Influence of Gallogen and Diethanolamine Upon Induced Hypercholesterolemia in the Rat

By SEYMOUR DAYTON, M.D.

The diethanolamine salt of the mono (α,p-dimethylbenzyl) ester of d-caniphoric acid (Gallogen) augmented diet-induced hypercholesterolemia and hyperlipemia in the rat, while depressing liver cholesterol and total lipid concentrations. Diethanolamine had virtually identical effects. Gallogen, given orally to rats with biliary fistulas had a slight hydrocholeretic effect, but did not influence biliary excretion of cholesterol, cholic acid, or chenodeoxycholic acid.

The diethanolamine salt of the mono (α,p-dimethylbenzyl) ester of d-caniphoric acid (Gallogen) was introduced in 1941 as a “true” choleretic. In 1956 Clarkson et al. observed that Gallogen partly prevented the hypercholesterolemia which results from feeding cholesterol to cockerels, and that it potentiated the hypocholesterolizing effects of soybean sterols in this species.

The present investigation was intended to study the mechanism of the reported hypocholesterolizing effect of this drug employing the rat. It was found, however, that contrary to the reported effects in cockerels, Gallogen exaggerated the induced hypercholesterolemia of rats while depressing liver cholesterol concentrations. Diethanolamine had the same effect, suggesting that it may be the active moiety of the Gallogen molecule. The choleretic effect attributed to Gallogen has not been observed. These findings form the basis of the present report.

METHODS

Experiments on Intact Animals. Male Long-Evans rats were divided into 3 groups, and fed the diets outlined in table 1. Food and water were given ad libitum. The animals were weighed twice weekly, and food consumption was determined daily.

The animals were sacrificed by exsanguination after 5 weeks on the experimental diets. Total lipid concentrations of plasma and liver samples were determined gravimetrically, employing the extraction and washing procedures of Folch. Plasma cholesterol was determined by the method of Abell et al. Liver cholesterol was determined by applying the Sperry-Webb procedure to the washed total lipid extract.

Experiments on Rats with Biliary Fistulas. Biliary fistulas were produced in male Long-Evans rats weighing 280 to 370 Gm., employing polyethylene catheters, and the animals were kept in restraining cages. They were fed Purina Laboratory Chow and given 0.9 per cent aqueous NaCl ad libitum. Beginning 3 or more days after operation, collection of control bile samples was carried out for 2 days. The salt solution was then supplemented with 0.2 per cent aqueous NaCl for 2 or 3 days, followed by another 2 or 3 day period on 0.9 per cent NaCl alone.

Bile was collected in 24-hour fractions under toluene. Cholic and chenodeoxycholic acids were assayed by the method of Mosbach et al., and biliary cholesterol by a modification of the Sperry-Webb procedure.

Table 1.—Composition of Diets

<table>
<thead>
<tr>
<th>Group</th>
<th>Control (parts)</th>
<th>Gallogen (parts)</th>
<th>Diethanolamine (parts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purina Laboratory Chow (ground)</td>
<td>80.7</td>
<td>80.7</td>
<td>80.7</td>
</tr>
<tr>
<td>Sucrose</td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Sodium cholate</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Thiouracil</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Gallogen*</td>
<td>—</td>
<td>0.2†</td>
<td>—</td>
</tr>
<tr>
<td>Diethanolamine</td>
<td>—</td>
<td>—</td>
<td>0.05†</td>
</tr>
</tbody>
</table>

* Gallogen was used as received. Purity was stated by the manufacturer as >97.5 per cent.
† The concentrations of Gallogen and diethanolamine are equimolecular.
Table 2—Effects of Gallogen and Diethanolamine upon Plasma and Liver Lipid Concentrations

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Gallogen</th>
<th>Diethanolamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>9</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Plasma cholesterol, mg./100 ml.</td>
<td>840 ±70*</td>
<td>1590 ±260</td>
<td>1270 ±160</td>
</tr>
<tr>
<td>Plasma total lipid, mg./100 ml.</td>
<td>1930 ±150</td>
<td>3130 ±360</td>
<td>2080 ±300</td>
</tr>
<tr>
<td>Liver cholesterol, per cent of wet weight</td>
<td>6.50 ±0.37</td>
<td>3.72 ±0.33</td>
<td>3.37 ±0.27</td>
</tr>
<tr>
<td>Liver total lipid, per cent of wet weight</td>
<td>22.6 ±0.5</td>
<td>14.1 ±0.8</td>
<td>13.5 ±0.6</td>
</tr>
<tr>
<td>Body weight, Gm.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>409 ±10</td>
<td>407 ±20</td>
<td>414 ±17</td>
</tr>
<tr>
<td>Final</td>
<td>390 ±7</td>
<td>389 ±14</td>
<td>390 ±14</td>
</tr>
<tr>
<td>Liver weight, per cent of body weight</td>
<td>4.82 ±0.15</td>
<td>5.44 ±0.28</td>
<td>5.42 ±0.17</td>
</tr>
</tbody>
</table>

* The values given in this table are mean ± standard error of the mean.
† The p value in each case signifies the probability, from Fisher's table of t, that the difference between the indicated value and the control value is due to chance.

RESULTS

The effects of Gallogen and diethanolamine feeding upon cholesterol and total lipid concentrations of plasma and liver are shown in table 2. Food consumption of all groups was poor for the first 4 or 5 days, but subsequently rose. Curves of mean weight and mean food consumption were essentially identical for all 3 groups. Initial and final weights are shown in table 2.

The animals fed Gallogen developed substantially greater hypercholesterolemia and hyperlipemia than did the control group. On the other hand, liver cholesterol and total lipid concentrations were substantially lower in the Gallogen-treated animals. In all instances, the results of diethanolamine feeding were concordant with, and in fact indistinguishable from, those observed in the Gallogen-fed group. Not shown in the table are p values for difference between Gallogen and diethanolamine feeding; these are in all instances >0.3.

The results of the experiments on animals with biliary fistulas are shown in table 3. It was not possible, because of spillage, to quantitate fluid consumption. However, employing the daily bile volume as a minimum figure for water intake, it is calculated that the minimum Gallogen dosage was about 120 mg./Kg./day. These animals therefore received a larger dosage than the Gallogen-fed group in table 2, who had a mean intake of 107 mg./Kg./day.

The results summarized in table 3 demonstrate that Gallogen had a small hydrocholeretic effect. There was no apparent effect upon the biliary excretion of cholesterol nor of the two major bile acids.

DISCUSSION

Investigation of a possible hypocholesterolizing effect of Gallogen was initially undertaken by Clarkson et al. because of its supposed choleretic properties. However, the same workers subsequently reported that the drug is only weakly hydrocholeretic in the cockerel, and that it has no effect upon biliary cholesterol excretion in that species. A similar absence of choleretic effect upon oral administration in the rat is demonstrated by the present study.

While a number of other derivatives of 2, p-dimethylbenzyl alcohol have been reported to be choleretic in the rat, there appear to be no published reports on the effects of Gallogen in this species. The present data do not exclude the possibility of a choleretic effect in larger dosage or via another route. It is apparent, however, that the effects of Gallogen upon plasma and liver lipid concentrations observed in these rats are not mediated via alterations in biliary excretion of cholesterol or its major products.

The quantitative similarity of the effects of Gallogen and diethanolamine suggests that the latter moiety is responsible for all the effects of Gallogen observed in these experiments. Studies of the metabolic effects of...
diethanolamine have hitherto been rather limited. Annau and his associates have reported that diethanolamine lowered liver total lipid concentrations in a small group of mice on a stock ration. Annau and his associates have reported that diethanolamine lowered liver total lipid concentrations in a small group of mice on a stock ration.10 Artom et al.11 had earlier reported experiments suggesting that diethanolamine is incorporated into phospholipids in rats. Hepatic synthesis of both cephalins and lecithins was depressed after continued ingestion of diethanolamine, pointing to possible behavior as a metabolic antagonist. Such information suggests that the influence of Gallogen and diethanolamine upon liver lipid concentrations in the present experiments may have been due to lipotropic activity of a diethanolamine-containing phosphatide. Such behavior would be in contrast to the reported absence of lipotropic activity on the part of ethanolamine.12,13 It is perhaps relevant that choline has been found capable of partially preventing accumulations of lipid in livers of cholesterol-fed animals.14

On the other hand, the enhancement of elevated plasma lipid levels by diethanolamine and Gallogen has no obvious physiologic counterpart. One may speculate that this finding might be related in some manner to an anti-metabolic effect of an abnormal (diethanolamine-containing) phospholipid.

The diet employed for the production of hypercholesterolemia, patterned after that described by Fillios et al.,15 was adopted with a view toward establishing a marked degree of plasma cholesterol elevation in order to facilitate demonstration of possible hypocholesterolizing effects of the drugs. It is possible that future experiments with simpler diets may shed some light on the mechanism of the effects observed in this study.

**SUMMARY**

Hypercholesterolemia was induced in rats by feeding a diet containing cholesterol, sodium cholate and thiouracil. Addition to this diet of the diethanolamine salt of the mono (α,β-dimethylbenzyl ester of d-camphoric acid (Gallogen) resulted in higher plasma cholesterol and total lipid concentrations, and lower liver cholesterol and total lipid concentrations. Indistinguishable results were obtained by administration of diethanolamine in place of Gallogen. Oral administration of Gallogen to rats with biliary fistulas resulted in slight hydrocholeresis, but no detectable alteration in biliary excretion of cholesterol, cholic acid, or chenodeoxycholic acid.

**ACKNOWLEDGMENTS**

The valuable assistance of Mrs. Kathleen Houska is gratefully acknowledged. The Gallogen was donated by the S. E. Massengill Co.
Hypercholesterolemia esseva inducite in rattos per dietas continente cholesterol, cholato de natrium, e thiouracil. Le addition del sal diethanolaminic del mono-(a,p-dimethil-benzyl)-ester de acido d-camphoric (Gallogeno) resultava in plus alter concentrationes del cholesterol e del lipidos total in le plasma e in plus basse concentrationes del cholesterol e del lipidos total in le hepate. Le mesme resultatos esseva obtenite per le administration de diethanolamina in loco de Gallogeno. Le administration oral de Gallogeno a rattos con un fistula biliarri resultava in leve grades de hydrocholerese sed in nulle detegibile alteration del excretion biliarri de cholesterol, acido cholic, o acido chenodeoxycholic.

REFERENCES
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