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Alimentary Production of Gallstones in Hamsters

24. Influence of orally ingested chenodeoxycholic acid and hyodeoxycholic acid on formation of gallstones

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With 18 tables

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A previous report (1) from this laboratory dealt with the influence of orally ingested bile acids on the alimentary production of gallstones in hamsters. The bile acids examined were commercially available preparations of cholic, deoxycholic, dehydrocholic, lithocholic and hyodeoxycholic acids. They were added in the amount of 0.1 % to a cholesterol gallstone producing fat-free diet having all its carbohydrate in the form of glucose. *Hyodeoxycholic acid* (3α-, 6α-dihydroxy-5β-cholanoic acid) was found to exert a marked inhibition on the formation of gallstones whereas the other bile acids tested showed only slight inhibitory effect on gallstone formation if any at all.

When these experiments were carried out, *chenodeoxycholic acid* (3α-, 7α-dihydroxy-5β-cholanoic acid) was not available in quantities sufficient for testing, but recently it has become accessible in a fair degree of purity.

We therefore found it worthwhile to compare the influence of orally ingested chenodeoxycholic acid on gallstone formation with that of similarly ingested hyodeoxycholic acid; and since the first trial with this purpose showed that chenodeoxycholic acid promotes formation of gallstones – an effect which is the opposite of that of hyodeoxycholic acid – we carried out some further experiments with chenodeoxycholic acid alone.

Experimental

Chenodeoxycholic acid was obtained from dr. *B. E. Hébert* of Weddel Pharmaceutical Ltd., London EC 1. It was tested for purity by thin layer chromatography (50 μg on silicagel H, solvent system benzene : ethanol : glacial acetic acid 30 : 10 : 2, visualization by charring with 50 % sulfuric acid). The substance was found to be accompanied by a small amount of impurities migrating slower than chenodeoxycholic acid.

Hyodeoxycholic acid was provided by drs. *U. Gloor* and *O. Wiss* of F. Hoffmann-La Roche & Co. A.G., Basel, Switzerland. When tested for purity by thin layer chromatography as above only a very faint spot migrating slower than hyodeoxycholic acid could be seen besides the spot respresenting hyodeoxycholic acid. A gas-liquid-chromatogram of the trimethylsilyether of the substance carried out by drs. *Gloor* and *Wiss* revealed no impurity at all.

Table 1. Composition of the basal diets

	g	g
Casein, crude[1])	20.0	20.0
Glucose	74.3	0
Rice starch	0	74.3
Salt mixture[2])	5.0	5.0
Vitamin mixture[3])	0.5	0.5
Choline chloride	0.2	0.2
	100.0	100.0

[1]) "Dairinex", from Dansk Mejeri Industri & Export Kompagni, Stege, Denmark.
[2]) The salt mixture indicated in Reference 9.
[3]) The vitamin mixture indicated in Reference 9.

The compositions of the basal diets used are indicated in table 1.

Three experiments were carried out.

The first experiment (Experimental series G 139) was carried out with young hamsters (33–37 days of age at beginning of the experimental feeding), and the basal diet with glucose as carbohydrate. The effects of the diet without addition, with addition of 0.1 % chenodeoxycholic acid, and with addition of 0.1 % hyodeoxycholic acid were observed. The experimental feeding lasted 42–44 days.

The second experiment (Experimental series G 140) was also carried out with young hamsters (30–32 days of age at beginning of the experimental feeding) but with the basal diet having rice starch as carbohydrate. The effects of the diet without addition and with addition of 0.1 % chenodeoxycholic acid were observed. The experimental feeding lasted 42 days.

The third experiment (Experimental series G 141) was carried out with old hamsters (from 112 to about 320 days of age at beginning of the experimental feeding) and the basal diet with glucose as carbohydrate. The effects of the diet without and with addition of 0.1 % chenodeoxycholic acid were observed. The experimental feeding lasted 42 days.

The young hamsters used in the first and second experiment were newly weaned from our stock colony. The old hamsters used in the third experiment had served as breeding animals in our stock colony.

During the feeding experiments the hamsters were housed in wire screen cages, two hamsters of the same sex in one cage. Diet and water were available *ad libitum*. The hamsters were weighed weekly. 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.

Results and discussion

The individual results are presented in tables 2–15. Summaries of each experiment with respect to gallstone formation are given in tables 16–18.

None of the hamsters had diarrhea.

Experiment 1. Young hamsters, glucose diet (tables 1–7 and 16).

In the group of animals receiving the diet *without addition*, 15 out of 22 males, and 16 out of 26 females had cholesterol gallstones. Amorphous

Table 2 (Experiment 1)
Occurrence of gallstones, and other data for young male hamsters fed the glucose diet without addition
(Exp. series G 139, group 1337)

Animal number	Days on diet	Gall-stones[1])	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	45	60	15	3.83	0.15	0.13
2	42	O	48	63	15	3.58	0.09	0.06
3	42	C	49	63	14	3.25	0.09	0.09
4	42	C	51	68	17	3.82	0.11	0.12
9	42	C	58	69	11	3.60	0.13	0.12
10	42	C	62	76	14	3.88	0.20	0.18
13	42	C	48	66	18	3.34	0.62	0.63
14	42	C	48	65	17	3.45	0.13	0.14
15	42	O	58	68	10	3.71	0.15	0.14
16	42	C	60	69	9	3.72	0.10	0.10
17	42	C	65	83	18	4.69	0.12	0.15
18	42	C	47	56	9	3.09	0.12	0.11
19	42	C	49	60	11	3.35	0.09	0.09
20	42	C	44	58	14	2.93	0.09	0.09
21	42	C	56	68	12	3.60	0.24	0.24
22	42	C	50	68	18	3.74	0.11	0.11
23	44	O	48	59	11	2.97	0.07	0.08
33	44	C	42	65	23	3.65	0.07	0.07
34	44	O	36	57	21	3.13	0.04	0.03
35	44	O	38	57	19	3.48	0.08	0.08
36	44	O	43	60	17	3.26	0.08	0.09
37	44	O	46	66	20	3.46	0.13	0.12
Mean			49.6	64.7	15.1	3.52	0.14	0.14
st.d.			±1.6	±1.4	±2.1	±0.28	±0.02	±0.02

[1]) C = cholesterol gallstones, O = no gallstones

gallstones did not occur in this group. The bile, usually, was clear and yellow.

All the animals in the group receiving the diet with *0.1 % chenodeoxycholic acid*, 22 males and 26 females, had cholesterol gallstones. The gall bladders of these animals were completely filled up with undissolved cholesterol, partly in the form of single crystals, partly in the form of loose aggregates, and partly in the form of solid aggregates of crystals (the usual form of cholesterol gallstones seen in hamsters)[1]). The bile usually was clear and yellow; in a few cases rather viscous (gelatinous).

[1]) From the gall bladders of 8 males and 12 females in the group receiving chenodeoxycholic acid the precipitated material was collected (and pooled for each sex) into small centrifuge tubes, washed with 0.9 % NaCl solution and with distilled water, dried *in vacuo* over P_2O_5, weighed and extracted with ether, whereafter the content of cholesterol in the ether extractable fraction was deter-

Table 3 (Experiment 1)
Occurrence of gallstones, and other data for young female hamsters fed the glucose diet without addition
(Exp. series G 139, group 1337)

Animal number	Days on diet	Gall-stones[1])	Weight at start g	Weight after 6 weeks g	Weight gain 6 weeks g	Weight of liver g
2	42	C	47	66	19	3.77
3	42	C	51	73	22	4.30
5	42	C	53	62	9	3.28
6	42	C	48	64	16	3.45
7	42	C	41	60	19	3.15
9	42	C	60	79	19	4.02
11	42	C	56	72	16	4.32
12	42	C	48	59	11	3.23
13	42	C	52	66	14	3.23
17	42	C	56	72	16	3.97
18	42	O	62	77	15	3.94
19	42	O	47	57	10	3.08
20	42	O	52	66	14	3.79
22	42	O	44	57	13	3.30
23	42	O	52	64	12	3.33
24	42	O	40	52	12	3.06
25	43	C	43	56	13	3.18
26	43	C	52	64	12	3.96
27	44	C	42	57	15	3.00
33	44	C	37	53	16	3.28
34	44	O	45	64	19	3.35
46	44	O	39	49	10	2.33
53	44	O	39	48	9	2.23
87	44	C	43	58	15	3.03
92	44	O	46	52	6	1.70
104	44	C	41	64	23	3.34
Mean st.d.			47.5 ±1.3	62.0 ±1.5	14.4 ±2.0	3.33 ±0.39

[1]) C = cholesterol gallstones, O = no gallstones

In the group of animals receiving the diet with *0.1 % hyodeoxycholic acid*, only 1 out of 22 males and 1 out of 26 females had cholesterol gallstones. Amorphous gallstones did not occur. In many cases, the bile was opaque, whitish, and under the microscope showed small filiform particles not exhibiting birefringence when viewed between crossed Nicols.

mined by the *Liebermann-Burchard* reaction. The percentage of cholesterol in the washed and dried material amounted to 96 % for the sample from the males and 93 % in the sample from the females. A correspondingly treated sample from 9 males in the group receiving the unsupplemented diet contained 98 % cholesterol.

Table 4 (Experiment 1)
Occurrence of gallstones, and other data, for young male hamsters fed the glucose diet with additions of 0.1% chenodeoxycholic acid (Exp. series G 139, group 1338)

Animal number	Days on diet	Gall-stones[1])	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	C	51	56	5	3.52	0.22	0.20
6	42	C	62	69	7	4.33	0.16	0.13
7	42	C	54	63	9	4.07	0.15	0.12
8	42	C	51	67	16	4.64	0.12	0.11
11	42	C	51	61	10	3.92	0.10	0.11
12	42	C	52	73	21	4.54	0.13	0.12
24	42	C	52	64	12	4.76	0.42	0.38
25	42	C	41	49	8	3.99	0.15	0.14
26	42	C	54	75	21	5.43	0.15	0.14
27	42	C	46	67	21	4.70	0.59	0.58
28	42	C	57	67	10	5.05	1.00	0.97
29	42	C	34	50	16	4.02	0.11	0.11
30	42	C	36	64	28	4.56	0.08	0.07
38	42	C	59	67	8	4.75	0.88	0.92
39	42	C	51	70	19	4.90	0.16	0.17
40	42	C	47	67	20	5.51	0.15	0.14
41	44	C	42	56	14	4.53	0.06	0.08
42	44	C	52	66	14	4.77	0.20	0.22
43	44	C	50	65	15	4.67	0.05	0.06
44	44	C	50	62	12	4.82	0.23	0.21
45	44	C	45	55	10	4.03	0.05	0.07
46	44	C	53	61	8	3.73	0.79	0.76
Mean			49.5	63.4	13.8	4.51	0.27	0.26
st.d.			±1.5	±1.5	±2.1	±0.35	±0.06	±0.05

[1]) C = cholesterol gallstones

The hamsters receiving the diets supplemented with bile acids showed lower gain in weight during 6 weeks than did the hamsters receiving the unsupplemented diet.

For the hamsters on the diet with hyodeoxycholic acid the difference in weight gain was significant ($0.01 > P$ for the males, $0.05 > P$ for the females) whereas for the hamsters on the diet with chenodeoxycholic acid the difference was of very low significance[1]).

The bile acid supplements tended to increase the weight of the liver, but the increase in liver weight was only significant for the animals on chenodeoxycholic acid ($0.05 > P$ for the males, $0.1 > P$ for the females).

[1]) Significances were estimated by determination of $t = \frac{\text{mean}_1 - \text{mean}_2}{\sqrt{\Delta_1^2 + \Delta_2^2}}$ (reference 2) and interpretation of the probability on the basis of t and the number of individual data as indicated in table 2. 4. of reference 3.

Table 5 (Experiment 1)
Occurrence of gallstones, and other data, for young female hamsters fed the glucose diet with additions of 0.1% chenodeoxycholic acid (Exp. series G 139, group 1338)

Animal number	Days on diet	Gall-stones[1])	Weight at start g	Weight after 6 weeks g	Weight gain 6 weeks g	Weight of liver g
1	42	C	47	60	13	4.20
4	42	C	60	80	20	5.33
15	42	C	56	68	12	4.89
21	42	C	49	55	6	3.88
29	42	C	46	47	1	3.58
30	42	C	46	51	5	4.06
31	42	C	43	45	2	4.21
32	42	C	42	45	3	3.94
35	42	C	46	64	18	4.89
36	42	C	44	60	16	4.57
37	42	C	57	77	20	5.32
38	42	C	46	55	9	4.12
39	42	C	52	62	10	4.29
40	42	C	48	56	8	4.26
41	42	C	52	50	−2	3.95
42	42	C	55	71	16	5.47
43	43	C	55	75	20	6.27
44	43	C	44	58	14	5.02
45	44	C	37	54	17	3.87
47	44	C	44	52	8	3.99
51	44	C	41	54	13	4.04
52	44	C	41	54	13	4.65
58	44	C	44	52	8	4.03
75	44	C	51	58	7	3.70
107	44	C	46	53	7	3.58
108	44	C	44	56	12	4.09
Mean			47.5	58.2	10.6	4.39
st.d.			±1.1	±1.9	±2.2	±0.43

[1]) C = cholesterol gallstones

In the males, the bile acid supplements tended to increase the weight of the testes. For both bile acids the increase in weight of the testes was moderatley significant ($0.1 > P > 0.05$).

Experiment 2. Young hamsters, rice starch diet (tables 8–11 and 17).

With the unsupplemented rice starch diet, formation of gallstones usually is absent or rare. In the present experiment none of the hamsters on the unsupplemented rice starch diet, 5 males and 6 females, had gallstones, whereas in the group receiving the rice starch diet with 0.1% chenodeoxycholic acid, 5 out of 6 males and 5 out of 5 females had cholesterol gallstones. Other forms of gallstones did not occur.

Table 6 (Experiment 1)
Occurrence of gallstones, and other data, for young male hamsters fed the glucose diet with addition of 0.1% hyodeoxycholic acid
(Exp. series G 139, group 1339)

Animal number	Days on diet	Gall-stones[1])	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
47	42	O	43	50	7	3.95	0.23	0.23
50	42	O	56	53	−3	3.76	0.35	0.42
51	42	O	49	50	1	3.70	0.18	0.16
52	42	O	64	68	4	4.06	0.90	0.88
53	42	O	57	65	8	4.38	0.25	0.24
54	42	C	57	58	1	4.46	0.23	0.19
55	42	O	60	61	1	4.07	0.25	0.21
56	42	O	51	57	6	3.89	0.15	0.13
57	42	O	56	62	6	4.25	0.16	0.19
58	42	O	56	63	7	4.24	0.17	0.15
60	42	O	48	55	7	3.90	0.74	0.70
61	42	O	37	48	11	4.11	0.14	0.14
62	42	O	36	47	11	3.49	0.11	0.09
63	42	O	55	60	5	3.60	0.19	0.15
64	42	O	55	64	9	4.32	0.38	0.32
65	42	O	40	47	7	3.71	0.12	0.11
66	44	O	43	53	10	3.45	0.05	0.07
67	44	O	43	50	7	3.68	0.18	0.18
68	44	O	49	50	1	3.49	0.07	0.07
69	44	O	48	56	8	3.82	0.14	0.14
70	44	O	45	60	15	4.49	0.06	0.07
71	44	O	42	51	9	3.78	0.05	0.05
Mean			49.5	55.8	6.3	3.98	0.23	0.22
st.d.			±1.7	±1.4	±2.2	±0.35	±0.05	±0.04

[1]) O = no gallstones, C = cholesterol gallstones

The difference with respect to occurrence of gallstones between the male hamsters receiving the diet containing chenodeoxycholic acid and the male hamsters receiving the unsupplemented basal diet is significant with 95 % probability [cf. reference (4)]. For the females the difference was significant with 99 % probability.

In the hamsters on the rice starch diet, addition of 0.1 % chenodeoxycholic acid did not decrease the weight gain during 6 weeks; but rather seemed to have had the opposite effect.

The weight of the liver in both sexes, and the weight of the testes of the males were increased by addition of 0.1 % chenodeoxycholic acid as in experiment 1.

The difference in liver weight was highly significant for the females ($0.01 > P > 0.001$) but not for the males.

Among the males the difference with respect to weight of testes was significant ($0.05 > P > 0.02$).

Table 7 (Experiment 1)
Occurrence of gallstones, and other data, for young female hamsters fed the glucose diet with addition of 0.1% hyodeoxycholic acid
(Exp. series G.139, group 1339)

Animal number	Days on diet	Gall-stones[1])	Weight at start g	Weight after 6 weeks g	Weight gain 6 weeks g	Weight of liver g
28	42	O	49	47	−2	3.52
48	42	O	34	45	11	3.29
54	42	O	42	51	9	3.58
55	42	O	49	62	13	4.26
56	42	C	44	52	8	3.79
57	42	O	43	57	14	4.12
59	42	O	42	47	5	3.53
60	42	O	48	65	17	4.52
61	42	O	49	62	13	4.27
62	42	O	48	55	7	4.16
63	42	O	41	50	9	4.14
64	42	O	50	61	11	3.75
65	42	O	52	59	7	4.08
66	42	O	53	57	4	4.48
67	42	O	53	62	9	4.50
68	42	O	49	60	11	4.02
69	43	O	46	52	6	4.20
70	43	O	48	55	7	3.98
71	44	O	50	64	14	4.72
72	44	O	59	60	1	4.12
73	44	O	58	63	5	4.41
74	44	O	60	67	7	5.46
76	44	O	51	56	5	3.24
77	44	O	46	58	12	3.38
78	44	O	37	45	8	2.93
79	44	O	35	44	9	3.24
Mean st.d.			47.5 ±1.3	56.0 ±1.3	8.5 ±1.9	3.99 ±0.33

[1]) O = no gallstones, C = cholesterol gallstones

Experiment 3. Old hamsters, glucose diet (tables 12–15 and 18).

When hamsters are transferred from the stock diet to the fat-free glucose diet at a more advanced age, e.g. 107–116 days, the tendency to formation of cholesterol gallstones as a consequence of the dietary change is greatly reduced and may be replaced by a tendency to formation of amorphous pigmented gallstones (5). Amorphous pigmented gallstones are also sometimes observed in old hamsters on the stock diet.

In the present experiment with old hamsters, only 1 of the 12 males and none of the 10 females receiving the unsupplemented glucose diet had cholesterol gallstones. With the diet containing 0.1 % chenodeoxycholic acid 6 out of the 12 males and 6 out of the 10 females had cholesterol gallstones.

Table 8 (Experiment 2)
Occurrence of gallstones, and other data for young male hamsters fed the rice starch diet without addition
(Exp. series G 140, group 1341)

Animal number	Days on diet	Gall-stones[1])	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
2	42	O	51	58	7	2.81	0.85	0.95
6	42	O	50	54	4	2.65	0.92	0.93
10	42	O	40	54	14	2.70	0.68	0.72
12	42	O	60	71	11	3.43	1.09	1.17
20	42	O	41	60	19	3.23	0.91	0.91
Mean			48.4	59.4	11.0	2.96	0.89	0.94
st.d.			±3.7	±3.1	±4.8	±0.17	±0.07	±0.07

[1]) O = no gallstones

Table 9 (Experiment 2)
Occurrence of gallstones, and other data for young female hamsters fed the rice starch diet without addition
(Exp. series G 140, group 1341)

Animal number	Days on diet	Gall-stones[1])	Weight at start g	Weight after 6 weeks g	Weight gain 6 weeks g	Weight of liver g
3	42	O	51	65	14	3.38
4	42	O	41	54	13	2.86
5	42	O	43	62	19	2.58
7	42	O	45	59	14	2.51
8	42	O	48	54	6	2.31
18	42	O	41	47	6	2.09
Mean			44.8	56.8	12.0	2.62
st.d.			±1.6	±2.7	±3.1	±0.19

[1]) O = no gallstones

When the two sexes are considered as one group, the difference with respect to occurrence of cholesterol gallstones between the animals on the diet with chenodeoxycholic acid and the animals on the unsupplemented basal diet is significant with 95 % probability.

(Because of the somewhat irregular occurrence of amorphous gallstones in old hamsters on stock diet, it is less certain to draw conclusions from the observed difference with respect to the prevalence of this type of gallstones.)

The main conclusion from all three experiments is that addition of 0.1 % chenodeoxycholic acid to the basal diet greatly increases the

Table 10 (Experiment 2)
Occurrence of gallstones, and other data for young male hamsters fed the rice starch diet + addition of 0.1% chenodeoxycholic acid (Exp. series G 140, group 1342)

Animal number	Days on diet	Gall-stones[1])	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	53	63	10	3.78	1.07	1.14
13	42	C	50	73	23	4.73	1.05	1.06
14	42	C	49	74	25	4.09	1.14	1.16
16	42	C	51	72	21	4.53	1.12	1.09
21	42	C	44	63	19	3.66	1.08	1.11
22	42	O	43	70	27	4.38	1.07	1.18
Mean st.d.			48.3 ±1.7	69.2 ±1.8	20.8 ±2.4	4.20 ±0.95	1.09 ±0.02	1.12 ±0.02

[1]) C = cholesterol gallstones, O = no gallstones

Table 11 (Experiment 2)
Occurrence of gallstones, and other data for young female hamsters fed the rice starch diet with addition of 0.1% chenodeoxycholic acid (Exp. series G 140, group 1342)

Animal number	Days on diet	Gall-stones[1])	Weight at start g	Weight after 6 weeks g	Weight gain 6 weeks g	Weight of liver g
9	42	C	51	67	16	5.17
11	42	C	42	56	14	3.71
15	42	C	55	97	42	6.23
17	42	C	44	60	16	4.66
19	42	C	47	58	11	3.63
Mean st.d.			47.8 ±2.4	67.6 ±7.6	19.8 ±8.0	4.68 ±0.48

[1]) C = cholesterol gallstones, O = no gallstones

tendency to formation of cholesterol gallstones not only among young hamsters on the glucose diet (which in itself produces cholesterol gallstones in young hamsters), but also among young hamsters on the rice starch diet (with which production of cholesterol gallstones usually is absent or rare), and among old hamsters on the glucose diet (which usually is much less effectiv in producing cholesterol gallstones in old hamsters than in young hamsters). These results are different from those of *Thistle & Schoenfield* (6), according to which ingestion of 1 g chenodeoxycholic acid per day to women through 4 months increased the solubilizing capacity of bile for cholesterol thus decreasing lithogenic potential.

Table 12 (Experiment 3)
Occurrence of gallstones, and other data for old male hamsters fed the glucose diet without addition
(Exp. series G 141, group 1343)

Animal number	Days on diet	Gall-stones[1])	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
47	42	O	71	77	6	5.02	1.29	1.23
48	42	O	106	86	−20	4.31	0.90	0.79
49	42	O	73	87	14	4.95	1.06	1.26
50	42	O	110	92	−18	4.42	1.25	1.22
55	42	O	112	116	4	9.47	2.73	1.94
56	42	O	97	110	13	8.01	1.58	1.51
57	42	O	114	102	−12	7.66	1.68	1.49
58	42	O	142	124	−18	7.01	1.82	1.73
63	42	A	102	107	5	5.54	1.87	1.92
11	42	O	113	93	−20	4.52	1.45	1.45
59	42	O	70	60	−10	2.61	1.21	1.18
92	42	C	67	73	6	3.56	1.21	1.20
Mean			98.1	93.9	− 4.2	5.59	1.50	1.41
st.d.			±6.7	±5.4	± 8.6	±0.58	±0.13	±0.10

[1]) O = no gallstones, C = cholesterol gallstones, A = amorphous gallstones

Table 13 (Experiment 3)
Occurrence of gallstones, and other data for old female hamsters fed the glucose diet without addition
(Exp. series G 141, group 1343)

Animal number	Days on diet	Gall-stones[1])	Weight at start g	Weight after 6 weeks g	Weight gain 6 weeks g	Weight of liver g
45	42	A	176	144	−32	6.69
47	42	A	135	113	−22	5.78
51	42	A	160	143	−17	7.77
53	42	O	139	118	−21	7.46
55	42	O	125	127	2	7.04
56	42	A	141	128	−13	6.91
57	42	O	93	88	− 5	5.02
58	42	A	131	129	− 2	7.78
65	42	O	146	145	− 1	9.98
66	42	O	130	122	− 8	6.31
Mean			137.6	125.7	−11.9	7.07
st.d.			±6.9	±5.5	±8.8	±0.42

[1]) O = no gallstones, A = amorphous gallstones

Table 14 (Experiment 3)
Occurrence of gallstones, and other data for old male hamsters fed the glucose diet with addition of 0.1% chenodeoxycholic acid (Exp. series G 141, group 1344)

Animal number	Days on diet	Gall-stones[1])	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
51	42	C	83	85	2	5.34	1.31	1.33
52	42	C	92	87	−5	5.67	1.34	1.36
53	42	C	93	90	−3	5.77	1.14	1.34
54	42	C	117	101	−16	4.81	1.49	1.81
60	42	O	129	112	−17	6.54	1.69	1.65
61	42	O	122	115	−7	5.48	1.77	1.76
62	42	A	118	101	−17	5.48	1.38	1.30
12	42	O	107	98	−9	5.06	1.51	1.35
13	42	C	77	70	−7	3.87	1.16	0.34
31	42	C	68	72	4	3.91	1.58	1.35
32	42	O	79	80	1	4.04	1.04	1.02
100	42	O	73	52	−21	3.56	0.60	0.58
Mean st.d.			96.5 ±6.1	88.6 ±5.3	−7.9 ± 8.1	4.96 ±0.27	1.33 ±0.09	1.27 ±0.13

[1]) C = cholesterol gallstones, A = amorphous gallstones, O = no gallstones

Table 15 (Experiment 3)
Occurrence of gallstones, and other data for old female hamsters fed the glucose diet with addition of 0.1% chenodeoxycholic acid (Exp. series G 141, group 1345)

Animal number	Days on diet	Gall-stones[1])	Weight at start g	Weight after 6 weeks g	Weight gain 6 weeks g	Weight of liver g
43	42	C	110	102	−8	5.42
44	42	O	121	101	−20	6.34
46	42	O	99	92	−7	9.50
49	42	C	126	89	−37	7.00
50	42	C	118	86	−32	4.33
54	42	C	117	95	−22	5.61
59	42	O	139	122	−17	7.70
60	42	C	151	139	−12	6.80
63	42	O	113	94	−19	5.34
64	42	C	148	121	−27	7.41
Mean st.d.			124.2 ±5.4	104.1 ±5.5	−20.1 ± 7.7	6.55 ±0.46

[1]) C = cholesterol gallstones, O = no gallstones

Table 16. Summary of occurrence and non-occurrence of gallstones in Experiment 1 (young hamsters, characteristics of basal diet: glucose, no added fat)

	number of animals in group		animals with cholesterol gallstones		animals without gallstones	
			no.	%	no.	%
Basal diet without addition	males	22	15	68.2	7	31.8
	females	26	16	61.5	10	38.5
	total	48	31	64.6	17	35.4
Basal diet plus 0.1% cheno-deoxycholic acid	males	22	22	100.0	0	0.0
	females	26	26	100.0	0	0.0
	total	48	48	100.0	0	0.0
Basal diet plus 0.1% hyo-deoxycholic acid	males	22	1	4.5	21	95.5
	females	26	1	3.8	25	96.2
	total	48	2	4.2	46	95.9

The finding of a more or less pronounced increase in weight of the liver and weight of the testes as a result of adding chenodeoxycholic acid to the diet reminds of the finding by other investigators [e.g. *Hunt* et al. (7)] of proliferation of bile ductules caused by dietary lithocholic acid. Since lithocholic acid may be formed by the action of intestinal bacteria on chenodeoxycholic acid, it will be of interest to examine how far the effects of chenodeoxycholic acid observed in the present experiments are caused by the conversion product lithocholic acid.

The finding in exp. 1 of a greatly reduced incidence of gallstones among the hamsters receiving hyodeoxycholic acid confirms the result of the earlier study (1) carried out with an impure preparation of this bile acid. The bile of the hamsters receiving hyodeoxycholic acid was not normal, however, and hyodeoxycholic acid was found to retard growth and moderately increase weight of the testes.

Beher et al. (8) have found that hyodeoxycholic acid has a hypocholesteremic effect in mice fed cholesterol. Whether this observation has any relation to the antilithogenic effect of hyodeoxycholic acid in hamsters is not known.

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Summary

Chenodeoxycholic acid added at the level of 0.1 % to the diet:

1. *enhanced* the production of cholesterol gallstones in young hamsters reared on a diet (having glucose as carbohydrate and containing no added fat) which *per se* produces cholesterol gallstones in young hamsters,

Table 17. Summary of occurrence and non-occurrence of gallstones in Experiment 2
(young hamsters, characteristics of basal diet: rice starch, no added fat)

	number of animals in group		animals with cholesterol gallstones no.	%	animals without gallstones no.	%
Basal diet without addition	males	5	0	0.0	5	100.0
	females	6	0	0.0	6	100.0
	total	11	0	0.0	11	100.0
Basal diet plus 0.1% cheno-deoxycholic acid	males	6	5	83.3	1	16.7
	females	5	5	100.0	0	0.0
	total	11	10	90.9	1	9.1

2. *produced* cholesterol gallstones in young hamsters reared on a diet (having rice starch as carbohydrate and containing no added fat) with which production of gallstones otherwise is rare or absent,

3. *produced* cholesterol gallstones in old hamsters which usually exhibit a considerable degree of resistance to induction of cholesterol gallstones when shifted from the stock diet to the diet having glucose as carbohydrate and containing no added fat, and

4. tended to increase the weight of liver and testes in the young hamsters.

Pure *hyodeoxycholic acid* added at the level of 0.1 % to a diet having glucose as carbohydrate and containing no added fat:

1. greatly *inhibited* formation of cholesterol gallstones in young hamsters (thereby confirming the result previously obtained with impure hyodeoxycholic acid) but introduced certain macroscopic and microscopic abnormalities in the appearance of the bile,

2. reduced growth rate, and

3. tended to increase the weight of the testes.

Zusammenfassung

Die Beigabe von 0,1 % *Chenodeoxycholsäure* zur Nahrung:

1. *verstärkte* die Bildung von Cholesterin-Gallensteinen bei jungen Hamstern, wenn die Basalnahrung (Charakteristika: Glukose als Kohlenhydrat, kein Fettzusatz) an sich die Bildung von solchen Gallensteinen bei jungen Hamstern herbeiführt,

2. *induzierte* Bildung von Cholesterin-Gallensteinen bei jungen Hamstern, wenn die Basalnahrung (Charakteristika: Reisstärke als Kohlenhydrat, kein Fettzusatz) an sich nicht oder nur selten zur Bildung von Gallensteinen Anlaß gibt,

3. *induzierte* Bildung von Cholesterin-Gallensteinen bei alten Hamstern, welche sonst eine beträchtliche Resistenz gegen Bildung von Cholesterin-Gallensteinen aufweisen, wenn sie von der „Stocknahrung" zu der Nahrung, welche Glukose als Kohlenhydrat und keinen Fettzusatz enthält, überführt werden und

4. tendierte zu Erhöhung des Gewichts der Leber und der Testes der jungen Hamster

Table 18. Summary of occurrence and non-occurrence of gallstones in Experiment 3
(old hamsters, characteristics of basal diet: glucose, no added fat)

	number of animals in group		animals with cholesterol gallstones		animals with amorphous gallstones		animals without gallstones	
			no.	%	no.	%	no.	%
Basal diet without addition	males	12	1	8.3	1	8.3	10	83.3
	females	10	0	0.0	5	50.0	5	50.0
	total	22	1	4.5	6	27.3	15	68.2
Basal diet plus 0.1% chenodeoxycholic acid	males	12	6	50.0	1	8.3	5	41.7
	females	10	6	60.0	0	0.0	4	40.0
	total	22	12	54.5	1	4.5	9	41.0

Die Beigabe von 0,1 % reiner *Hyodeoxycholsäure* zur Nahrung:

1. *verhinderte* weitgehend die Bildung von Cholesterin-Gallensteinen bei jungen Hamstern, wenn die Basalnahrung (Charakteristika: Glukose als Kohlenhydrat, kein Fettzusatz) sonst die Bildung von solchen Gallensteinen bei jungen Hamstern herbeiführt (wodurch das Ergebnis früherer mit unreiner Hyodeoxycholsäure angestellten Versuche bestätigt wurde) führte aber gewisse makroskopisch und mikroskopisch erkennbare Anomalitäten der Galle herbei,

2. verminderte die Gewichtszunahme der jungen Hamster während des Versuches und

3. tendierte zu Erhöhung des Gewichts der Leber und der Testes der jungen Hamster.

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