Alimentary production of gallstones in hamsters.
26. The influence of orally ingested lithocholic, cholic, dehydrocholic and deoxycholic acids on gallstone production compared with the influence of chenodeoxycholic acid

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With 23 tables

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In the hamster, chenodeoxycholic acid ingested at the level of 0.1% of the diet markedly intensifies production of cholesterol gallstones (1), whereas hyodeoxycholic acid ingested at the same level markedly inhibits formation of this type of gallstones (1, 2) but induces other anomalies of the bile (1).

The first study from this laboratory on the influence of bile acids on production of gallstones in the hamster (2) included besides hyodeoxycholic acid also lithocholic, cholic, dehydrocholic and deoxycholic acids, but the production of cholesterol gallstones in the control group of hamsters receiving the basal diet without addition was so marked that a possible intensifying effect of certain of the four last-mentioned bile acids would have gone unnoticed. Furthermore, an intensifying effect was not anticipated at the time when the study was carried out.

It is, therefore, of interest to retest the effects of lithocholic, cholic, dehydrocholic and deoxycholic acids under circumstances in which production of cholesterol gallstones in the control group is moderate or absent. Of particular interest is the question whether a possible intensifying effect of lithocholic acid on gallstone production is more marked than the effect of chenodeoxycholic acid, since it could be thought that chenodeoxycholic acid exerts its effect on gallstone production through lithocholic acid formed from chenodeoxycholic acid by bacteria in the intestine.

Experimental

Bile acids

Chenodeoxycholic acid, lithocholic acid, cholic acid and deoxycholic acid were obtained from Weddel Pharmaceuticals Ltd., London. Dehydrocholic acid was obtained from Nutritional Biochemicals, Cleveland, Ohio.

Samples of 50 microgram of each of the bile acids were examined for impurities by thin layer chromatography; solvent system benzene: ethanol:glacial acetic acid, 30:10:2 (v:v:v). The samples of chenodeoxycholic and deoxycholic
acids showed an extra spot of relatively small size migrating slower than the pure compounds (and probably representing cholic acid). Lithocholic acid, dehydrocholic acid and cholic acid showed, practically, only one spot.

**Experimental diets**

In the tests for influence on gallstone production, the bile acids were added at the 0.1% level to the basal diet indicated in table 1.

<table>
<thead>
<tr>
<th>Tab. 1. Composition of the basal diet.</th>
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<tr>
<td>Casein&lt;sup&gt;1&lt;/sup&gt;</td>
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<tr>
<td>Glucose</td>
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<tr>
<td>Rice starch</td>
</tr>
<tr>
<td>Salt mixture&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin mixture&lt;sup&gt;3&lt;/sup&gt;</td>
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<tr>
<td>Choline chloride</td>
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<td>100.00 g</td>
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<sup>1</sup> “Dairinex”, from Dansk Mejeri Industri & Export Kompagni, Stege, (Denmark.)

<sup>2</sup> The salt mixture indicated in reference 3.

<sup>3</sup> The vitamin mixture indicated in reference 3.

The essential feature of this diet is that the carbohydrate, instead of being all glucose, is represented by equal parts of glucose and rice starch, whereby the ability of the unsupplemented diet to produce cholesterol gallstones is substantially reduced.

**Animals, housing and treatment**

The hamsters were newly weaned from our stock colony. During the experimental feeding they were housed in wire screen cages, two of the same sex in one cage. Diet and tap water were available ad libitum. At the end of the feeding period the animals were killed with chloroform, autopsied and inspected for gallstones as previously described, the type of gallstones being determined by the aid of dissecting and polarizing microscopes. The livers of all the animals and the testes of the males were weighed, and the livers stored at minus 20°C, individually wrapped in aluminium foil for later chemical examination.

Two experiments were carried out.

In the first experiment the effects of chenodeoxycholic acid and lithocholic acid were compared, using 27 male and 30 female hamsters, 33–38 days old at beginning of the feeding period, which lasted 42–43 days.

In the second experiment the effects of cholic acid, dehydrocholic acid and deoxycholic acid were compared with the effect of chenodeoxycholic acid, using 55 male and 47 female hamsters, 30–35 days old at beginning of the feeding which likewise lasted 42–43 days.

**The stock diet**

Due to certain practical circumstances, the stock diet to which the hamsters had access before beginning of the experimental feeding, was not the same in the two experiments. The hamsters used in the first experiment had been reared on the stock diet, which was in use in our hamster colony since January 1964,