# Dissolution of gallstones in hamsters by 3-hydroxy-3-methylglutaric acid[1]

D. Kritchevsky, J.A. Story[2] and D.M. Klurfeld

*The Wistar Institute of Anatomy and Biology, 36th Street at Spruce, Philadelphia (PA 19104, USA), 1 March 1978*

*Summary.* Addition of 0.05–0.20% of 3-hydroxy-3-methylglutaric acid to a lithogenic hamster diet resulted in a marked reduction in the incidence of gallstones in hamsters.

Cholesterol gallstones in hamsters can be induced by feeding a semipurified fat-free diet whose major components are sucrose and casein[3]. Appearance of gallstones in hamsters can be inhibited by substitution of whole wheat or rolled oats for sucrose[4], by addition of pectin, lignin or psyllium[5] or by administration of a cholesterol lowering agent such as cholestyramine[6] or clofibrate[7]. We have investigated the effects of 3-hydroxy-3-methylglutaric acid (HMG), an inhibitor of cholesterol synthesis[8], on development and regression of gallstones in hamsters. HMG has been shown to be hypocholesteremic in rats[9,10], rabbits[11,12] and man[13]. The results of our experiments with HMG are reported here.

*Materials and methods.* Male Syrian hamsters were used in all experiments. The lithogenic diet (LD) which was first described by Dam and Christensen[3], consisted of sucrose (74.3%), casein (20%), salt mix USP XIV (5%), vitamin mix (0.5%) and choline chloride (0.2%). Additions were made at the expense of the sucrose.

In the 1st experiment 180 hamsters were fed the LD for 30 days. At this time 11 of the surviving animals were killed and their gall bladders examined for opacity of bile (which we took to be a pre-precipitation condition) or for presence of gallstones. The remaining hamsters were separated into 6 groups which were fed one of the following diets: laboratory ration (LR); LR plus 0.05% HMG; LD plus 0.07% chenodeoxycholic acid (LDC); LDC plus 0.05% HMG; LD plus 0.05% HMG and LD plus 0.2% HMG. Chenodeoxycholic acid was used because of its ability to dissolve gallstones in man[14]. 8 animals from each group were autopsied after 30 days and the remainder after 90 days.

In a 2nd experiment 100 hamsters were fed the LD for 42 days. 26 of the 86 surviving hamsters were killed at this time and their gall bladders examined. The remaining 60 animals were divided into 2 groups of 30 each and were fed either the LD or LD plus 0.2% HMG for another 50 days, at which time they were killed and their gall bladders examined. We are indebted to Calbiochem, San Diego, California for the generous gifts of chenodeoxycholic acid and HMG used in these studies.

*Results and discussion.* The results of the 1st experiment are summarized in table 1. It is evident that in the case of every test diet, save that containing chenodeoxycholic acid plus HMG, the percentage of animals with gallstones was lower than that observed on the lithogenic diet. Laboratory ration has been shown to cause regression of cholesterol-induced gallstones in hamsters[15]. In this experiment, after 60 days, laboratory ration resulted in only 35% of the test animals exhibiting gallstones. Addition of HMG (0.05%) to the lab ration reduced incidence of gallstones to 22%. Among the test diets, that containing 0.2% HMG resulted in the smallest percentage of animals with gallstones and the greatest percentage with normal, non-lithogenic bile.

In the 2nd experiment (table 2), administration of a lithogenic diet to hamsters for 42 days resulted in a 39% incidence of gallstones. Continuation of this regimen for another 50 days resulted in 74% of animals exhibiting gallstones. Addition of 0.2% HMG to the lithogenic diet resulted in a 27% incidence of gallstones and 23% of animals with normal bile. The data indicate that HMG fed at a level of 0.2% of the diet will cause dissolution of gallstones even when it is fed as a component of a lithogenic diet.

Table 1. Influence of 3-hydroxy-3-methylglutaric acid (HMG) and chenodeoxycholic (CDCA) on gallstone dissolution in hamsters

| Diet | Duration (days) | No. | Gall bladder findings: Stones | Biliary opacity | Normal bile |
|---|---|---|---|---|---|
| LD* | 30 | 11 | 8 | 2 | 1 |
| Lab. Ration (LR) | 30 | 8 | 1 | 7 | 0 |
| | 60 | 21 | 8 | 9 | 4 |
| LR + 0.05% HMG | 30 | 8 | 4 | 4 | 0 |
| | 60 | 18 | 4 | 11 | 3 |
| LD + 0.07% CDCA (LDC) | 30 | 8 | 6 | 2 | 0 |
| | 60 | 16 | 10 | 6 | 0 |
| LDC + 0.05% HMG | 30 | 8 | 4 | 0 | 4 |
| | 60 | 15 | 12 | 3 | 0 |
| LD + 0.05% HMG | 30 | 8 | 0 | 4 | 4 |
| | 60 | 17 | 9 | 7 | 1 |
| LD + 0.20% HMG | 30 | 8 | 3 | 3 | 2 |
| | 60 | 13 | 6 | 4 | 3 |

* LD, lithogenic diet of Dam and Christensen[3]: sucrose, 74.3%; casein, 20%; salt mix, 5%; vitamin mix, 0.5%; and choline chloride, 0.2%.

Table 2. Influence of 3-hydroxy-3-methylglutaric acid (HMG) (0.2%) on dissolution of gallstones in hamsters

| Diet | Duration (days) | No. | Gall bladder findings: Stones (%) | Opacity (%) | Normal (%) |
|---|---|---|---|---|---|
| LD* | 42 | 26 | 10 (39) | 15 (57) | 1 (4) |
| LD | 92 | 19/30** | 14 (74) | 5 (26) | 0 |
| LD + 0.2% HMG | 92 | 22/30** | 6 (27) | 11 (50) | 5 (23) |

* LD, lithogenic diet (see footnote, table 1); ** survival ratio.

1 Supported, in part, by grants HL-03299, AG-00076 and T32 CA-09171 and a Research Career Award, HL-0734 from the National Institutes of Health.

2 Present address: Dept Foods and Nutrition, Purdue University, W. Lafayette, Indiana 47907.

3 H. Dam and F. Christensen, Acta path. microbiol. scand. *30*, 236 (1952).
4 F. Christensen, H. Dam and I. Prange, Acta physiol. scand. *27*, 315 (1952).
5 F. Bergman and W. van der Linden, Z. Ernährungswiss. *14*, 217 (1975).
6 F. Bergman and W. van der Linden, Gastroenterology *53*, 418 (1967).
7 H. Dam, Am. J. Med. *51*, 597 (1971).
8 Z.H. Beg and P.J. Lupien, Biochim. biophys. Acta *260*, 439 (1972).
9 Z.H. Beg and M. Siddiqi, Experientia *23*, 380 (1967).
10 Z.H. Beg and M. Siddiqi, Experientia *24*, 791 (1968).
11 P.J. Lupien, M. Tremblay and Z.H. Beg, Atherosclerosis *18*, 407 (1973).
12 A.N.K. Yusufi, and M. Siddiqi, Atherosclerosis *20*, 517 (1974).
13 P.J. Lupien, D. Brun and M. Moorjani, Lancet *2*, 101 (1973).
14 J.I. Thistle and A.F. Hofmann, New Engl. J. Med. *289*, 655 (1973).
15 F. Bergman, A.H. Juul and W. van der Linden, Acta path. microbiol. scand. *78*, 179 (1970).