0.05% Gallogen</td>
<td>10</td>
</tr>
</tbody>
</table>

* Obtained from the Glidden Company, Soya Products Division, Chicago, Ill.

to 3.5 ml. with acetic acid, and a 0.5 ml. aliquot was assayed for cholesterol by the method of Zlatkis and co-workers. The resultant colors gave no indication of any interference.

RESULTS AND DISCUSSION

Results of the weekly serum cholesterol determinations are shown in figure 1. The feeding of cholesterol and oil produced the expected rise in serum cholesterol. Indeed, the rise exceeded that which usually would be expected. Since this may in part be a seasonal effect, it should be mentioned that the experiment was performed between February and April and that in subsequent experiments the more generally reported values have been obtained both in normal and in cholesterol-fed cockerels. The newer chemical procedure, which was quite satisfactory, also avoided the inevitable manipulatory losses in the classical digitonin precipitation and accounts in some part for the higher values. Soybean sterol was effective in inhibiting the rise in serum cholesterol, although not to the degree described by Peterson and co-workers. It is obvious from figure 1 that Gallogen also produced a considerable potentiation of the hypcholesterolizing effect of the soybean sterols, suggesting that it acts by some different mechanism. Growth curves and food consum-

FIG. 1. Effect of Gallogen, soybean sterols and Gallogen-soybean sterols in combination on serum cholesterol levels. Standard deviations are indicated. Deaths during the experiment: One each from cannibalism in Groups G-2, G-3 and G-4, weeks 3, 2, and 6, respectively; one each of osteopetrotic lymphomatosis in Groups G-1 and G-2, weeks 9 and 3, respectively; one from heart puncture injury, G-4, week 3.

TABLE 2.—Effect of Soybean Sterol, Gallogen and Soybean Sterol-Gallogen in Combination on Aortic Cholesterol.

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>Aortic Cholesterol in /mg. Dry Weight</th>
<th>Significance between Group and Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-1</td>
<td>Control</td>
<td>18.30 ± 3.00*</td>
<td>—</td>
</tr>
<tr>
<td>G-2</td>
<td>Soy Sterol</td>
<td>12.1 ± 0.87</td>
<td>t = 1.96</td>
</tr>
<tr>
<td>G-3</td>
<td>Gallogen</td>
<td>13.2 ± 1.24</td>
<td>t = 1.59</td>
</tr>
<tr>
<td>G-4</td>
<td>Gallogen plus</td>
<td>9.1 ± 0.55</td>
<td>t = 3.00</td>
</tr>
<tr>
<td></td>
<td>Soy Sterol</td>
<td>0.1 ± 0.55</td>
<td>(P &lt; 0.05)</td>
</tr>
</tbody>
</table>

* ± Standard error of the mean.

tion showed no noteworthy differences among the four groups.

Results of the aortic analyses are shown in table 2. A statistically significant difference did not exist between the soybean sterol-treated group or the Gallogen-treated group and the
controls. Such significance ($P < 0.05$) did exist between the controls and the group receiving a combination of soybean sterol and Gallogen. A statistically significant difference ($P < 0.05$) was also found in aortic cholesterol levels between Groups G-4 and G-3 and between G-4 and G-2.

The mechanism whereby Gallogen potentiates the soybean sterol effect is not known; however, stimulation of the excretion of bile salts and biliary cholesterol may somehow account for its effect. The possibility exists that Gallogen might also have some direct effect on cholesterol levels.

**SUMMARY**

Evidence is presented that Gallogen has a serum-cholesterol-lowering effect and also a significant potentiating effect on the ability of soybean sterols to reduce hypercholesterolemia in the cholesterol-fed cockerel.

Neither soybean sterols nor Gallogen, administered singly, produced a statistically significant difference in aortic cholesterol, as compared with controls.

Soybean sterols and Gallogen in combination did produce a statistically significant difference.

**SUMMARIO IN INTERLINGUA**

Es presentate datos que mostra que Gallogen ha un effetto depressive super le nivello serumal de cholesterol e etiam un significative effetto potentiante super le capacitate de sterole de soja a reducere hypercholesterolemia in gallos mantenite super dietas cholesterolisate.

Ni sterole de soja ni Gallogen in administrationes non-combine producereva un statisticamente significative differencia del cholesterol aortic in comparation con animales de controlo.

Steroles de soja e Gallogen in combination, del altere latere, producereva un tal differentia significacion statistic.

**REFERENCES**


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