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Alimentary Production of Gallstones in Hamsters

25. Inhibition of production of cholesterol gallstones by 2-(p-chlorophenoxy)-isobutyric acid ethyl ester (Clofibrate)

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With 10 tables

(Received May 21, 1971)

The abundant formation of cholesterol gallstones in young hamsters receiving a diet in which the carbohydrate is glucose in contrast to the absence or rare occurrence of such gallstones in young hamsters receiving a diet in which the carbohydrate is rice starch is related to the fact that in the hamsters on the glucose diet more cholesterol is synthesized from acetate than in the hamsters on the rice starch diet [*Jensen* and *Dam* (1), *Muroya* et al. (2)].

According to the last-mentioned investigators the enhanced activity of cholesterol synthesis from acetate in hamsters on the glucose diet appears to be due to increase of enzymic activities before mevalonate in the biosynthetic pathway of cholesterol in the liver.

It is, therefore, of interest to examine whether formation of cholesterol gallstones in hamsters receiving the glucose diet can be prevented or at least greatly reduced by ingestion of substances capable of inhibiting the biosynthesis of cholesterol at a suitable stage.

Certain observations concerning this question have already been made as far as one of our earlier experiments (3) showed that addition of 1 % cholesterol to a gallstone producing diet for hamsters diminished the incidence of cholesterol gallstones in both sexes. Dietary cholesterol has been shown to act as an inhibitor of the biosynthesis of cholesterol in several species, e.g. rats [*Tomkins* et al (4)], dogs [*Gould* et al. (5)], and monkeys [*Cox* et al. (6)] and probably has the same effect in hamsters, although this has not yet been proved directly. Furthermore, some investigators, e.g. *Byers* and *Friedman* [working with rats (7)], assume that biliary cholesterol originates from endogenously synthesized cholesterol even when the diet contains cholesterol[1]). Therefore, the observed reduction of the incidence of cholesterol gallstones in the cholesterol-fed hamsters could have been an effect of reduced synthesis of cholesterol.

[1]) Under certain circumstances (species and dosage), transfer of dietary cholesterol to bile cholesterol may be marked, however. In chicks it has been found that the amount of cholesterol per g dry matter of the bladder bile can be increased considerably by incorporation of cholesterol into the diet (8), and *Osuga* and *Portman* (9) have produced cholesterol gallstones in squirrel monkeys by feeding cholesterol.

Table 1 (Experiment 1)
Occurrence of gallstones, and other data for young male hamsters fed the glucose diet without additions

Animal number	Days on diet	Gall-stones[1])	Weight at start g	Weight after 6 weeks g	Weight gain, 6 weeks g
2	42	C	67	82	15
6	42	C	67	76	9
12	42	C	57	66	9
15	42	O	29	39	10
16	42	C	48	59	11
20	42	C	50	61	11
21	42	C	50	63	13
23	42	C	46	53	7
Mean			51.8	62.4	10.6
st.d.			±4.3	±4.6	±6.3

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

However, other effects of dietary cholesterol, especially a possible increased production of bile acids, might also have contributed to the observed result[2]).

Our present study deals with the influence of the ethyl ester of 2-(p-chlorophenoxy)-isobutyric acid (Clofibrate) on the formation of cholesterol gallstones in hamsters.

In studies with rats, *Avoy* et al. (10) have found that this substance – probably in an indirect way – inhibits one or more steps located on the acetyl to mevalonate pathway beyond the point at which fatty acid and ketone body synthesis branch off.

Schweppe and *Jungmann* (11) made the additional observation that Clofibrate stimulates the *in vitro* synthesis of cholesterol esters from free cholesterol by a rat liver preparation, particularly the oleate and linoleate.

In the experiments to be reported here, Clofibrate was tested at two different levels, 0.4 % and 1.0 %, as addition to the fat-free basal diet having glucose as the only carbohydrate. At the higher level a very marked protection against formation of cholesterol gallstones was observed without induction of formation of amorphous pigmented gallstones, but growth of the animals was markedly impeded.

[2]) The use of cholesterol in dietary experiments, generally, is complicated by the circumstance that absorption of a substantial amount of cholesterol from the intestine requires the presence of a certain amount of fat in the diet. In our study (3) the basal diet contained 12 % rice starch and 10 % butter-fat. In hamsters, such a diet usually produces some cases of amorphous pigmented gallstones, and it was noted that dietary cholesterol increased the incidence of this type of gallstones in the females. This effect, however, is probably unrelated to an influence of the biosynthesis of cholesterol.

Table 2 (Experiment 1)
Occurrence of gallstones, and other data for young female hamsters fed the glucose diet without addition

Animal number	Days on diet	Gall-stones[1])	Weight at start g	Weight after 6 weeks g	Weight gain, 6 weeks g
3	42	C	44	55	11
4	42	C	51	59	8
7	42	C	58	70	12
10	42	C	58	66	8
11	42	C	54	61	7
13	42	C	56	63	7
14	42	C	50	65	15
17	42	C	39	53	14
18	42	C	49	60	11
19	42	C	45	53	8
31	42	C	45	63	18
Mean			49.9	60.7	10.8
st.d.			±1.9	±1.7	±2.5

[1]) C = cholesterol gallstones

Experimental

Housing and treatment of the hamsters were as described in our previous paper (12). The basal diet was the glucose diet without addition of fat indicated in our paper (12).

Ethyl 2-(p-chlorophenoxy)-isobutyrate (Clofibrate) was obtained from Scanmeda Ltd., Copenhagen, under the trade name Atromidin, and from Alfred Benzon, Copenhagen, under the trade name Recolip, in both cases in the form of capsules containing 250 mg each. The content of a sufficient number of capsules was quantitatively brought into solution in ether and the ethereal solution distributed over a weighed amount of the basal diet. After evaporation of the ether the mixture was thoroughly stirred in a mechanical mixing machine.

In the first experiment (Exp. Series G 138) Clofibrate was incorporated into the diet at the level of 0.4 %. Eight male and 11 female hamsters received the basal diet without addition. Eight males and 12 females received the diet with 0.4 % Clofibrate. The animals were 34–41 days old at the beginning of the feeding period which lasted 42 days.

In the second experiment (Exp. Series G 139) Clofibrate was incorporated into the diet at the level of 1.0 %. Twentytwo male and 26 female hamsters received the basal diet without addition. Twenty males and 26 females received the diet with 1 % Clofibrate. The animals were 33–37 days old at the beginning of the experimental feeding which lasted 42–44 days[3]).

[3]) The experiment with 1 % Clofibrate was carried out simultaneously with the experiment with 0.1 % chenodeoxycholic acid in our previous study (12). Therefore, the control group without addition with which the effect of 1 % Clofibrate is to be compared is identical with the control group with which the effect of 0.1 % chenodeoxycholic acid was compaired in our study (12).

Table 3 (Experiment 1)
Occurrence of gallstones, and other data for young male hamsters fed the glucose diet with addition of 0.4% Clofibrate

Animal number	Days on diet	Gall-stones[1])	Weight at start g	Weight after 6 weeks g	Weight gain, 6 weeks g
1	42	O	59	67	8
8	42	C	57	69	12
24	42	C	51	57	6
26	42	C	41	50	9
27	42	C	49	59	10
29	42	C	44	58	14
33	42	C	54	59	5
37	42	O	61	63	2
Mean			52.0	60.3	8.3
st.d.			±2.5	±1.9	±3.1

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

Table 4 (Experiment 1)
Occurrence of gallstones, and other data for young female hamsters fed the glucose diet with addition of 0.4% Clofibrate

Animal number	Days on diet	Gall-stones[1])	Weight at start g	Weight after 6 weeks g	Weight gain, 6 weeks g
5	42	C, A	57	64	7
9	42	C	56	60	4
22	42	C	31	59	28
25	42	C	41	45	4
28	42	O	42	56	14
30	42	O	41	51	10
32	42	O	63	60	−3
34	42	C	59	61	2
35	42	O	56	61	5
36	42	C	49	54	5
38	42	O	50	56	6
39	42	O	56	55	−1
Mean			50.1	56.8	7.3
st.d.			±2.7	±1.6	±3.1

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

Table 5 (Experiment 2)
Occurrence of gallstones, and other data for young male hamsters fed the glucose diet without addition

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*

Results and discussion

The individual results are presented in tables 1–8. Summaries of the results with respect to gallstone formation are given in tables 9 and 10.

None of the hamsters had diarrhea.

In Experiment 1, one out of the 8 male hamsters receiving the diet without addition had no gallstones, whereas the 7 others had cholesterol gallstones.

Two out of the 8 males receiving the diet supplemented with 0.4 % Clofibrate had no cholesterol gallstones. The six others had cholesterol gallstones. Obviously, the difference of the percentages of animals not having gallstones in the two groups of males is not significant.

All the 11 females receiving the diet without addition had cholesterol gallstones.

Table 6 (Experiment 2)
Occurrence of gallstones, and other data for young female hamsters fed the glucose diet without addition

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			47.5	62.0	14.4	3.33
st.d.			±1.3	±1.5	±2.0	±0.39

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

Six out of the 12 females receiving the diet supplemented with 0.4 % Clofibrate had no gallstones, the other 6 had cholesterol gallstones and one of them had also amorphous pigmented gallstones.

The difference of the percentages of animals not having gallstones in the two groups of females is significant with a probability of 95 %.

The addition of 0.4 % Clofibrate seems to have reduced weight gain in males as well as in females but the difference is not significant.

It was, therefore, desirable to repeat the trials with a larger number of males and females and a higher level of Clofibrate.

In Experiment 2, seven out of the 22 males receiving the diet without addition had no gallstones, the 15 others had cholesterol gallstones.

Table 7 (Experiment 2)
Occurrence of gallstones. and other data for young male hamsters fed the glucose diet with addition of 1% Clofibrate

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
48	42	O	51	50	— 1	2.51	0.10	0.10
49	42	O	59	37	—22	3.12	0.16	0.15
72	42	O	47	38	— 9	2.39	0.08	0.06
73	42	O	49	50	1	3.70	0.19	0.14
74	42	O	44	44	0	3.04	0.09	0.08
75	42	O	51	51	0	3.71	0.10	0.08
76	42	O	52	51	— 1	3.49	0.13	0.15
77	42	O	46	48	2	3.39	0.14	0.15
78	42	O	45	49	4	3.17	0.08	0.08
79	42	O	52	56	4	3.68	0.10	0.10
80	42	O	57	58	1	3.42	0.12	0.11
81	42	O	58	56	— 2	3.76	0.11	0.11
82	42	O	57	50	— 7	3.37	0.34	0.33
83	42	C	50	45	— 5	3.21	0.08	0.08
84	42	O	46	41	— 5	2.39	0.10	0.09
85	42	O	42	46	4	3.18	0.10	0.09
86	44	O	55	55	0	3.82	0.21	0.19
87	44	O	50	50	0	3.47	0.05	0.06
88	44	O	59	44	—15	3.33	0.07	0.08
89	44	O	42	47	5	2.87	0.08	0.09
Mean			50.6	48.3	— 2.3	3.25	0.12	0.12
st.d.			±1.2	±1.3	± 1.8	±0.31	±0.02	±0.02

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

Nineteen out of the 20 males receiving the diet with 1% Clofibrate had no gallstones, one had cholesterol gallstones.

The difference between the two groups of males with respect to percentage of animals not having gallstones is significant with 99% probability.

Ten out of the 26 females receiving the diet without addition had no gallstones, 16 had cholesterol gallstones.

None of the 26 females receiving the diet with 1% Clofibrate had gallstones. The difference with respect to the percentage of the animals not having gallstones in the two groups of females is also significant with a probability of 99%.

Thus, it is evident that Clofibrate given at the 1% level affords a marked degree of protection against formation of cholesterol gallstones.

There was no significant difference with respect to the weight of the liver between the groups receiving the unsupplemented diet and the

Table 8 (Experiment 2)
Occurrence of gallstones, and other data for young female hamsters fed the glucose diet with addition of 1% Clofibrate

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
8	42	O	64	55	— 9	3.92
14	42	O	57	51	— 6	3.54
16	42	O	67	56	—11	3.80
80	42	O	41	37	— 4	2.89
82	42	O	37	40	3	2.89
83	42	O	46	52	6	3.56
84	42	O	45	46	1	3.11
85	42	O	50	54	4	4.10
86	42	O	43	50	7	3.17
88	42	O	37	44	7	3.13
89	42	O	38	43	5	3.06
90	42	O	44	48	4	3.81
91	42	O	46	42	— 4	2.92
93	42	O	46	34	—12	2.90
94	42	O	46	49	3	3.21
95	42	O	53	49	— 4	3.51
96	43	O	44	42	— 2	2.95
97	43	O	48	39	— 9	3.11
98	44	O	43	46	3	3.04
99	44	O	49	44	— 5	3.15
100	44	O	56	64	8	4.35
101	44	O	58	54	— 4	4.16
102	44	O	53	54	1	3.66
103	44	O	39	28	—11	2.24
105	44	O	47	49	— 2	3.64
106	44	O	39	41	2	2.81
Mean			47.5	46.6	— 1.1	3.33
st.d.			± 1.6	± 1.5	± 2.2	±0.31

[1]) O = no gallstones

groups receiving the diet with 1% Clofibrate. Neither was there a significant difference with respect to the weight of the testes in the males receiving the diet without or with 1% Clofibrate.

The weight gain, however, was greatly reduced in the groups receiving the diet with 1% Clofibrate, even to the degree that the mean weight gain became slightly negative in these groups. The significance of the difference in weight gain without or with 1% Clofibrate is very high ($0.001 > P$) for both sexes. Since weight curves are not given it ought to be mentioned that after the moderate drop in body weight usually occurring during the first week on an artificial diet, the mean body weight increased gradually both in the groups on the unsupplemented diet and in the groups receiving

Table 9. Summary of occurrence and non-occurrence of gallstones in *Experiment 1*

	Number of animals in group		Animals with cholesterol gallstones		Animals with amorphous gallstones		Animals without gallstones	
			no.	%	no.	%	no.	%
Basal diet without addition	males	8	7	87.5	0	0	1	12.5
	females	11	11	100.0	0	0	0	0
Basal diet plus 0.4% Clofibrate	males	8	6	75.0	0	0	2	25.0
	females	12	6	50.0	1*)	8.3	6	50.0

*) This animal also had cholesterol gallstones.

Table 10. Summary of occurrence and non-occurrence of gallstones in *Experiment 2*

	Number of animals in group		Animals with cholesterol gallstones		Animals with amorphous gallstones		Animals without gallstones	
			no.	%	no.	%	no.	%
Basal diet without addition	males	22	15	68.2	0	0	7	31.8
	females	26	16	61.5	0	0	10	38.5
Basal diet plus 1% Clofibrate	males	20	1	5.0	0	0	19	95.5
	females	26	0	0	0	0	26	100.0

diet with 1% Clofibrate, but much less in the latter groups which did not regain the initial mean body weight completely.

It could be thought that the impairment of growth had a causal relationship to the marked protection against gallstones afforded by 1% Clofibrate. This is not likely, however, since in several of our earlier studies [(13, 14) and unpublished experiments] it has occurred that some of the hamsters on unsupplemented glucose diet failed to grow, and yet these animals had cholesterol gallstones.

Acknowledgement

Thanks are due to Scanmeda Ltd., Copenhagen for gifts of Atromidin (Clofibrate) and to *Alfred Benzon*, Copenhagen for gift of Recolip (Clofibrate).

Summary

At the dietary level of 1%, 2-(p-chlorophenoxy)-isobutyric acid ethyl ester (Clofibrate) afforded a marked protection against formation of cholesterol gallstones in both sexes of young hamsters receiving a fat-free diet in which the

total carbohydrate was glucose. This effect was accompanied by a marked inhibition of growth of the young hamsters.

At the dietary level of 0.4 %, Clofibrate afforded a much lesser and less significant protective effect against gallstone formation and no significant inhibition of growth was observed.

Zusammenfassung

Zulage von 2-(p-chlorophenoxy)-isobuttersäure-äthylester (Clofibrat) in der Höhe von 1 % der Nahrung schützte weitgehend gegen Bildung von Cholesterin-Gallensteinen bei jungen Hamstern beider Geschlechter, welche mit einer fettfreien Nahrung, deren Kohlenhydrat aus Glucose bestand, gefüttert wurden.

Diese Wirkung war von markierter Wachstumshemmung begleitet.

Wenn die Höhe der Zulage von Clofibrat nur 0,4 % der Nahrung betrug, war die Schutzwirkung gegen Bildung von Cholesterin-Gallensteinen viel geringer und weniger signifikant, und signifikante Wachstumshemmung wurde nicht beobachtet.

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