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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

(Received December 15, 1972)

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 os gallstones (1, 2) but induces other anomalities 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.

Tab. 1. Composition of the basal diet.

Casein[1])	20.00 g
Glucose	37.15 g
Rice starch	37.15 g
Salt mixture[2])	5.00 g
Vitamin mixture[3])	0.50 g
Choline chloride	0.20 g
	100.00 g

[1]) "Dairinex", from Dansk Mejeri Industri & Export Kompagni, Stege, (Denmark.)
[2]) The salt mixture indicated in reference 3.
[3]) 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, but before the breeding of the hamsters used in the second experiment took place, a new stock diet was introduced.

Since the stock diet, especially through its content of polyunsaturated fatty acids, has an influence on the production of gallstones in the experimental period, the composition of the two stock diets and their content of fatty acids are recorded (tables 2, 3, 4 and 5).

Tab. 2. Composition of "Stock diet, January 1964"[1]) to which the hamsters used in experiment 1 had access before beginning of the experiment.

	g
Ground whole wheat	16.00
Corn starch	12.00
Ground yellow maize	19.00
Rice flour	12.00
Crude casein[2])	2.00
Soybean meal[3])	3.00
Dry brewer's yeast	12.00
Green alfalfa meal	2.22
Skim milk powder	12.00
Calcium carbonate	1.38
Salt mixture[4])	0.905
Vitamin mixture[5])	0.50
Total	100.005

[1]) Prepared in our laboratory.
[2]) Dairinex, from Dansk Mejeri Industri & Export Kompagni, Stege (Denmark).
[3]) Thermally debittered but not defatted, brand "Nurupan", obtained from Hans Lundbeck A/S, Copenhagen (Denmark).

[4])

Sodium chloride	0.40000 g
Potassium dihydrogen phosphate	0.33000 g
Magnesium carbonate, basic	0.11000 g
Ferric citrate	0.06000 g
Calcium iodate	0.00073 g
Zinc carbonate, basic	0.01145 g
Manganese carbonate, basic	0.02670 g
Cupric carbonate, basic	0.00381 g
Sodium fluoride	0.00635 g
Sodium molybdate, 2 H_2O	0.00064 g
Chromic sulfate, 15 H_2O	0.00021 g
Selenium dioxide	0.00005 g
Total	0.90500 g

[5])

Vitamin A 1000 i.u., Vitamin D_3 100 i.u. "Rovimix 50/5", Roche	20.0 mg
All-rac.-α-tocopherol acetate, "Ephynal", Roche	5.0 mg
Thiamin hydrochloride	1.5 mg
Riboflavin	1.5 mg
Pyridoxin hydrochloride	1.5 mg
Calcium pantothenate	3.0 mg
Nicotinamide	4.0 mg
Ascorbic acid	10.0 mg
Sucrose	453.5 mg
Total	500.0 mg

Tab. 3. Composition of the new stock diet, "Rostock mixture"[1]) to which the hamsters used in experiment 2 had access before beginning of the experiment.

	g
Ground whole wheat	12.0
Oatmeal	30.0
Ground yellow maize	30.0
Fish meal	8.0
Soybean meal	9.0
Dry brewer's yeast	3.0
Green alfalfa meal	2.0
Skim milk powder	3.0
Salt mixture[2])	2.0
Vitamin mixture[3])	1.0
Total	100.0

[1]) According to the declaration of the manufacturer: Korn og Foderstof Kompagniet A/S, Copenhagen (Denmark).

[2])

Calcium phosphate, dibasic	1.6600 g
Sodium chloride	0.3000 g
Ferrous sulfate	0.0280 g
Manganese sulfate	0.0060 g
Cupric sulfate	0.0040 g
Cobaltous sulfate	0.0012 g
Zinc sulfate	0.0006 g
Potassium iodide	0.0002 g
Total	2.0000 g

[3]) Furnishes 100 g of diet with:

Vitamin A	4000 i. u.
Vitamin D_3	1000 i. u.
Vitamin E[4])	20.0 mg
Vitamin K[4])	40.0 mg
Thiamine hydrochloride	0.6 mg
Riboflavin	1.8 mg
Nicotinamide	5.0 mg
Pantothenic acid	1.8 mg
Vitamin B_{12}	0.0025 mg
Choline chloride	100.0 mg
"Ethoxyquin", Monsanto	25.0 mg
Powdered Oyster shells	850.0 mg

[4]) Preparation not specified.

Results and Discussion

The individual results are presented in tables 6–21. Summaries of each experiment with respect to occurrence of gallstones are given in tables 22 and 23.

None of the hamsters in the two experiments had diarrhoea.

Experiment 1

Amorphous gallstones did not occur in any of the three groups.

In the group of animals receiving the diet *without addition,* 3 out of the 9 males and 1 out of the 10 females had cholesterol gallstones. In the group

Tab. 4. Individual fatty acids in "Stock diet, January 1964"[1]).

Fatty acid[2])	As percent of total fatty acids	As percent of diet
12:0	0.47	0.01
14:0	1.36	0.04
16:0	16.47	0.45
16:1 ω 7	1.81	0.05
18:0	3.91	0.11
18:1 ω 9	24.75	0.68
18:2 ω 6	44.40	1.23
18:3 ω 3	6.52	0.18
22:1	0.27	0.01

[1]) Total fat content of diet 2.90%.
[2]) Number of carbon atoms and double bonds.

Tab. 5. Individual fatty acids in the new stock diet, "Rostock mixture"[1]).

Fatty acid[2])	As percent of total fatty acids	As percent of diet
14:0	0.28	0.01
16:0	14.44	0.63
16:1 ω 7	0.46	0.02
18:0	1.98	0.09
18:1 ω 9	28.80	1.27
18:2 ω 6	45.87	2.02
18:3 ω 3	3.30	0.15
20:1	1.06	0.05
22:1	2.26	0.10
20:5 ω 3	0.57	0.03
22:6 ω 3	0.95	0.04

[1]) Total fat content of diet 4.62%.
[2]) Number of carbon atoms and double bonds.

receiving the diet with *0.1% chenodeoxycholic acid,* 8 out of 9 males and all the 10 females had cholesterol gallstones. In the group receiving the diet with *0.1% lithocholic acid,* 7 out of the 9 males and 7 out of the 10 females had cholesterol gallstones.

The difference between occurrence of cholesterol gallstones in the group receiving lithocholic acid and occurrence of cholesterol gallstones in the group receiving the unsupplemented basal diet is significant for the females and also for males plus females, the probability being higher than 95% in both cases (mode of evaluation as in reference 4).

Thus, it is evident, that not only chenodeoxycholic acid but also lithocholic acid stimulates production of cholesterol gallstones.

The number of animals having cholesterol gallstones was lower in the group receiving lithocholic acid than in the group receiving chenodeoxycholic acid, but the difference was not significant with a probability of

Tab. 6 (Experiment 1). Occurrence of gallstones and other data for male hamsters fed the basal diet without addition.

Animal no.	Days on diet	Gall-stones	Weight at start g	Weight after 6 weeks g	Weight gain 6 weeks g	Weight of liver g	Weight of l. testis g	Weight of r. testis g
4	42	0	60	73	13	3.28	1.21	1.22
5	42	0	59	72	13	3.44	1.31	1.31
13	42	C	51	70	19	3.39	1.33	1.27
20	42	C	49	69	20	4.21	1.17	1.16
24	42	C	43	65	22	3.09	1.33	1.09
27	42	0	52	67	15	2.89	1.24	1.15
37	42	0	72	82	10	4.05	1.37	1.36
40	42	0	64	85	21	3.74	1.28	1.21
46	42	0	58	78	20	2.92	1.17	1.11
Mean			56.4	73.4	17.0	3.42	1.25	1.21
st. d.			±2.9	±2.3	±1.4	±1.53	±0.02	±0.02

0 = no gallstones
C = cholesterol gallstones

Tab. 7 (Experiment 1). Occurrence of gallstones and other data for female hamsters fed the basal diet without addition.

Animal no.	Days on diet	Gall-stones	Weight at start g	Weight after 6 weeks g	Weight gain 6 weeks g	Weight of liver g
60	42	0	62	79	17	4.11
90	42	0	52	75	23	4.21
15	42	0	45	61	16	3.17
17	42	0	64	83	19	5.16
29	42	C	60	72	12	3.46
35	42	0	42	55	13	2.66
41	42	0	69	87	18	4.39
48	42	0	49	66	17	3.60
51	43	0	46	61	15	4.25
54	43	0	55	73	18	4.39
Mean			54.4	71.2	16.8	3.94
st. d.			±2.9	±3.3	±1.0	±0.22

0 = no gallstones
C = cholesterol gallstones

95 %. From inspection of the gall bladders it was evident, however, that the amount of gallstones and cholesterol crystals in the cases where gallstones occurred was greater in the group receiving chenodeoxycholic acid than in the group receiving lithocholic acid.

This circumstance together with the difference in incidence is taken to indicate that chenodeoxycholic acid is somewhat more efficient as promoter

Tab. 8 (Experiment 1). Occurrence of gallstones and other data for male hamsters fed the basal diet with addition of 0.1% chenodeoxycholic acid.

Animal no.	Days on diet	Gall-stones	Weight at start g	Weight after 6 weeks g	Weight gain 6 weeks g	Weight of liver g	Weight of l. testis g	Weight of r. testis g
1	42	C	57	67	10	3.83	1.07	1.06
2	42	0	55	69	14	4.49	1.19	1.11
14	42	C	60	77	17	4.80	1.32	1.29
21	42	C	50	70	20	4.42	1.31	1.20
25	42	C	58	71	13	4.16	1.18	1.20
30	42	C	55	76	21	4.69	1.18	1.17
34	42	C	49	68	19	4.28	1.29	1.33
42	42	C	69	90	21	5.03	1.30	1.29
47	42	C	57	78	21	3.72	1.25	1.23
Mean			56.7	74.0	17.3	4.38	1.23	1.21
st. d.			±2.0	±2.4	±3.1	±0.14	±0.03	±0.03

0 = no gallstones
C = cholesterol gallstones

Tab. 9 (Experiment 1). Occurrence of gallstones and other data for female hamsters fed the basal diet with addition of 0.1% chenodeoxycholic acid.

Animal no.	Days on diet	Gall-stones	Weight at start g	Weight after 6 weeks g	Weight gain 6 weeks g	Weight of liver g
7	42	C	60	67	7	4.59
10	42	C	55	63	8	3.49
16	42	C	58	68	10	4.10
28	42	C	47	62	15	3.95
31	42	C	52	72	20	5.24
36	42	C	60	77	17	4.63
43	42	C	55	64	9	4.24
49	42	C	52	73	21	4.38
52	43	C	48	66	18	4.93
55	43	C	57	76	19	5.31
Mean			54.4	68.8	14.4	4.49
st. d.			±1.4	±1.7	±2.2	±0.17

C = cholesterol gallstones

of formation of cholesterol gallstones in the hamster than is lithocholic acid. If the effect of chenodeoxycholic acid were dependent upon partial conversion to lithocholic acid, then lithocholic acid should have been found to be definitely more efficient than chenodeoxycholic acid.

Both chenodeoxycholic acid and lithocholic acid tended to increase the weight of the liver, but had no influence on the weight of the testes. In

Tab. 10 (Experiment 1). Occurrence of gallstones and other data for male hamsters fed the basal diet with addition of 0.1% lithocholic acid.

Animal no.	Days on diet	Gall-stones	Weight at start g	Weight after 6 weeks g	Weight gain 6 weeks g	Weight of liver g	Weight of l. testis g	Weight of r. testis g
3	42	0	68	80	12	4.51	1.30	1.35
11	42	C	55	65	10	4.00	1.21	1.15
18	42	C	58	75	17	3.63	1.24	1.25
23	42	C	50	67	17	3.40	1.11	1.07
26	42	C	58	81	23	4.29	1.48	1.46
32	42	C	56	70	14	3.65	1.13	1.09
39	42	C	62	83	21	4.13	1.45	1.39
44	42	0	52	64	12	3.19	0.92	0.88
57	42	C	51	74	23	3.34	1.17	1.16
Mean			56.7	73.2	16.6	3.79	1.22	1.20
st. d.			±1.9	±2.4	±3.1	±0.14	±0.06	±0.06

0 = no gallstones
C = cholesterol gallstones

Tab. 11 (Experiment 1). Occurrence of gallstones and other data for female hamsters fed the basal diet with addition of 0.1% lithocholic acid.

Animal no.	Days on diet	Gall-stones	Weight at start g	Weight after 6 weeks g	Weight gain 6 weeks g	Weight of liver g
8	42	C	55	72	17	4.52
12	42	C	55	75	20	4.58
19	42	C	45	75	30	4.87
22	42	C	43	71	28	4.38
33	42	C	51	71	20	4.19
38	42	0	65	83	18	5.27
45	42	C	57	86	29	4.95
50	42	C	57	77	20	3.99
53	43	0	58	79	21	4.44
56	43	0	57	77	20	4.48
Mean			54.3	76.6	22.3	4.57
st. d.			±2.0	±1.6	±2.6	±0.04

C = cholesterol gallstones
0 = no gallstones

our previous study with chenodeoxycholic acid and hyodeoxycholic acid (1), the weight of the testes was increased by adding the bile acids to the diet, and the testes were much smaller than in the animals of comparable age used in the present experiment. It is a common experience that the size of the testes of the hamster can vary considerably from time to time.

Tab. 12 (Experiment 2). Occurrence of gallstones and other data for male hamsters fed the basal diet without addition.

Animal no.	Days on diet	Gall-stones	Weight at start g	Weight after 6 weeks g	Weight gain 6 weeks g	Weight of liver g	Weight of l. testis g	Weight of r. testis g
5	42	0	67	92	25	5.23	1.25	1.23
10	42	A	61	97	36	4.98	1.27	1.30
24	42	0	57	85	28	4.11	1.27	1.20
41	42	0	63	87	24	4.99	1.48	1.39
47	42	0	55	81	26	3.75	1.18	1.14
56	42	0	69	98	29	4.83	1.40	1.39
64	43	A	57	96	39	4.78	1.27	1.22
71	43	0	78	111	33	5.63	1.38	1.46
90	43	0	57	72	15	3.83	1.05	1.16
96	43	0	47	62	15	3.32	0.88	0.85
101	43	A	72	93	21	5.67	1.43	1.37
Mean			62.1	88.5	26.5	4.65	1.26	1.25
st. d.			±2.7	±4.1	±4.9	±0.24	±0.05	±0.05

0 = no gallstones
A = amorphous gallstones

Tab. 13 (Experiment 2). Occurrence of gallstones and other data for female hamsters fed the basal diet without addition.

Animal no.	Days on diet	Gall-stones	Weight at start g	Weight after 6 weeks g	Weight gain 6 weeks g	Weight of liver g
13	42	0	50	79	29	4.59
20	42	0	52	79	27	5.02
33	42	0	50	71	21	3.82
46	42	A	65	93	28	5.75
57	42	A	66	92	26	4.10
68	42	A	54	82	28	3.88
70	43	0	82	100	18	5.76
78	43	0	54	71	17	4.39
86	43	0	54	64	10	3.68
Mean			58.6	81.2	22.7	4.50
st. d.			±3.5	±4.0	±5.3	±0.29

0 = no gallstones
A = amorphous gallstones

Experiment 2

Amorphous pigmented gallstones occurred in this experiment, in which the hamsters had been reared on the new stock diet, "Rostock Mixture", before they received the artificial basal diet with and without addition of bile acids.

Tab. 14 (Experiment 2). Occurrence of gallstones and other data for male hamsters fed the basal diet with addition of 0.1% chenodeoxycholic acid.

Animal no.	Days on diet	Gall-stones	Weight at start g	Weight after 6 weeks g	Weight gain 6 weeks g	Weight of liver g	Weight of l. testis g	Weight of r. testis g
6	42	C	70	96	26	5.22	1.29	1.40
11	42	C	64	98	34	5.56	1.45	1.39
27	42	C	65	94	29	4.62	1.31	1.19
31	42	C	59	99	40	6.47	1.58	1.52
42	42	C	64	93	29	5.11	1.52	1.42
50	42	C	66	88	22	4.86	1.29	1.33
60	43	C	56	84	28	5.08	1.17	1.10
65	43	C	43	74	31	4.65	1.04	1.02
72	43	C	77	95	18	4.95	1.48	1.43
79	43	C	61	69	8	4.11	0.91	0.89
91	43	CA	59	66	7	4.21	0.96	0.89
Mean			62.2	86.9	24.7	4.99	1.27	1.24
st. d.			±2.6	±3.6	±4.5	±0.20	±0.07	±0.07

C = cholesterol gallstones
CA = cholesterol gallstones and amorphous gallstones occurring together

Tab. 15 (Experiment 2). Occurrence of gallstones and other data for female hamsters fed the basal diet with addition of 0.1% chenodeoxycholic acid.

Animal no.	Days on diet	Gall-stones	Weight at start g	Weight after 6 weeks g	Weight gain 6 weeks g	Weight of liver g
2	42	C	71	93	22	5.57
14	42	C	62	78	16	4.94
22	42	C	49	77	28	4.64
34	42	0	53	81	28	5.84
48	42	C	62	82	20	5.11
58	42	C	64	80	16	5.20
69	43	C	55	70	15	3.73
80	43	0	56	73	17	5.16
88	43	C	56	83	27	5.96
Mean			58.7	79.7	21.0	5.13
st. d.			±2.2	±2.2	±3.0	±0.23

C = cholesterol gallstones
0 = no gallstones

In the group receiving the basal diet without addition, 3 out of the 11 males and 3 out of the 9 females had amorphous gallstones, and none of the animals in the group had cholesterol gallstones.

In the group receiving the diet with 0.1 % chenodeoxycholic acid, 10 out of the 11 males, and 7 out of the 9 females had cholesterol gallstones,

Tab. 16 (Experiment 2). Occurrence of gallstones and other data for male hamsters fed the basal diet with addition of 0.1% cholic acid.

Animal no.	Days on diet	Gall-stones	Weight at start g	Weight after 6 weeks g	Weight gain 6 weeks g	Weight of liver g	Weight of l. testis g	Weight of r. testis g
7	42	0	71	88	17	5.96	1.34	1.29
26	42	0	62	82	20	5.39	1.25	1.27
38	42	0	64	88	24	5.38	1.36	1.38
43	42	0	63	87	24	5.28	1.37	1.31
51	42	0	58	89	31	4.68	1.16	1.10
61	42	0	49	76	27	4.79	1.25	1.16
74	43	0	69	92	23	6.23	1.51	1.46
84	43	0	72	93	21	6.63	1.40	1.35
93	43	0	57	82	25	4.59	1.06	1.00
95	43	0	55	81	26	4.85	1.15	1.12
100	43	A	64	66	2	5.04	1.23	1.27
Mean			62.2	84.0	21.8	5.35	1.28	1.25
st. d.			±2.1	±2.4	±3.2	±0.20	±0.04	±0.04

0 = no gallstones
A = amorphous gallstones

Tab. 17 (Experiment 2). Occurrence of gallstones and other data for female hamsters fed the basal diet with addition of 0.1% cholic acid.

Animal no.	Days on diet	Gall-stones	Weight at start g	Weight after 6 weeks g	Weight gain 6 weeks g	Weight of liver g
1	42	A	62	78	16	6.38
30	42	0	66	80	14	6.01
12	42	A	58	85	27	5.51
35	42	0	50	85	35	5.68
49	42	0	54	74	20	4.82
59	42	A	64	82	18	5.07
66	43	0	44	63	19	4.38
81	43	0	69	91	22	6.44
89	43	0	62	87	25	6.16
Mean			58.8	80.6	21.8	5.61
st. d.			±2.7	±2.8	±3.9	±0.24

A = amorphous gallstones
0 = no gallstones

but only 1 animal, 1 of the males, had amorphous gallstones, occurring together with cholesterol gallstones.

In the group receiving the diet with 0.1 % cholic acid, none of the animals had cholesterol gallstones, but 1 out of the 11 males and 3 out of the 9 females had amorphous gallstones.

Tab. 18 (Experiment 2). Occurrence of gallstones and other data for male hamsters fed the basal diet with addition of 0.1% dehydrocholic acid.

Animal no.	Days on diet	Gall-stones	Weight at start g	Weight after 6 weeks g	Weight gain 6 weeks g	Weight of liver g	Weight of l. testis g	Weight of r. testis g
8	42	0	64	77	13	3.95	1.32	1.21
16	42	A	61	84	23	4.63	1.27	1.16
28	42	0	55	81	26	4.38	1.28	1.34
30	42	0	56	82	26	4.60	1.29	1.22
39	42	0	65	97	32	4.60	1.43	1.43
44	42	0	61	84	23	4.60	1.36	1.31
53	43	0	71	84	13	5.91	1.29	1.26
62	43	0	75	93	18	6.08	1.34	1.27
75	43	0	65	75	10	4.68	1.07	1.04
85	43	C	58	76	18	4.89	1.11	1.09
94	43	CAM	52	61	9	4.24	0.52	0.59
Mean			62.1	81.3	19.2	4.78	1.21	1.18
st. d.			±2.1	±2.9	±3.5	±0.20	±0.07	±0.07

0 = no gallstones
A = amorphous gallstones
C = cholesterol gallstones
CAM = cholesterol gallstones, amorphous gallstones and "mixed gallstones" occurring together

Tab. 19 (Experiment 2). Occurrence of gallstones and other data for female hamsters fed the basal diet with addition of 0.1% dehydrocholic acid.

Animal no.	Days on diet	Gall-stones	Weight at start g	Weight after 6 weeks g	Weight gain 6 weeks g	Weight of liver g
4	42	0	66	82	16	5.61
18	42	0	50	83	33	5.01
23	42	0	50	63	13	4.19
25	42	0	53	79	26	5.53
36	43	0	58	83	25	6.78
52	43	0	73	99	26	7.83
73	43	0	69	84	15	5.84
82	43	A	44	79	35	5.77
92	43	0	58	73	15	4.81
102	43	0	65	83	18	5.79
Mean			58.6	80.8	22.2	5.72
st. d.			±3.0	±2.9	±4.0	±0.32

0 = no gallstones
A = amorphous gallstones

In the group receiving the diet with 0.1 % dehydrocholic acid, 1 out of the 11 males had cholesterol gallstones, another had amorphous gallstones,

Tab. 20 (Experiment 2). Occurrence of gallstones and other data for male hamsters fed the basal diet with addition of 0.1% deoxycholic acid.

Animal no.	Days on diet	Gall-stones	Weight at start g	Weight after 6 weeks g	Weight gain 6 weeks g	Weight of liver g	Weight of l. testis g	Weight of r. testis g
9	42	0	70	92	22	5.91	1.40	1.23
15	42	A	60	88	28	5.51	1.26	1.25
21	42	0	61	96	35	6.03	1.22	1.17
29	42	0	54	80	26	4.14	1.18	1.14
40	42	0	68	98	30	5.06	1.38	1.38
45	42	0	49	72	23	4.16	1.18	1.14
55	43	0	78	105	27	6.22	1.45	1.39
63	43	0	50	84	34	5.60	1.13	1.15
77	43	0	69	85	16	5.68	1.30	1.24
87	43	C	57	61	4	4.23	0.84	0.95
98	43	0	68	81	13	6.15	1.16	1.06
Mean			62.2	85.6	23.4	5.34	1.23	1.19
st. d.			±2.8	±3.8	±4.6	±0.25	±0.06	±0.04

0 = no gallstones
A = amorphous gallstones
C = cholesterol gallstones

Tab. 21 (Experiment 2). Occurrence of gallstones and other data for female hamsters fed the basal diet with addition of 0.1% deoxycholic acid.

Animal no.	Days on diet	Gall-stones	Weight at start g	Weight after 6 weeks g	Weight gain 6 weeks g	Weight of liver g
17	42	0	64	98	34	5.91
19	42	0	49	79	30	4.73
32	42	A	66	92	26	4.66
37	42	0	56	81	25	4.77
54	43	A	68	106	38	7.43
67	43	0	58	93	35	4.92
76	43	0	49	73	24	4.68
83	43	A	66	91	25	6.84
97	43	0	43	82	39	4.90
99	43	0	68	98	30	6.78
Mean			58.7	89.3	30.6	5.57
st. d.			±2.9	±3.2	±4.3	±0.34

0 = no gallstones
A = amorphous gallstones

and a third had cholesterol gallstones occurring together with amorphous gallstones and "mixed gallstones", i.e., gallstones containing both cholesterol and amorphous material. One out of the 10 females had amorphous gallstones.

Tab. 22. Summary of occurrence and non-occurrence of gallstones in experiment 1.

	Number of animals in group		Animals having cholesterol gallstones no.	%	Animals not having gallstones no.	%
Basal diet without addition	males	9	3	33.3	6	66.7
	females	10	1	10.0	9	90.0
	total	19	4	21.0	15	79.0
Basal diet plus 0.1% chenodeoxycholic acid	males	9	8	89.0	1	11.0
	females	10	10	100.0	0	0.0
	total	19	18	94.8	1	5.2
Basal diet plus 0.1% lithocholic acid	males	9	7	77.8	2	22.2
	females	10	7	70.0	3	30.0
	total	19	14	73.7	5	26.3

In the group receiving the diet with 0.1 % deoxycholic acid, 1 out of the 11 males had cholesterol gallstones and another had amorphous gallstones. Three out of the 10 females had amorphous gallstones.

Thus, among the four bile acids tested in this experiment, only chenodeoxycholic acid behaved as an efficient promoter of formation of cholesterol gallstones. The few cases of cholesterol containing gallstones found in the groups receiving dehydrocholic acid and deoxycholic acid do not represent a significant difference from the non-occurrence of this type of gallstones in the group receiving the basal diet without addition.

The occurrence of amorphous gallstones was less in all the groups receiving bile acids, than in the group receiving the unsupplemented basal diet, but only in the group receiving chenodeoxycholic acid was this difference marked, and even in this case not significant with a probability of 95 %.

As in experiment 1, all the bile acids tended to increase the weight of the liver and had no influence on the weight of the testes. The testes were of larger size than in our previous study on the influence of chenodeoxycholic acid and hyodeoxycholic acid on gallstone production (1).

Summary

Both chenodeoxycholic acid and lithocholic acid intensified the production of cholesterol gallstones when added at the level of 0.1 % to a basal diet under conditions in which production of cholesterol gallstones by the unsupplemented basal diet was low or moderate. But the effect of chenodeoxycholic acid was somewhat more marked than the effect of lithocholic acid.

When added at the 0.1 % level to a basal diet under conditions in which the unsupplemented basal diet produced no cholesterol gallstones but a moderate incidence of amorphous pigmented gallstones, chenodeoxycholic acid gave rise to abundant formation of cholesterol gallstones, whereas only slight production of cholesterol gallstones was seen with dehydrocholic acid and deoxycholic acid and none with cholic acid. Occurrence of amorphous pigmented gallstones

Tab. 23. Summary of occurrence and non-occurrence of gallstones in Experiment 2[1]).

		Number of animals in group	Animals having C-gallstones only no.	%	Animals having A-gallstones only no.	%	Animals having C- and A-gallstones no.	%	Animals having C-, A-, and M-gallstones no.	%	Animals not having gallstones no.	%
Basal diet without addition	m	11			3	27					8	73
	f	9			3	33					6	67
	total	20			6	30					14	70
Basal diet plus 0.1% chenodeoxycholic acid	m	11	10	91			1	9			0	0
	f	9	7	78			0	0			2	22
	total	20					1	5			2	10
Basal diet plus 0.1% cholic acid	m	11			1	9					10	91
	f	9			3	33					6	67
	total	20			4	20					16	80
Basal diet plus 0.1% dehydrocholic acid	m	11	1	9	1	9			1	9	8	73
	f	10	0	0	1	10			0	0	9	90
	total	21	1	4.7	2	9.5			1	4.7	17	81
Basal diet plus 0.1% deoxycholic acid	m	11	1	9	1	9					9	82
	f	10	0	0	3	30					7	70
	total	21	1	4.7	4	19					16	76

[1]) C-gallstones = cholesterol gallstones
A-gallstones = amorphous gallstones
M-gallstones = "mixed gallstones"
m = males
f = females

was more frequent in the group of animals receiving the unsupplemented basal diet than in the groups receiving additions of dehydrocholic, deoxycholic, cholic, and – especially – chenodeoxycholic acid.

The importance of the stock diet used in the hamster colony to the development of gallstones occurring when the animals are exposed to the artificial experimental regimen is mentioned.

Zusammenfassung

Nicht nur Chenodeoxycholsäure, sondern auch Lithocholsäure intensivierte die Bildung von Cholesterin-Gallensteinen, wenn als Zulage in der Höhe von 0,1 % zu einer künstlichen Basal-Nahrung gegeben unter Bedingungen, unter denen die Bildung von Cholesterin-Gallensteinen durch die Basal-Nahrung allein nur gering oder mäßig war. Die Wirkung von Chenodeoxycholsäure war etwas stärker als die Wirkung von Lithocholsäure.

Wenn als Zulage in der Höhe von 0,1 % zu einer Basal-Nahrung gegeben unter Bedingungen, unter denen die Basal-Nahrung allein nicht zur Bildung von Cholesterin-Gallensteinen, sondern – in mäßigem Umfange – zur Bildung von amorphen, pigmentierten Gallensteinen Anlaß gab, führte Chenodeoxycholsäure zu reichlicher Bildung von Cholesterin-Gallensteinen, während Bildung von Cholesterin-Gallensteinen unter dem Einfluß von Dehydrocholsäure und Deoxycholsäure nur gering war, und unter dem Einfluß von Cholsäure nicht vorkam. Das Vorkommen von amorphen, pigmentierten Gallensteinen war häufiger in der mit der Basal-Nahrung ohne Zulage von Gallensäuren gefütterten Gruppe von Hamstern als in den mit Zulage von Dehydrocholsäure, Deoxycholsäure, Cholsäure und Chenodeoxycholsäure gefütterten Gruppen, besonders in der zuletzt erwähnten Gruppe.

Die Bedeutung der in der Hamster-Kolonie benutzten „Stock-Nahrung“ für die Entwicklung von Gallensteinen unter dem Einfluß der künstlichen Experimental-Nahrung wird erwähnt.

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