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# Alimentary Production of Gallstones in Hamsters

## 14. Relation between Diet and Composition of Bladder Bile. 2.*)**)

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With 11 tables

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In a previous communication (1) we reported analyses ($p_H$, cholesterol, bile acids and lipid-soluble phosphorus) of bladder bile of young hamsters reared for various lengths of time (until about 100 days) on diets with known influence on gallstone formation in young individuals of this species.

The general trend of the analytical data showed a fair parallelism to the incidence of cholesterol gallstones in the groups fed the various diets. Compared with a fat-free diet having glucose as the sole carbohydrate, a similar diet with rice starch as carbohydrate caused a marked rise of the ratio total bile acids/cholesterol, whereas the "curative diet", capable of dissolving already developed cholesterol gallstones in the animal, greatly increased both the ratio total bile acids/cholesterol and the ratio lipid-soluble phosphorus/cholesterol. Further, in hamsters transferred to the fat-free glucose diet at a more advanced age the two ratios were found to be higher than in young hamsters transferred to the same diet, corresponding to the lesser tendency for the older hamsters to development of cholesterol gallstones.

The present work deals with the composition of bladder bile of young hamsters reared (from the age of 30–44 days) for 6 to 5 weeks on the following diets[1]):

1. A fat-free basal diet containing 20% casein, 62.3% glucose and 12% rice starch as protein and carbohydrate components, producing a high incidence of cholesterol gallstones in young hamsters (2).
2. The basal diet in which 3% glucose is replaced by 3% filtrol-treated cod liver oil which suppresses the formation of cholesterol gallstones and favors development of amorphous pigmented gallstones (2).
3. The basal diet supplemented with certain trace elements (Cu, Mn, I) which apparently do not alter the tendency to formation of cholesterol gallstones (2).
4. The stock diet (3) hitherto used for our breeding colony of hamsters. The stock diet prevents formation of cholesterol gallstones but does not completely prevent formation of amorphous pigmented gallstones.

Further, the present work deals with the changes of the ratio lipid-soluble phosphorus/cholesterol resulting from replacing the 10% lard in the "curative

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[1]) Before start of the experimental feeding, all the hamsters had access to the stock diet plus water-milk (1:1).

diet" by rice starch, and by introducing 20% lard into a diet with rice starch as the sole carbohydrate at the expense of rice starch.

## Experimental

Housing and care of the animals, sampling of bile and analytical procedures were as previously described (1, 2, 3, 4).

Animals from the experimental series G 96 and G 94 in our study (2) were used for that part of the present work which deals with the influence of filtrol-treated cod liver oil and of supplementation with Cu, Mn and I. The composition of the respective diets as well as the filtrol treatment of the cod liver oil and its purpose are described in (2). It was not possible to obtain bile from all the surviving animals in the groups, and in some cases it was necessary to pool bile from several animals in order to get enough material for determination of five fractions of bile acids besides cholesterol and lipid-soluble phosphorus. Pooling of bile was also necessary in the group fed the stock diet.

In the groups fed the "curative diet" with and without lard and in the groups fed the rice starch diet with and without lard, the bile analyses were limited to cholesterol and lipid-soluble phosphorus, whereby it was possible to carry out the determination on individual bile samples, except in one case.

Since in our previous study (1) $p_H$ of the bladder bile seemed to be essentially the same on all the diets examined, $p_H$ was left out of consideration in the present work.

## Results and Discussion

The results are presented in tables 1–11.

In series G 96 (Tables 1a, 1b, 2, 3) it is seen that the addition of 3% filtrol-treated cod liver oil has caused an increase of the ratio total bile acids/cholesterol in both sexes. The increase is assumed to be significant ($P < 0.001$ for the females; $P < 0.01$ for the males[1]), but the value obtained for this ratio is not as high as that found in our previous study (1) with the "curative" diet or with the rice starch diet.

The addition of 3% filtrol-treated cod liver oil has also increased the ratio lipid-soluble phosphorus/cholesterol ($P < 0.001$ for both sexes), although not to the level obtained with the curative diet.

The change of the two ratios caused by the filtrol-treated cod liver oil favors solubilization of cholesterol.

The change of the ratio dihydroxycholanic acids/trihydroxycholanic acids goes in the opposite direction. The dihydroxycholanic acids are generally assumed to be better solubilizers for cholesterol than cholic acid is. But the ratio is decreased by filtrol-treated cod liver oil (although with less significance: $P < 0.05$ for the females, $P < 0.01$ for the males).

The ratio glycine/taurine seems to be lowered by filtrol-treated cod liver oil, but the significance of the observed difference is low ($P < 0.2$ for the females, $P < 0.01$ for the males).

The filtrol-treated cod liver oil seems to lower the cholesterol concentration in both sexes ($P < 0.05$), the lowest value being reached in the females.

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[1]) An exact evaluation of the significance of differences between corresponding mean values is complicated by the fact that some of the analyses were carried out on pooled samples of bile. In order to get at least a rough estimate, probabilities are calculated as if all the single determinations had the same weight.

*Table 1a.* Data for bladder bile of female hamsters reared on the *fat-free basal diet* from the age of 33–39 days (Experimental series G 96). All concentrations are given in millimolarities

| Group no. hamster no. | Type of stone | Days on diet | Chole-sterol | Lipid phos-phorus | GC | GCD | GD | TC | TCD + TD | Total bile acids | G | T | Tri | Di | G/T | Di/Tri | Total bile acids/ cholest. | Lipid phosphor./ cholest. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 930/135 | o | 43 | 6.3 | 16.9 | 41.6 | 16.7 | 19.4 | 3.7 | 8.2 | 89.6 | 77.7 | 11.9 | 45.3 | 44.3 | 6.5 | 1.0 | 14.2 | 2.7 |
| 933/133 | m | 43 | 1.0 | 3.7 | 3.9 | 1.2 | 6.6 | 1.2 | 2.2 | 15.1 | 11.7 | 3.4 | 5.1 | 10.3 | 3.4 | 2.0 | 15.1 | 3.7 |
| 934/ 56<br>934/ 58 | a, c<br>m | 43 | 5.2 | 16.0 | 33.2 | 12.2 | 20.5 | 8.4 | 10.2 | 84.5 | 65.9 | 18.6 | 41.5 | 42.9 | 3.5 | 1.0 | 16.3 | 3.1 |
| 930/ 82 | c | 43 | 3.3 | 8.9 | 7.9 | 7.5 | 14.7 | 1.7 | 2.1 | 33.9 | 30.1 | 3.8 | 9.6 | 24.3 | 7.9 | 2.5 | 10.3 | 2.7 |
| 931/ 98<br>934/ 70 | o<br>o | 43 | 4.7 | 17.4 | 34.6 | 21.5 | 22.5 | 7.0 | 12.9 | 98.5 | 78.6 | 19.9 | 41.6 | 56.9 | 3.9 | 1.4 | 21.0 | 3.7 |
| 934/ 49 | c, m | 45 | 4.5 | 25.2 | 54.3 | 18.8 | 44.9 | 7.4 | 13.4 | 138.8 | 118.0 | 20.8 | 61.7 | 77.1 | 5.7 | 1.2 | 30.8 | 5.6 |
| 936/ 18 | c | 45 | 8.8 | 23.1 | 47.2 | 11.2 | 47.2 | 3.8 | 9.7 | 119.1 | 105.6 | 13.5 | 51.0 | 68.1 | 7.8 | 1.3 | 13.5 | 2.6 |
| | | 43–45 | 4.8[7]<br>± 0.9 | 15.9[7]<br>± 2.8 | 31.8[7] | 12.7[7] | 25.1[7] | 4.7[7] | 8.4[7] | 82.8[7]<br>± 16.7 | 69.7[7] | 13.1[7] | 36.5[7] | 46.2[7] | 5.5[7]<br>± 0.7 | 1.5[7]<br>± 0.2 | 17.3[7]<br>± 2.5 | 3.4[7]<br>± 0.4 |

GC = Glycocholic acid
GCD = Glycochenodeoxycholic acid
GD = Glycodeoxycholic acid
TC = Taurocholic acid
TCD + TD = Taurochenodeoxycholic acid + taurodeoxycholic acid
G = Glycine conjugated bile acids
T = Taurine conjugated bile acids
Tri = Trihydroxycholanic acids = Glycocholic acid + taurocholic acid
Di = Dihydroxycholanic acids = glycochenodeoxycholic acid + glycodeoxycholic acid + taurochenodeoxycholic acid + taurodeoxycholic acid.

c = cholesterol gallstones
a = amorphous pigmented gallstones
m = mixed stones
c, a = c and a occurring together in the same animal
c, m, a = c, m and a *occurring* together in the same animal
o = no gallstones

*Table 1b.* Data for bladder bile of male hamsters reared on the *fat-free basal diet* from the age of 33–39 days (Experimental series G 96). All concentrations are given in millimolarities.

| Group no., hamster no. | Type of stone | Days on diet | Chole-sterol | Lipid phos-phorus | GC | GCD | GD | TC | TCD + TD | Total bile acids | G | T | Tri | Di | G/T | Di/Tri | Total bile acids/ cholest. | Lipid phosphor. cholest. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 930/ 80<br>930/ 89 | o<br>o | 42 | 4.6 | 9.4 | 19.2 | 9.1 | 36.6 | 2.0 | 5.8 | 72.7 | 64.9 | 7.8 | 21.2 | 51.5 | 8.3 | 2.4 | 15.8 | 2.0 |
| 930/ 95<br>930/ 93<br>931/ 71<br>931/105 | c<br>c<br>c<br>c | 42 | 5.8 | 14.1 | 29.9 | 14.5 | 33.3 | 4.0 | 8.2 | 89.9 | 77.7 | 12.2 | 33.9 | 56.0 | 6.4 | 1.7 | 15.5 | 2.4 |
| 931/ 55 | c | 42 | 4.7 | 15.0 | 21.1 | 8.1 | 22.7 | 5.7 | 11.1 | 68.7 | 51.9 | 16.8 | 26.8 | 41.9 | 3.1 | 1.6 | 14.6 | 3.2 |
| 932/ 40<br>932/ 72 | c<br>c | 42 | 4.0 | 6.7 | 9.7 | 5.6 | 9.2 | 1.2 | 0.8 | 26.5 | 24.5 | 2.0 | 10.9 | 15.6 | 12.3 | 1.4 | 6.6 | 1.7 |
| 932/ 63 | o | 42 | 2.6 | 6.3 | 13.8 | 8.5 | 19.3 | 2.2 | 4.1 | 47.9 | 41.6 | 6.3 | 16.0 | 31.9 | 6.6 | 2.0 | 18.4 | 2.4 |
| 933/ 3<br>933/ 28 | c<br>c | 44 | 7.6 | 25.2 | 48.3 | 38.4 | 23.5 | 7.8 | 17.6 | 135.6 | 110.2 | 25.4 | 56.1 | 79.5 | 4.3 | 1.4 | 17.8 | 3.3 |
| 935/ 1<br>935/ 16<br>935/136 | c<br>c<br>c | 44 | 3.9 | 14.7 | 46.2 | 22.1 | 24.0 | 12.3 | 15.0 | 119.6 | 92.3 | 27.3 | 58.5 | 61.1 | 3.4 | 1.0 | 30.7 | 3.8 |
| 935/ 53 | o | 44 | 8.3 | 16.8 | 18.9 | 21.2 | 28.0 | 4.5 | 10.5 | 83.1 | 68.1 | 15.0 | 23.4 | 59.7 | 4.5 | 2.6 | 10.2 | 2.0 |
| 936/ 96 | c | 44 | 2.9 | 8.8 | 15.7 | 6.4 | 30.7 | 1.7 | 4.8 | 59.3 | 52.8 | 6.5 | 17.4 | 41.9 | 8.1 | 2.4 | 20.4 | 3.1 |
| 936/ 54 | c, m, a | 44 | 9.1 | 22.3 | 25.0 | 18.9 | 39.8 | 3.6 | 7.5 | 94.8 | 83.7 | 11.1 | 28.6 | 66.2 | 7.5 | 2.3 | 10.4 | 2.5 |
| 934/ 75<br>936/145 | c,m, a<br>c, a | 44 | 8.1 | 32.8 | 26.2 | 17.2 | 11.3 | 23.9 | 28.3 | 106.9 | 54.7 | 52.2 | 50.1 | 56.8 | 1.0 | 1.1 | 13.4 | 4.0 |
| | | 42-44 | 5.6[11] ± 0.7 | 15.6[11] ± 2.4 | 24.9[11] | 15.4[11] | 25.3[11] | 6.3[11] | 10.3[11] | 82.2[11] ± 9.6 | 65.7[11] | 16.6[11] | 31.2[11] | 51.1[11] | 5.9[11] ± 0.9 | 1.8[11] ± 0.2 | 15.8[11] ± 1.9 | 2.8[11] ± 0.3 |

*Table 2.* Data for female hamsters reared from the age of 33–39 days on the *basal diet* in which *3% glucose was replaced by 3% filtrol-treated cod liver oil* (Experimental series G 96). All concentrations are given in millimolarities

| Group no. hamster no. | Type of stone | Days on diet | Cholesterol | Lipid phosphorus | GC | GCD | GD | TC | TCD + TD | Total bile acids | G | T | Tri | Di | G/T | Di/Tri | Total bile acids/ cholest. | Lipid phosphor./ cholest. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 937/ 32 | o | 43 | 1.7 | 18.4 | 47.6 | 22.0 | 6.7 | 18.2 | 9.0 | 103.5 | 76.3 | 27.2 | 65.8 | 37.7 | 2.8 | 0.6 | 60.9 | 10.8 |
| 937/ 91 | a | 43 | 1.6 | 11.4 | 20.7 | 21.7 | 7.4 | 5.2 | 5.8 | 60.8 | 49.8 | 11.0 | 25.9 | 34.9 | 4.5 | 1.3 | 38.0 | 7.1 |
| 937/100 | a | 43 | 3.5 | 19.6 | 59.0 | 48.0 | 13.8 | 5.4 | 4.4 | 130.6 | 120.8 | 9.8 | 64.4 | 66.2 | 12.3 | 1.0 | 37.3 | 5.6 |
| 937/ 81<br>938/108 | a<br>a | 43 | 3.7 | 21.8 | 42.3 | 15.7 | 13.4 | 15.4 | 10.9 | 97.7 | 71.4 | 26.3 | 57.7 | 40.0 | 2.7 | 0.7 | 26.4 | 5.9 |
| 938/138 | o | 43 | 1.8 | 7.8 | 11.6 | 15.6 | 4.6 | 3.2 | 4.7 | 39.7 | 31.8 | 7.9 | 14.8 | 24.9 | 4.0 | 1.7 | 22.1 | 4.3 |
| 939/ 57 | o | 43 | 3.3 | 19.5 | 51.3 | 17.7 | 22.2 | 8.6 | 5.4 | 105.2 | 91.2 | 14.0 | 59.9 | 45.3 | 6.5 | 0.8 | 31.9 | 5.9 |
| 938/ 78<br>939/ 61 | o<br>o | 43 | 3.4 | 13.4 | 45.9 | 33.5 | 11.9 | 7.8 | 8.1 | 107.2 | 91.3 | 15.9 | 53.7 | 53.5 | 5.7 | 1.0 | 31.5 | 3.9 |
| 940/ 17 | a | 45 | 2.5 | 10.0 | 22.1 | 7.9 | 9.4 | 9.2 | 8.0 | 56.6 | 39.4 | 17.2 | 31.3 | 25.3 | 2.3 | 0.8 | 22.6 | 4.0 |
| 940/ 39 | a | 45 | 4.2 | 19.7 | 31.8 | 17.8 | 16.9 | 10.5 | 9.7 | 86.7 | 66.5 | 20.2 | 42.3 | 44.4 | 3.3 | 1.0 | 20.6 | 4.7 |
| 942/120 | a | 45 | 2.4 | 14.2 | 25.4 | 16.0 | 6.9 | 12.6 | 14.4 | 75.3 | 48.3 | 27.0 | 38.0 | 37.3 | 1.8 | 1.0 | 31.4 | 5.9 |
| 942/115 | o | 45 | 2.3 | 9.2 | 19.2 | 7.9 | 29.8 | 2.0 | 4.6 | 63.5 | 56.9 | 6.6 | 21.2 | 42.3 | 8.6 | 2.0 | 27.6 | 4.0 |
| 943/ 94 | o | 45 | 1.1 | 5.7 | 8.3 | 3.9 | 3.0 | 4.1 | 4.7 | 24.0 | 15.2 | 8.8 | 12.4 | 11.6 | 1.7 | 0.9 | 21.8 | 5.2 |
| 943/114 | a | 45 | 4.2 | 20.5 | 53.0 | 18.2 | 28.5 | 14.1 | 6.9 | 120.7 | 99.7 | 21.0 | 67.1 | 53.6 | 4.7 | 0.8 | 28.7 | 4.9 |
| 943/ 69 | o | 45 | 2.7 | 18.8 | 41.2 | 13.0 | 12.9 | 16.5 | 11.6 | 95.2 | 67.1 | 28.1 | 57.7 | 37.5 | 2.4 | 0.7 | 35.3 | 7.0 |
| 942/ 97 | o | 45 | 2.7 | 17.4 | 39.4 | 24.7 | 12.2 | 10.7 | 12.6 | 99.6 | 76.3 | 23.3 | 50.1 | 49.5 | 3.3 | 1.0 | 36.9 | 6.4 |
| 940/ 36<br>943/ 83 | a<br>a | 45 | 3.1 | 23.9 | 56.1 | 25.1 | 15.2 | 14.4 | 12.1 | 122.9 | 96.4 | 26.5 | 70.5 | 52.4 | 3.6 | 0.7 | 39.6 | 7.7 |
| 942/ 66 | o | 45 | 2.5 | 14.5 | 35.6 | 23.1 | 8.0 | 7.8 | 11.9 | 86.4 | 66.7 | 19.7 | 43.4 | 43.0 | 3.4 | 1.0 | 34.6 | 5.8 |
| | | 43–45 | $2.8^{17}$ ± 0.2 | $15.7^{17}$ ± 0.4 | $35.9^{17}$ | $19.5^{17}$ | $13.1^{17}$ | $9.7^{17}$ | $8.5^{17}$ | $86.8^{17}$ ± 7.2 | $68.5^{17}$ | $18.3^{17}$ | $45.6^{17}$ | $41.1^{17}$ | $4.3^{17}$ ± 0.2 | $1.0^{17}$ ± 0.1 | $32.2^{17}$ ± 2.4 | $5.8^{17}$ ± 0.4 |

*Table 3.* Data for male hamsters reared from the age of 33–39 days on the *basal diet* in which 3% *glucose was replaced by 3% filtrol-treated cod liver oil* (Experimental series G 96). All concentrations are given as millimolarities

| Group no. hamster no. | Type of stone | Days on diet | Chole-sterol | Lipid phos-phorus | GC | GCD | GD | TC | TCD + TD | Total bile acids | G | T | Tri | Di | G/T | Di/Tri | Total bile acids/ cholest. | Lipid phosphor./ cholest. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 937/ 4 | o | 42 | 4.5 | 23.0 | 33.9 | 26.1 | 14.5 | 10.0 | 8.4 | 92.9 | 74.5 | 18.4 | 43.9 | 49.0 | 4.0 | 1.1 | 20.6 | 5.1 |
| 937/132 | o | 42 | 5.5 | 24.6 | 38.9 | 20.2 | 22.1 | 15.0 | 21.4 | 117.6 | 81.2 | 36.4 | 53.9 | 63.7 | 2.2 | 1.2 | 21.4 | 4.5 |
| 937/113<br>938/ 90 | o<br>o | 42 | 5.6 | 19.1 | 28.4 | 11.9 | 8.5 | 23.9 | 15.8 | 88.5 | 48.8 | 39.7 | 52.3 | 36.2 | 1.2 | 0.7 | 15.8 | 3.4 |
| 937/134<br>938/125 | o<br>o | 42 | 4.9 | 30.9 | 53.9 | 21.9 | 16.5 | 31.6 | 23.3 | 147.2 | 92.3 | 54.9 | 85.5 | 61.7 | 1.7 | 0.7 | 30.0 | 8.0 |
| 938/111<br>939/ 26 | o<br>o | 42 | 4.3 | 25.5 | 45.1 | 24.3 | 14.4 | 18.1 | 18.4 | 120.3 | 83.8 | 36.5 | 63.2 | 57.1 | 2.2 | 0.9 | 28.0 | 5.9 |
| 939/ 59 | a | 42 | 3.6 | 25.2 | 25.1 | 31.0 | 5.9 | 9.6 | 24.4 | 96.0 | 62.0 | 34.0 | 34.7 | 61.3 | 1.8 | 1.7 | 26.7 | 7.0 |
| 939/ 10 | o | 42 | 1.5 | 11.5 | 13.1 | 8.3 | 6.3 | 5.8 | 7.2 | 40.7 | 27.7 | 13.0 | 18.9 | 21.8 | 2.1 | 1.2 | 27.1 | 7.7 |
| 939/ 92<br>940/ 7 | o<br>o | 44 | 4.6 | 18.7 | 28.7 | 29.3 | 11.7 | 6.7 | 5.9 | 82.3 | 69.7 | 12.6 | 35.4 | 46.9 | 5.5 | 1.3 | 19.5 | 4.1 |
| 940/ 85 | o | 44 | 3.1 | 13.6 | 21.0 | 16.5 | 9.6 | 6.1 | 6.9 | 60.1 | 47.1 | 13.0 | 27.1 | 33.0 | 3.6 | 1.3 | 19.4 | 4.4 |
| 940/ 19<br>940/140 | o<br>o | 44 | 2.6 | 13.7 | 18.8 | 12.5 | 9.4 | 4.4 | 5.4 | 50.5 | 40.7 | 9.8 | 23.2 | 27.3 | 4.2 | 1.2 | 19.4 | 5.3 |
| 940/141 | o | 44 | 3.4 | 15.4 | 23.7 | 13.3 | 19.7 | 4.4 | 5.0 | 66.1 | 56.7 | 9.4 | 28.1 | 38.0 | 6.0 | 1.4 | 19.4 | 4.5 |
| 942/ 23 | o | 44 | 3.3 | 16.1 | 29.2 | 10.5 | 13.9 | 10.9 | 9.2 | 73.7 | 53.6 | 20.1 | 40.1 | 33.6 | 2.7 | 0.8 | 22.3 | 4.9 |
| 942/102<br>942/ 8 | o<br>o | 44 | 3.7 | 12.5 | 20.7 | 10.3 | 6.3 | 27.8 | 24.2 | 89.3 | 37.3 | 52.0 | 48.5 | 40.8 | 0.7 | 0.9 | 24.1 | 3.4 |
| 943/131 | o | 44 | 4.3 | 22.1 | 29.0 | 30.7 | 13.4 | 14.0 | 21.6 | 108.7 | 73.1 | 35.6 | 43.0 | 65.7 | 2.1 | 1.5 | 25.3 | 5.1 |
| 939/ 29<br>943/144 | a<br>a | 44 | 4.3 | 23.3 | 57.3 | 47.6 | 7.6 | 15.3 | 20.7 | 148.5 | 112.5 | 56.0 | 72.6 | 75.9 | 3.1 | 1.0 | 34.5 | 5.4 |
| | | 42-44 | $3.9^{15}$<br>± 0.3 | $20.3^{15}$<br>± 2.4 | $31.1^{15}$ | $21.0^{15}$ | $12.0^{15}$ | $13.6^{15}$ | $14.5^{15}$ | $92.2^{15}$<br>± 8.4 | $64.1^{15}$ | $28.1^{15}$ | $44.7^{15}$ | $47.5^{15}$ | $2.9^{15}$<br>± 0.4 | $1.1^{15}$<br>± 0.1 | $23.6^{15}$<br>± 1.1 | $5.2^{15}$<br>± 0.4 |

*Table 4.* Data for bladder bile from hamsters reared on the *fat-free basal diet* from the age of 39–44 days (Experimental series G 94). All concentrations are given as millimolarities

| Group no. hamster no. | Type of stone | Days on diet | Cholesterol | Lipid phosphorus | GC | GCD | GD | TC | TCD + TD | Total bile acids | G | T | Tri | Di | G/T | Di/Tri | Total bile acids/ cholest. | Lipid phosphor./ cholest. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *females:* | | | | | | | | | | | | | | | | | | |
| 897/ 74 | o | 44 | 2.8 | 10.9 | 15.7 | 12.5 | 32.8 | 0.8 | 3.9 | 65.7 | 61.0 | 4.7 | 16.5 | 49.2 | 13.0 | 3.0 | 23.5 | 3.9 |
| 897/ 17 | c, m | | | | | | | | | | | | | | | | | |
| 897/ 37 | c | 44 | 5.0 | 19.2 | 42.5 | 44.4 | 23.6 | 3.9 | 14.7 | 129.1 | 110.5 | 18.6 | 46.4 | 82.7 | 5.9 | 1.8 | 25.8 | 3.8 |
| 897/ 41 | c | | | | | | | | | | | | | | | | | |
| 898/ 78 | c | 44 | 4.1 | 10.4 | 13.7 | 7.0 | 10.7 | 2.8 | 7.2 | 41.4 | 31.4 | 10.0 | 16.5 | 24.9 | 3.1 | 1.5 | 10.1 | 2.5 |
| 900/ 86 | c | 44 | 3.3 | 14.8 | 52.0 | 45.8 | 34.3 | 3.0 | 11.7 | 146.8 | 132.1 | 14.7 | 55.0 | 91.8 | 8.9 | 1.7 | 44.5 | 4.5 |
| 900/ 90 | c | | | | | | | | | | | | | | | | | |
| 900/ 12 | c | | | | | | | | | | | | | | | | | |
| 900/ 23 | c | 44 | 5.0 | 21.4 | 79.3 | 35.6 | 33.6 | 10.3 | 19.1 | 177.9 | 148.5 | 29.4 | 89.6 | 88.3 | 5.1 | 0.9 | 35.6 | 4.3 |
| 900/ 39 | c | | | | | | | | | | | | | | | | | |
| 899/ 5 | a | 45 | 2.1 | 10.1 | 15.3 | 21.8 | 8.6 | 2.8 | 3.7 | 52.2 | 45.7 | 6.5 | 18.1 | 34.1 | 7.0 | 1.9 | 24.9 | 4.8 |
| 899/ 84 | c, m, a | | | | | | | | | | | | | | | | | |
| 899/ 80 | c | 45 | 5.4 | 13.2 | 39.2 | 23.4 | 20.4 | 10.4 | 10.6 | 104.0 | 83.0 | 21.0 | 49.6 | 54.4 | 4.0 | 1.1 | 19.3 | 2.4 |
| 899/ 26 | c | | | | | | | | | | | | | | | | | |
| | | 44–45 | $4.0^{7}$ $\pm 0.5$ | $14.3^{7}$ $\pm 1.7$ | $36.8^{7}$ | $27.2^{7}$ | $23.4^{7}$ | $4.9^{7}$ | $10.1^{7}$ | $102.4^{7}$ $\pm 19.5$ | $87,4^{7}$ | $15.0^{7}$ | $41.7^{7}$ | $60.8^{7}$ | $6.7^{7}$ $\pm 1.3$ | $1.7^{7}$ $\pm 0.3$ | $26.2^{7}$ $\pm 4.2$ | $3.7^{7}$ $\pm 0.4$ |
| *males:* | | | | | | | | | | | | | | | | | | |
| 897/ 34 | c | 43 | 3.7 | 8.3 | 26.3 | 28.0 | 16.5 | 3.7 | 9.5 | 84.0 | 70.8 | 13.2 | 30.0 | 54.0 | 5.4 | 1.8 | 22.7 | 2.2 |
| 897/ 66 | c | | | | | | | | | | | | | | | | | |
| 897/ 4 | c | 43 | 3.9 | 12.8 | 40.6 | 27.8 | 15.3 | 1.8 | 4.1 | 89.6 | 83.7 | 5.9 | 42.4 | 14.2 | 14.2 | 1.1 | 18.3 | 3.3 |
| 898/ 46 | c | | | | | | | | | | | | | | | | | |
| 899/ 45 | c | 43 | 9.1 | 18.8 | 53.6 | 33.9 | 25.8 | 3.3 | 14.6 | 131.2 | 113.3 | 17.9 | 56.9 | 74.3 | 6.3 | 1.3 | 13.0 | 2.1 |
| 899/ 76 | c | | | | | | | | | | | | | | | | | |
| | | 43 | $5.6^{3}$ | $13.3^{3}$ | $40.2^{3}$ | $29.9^{3}$ | $19.2^{3}$ | $2.9^{3}$ | $9.4^{3}$ | $101.6^{3}$ | $89.3^{3}$ | $12.3^{3}$ | $43.1^{3}$ | $58.5^{3}$ | $8.7^{3}$ | $1.4^{3}$ | $18.0^{3}$ | $2.5^{3}$ |

*Table 5.* Data for bladder bile of hamsters reared from the age of 39–44 days on the *fat-free basal diet* supplemented with Cu, Mn and I (Experimental series G 94). All concentrations are given as millimolarities

| Group no. hamster no. | Type of stone | Days on diet | Cholesterol | Lipid phosphorus | GC | GCD | GD | TC | TCD + TD | Total bile acids | G | T | Tri | Di | G/T | Di/Tri | Total bile acids/ cholest. | Lipid phosphor./ cholest. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *females:* | | | | | | | | | | | | | | | | | | |
| 901/ 8 | c | 43 | 4.5 | 14.2 | 8.7 | 5.2 | 1.5 | 23.3 | 19.2 | 57.9 | 15.4 | 42.5 | 32.0 | 25.9 | 0.4 | 0.8 | 12.9 | 3.2 |
| 901/ 72 | o | 44 | 2.3 | 22.0 | 2.7 | 0.4 | 0.4 | 40.1 | 84.0 | 127.6 | 3.5 | 124.1 | 42.8 | 84.8 | 0.03 | 2.0 | 66.1 | 9.6 |
| 901/ 28<br>901/ 63 | c<br>c | 44 | 6.3 | 19.0 | 20.6 | 3.4 | 1.4 | 85.5 | 74.0 | 184.9 | 25.4 | 159.5 | 106.1 | 78.8 | 0.2 | 0.7 | 29.3 | 3.0 |
| 901/ 40<br>901/ 62 | c<br>c | 44 | 4.7 | 15.7 | 20.4 | 9.7 | 11.6 | 18.5 | 26.0 | 86.2 | 41.7 | 44.5 | 38.9 | 47.3 | 0.9 | 1.2 | 18.3 | 3.3 |
| 902/ 48<br>902/ 49 | c<br>c | 44 | 2.4 | 7.4 | 10.5 | 17.1 | 10.7 | 2.1 | 5.1 | 45.5 | 38.3 | 7.2 | 12.6 | 32.9 | 5.3 | 2.6 | 19.0 | 3.1 |
| 903/ 47 | c | 45 | 3.5 | 13.8 | 34.1 | 19.2 | 12.9 | 14.4 | 19.9 | 100.5 | 66.2 | 34.3 | 48.5 | 52.0 | 1.9 | 1.1 | 28.2 | 3.9 |
| 902/ 30<br>903/ 35<br>903/ 43 | c<br>c<br>c,m | 45 | 4.7 | 13.4 | 25.3 | 10.6 | 8.1 | 14.0 | 20.1 | 78.1 | 44.0 | 34.1 | 39.3 | 38.8 | 1.3 | 0.9 | 16.6 | 2.9 |
| 904/ 9 | o | 45 | 1.7 | 6.3 | 13.7 | 11.9 | 8.2 | 4.7 | 6.8 | 45.3 | 33.8 | 11.5 | 18.4 | 26.9 | 2.9 | 1.5 | 26.6 | 3.7 |
| 904/ 24 | c | 45 | 1.5 | 7.5 | 18.5 | 9.1 | 10.1 | 2.8 | 3.6 | 44.1 | 37.7 | 6.4 | 21.3 | 22.8 | 5.9 | 1.1 | 29.4 | 5.0 |
| 904/ 50 | c | 45 | 0.9 | 5.2 | 11.8 | 11.5 | 10.1 | 2.2 | 3.8 | 39.4 | 33.4 | 6.0 | 14.0 | 25.4 | 5.6 | 1.8 | 43.8 | 5.8 |
| 904/ 52<br>904/ 7 | c<br>c | 45 | 1.8 | 5.2 | 7.5 | 4.3 | 4.1 | 49.6 | 22.9 | 88.4 | 15.9 | 72.5 | 57.1 | 31.3 | 0.2 | 0.5 | 49.1 | 2.9 |
| 902/ 70 | c | 44 | 3.7 | 14.8 | 25.7 | 32.9 | 6.3 | 18.6 | 28.8 | 112.3 | 64.9 | 47.4 | 44.3 | 68.0 | 1.4 | 1.5 | 30.4 | 4.0 |
| | | 43-45 | 3.2[12] ± 0.5 | 12.0[12] ± 1.6 | 16.6[12] | 11.3[12] | 7.1[12] | 23.0[12] | 26.2[12] | 84.2[12] ± 12.5 | 35.0[12] | 49.2[12] | 39.6[12] | 44.6[12] | 2.2[12] ± 0.6 | 1.3[12] ± 0.2 | 30.9[12] ± 4.5 | 4.2[12] ± 0.6 |
| *males:* | | | | | | | | | | | | | | | | | | |
| 901/ 95<br>903/ 32<br>903/ 75 | c<br>c<br>c | 43 | 4.5 | 12.0 | 34.5 | 15.4 | 8.7 | 5.3 | 7.1 | 71.0 | 58.6 | 12.4 | 39.8 | 31.2 | 4.7 | 0.8 | 15.8 | 2.7 |

*Table 6.* Data for bladder bile of hamsters reared on the *stock diet* from the age of 30–33 days (Experimental series G 91). All concentrations are given as millimolarities

| Group no. hamster no. | Type of stone | Days on diet | Cholesterol | Lipid phosphorus | GC | GCD | GD | TC | TCD + TD | Total bile acids | G | T | Tri | Di | G/T | Di/Tri | Total bile acids/ cholest. | Lipid phosphor./ cholest. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *females:* | | | | | | | | | | | | | | | | | | |
| 833/ 10 | o | | | | | | | | | | | | | | | | | |
| 833/ 19 | o | 44 | 1.7 | 16.8 | 1.7 | 16.2 | 16.1 | 3.9 | 10.5 | 48.4 | 34.0 | 14.4 | 5.6 | 42.8 | 2.4 | 7.6 | 28.5 | 9.9 |
| 833/ 70 | o | | | | | | | | | | | | | | | | | |
| 833/ 73 | o | 45 | 0.6 | 5.0 | 4.5 | 6.4 | 3.0 | 2.3 | 10.0 | 26.1 | 13.8 | 12.3 | 6.7 | 19.8 | 1.1 | 2.9 | 43.5 | 8.3 |
| 833/114 | o | | | | | | | | | | | | | | | | | |
| 834/ 1 | o | 48 | 0.9 | 5.2 | 14.7 | 3.7 | 1.3 | 16.9 | 5.7 | 47.0 | 19.7 | 22.6 | 31.6 | 10.7 | 0.9 | 0.3 | 47.0 | 5.8 |
| 834/ 39 | o | | | | | | | | | | | | | | | | | |
| 834/ 48 | o | 49 | 2.0 | 11.4 | 54.5 | 16.7 | 3.5 | 6.5 | 14.6 | 95.8 | 74.7 | 21.1 | 61.0 | 34.8 | 3.5 | 0.6 | 47.9 | 5.7 |
| 834/ 92 | o | | | | | | | | | | | | | | | | | |
| | | 44–49 | 1.3[4] ± 0.3 | 9.6[4] ± 2.5 | 18.9[4] | 10.7[4] | 5.9[4] | 7.4[4] | 10.2[4] | 53.2[4] ± 16.1 | 35.6[4] | 17.6[4] | 26.2[4] | 26.9[4] | 2.0[4] ± 0.6 | 2.9[4] ± 1.4 | 41.9[4] | 7.4[4] |
| *males:* | | | | | | | | | | | | | | | | | | |
| 833/ 21 | o | 44 | 2.6 | 11.7 | 41.3 | 27.4 | 4.5 | 13.1 | 6.2 | 92.5 | 73.2 | 19.3 | 54.4 | 38.1 | 3.8 | 0.7 | 35.6 | 4.5 |
| 833/ 30 | o | | | | | | | | | | | | | | | | | |
| 833/ 89 | a | 44 | 1.7 | 9.4 | 20.4 | 1.7 | 4.3 | 14.0 | 5.5 | 45.9 | 26.4 | 19.5 | 34.4 | 11.5 | 1.4 | 0.3 | 27.0 | 5.5 |
| 834/ 37 | o | 45 | 1.0 | 10.1 | 4.2 | 39.1 | 18.7 | 8.3 | 6.8 | 77.1 | 62.0 | 15.1 | 12.5 | 64.6 | 4.1 | 5.2 | 77.1 | 10.1 |
| 834/ 16 | o | | | | | | | | | | | | | | | | | |
| 834/ 96 | o | 48 | 1.6 | 12.5 | 24.8 | 17.6 | 3.9 | 9.3 | 2.5 | 58.1 | 46.3 | 11.8 | 34.1 | 24.0 | 3.9 | 0.7 | 36.3 | 7.8 |
| 834/107 | o | | | | | | | | | | | | | | | | | |
| | | 44–48 | 1.7[4] ± 0.3 | 10.9[4] ± 0.9 | 22.6[4] | 21.4[4] | 7.8[4] | 11.2[4] | 5.2[4] | 68.4[4] ± 10.3 | 51.9[4] | 16.4[4] | 33.8[4] | 34.6[4] | 3.3[4] ± 0.6 | 1.7[4] ± 1.1 | 44.0[4] ± 11.3 | 7.0[4] ± 1.3 |

In the fat-free group in series G 96 (Table 1a, 1b), all the average values seem to be independent of the sex.

The abundant occurrence of amorphous pigmented gallstones in the group receiving filtrol-treated cod liver oil does not show any obvious causative correlation to the analytical data in the tables. The increase of the ratios bile acids/cholesterol and lipid-soluble phosphorus/cholesterol do not seem to afford an explanation since these ratios are also increased in the bile of animals on the "curative diet", and in these animals amorphous pigmented gallstones do not occur. The tendency to formation of gallstones of this type is, therefore, probably determined by concentration of other bile constituents than those analyzed in the present study

In the experiment in which the influence of the two salt mixtures are compared (series G 94, Tables 4 and 5), the only change which seems to be significant is that of the ratio glycine/taurine which is low although widely scattered on the diet with added Cu, Mn and I ($P < 0.01$ for the females). The number of analyses for the males in G 94 is too small to allow a comparison of the figures for the two sexes in that series.

Comparison of the figures for series G 94 with those for series G 96 leads to the following considerations:

*A priori*, the fat-free diets with 12% rice starch could be expected to cause some increase of the ratio total bile acids/cholesterol over the value previously found with corresponding diets having glucose as the sole source of carbohydrate (1). This was not found to be the case in the fat-free group in G 96, but in G 94 both groups on fat-free diets had a somewhat higher value for this ratio which was nearly the same as that found for the group receiving filtrol-treated cod liver oil in G 96. The reason for this difference between the fat-free groups in the two series cannot be stated with certainty, but whatever the cause may be, the figures clearly indicate that the increase of the ratio total bile acids/cholesterol in the cod liver oil-fed group in G 96 cannot be the only cause of the absence of cholesterol gallstones in this group, since about the same value for this ratio has been found in groups of animals on fat-free diets (G 94) having cholesterol gallstones.

The cause of the absence of cholesterol gallstones in the cod liver oil-fed group may be the combination of the increased ratios bile acids/cholesterol and lipid-soluble phosphorus/cholesterol, possibly together with changes in other bile constituents, e.g., amount of free fatty acids, glycerides, degree of saturation or chain length of the fatty acids of the bile lipids, etc., which have not been determined in the present study. Some of these other constituents may also be involved in the formation of amorphous gallstones.

In the animals receiving the stock diet (G 91, Table 6), the absence of cholesterol gallstones is probably related to the relatively high figures for the ratios total bile acids/cholesterol and lipid-soluble phosphorus/cholesterol, possibly together with constituents not yet analyzed.

In the groups receiving the "curative diet" with and without its content of 10% lard (series G 91, Tables 7 and 8) it is evident from the figures for both sexes that the ratio lipid-soluble phosphorus/cholesterol is higher when the diet contains the 10% lard than when the lard is replaced by the same weight of rice starch ($P < 0.001$ for the females, $P < 0.01$ for the males). The difference seems to depend more on the lowering of the cholesterol concentration than on the increase of the concentration of lipid-soluble phosphorus.

*Table 7.* Cholesterol and lipid phosphorus in bladder bile of hamsters reared on the "*curative diet*" from the age of 30–33 days (Experimental series G 91). The concentrations are given as millimolarities

| Group no./ hamster no. | Type of stone | Days on diet | Cholesterol | Lipid phosphorus | Lipid phosphor./ cholest. | Group no./ hamster no. | Type of stone | Days on diet | Cholesterol | Lipid phosphorus | Lipid phosphor./ cholest. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *females:* | | | | | | *males:* | | | | | |
| 825/ 24 | 0 | 44 | 1.5 | 12.5 | 8.3 | 823/ 67 | 0 | 44 | 1.3 | 15.9 | 12.2 |
| 824/111 | 0 | 45 | 1.3 | 9.5 | 7.3 | 823/ 18 | 0 | 44 | 2.2 | 18.7 | 8.5 |
| 826/ 23 | 0 | 45 | 1.1 | 10.2 | 9.3 | 823/104 | 0 | 45 | 0.6 | 7.6 | 12.7 |
| 825/ 79 | 0 | 48 | 1.2 | 7.8 | 6.5 | 823/ 94 | 0 | 45 | 2.2 | 14.2 | 6.5 |
| 825/ 84 | 0 | 48 | 1.2 | 6.6 | 5.5 | 823/ 46 | 0 | 48 | 2.9 | 26.1 | 9.0 |
| 825/ 98 | 0 | 49 | 1.8 | 14.5 | 8.1 | 823/106 | 0 | 48 | 1.8 | 6.6 | 3.7 |
| 826/ 65 | 0 | 49 | 2.6 | 15.0 | 5.8 | 824/ 63 | 0 | 49 | 2.1 | 10.1 | 4.8 |
| 826/108 | 0 | 49 | 1.8 | 12.6 | 7.0 | 824/ 50 | 0 | 49 | 2.7 | 21.5 | 7.9 |
| 826/ 83 | 0 | 49 | 2.4 | 17.0 | 7.1 | 826/112 | 0 | 49 | 1.4 | 10.9 | 7.5 |
| | | | | | | 824/ 86 | 0 | 49 | 2.4 | 12.1 | 5.1 |
| | | | | | | 824/ 66 | 0 | 49 | 1.7 | 12.9 | 7.6 |
| | | | | | | 826/ 66 | 0 | 46 | 0.9 | 9.9 | 10.9 |
| | | 44-49 | $1.7^{9}$ $\pm 0.2$ | $11.7^{9}$ $\pm 1.2$ | $7.2^{9}$ $\pm 0.4$ | | | 44-49 | $1.9^{12}$ $\pm 0.2$ | $16.7^{12}$ $\pm 1.8$ | $8.0^{12}$ $\pm 0.8$ |

*Table 8.* Cholesterol and lipid phosphorus in bladder bile of hamsters reared from the age of 30–33 days on the "*curative diet*" *in which lard was replaced by rice starch* (Experimental series G 91). The concentrations are given as millimolarities

| Group no./ hamster no. | Type of stone | Days on diet | Cholesterol | Lipid phosphorus | Lipid phosphor./ cholest. | Group no./ hamster no. | Type of stone | Days on diet | Cholesterol | Lipid phosphorus | Lipid phosphor./ cholest. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *females:* | | | | | | *males:* | | | | | |
| 827/ 34 | 0 | 44 | 6.3 | 12.9 | 2.0 | 827/ 61 | 0 | 44 | 2.0 | 6.6 | 3.3 |
| 827/ 5 | 0 | 44 | 7.2 | 15.4 | 2.1 | 827/121 | 0 | 44 | 1.2 | 11.7 | 9.8 |
| 827/103 | 0 | 45 | 4.2 | 14.2 | 3.4 | 827/122 | 0 | | | | |
| 827/ 38 | 0 | 45 | 3.0 | 10.7 | 3.6 | 828/ 11 | 0 | 45 | 3.9 | 10.9 | 2.8 |
| 828/ 82 | 0 | 48 | 1.4 | 5.8 | 4.1 | 827/ 71 | 0 | 45 | 2.0 | 11.2 | 5.6 |
| 828/ 85 | 0 | 48 | 1.2 | 5.1 | 4.3 | 828/ 68 | 0 | 48 | 2.5 | 9.4 | 3.8 |
| 828/115 | 0 | 49 | 4.1 | 9.4 | 2.3 | 828/ 49 | 0 | 48 | 5.8 | 19.2 | 3.3 |
| 828/101 | 0 | 49 | 1.5 | 6.5 | 4.3 | 828/113 | 0 | 48 | 4.4 | 13.4 | 3.1 |
| | | | | | | 828/124 | 0 | 48 | 2.0 | 8.4 | 4.2 |
| | | 44-49 | $3.6^{8}$ $\pm 0.8$ | $10.0^{8}$ $\pm 1.4$ | $3.3^{8}$ $\pm 0.4$ | | | 44-48 | $3.0^{8}$ $\pm 0.6$ | $11.4^{8}$ $\pm 1.3$ | $4.5^{8}$ $\pm 0.8$ |

The data for the females on the rice starch diet with 20% lard and without lard (series G 91, Tables 9 and 10), also show that the ratio lipid-soluble phosphorus/cholesterol is higher on the diet with lard and the difference is significant ($P < 0.001$ for the females).

The difference of the ratio is determined both by a decrease of the cholesterol concentration and by an increase of the concentration of lipid phosphorus.

*Table 9.* Cholesterol and lipid phosphorus in bladder bile of hamsters reared from the age of 30–33 days on *the rice starch diet in which 20% rice starch was replaced by lard* (Experimental series G 91). All concentrations are given as millimolarities

| Group no./ hamster no. | Type of stone | Days on diet | Cholesterol | Lipid phosphorus | Lipid phosphor./ cholest. | Group no./ hamster no. | Type of stone | Days on diet | Cholesterol | Lipid phosphorus | Lipid phosphor./ cholest. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *females:* | | | | | | *males:* | | | | | |
| 831/ 32 | 0 | 44 | 2.8 | 18.2 | 6.5 | 832/ 58 | 0 | 44 | 2.0 | 22.8 | 11.4 |
| 831/ 33 | 0 | 45 | 1.2 | 13.5 | 11.3 | 831/ 51 | 0 | 45 | 2.5 | 22.8 | 9.1 |
| 831/ 45 | 0 | 45 | 1.2 | 9.4 | 7.8 | 832/ 12 | 0 | 45 | 2.2 | 27.9 | 12.7 |
| 832/ 35 | 0 | 48 | 3.7 | 33.8 | 9.1 | | | | | | |
| 832/ 43 | 0 | 48 | 2.1 | 10.9 | 5.2 | | | | | | |
| 832/ 42 | 0 | 48 | 2.8 | 20.4 | 7.3 | | | | | | |
| 832/ 99 | 0 | 48 | 0.8 | 10.1 | 12.6 | | | | | | |
| 831/119 | 0 | 49 | 3.5 | 27.6 | 7.9 | | | | | | |
| 832/116 | 0 | 49 | 2.1 | 19.4 | 9.2 | | | | | | |
| | | 44–49 | $2.2^{9}$ $\pm 0.4$ | $18.1^{9}$ $\pm 2.8$ | $8.5^{9}$ $\pm 0.8$ | | | 44–45 | $2.2^{3}$ $\pm 0.5$ | $24.5^{3}$ $\pm 1.7$ | $11.1^{3}$ $\pm 1.1$ |

*Table 10.* Cholesterol and lipid phosphorus in bladder bile of hamsters reared from the age of 30–33 days on the *"fat-free" rice starch diet* (Experimental series G 91). The concentrations are given as millimolarities

| Group no./ hamster no. | Type of stone | Days on diet | Cholesterol | Lipid phosphorus | Lipid phosphor./ cholest. | Group no./ hamster no. | Type of stone | Days on diet | Cholesterol | Lipid phosphorus | Lipid phosphor./ cholest. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *females:* | | | | | | *males:* | | | | | |
| 829/109 | 0 | 44 | 1.6 | 7.1 | 4.4 | 829/ 31 | 0 | 44 | 1.7 | 5.9 | 3.4 |
| 829/ 9 | 0 | 44 | 6.7 | 11.4 | 1.7 | 829/ 27 | 0 | 44 | 2.8 | 12.9 | 4.6 |
| 830/ 7 | 0 | 45 | 3.6 | 17.4 | 4.8 | 829/ 53 | 0 | 45 | 2.2 | 17.3 | 7.7 |
| 830/ 20 | 0 | 45 | 2.3 | 7.9 | 3.4 | 829/ 52 | 0 | 45 | 6.8 | 24.4 | 3.6 |
| 830/ 62 | 0 | 48 | 1.5 | 6.1 | 4.1 | 830/ 13 | 0 | 48 | 3.7 | 7.0 | 1.9 |
| 830/ 69 | 0 | 48 | 4.7 | 14.5 | 1.3 | 830/ 97 | 0 | 48 | 2.5 | 13.7 | 5.5 |
| | | | | | | 830/ 59 | 0 | 49 | 2.7 | 8.4 | 3.1 |
| | | | | | | 830/ 25 | 0 | 49 | 1.5 | 4.8 | 3.2 |
| | | | | | | 830/118 | 0 | 49 | 1.7 | 6.6 | 3.9 |
| | | | | | | 830/ 91 | 0 | 49 | 1.6 | 4.8 | 3.0 |
| | | 44–48 | $3.4^{6}$ $\pm 0.8$ | $10.7^{6}$ $\pm 1.8$ | $3.3^{6}$ $\pm 0.6$ | | | 44–49 | $2.7^{10}$ $\pm 0.5$ | $10.5^{10}$ $\pm 2.1$ | $4.0^{10}$ $\pm 0.5$ |

The number of bile analyses for the males in the group with rice starch and lard is not sufficient for a comparison with the males in the rice starch group without lard. Corresponding figures for males and females in the rice starch group without lard are not significantly different from each other.

Table 11 shows the average gain in weight during six weeks for the animals from which bile was taken and analyzed. As expected, these figures as well as the corresponding figures for all the survivors in our previous study (2) demonstrate that, on the average, growth is faster when the diet contains fat than when it is fat-free (compare also (3)). The influence of the dietary fat on bladder bile composition and the ensuing protection against cholesterol gallstones cannot, however, be merely a result of faster growth and development. The composition of the bladder bile from the hamsters receiving 3% filtrol-treated cod liver oil is not the same as that of the bladder bile from hamsters fed a fat-free diet with starch as the sole carbohydrate (1) which also suppresses gallstone formation, and, when fed *ad libitum,* improves growth. Further, yet unpublished experiments have shown that gallstone formation could not be introduced by feeding the fat-free rice starch diet in restricted quantities, so adjusted that the weight of the animals remained nearly constant.

*Table 11.* Initial weight and weight gain during 6 weeks of the animals from which bladder bile was collected and analyzed

| Experimental series | Diet characteristics | Sex | Number of animals | Initial weight (g) | Weight gain in 6 weeks (g) |
|---|---|---|---|---|---|
| G 96 | fat-free | f | 11 | 56.3 | 8.3 |
| | | m | 22 | 53.3 | 15.7 |
| G 96 | 3% filtrol-treated | f | 21 | 51.9 | 23.0 |
| | cod liver oil | m | 23 | 54.8 | 20.6 |
| G 94 | fat-free | f | 14 | 46.8 | 15.4 |
| | | m | 6 | 57.0 | 16.5 |
| G 94 | fat-free + Cu, Mn, I | f | 19 | 46.4 | 25.6 |
| | | m | 3 | 55.3 | 15.7 |
| G 91 | stock diet | f | 9 | 35.8 | 30.2 |
| | | m | 7 | 39.3 | 26.9 |
| G 91 | "curative" | f | 10 | 37.2 | 23.7 |
| | | m | 12 | 39.0 | 24.3 |
| G 91 | "curative minus lard | f | 8 | 35.6 | 22.3 |
| | plus rice starch" | m | 9 | 37.6 | 16.1 |
| G 91 | "rice starch" | f | 6 | 34.3 | 14.7 |
| | | m | 10 | 38.1 | 19.9 |
| G 91 | "rice starch plus | f | 9 | 36.6 | 27.8 |
| | 20% lard" | m | 4 | 38.5 | 25.0 |

## *Summary*

Cholesterol, lipid-soluble phosphorus, glycine conjugated cholic, chenodeoxycholic and deoxycholic acids, and taurine conjugated cholic and chenodeoxycholic plus deoxycholic acids were determined in the bladder bile of young hamsters reared for 6–7 weeks from the age of 30–44 days on:

1. A fat-free basal diet containing 20% casein, 62.3% glucose, and 12% rice starch as protein and carbohydrate components, producing a high incidence of cholesterol gallstones,
2. The basal diet in which 3% glucose is replaced by 3% filtrol-treated cod liver oil which suppresses formation of cholesterol gallstones and favors development of amorphous pigmented gallstones,
3. The basal diet with addition of 6.4 mg Mn, 1.66 mg Cu and 0.5 mg J per 100 g, causing cholesterol gallstones as the unsupplemented diet,
4. The stock diet hitherto used for our breeding colony of hamsters, capable of preventing formation of cholesterol gallstones but not completely preventing formation of amorphous pigmented gallstones.

Further, cholesterol and lipid-soluble phosphorus were determined in bladder bile of young hamsters reared for 44–49 days from the age of 30–33 days on:

5. The previously described "curative diet" (1),
6. The "curative diet" in which lard (10% of the diet) was replaced by rice starch,
7. A fat-free diet containing 20% casein and 74.3% rice starch as protein and carbohydrate components,
8. A diet containing 20% casein, 54.3% rice starch, and 20% lard as protein, carbohydrate and fat-components.

The main results were as follows:

Addition of 3% filtrol-treated cod liver oil to the basal diet caused moderate increases of the ratios total bile acids/cholesterol and lipid-soluble phosphorus/cholesterol. The concentration of cholesterol was decreased, especially in the females.

The suppression of formation of cholesterol gallstones caused by 3% filtrol-treated cod liver oil may be due to the combined increases of the above mentioned ratios together with changes in other bile constituents which have not yet been quantitatively determined, e. g., concentration, chain length and degree of unsaturation of bile lipid fatty acids.

The increased tendency to formation of amorphous pigmented gallstones resulting from dietary filtrol-treated cod liver oil does not seem to be explained by the analytical data provided, and therefore, apparently depends upon variations in other bile constituents e. g. the fatty acids of bile lipids.

Addition of the minerals caused a decrease of the ratio glycine/taurine, but the scattering of the figures for this ratio was considerable.

The ratios total bile acids/cholesterol and lipid-soluble phosphorus/cholesterol in the bile of the animals on the stock diet were higher than the corresponding figures for the animals fed the diet with 3% filtrol-treated cod liver oil.

The data for the groups fed the "curative diet" with and without lard show that the 10% lard contained in the "curative diet" causes a high value of the ratio lipid-soluble phosphorus/cholesterol and a relatively low concentration of cholesterol. These effects of lard are confirmed by the data for the "rice starch diet" with and without lard, which, further, show that the lard causes a considerable increase of the concentration of lipid-soluble phosphorus in the bile.

## *Zusammenfassung*

Cholesterin, Lipid-löslicher Phosphor, Glycin-konjugierte Cholsäure, Chenodesoxycholsäure und Desoxycholsäure, sowie Taurin-konjugierte Cholsäure und Chenodesoxy- plus Desoxycholsäure wurden in Blasengalle von Hamstern bestimmt, welche von einem Alter von 30–44 Tagen an während 6–7 Wochen mit den folgenden Nahrungen gefüttert wurden:

1. Eine fettfreie Nahrung, deren Protein- und Kohlenhydratkomponenten aus 20% Casein, 62.3% Glucose und 12% Reisstärke bestanden, welche Nahrung an jungen Hamstern Cholesterin-Gallensteine hervorruft,
2. Die obenerwähnte Nahrung, in welcher 3% Glucose durch 3% Filtrol-behandelten Dorschlebertran ersetzt ist, wodurch die Entwicklung von Cholesterin-Gallensteinen unterdrückt und die Bildung von amorphen pigmentierten Gallensteinen gefördert wird.
3. Die obenerwähnte fettfreie Nahrung mit Zusatz von 6,4 mg Mn, 1,66 g Cu und 0,5 mg J per 100 g, wodurch die Entwicklung von Cholesterin-Gallensteinen unverändert bleibt.
4. Die für unsere Hamster-Zucht-Kolonie bisher benutzte Nahrung ("stock diet"), welche die Entwicklung von Cholesterin-Gallensteinen unterdrückt, die Bildung von amorphen pigmentierten Gallensteinen aber nicht ganz zu verhindern vermag.

Außerdem wurden Cholesterin und Lipid-löslicher Phosphor in der Blasengalle von Hamstern bestimmt, welche von einem Alter von 30–33 Tagen an während 44–49 Tagen mit den folgenden Nahrungen gefüttert wurden:

5. Die früher (1) beschriebene „kurative" Nahrung.
6. Die „kurative" Nahrung, in welcher der Gehalt an Schweineschmalz (10%) durch Reisstärke ersetzt ist.
7. Eine fettfreie Nahrung, deren Protein- und Kohlenhydratkomponenten aus 20% Casein und 74,3% Reisstärke bestanden.
8. Eine Nahrung, deren Protein-, Kohlenhydrat- und Fettkomponenten aus 20% Casein, 54,3% Reisstärke und 20% Schweineschmalz bestanden.

Die Hauptergebnisse waren:

Der Zusatz von 3% filtrol-behandelten Dorschlebertran zu der fettfreien Basal-Nahrung bewirkte moderate Erhöhungen der Verhältnisse von Gesamt-Gallensäuren zu Cholesterin und von Lipid-löslichem Phosphor zu Cholesterin. Die Konzentration des Cholesterins war erniedrigt, besonders bei den Weibchen.

Die Ursache der durch 3% filtrol-behandelten Dorschlebertran erreichten Unterdrückung der Bildung von Cholesterin-Gallensteinen ist in der gleichzeitigen Erhöhung der beiden obenerwähnten Konzentrations-Verhältnisse zu suchen. Wahrscheinlich zusammen mit noch nicht quantitativ bestimmten Veränderungen der Galle, z. B. Konzentration, Kettenlänge und Ungesättigkeitsgrad der Gallenfettsäuren.

Die durch 3% filtrol-behandelten Dorschlebertran bewirkte Erhöhung der Neigung zur Bildung von amorphen, pigmentierten Gallensteinen findet anscheinend keine unmittelbare Erklärung in den vorliegenden analytischen Daten, und scheint deshalb auf Veränderungen anderer Gallenbestandteile als die hier analysierten, z. B. der Gallenfettsäuren, zu beruhen.

Der Zusatz von den Mineralstoffen bewirkte eine Abnahme des Verhältnisses von Glycin zu Taurin. Die Zerstreuung der Werte von diesem Verhältnis war recht bedeutlich.

Die Verhältnisse von Gesamt-Gallensäuren zu Cholesterin und von Lipid-löslichem Phosphor zu Cholesterin in der Galle der mit "stock diet" gefütterten Tiere waren höher als die entsprechenden Verhältnisse für die mit 3% filtrol-behandelten Dorschlebertran gefütterten Tiere.

Aus den Daten für die „kurative" Nahrung mit und ohne Schweineschmalz ist ersichtlich, daß die Anwesenheit von 10% Schweineschmalz in der „kurativen" Nahrung ein hohes Verhältnis von Lipid-löslichem Phosphor zu Cholesterin und eine verhältnismäßig niedrige Konzentration von Cholesterin in der Blasengalle bedingt.

Diese Wirkungen von Schweineschmalz wurden durch die Daten für die Reisstärke-Nahrung mit und ohne Zulage von 20% Schweineschmalz bestätigt. Außerdem zeigte es sich hier, daß die Konzentration von Lipid-löslichem Phosphor in der Blasengalle durch das Schweineschmalz der Nahrung erhöht wurde.

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