Changes in bile composition during cholesterol gallstone formation were investigated in hamsters. Gallstones were found in the gall-bladder and/or bile duct after feeding on a lithogenic diet for two weeks or more. Treatment with the lithogenic diet caused a marked increase in cholesterol secretion and a significant decrease in bile acid secretion, without affecting phospholipid secretion. The increase in cholesterol secretion was greater in gallstone-present than in gallstone-absent animals. Analysis of bile acids in the bile revealed that cholic acid, a major primary bile acid, was markedly decreased by the lithogenic diet. A decrease in glycine-conjugated bile acids and a relative increase in taurine-conjugated bile acids were also observed. The critical period for gallstone formation produced by the lithogenic diet was one to two weeks after the start of the lithogenic diet. From the above findings, the following mechanisms for cholesterol gallstone formation are suggested. A decrease in bile acids and a marked increase in cholesterol in the bile may result in the reduction of cholesterol solubility in micells consisting of bile acids, phospholipids and cholesterol. Subsequently, insoluble cholesterol forms gallstones. In addition, an alteration of glycine-conjugation capability may also play a part in gallstone formation.

**Keywords**—cholesterol gallstone; bile acids; conjugated bile acid; biliary secretion; lithogenic diet

### INTRODUCTION

Cholesterol is dissolved in the bile, by forming micells with bile acids and phospholipids. The biliary excretion of cholesterol as well as phospholipids is dependent on bile acid concentration secreted into the bile.\(^1\) Admirand and Small\(^2\) explained the saturation phenomenon of cholesterol in the bile using a triangle correlation among concentrations of bile acids, phospholipids and cholesterol. Thereafter, Dam\(^3\) successfully produced cholesterol gallstones (CG) in hamsters experimentally with a lithogenic diet. However, changes in bile composition, particularly cholesterol and bile acid composition, during CG formation were not determined. We investigated the relationship between CG production and changes in bile composition.

### MATERIALS AND METHODS

Male golden hamsters weighing about 50 g

![](https://example.com/fig1.png)

**FIG. 1. Biliary Phospholipid, Bile Acid and Cholesterol Secretions during Cholesterol Gallstone Formation in Hamsters**

Each column represents the mean ± S.E. (mg/100 g/h) for a) 27, b) 7, c) 17, d) 13, e) 12, f) 18 and g) 7 animals.

Significantly different from the zero week value at * \( p < 0.05 \), ** \( p < 0.01 \) and *** \( p < 0.001 \).

- **Gallstone present**: □ **Gallstone absent**.
TABLE I. Changes in Bile Acid Composition during Cholesterol Gallstone Formation

<table>
<thead>
<tr>
<th></th>
<th>CA</th>
<th>DCA</th>
<th>CDCA</th>
<th>LCA</th>
<th>UDCA</th>
<th>Unknown</th>
<th>Total BA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0W</td>
<td>1.10±0.15</td>
<td>0.34±0.07</td>
<td>0.39±0.07</td>
<td>0.07±0.00</td>
<td>0.16±0.00</td>
<td>0.67±0.14</td>
<td>2.72±0.33</td>
</tr>
<tr>
<td>1W</td>
<td>0.35±0.05</td>
<td>0.10±0.02</td>
<td>0.19±0.04</td>
<td>0.05±0.01</td>
<td>0.02±0.00</td>
<td>0.45±0.10</td>
<td>1.13±0.13</td>
</tr>
<tr>
<td>2W</td>
<td>0.25±0.08</td>
<td>0.09±0.01</td>
<td>0.17±0.04</td>
<td>0.04±0.01</td>
<td>0.02±0.01</td>
<td>0.29±0.07</td>
<td>0.85±0.18</td>
</tr>
<tr>
<td>G.P.</td>
<td>0.17±0.03</td>
<td>0.15±0.04</td>
<td>0.17±0.03</td>
<td>0.05±0.01</td>
<td>0.03±0.01</td>
<td>0.39±0.10</td>
<td>0.93±0.17</td>
</tr>
<tr>
<td>G.A.</td>
<td>0.31±0.04</td>
<td>0.11±0.01</td>
<td>0.22±0.05</td>
<td>0.05±0.01</td>
<td>0.04±0.01</td>
<td>0.44±0.11</td>
<td>1.16±0.16</td>
</tr>
<tr>
<td>3W</td>
<td>0.40±0.12</td>
<td>0.15±0.04</td>
<td>0.14±0.03</td>
<td>0.05±0.01</td>
<td>0.06±0.04</td>
<td>0.56±0.16</td>
<td>1.29±0.25</td>
</tr>
<tr>
<td>G.P.</td>
<td>0.39±0.05</td>
<td>0.21±0.03</td>
<td>0.30±0.03</td>
<td>0.08±0.01</td>
<td>0.07±0.01</td>
<td>0.32±0.05</td>
<td>1.36±0.13</td>
</tr>
<tr>
<td>G.A.</td>
<td>0.27±0.08</td>
<td>0.15±0.04</td>
<td>0.23±0.10</td>
<td>0.05±0.01</td>
<td>0.12±0.08</td>
<td>0.31±0.04</td>
<td>1.36±0.33</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E. (mg/100 g/h). The numbers of experiments are as in Fig. 1. Significantly different from the zero week value at a) p < 0.05, b) p < 0.01 and c) p < 0.001.


were fed on the lithogenic diet according to Dam's method. The bile collections were made immediately before and on 1, 2, 3 and 4 weeks after the start of lithogenic feeding. Different animals were used for each bile collection. The animals were anesthetized with pentobarbital Na (40 mg/kg, i.p.). Then, the bile duct was cannulated with a polyethylene tube (PE-10), and a 3-hour bile was collected. Bile samples were immediately analyzed or frozen at -20°C for later analysis. Bile acid concentrations in the bile were measured using gas-liquid chromatography as described previously. Biliary cholesterol and conjugated bile acid concentrations were measured with thin-layer chromatography equipped with a flame ionization detector. Phospholipid concentration was determined with a phospholipid-test kit (Wako Pure Chemicals). The solvent systems used were petroleum ether : ethyl ether : acetic acid (40:20:3) for cholesterol, and chloroform : isopropanol : acetic acid : water (30:30:4:1) for conjugated bile acid determination.

RESULTS AND DISCUSSION

The incidence of gallstone production in hamsters fed on the lithogenic diet was 0% at 1 week, 30% at 2 weeks, 52% at 3 weeks and 72% at 4 weeks after a start of administration. A few gallstones were found in the gall-bladder and/or bile duct. The changes in bile composition are shown in Fig. 1. Biliary phospholipid secretion was not changed from pre-control value (0 week), except in a 2-week lithogenic feeding group. Total bile acid secretion was significantly decreased by lithogenic feeding, independent of

![FIG. 2. Changes in Conjugated Bile Acid Composition during Cholesterol Gallstone Formation in Hamsters](image-url)

Each column represents the mean ± S.E. The numbers of experiments are as in Fig. 1. TT: taurine-conjugated trihydroxy and unknown bile acids, TD: taurine-conjugated dihydroxy bile acids, GT: glycine-conjugated trihydroxy and unknown bile acids, GD: glycine-conjugated dihydroxy bile acids. Significantly different from the zero week value at *p < 0.05, **p < 0.01 and ***p < 0.001.

□ GD, ■ GT, ▲ TD, □ TT.
CG formation. On the other hand, cholesterol secretion increased more dramatically in the animals with CG, the peak value being at a 2-week feeding. The above findings indicate that CG formation may occur by an increase in cholesterol concentration and a decrease in bile acid concentration in the bile. That is, these changes in bile composition may result in a reduction of cholesterol solubility in micells consisted of bile acids, phospholipids and cholesterol. Subsequently, insoluble cholesterol forms gallstones. The critical period for CG formation in hamsters was one to two weeks after a start of lithogenic feeding.

The bile acid composition in the bile fed on the lithogenic diet, is shown in Table I. The normal composition (at 0 week) was cholic acid 40%, deoxycholic acid 13%, chenodeoxycholic acid 14%,ursodeoxycholic acid 6%, lithocholic acid 2.5% and unknown bile acids 24.5%. Cholic acid, a major primary bile acid, was markedly decreased in the animals fed on the lithogenic diet, and chenodeoxycholic acid, another primary bile acid, was also decreased. The decreases in primary bile acids (particularly, cholic acid) would result in a decreased synthesis of the secondary bile acids such as deoxycholic acid and lithocholic acid; the bile acid pool-size, thus, being decreased.5)

In Fig. 2 the percentage of taurine- and glycine-conjugated bile acids is shown. The normal composition of conjugated bile acids (at 0 week) was taurine-conjugated trihydroxy plus unknown bile acids 47%, taurine-conjugated dihydroxy bile acids 35%, glycine-conjugated trihydroxy plus unknown bile acids 12% and glycine-conjugated dihydroxy bile acids 6%. Glycine-conjugated bile acids decreased, but taurine-conjugated bile acids relatively increased in the bile after feeding the lithogenic diet. The CG formation may be caused, in a part, by a reduction of glycine-conjugation capability in the liver.

REFERENCES