The massive type is indefinite and also has no morphological features. Some of the black stones can be seen in this type. As to inorganic components, Ca, S, Cl, P, and Si are observed and regardless of the kinds of gallstones, Ca elements are the richest in all of them. Their distribution tends to be localized in the center. Bilirubin stones have more inorganic components than cholesterol stones. Each element is localized between crystals.

**DISCUSSION**

The developmental mode of gallstone from observations on their morphological features are illustrated in Table 2. Gallstones of the radial type develop directly from the center, but in the subradial type the core is formed at first and then radial development takes place.

These findings suggest the complicated growth formation.

The production route of the porous type is regarded that after formation of the external surface, condensing changes may occur during development to the center, so both outer and inner formation can be supposed. Many changes such as dissolution or recrystallization may take place in this type.

In the poly-core type, many granules are aggregated in the core and this suggests that gallstones of this type develop toward the outer side.

In the concentric type, growth layers are found and it shows that this type is produced from the inner to the outer side.

The massive type may develop to the external side, but because of having no typical features, its formation is obscure.

Many kinds of inorganic components are seen in gallstones and a large quantity of Ca elements are observed in the central region.

Their distributions are localized specifically between crystals. These facts indicate that inorganic components may have an important influence in unstabilization and coagulation of bile colloid and in formation of gallstone.

**SUMMARY**

Morphological classification and developmental mode of gallstones have been demonstrated.

The radial type grows toward the external side. In the porous type, both outer and inner development can be supposed and a complicated mechanism must be taken into consideration.

The poly-core, concentric and radial-concentric types develop from the center to the outer side and the massive type may be produced from the center.

A large quantity of Ca elements are contained in gallstone and they show a specific distribution and are presumed to take part in gallstone formation.

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(4) **Pathogenesis of Cholesterol Gallstone**

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The mechanism of cholesterol gallstone formation is discussed comparing human and alimentarily induced cholelithiasis in squirrel monkeys.

The process of cholesterol gallstone formation can be divided to three phases. 1) host reaction due to predisposition and risk factors 2) production of bile supersaturated...
with cholesterol 3) precipitation of cholesterol out of bile and crystal growth to macroscopic stones.

1) **Predisposition**

Twelve out of 15 squirrel monkeys, which had their gallstone removed by opening but not removing their gallbladder, after 13 months feeding of lithogenic diet, had a recurrence of stones, whereas nine others that were originally free of gallstones on the same diet, remained so. This fact suggests that some monkeys are more susceptible of forming cholesterol gallstone than others.

2) **Effects of diets on the rate of cholesterol gallstone formations and biliary lipid composition**

Many risk factors for gallstone formation have been described. As shown in Table 1, type of dietary composition affected the rate of cholesterol gallstone formations in squirrel monkeys. In the chow diet group there has been no occurrence of gallstones, whereas the group with the diet containing butter and cholesterol had high rate. Adding cholesterol to the chow diet resulted in the occurrence of gallstones, and the lithogenic diet with butter and cholesterol minus cholesterol could not lead to gallstone formation. Therefore, extragenous cholesterol is the responsible factor in the dietarily induced gallstone in the squirrel monkeys.

Biliary lipid composition, especially cholesterol molar percent relative to lecithin plus bile acids was found to be affected by the type of dietary composition in the squirrel monkeys, and the chow diet group with no stone had the lowest molar percent of cholesterol of gallbladder bile among various dietary groups.

Lithogenic diet group with butter and cholesterol had the increased and highest molar percent of cholesterol of bile and monkeys which exceeded the micelle zone of Admirand's triangle developed gallstones. Clearly type of diet affects the biliary lipid composition and excess of cholesterol in bile leads to gallstone formation.

3) **Turnover kinetics of bile acids**

The pool size of cholic acid was much greater in monkeys on a commercial chow diet than in any other group of semipurified diet. It was also greater in monkeys on a lithogenic diet but without gallstones than in monkeys from the same diet group with gallstones. The half-lives of cholic acid tended to be proportional to pool size, and absolute rates of cholic acid synthesis were, therefore, not much affected by diet. Dietary cholesterol increased concentrations of cholesterol relative to bile acids and phospholipids in hepatic bile as well as absolute secretory rates of all three components (Table 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>No. and sex of animals</th>
<th>Diet</th>
<th>Gallstones overall*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5, F</td>
<td>Purina Monkey Chow-25 + vitamin supplement + mashed banana</td>
<td>0/37</td>
</tr>
<tr>
<td>B</td>
<td>5, F</td>
<td>Same as A + butter (25%) and cholesterol (0.45%)</td>
<td>3/5</td>
</tr>
<tr>
<td>C</td>
<td>5, F</td>
<td>Semipurified diet with corn oil (8%)</td>
<td>9/36</td>
</tr>
<tr>
<td>D</td>
<td>5, F; 2, M</td>
<td>Semipurified diet with butter (19.5%), corn oil (0.7%), and cholesterol (0.45%)</td>
<td>30/56</td>
</tr>
<tr>
<td>E</td>
<td>3, F; 2, M</td>
<td>Same as D</td>
<td>30/56</td>
</tr>
<tr>
<td>F</td>
<td>3, F; 3, M</td>
<td>Same as D but without cholesterol</td>
<td>0/9</td>
</tr>
</tbody>
</table>

*Animals with gallstones/total animals.
Table 2. Constants for bile acid metabolism according to diet group.

<table>
<thead>
<tr>
<th>Group and diet</th>
<th>Cholic acid</th>
<th>Chenodeoxycholic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pool (mg./kg.)</td>
<td>Half-life (days)</td>
</tr>
<tr>
<td>A-Chow</td>
<td>86.0 ± 12.3*</td>
<td>4.18 ± 0.57</td>
</tr>
<tr>
<td>B-Chow + butter and cholesterol</td>
<td>57.4 ± 11.4</td>
<td>3.32 ± 0.28</td>
</tr>
<tr>
<td>C-Low corn oil</td>
<td>25.7 ± 6.0</td>
<td>2.16 ± 0.64</td>
</tr>
<tr>
<td>D-Butter and chol†</td>
<td>42.5 ± 7.4</td>
<td>1.87 ± 0.28</td>
</tr>
<tr>
<td>E-Butter and chol†</td>
<td>26.1 ± 3.7</td>
<td>1.38 ± 0.28</td>
</tr>
<tr>
<td>F-Butter</td>
<td>28.7 ± 2.0</td>
<td>3.00 ± 0.53</td>
</tr>
</tbody>
</table>

*Means ± S.E.M.
†Animals of Groups D and E were fed the same semipurified diet with butter and cholesterol (chol). The animals of Group E had stones.

4) Interruption of enterohepatic circulation (EHC)

Interruption of EHC of bile acids is another factor which affects the biliary lipid composition. External bile fistula increased cholesterol molar percent in hepatic bile both in humans and squirrel monkeys. EHC interruption in fasted monkeys with gallstone resulted in more rapid and marked increase in relative cholesterol concentration than occurred for monkeys without gallstones (Fig. 1). This was due to the low proportion of the bile acid pool outside the gallbladder and the low rate of new bile acid synthesis of the monkeys with gallstones during fasting. Recycling frequency of bile acid was increased in stone forming monkeys.

5) Lithogenic (supersaturated with cholesterol) bile production

Hepatic HMG-Co A reductase was increased and cholesterol 7α-hydroxylase was decreased in mice fed lithogenic diet which did not contain cholesterol as reported in the patients with cholelithiasis. All aforementioned data indicates that hepatic cholesterol synthesis and its secretion is increased in model cholelithiasis. By contrast, hepatic bile acid synthesis and secretion into hepatic bile is decreased as well as pool size, especially existing outside of gallbladder. Moreover, de novo synthesis of bile acids is decreased. Therefore, secreted bile from the liver of stone formers is supersaturated with cholesterol.
6) Growth of cholesterol crystal to macroscopic stones

Degree of cholesterol supersaturation in bile rich duodenal juice, hepatic and gallbladder bile of Japanese male and female without gallstone was lower than the value reported in Caucasians. There was no clear-cut distinction between normal subjects and patients with cholelithiasis with reference to cholesterol molar percent of bile. Calcium was often shown in the central core of the cut-surface of human stones, whereas no calcium was detected usually both in the central and peripheral area of the stones of squirrel monkeys when checked by X-ray microanalyzer. There is a possibility that the gallstone formation might occur due to the heterogenous nucleation in humans because of the relatively low degree of cholesterol supersaturation in bile, whereas the high supersaturation might lead to gallstone formation by means of the homogenous nucleation in monkeys. Postulated sequences of cholesterol crystal development to macroscopic stones were quite similar in both species, when their bile were observed with electron scanning microscope.

7) Role of gallbladder

Gallbladder is important for gallstone formation, since cholesterol stones form in gallbladder and removal of gallbladder prevents the recurrence. The gallbladder also provides favorable environment to crystal growth. Through its influences on EHC, gallbladder affects the biliary lipid composition. Viscous bile provides a similar environment to crystal growth in gel, including the factors such as supplying cholesterol of hepatic bile over the concentrated and stratified gallbladder bile, control of nucleation, adequate shaking and temperature.

8) Summary

For the cholesterol gallstone formation liver plays an major role through predisposition, diet, metabolic disturbances such as diabetes mellitus or hyperparathyroidism and drugs such as clofibrate and produces hepatic bile supersaturated with cholesterol. Gallbladder is also important through favorable environment to crystal growth and influence of EHC interruption. In each individual cases etiology may depend on complicated mixture of factors related to liver and gallbladder.

This work was done by the collaboration with Dr. Oscar W Portman, Oregon Regional Primate Research Center, USA

REFERENCES